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Alpha-adrenergic influences on myometrial contractility in cycling and pregnant sows

by

Chih-Huan Yang

A Dissertation Submitted to the

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LIST OF ABBREVIATIONS

ARs	Adrenoceptors
AUCC	Area under the contraction curve
B_{eq}	Binding at equilibrium state
B_{max}	Maximum binding density
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
CARB	Carbachol
CATs	Catecholamines
CL	Corpus luteum
CNS	Central nervous system
CR	Concentration ratio
EC_{50}	Concentration to produce 50% of the maximal response
EPG	Early pregnancy
EPI	Epinephrine
F	Follicular phase
FSH	Follicular stimulating hormone
GnRH	Gonadotropin-releasing hormone
IC_{50}	Concentration to inhibit 50% of the maximal response
K_B	Dissociation constant of antagonist
K_D	Equilibrium dissociation constant
K_i	Inhibition constant
k_{ob}	Pseudo-first order association rate constant or observed association rate constant
k_{+1}	Second order association rate constant
k_{-1}	Dissociation rate constant

L	Luteal phase
LPG	Late pregnancy
LH	Luteinizing hormone
MLCK	Myosin light chain kinase
MPG	Mid-pregnancy
n_H	Hill coefficient
NE	Norepinephrine
pD_2	Negative logarithm of EC_{50}
pK_B	Negative logarithm of K_B
pK_i	Negative logarithm of K_i
PPT	Prepartum period
PROP	Propranolol
PRZ	Prazosin
r	Correlation coefficient
RAU	Rauwolscine
VDCC	Voltage-dependent Ca^{2+} channel
VLPG	Very late pregnancy
SE	Standard error
YOH	Yohimbine

GENERAL INTRODUCTION

Dissertation Organization

This dissertation is written in an alternate thesis format, as permitted by Graduate College. It includes a research objective, a background and literature review, a rationale, five manuscripts to be published, a general discussion, a general summary, a list of references cited in the general introduction, literature review, rationale and general discussion, and acknowledgements.

This dissertation contains the experimental results obtained by the author during his graduate study under the supervision of his major professor, Dr. Walter H. Hsu.

Research Objectives

The purpose of this research was to study the α-adrenergic effects of natural catecholamines acting on myometrial contractility *in vitro* using the longitudinal layer and to identify and characterize myometrial α-adrenoceptors (ARs) using radioligand binding assays in sows in the estrous cycle and during pregnancy.

The myometrial contractility was monitored following the administration of the AR agonist and antagonist. Since Ca^{2+} is a major signal for triggering contraction of smooth muscles, including myometrium, the objective of this research was also to determine whether natural catecholamine-induced myometrial contractions are mediated through an increase in Ca^{2+} influx or release from intracellular stores. α_1- and α_2-ARs were characterized and quantified in porcine myometrium to understand the relationship between the density of α-ARs and myometrial contractility and between the density of α-ARs and different reproductive stages during the estrous

cycle and pregnancy.

Background and Literature Review

The objective of this section is to provide concise informational background for the study of α-adrenergic influences of myometrial contractility in the sow.

<u>Anatomy of the Uterus</u>

The uterus, one of the essential organs for reproduction and gestation, is a hollow muscular organ which is continuous with the oviducts cranially and opens into the vagina caudally. It consists of body, horns and neck (cervix). The uterine horn in the pig is bicornuate type, which is long and convoluted (Mossman, 1977). In this type, two cornua always join at their cervical ends to form the body, which opens by a single cervical canal into the vagina. The uterine horns are remarkable for their length in pigs. In the nongravid state, each is about one meter long; at the height of pregnancy this may easily be doubled in length (Dyce et al., 1987; Hafez, 1987). The horns lie cranial to the pelvic inlet midway between dorsal roof and ventral floor of the abdomen and are suspended by extensive broad ligaments. The broad ligaments, which contain much smooth muscle, enlarge considerably during pregnancy, allowing and supporting the horns to sink to the abdominal cavity.

The wall of the uterus has three distinct layers: endometrium, myometrium and perimetrium. Perimetrium or tunica serosa, the outmost layer, is a thin serous membrane, which is the extension of the peritoneum. The innermost layer is the endometrium or tunica mucosa, consisting of the epithelium of the lumen, the uterine glands and the connective tissues. The myometrium or tunica muscularis is located between the perimetrium and the endometrium. The myometrium consists of two main

layers of smooth muscle, the outer thinner longitudinal layer and the inner thicker

circular layer with respect to the uterine lumen. The outer longitudinal layer, an

extension of the smooth muscle present in the mesometrium, is continuous throughout

the length of the uterus. The inner circular layer is apposed to the endometrium and

separated from the outer layer by a vascular layer (pars vasculosa).

The smooth muscle bundles of the longitudinal layer are arranged parallel to the

long axis of the uterus, and upon contracting tend to shorten the uterus cephalo-

caudally. Because of the continuity of the myometrium with the more fibrous cervix

which is secured by the broad ligament, contraction of the longitudinal muscle layer

tends to pull the ovarian end of the uterus caudally. In the gravid uterus at term, such

contraction may assist in the dilation of the cervix. In the circular layer, the bundles

are arranged concentrically around the long axis. Contraction in this layer serves to

constrict the uterine lumen (Finn and Porter, 1975).

The ovary does not apparently influence the length of the uterus until the

prepubertal gilt is about 100 days of age (Wu and Dziuk, 1988). The uterus grows

gradually until puberty, then it doubles in length and weight at the first estrus.

Embryos cause growth of the uterus during pregnancy beginning at day 18, continuing

until about day 30 of pregnancy (Wu et al., 1988). From day 18 to 30 the uterus

doubles in length but grows relatively little during the remainder of gestation. Growth

of the uterus is stimulated by each embryo and is limited to that section of the uterus

occupied by an embryo. The length of each pregnant uterine horn is dependent on the

number of fetuses within that horn, independent of the number in the opposite horn

(Dziuk, 1991).

The uterine wall makes a slow but constant gain in weight throughout

pregnancy (McDonald, 1989). There is no noticeable difference in the thickness

between the longitudinal layer and circular layer of the myometrium in different phases

of the estrous cycle in pigs (Thilander and Rodriguez-Martinez, 1989a). In the early

pregnancy, the longitudinal layer is separated from the thicker circular layer by the

connective tissue. As the pregnancy proceeds, the thickness of the longitudinal layer

decreases. In contrast, the thickness of the circular layer does not vary throughout

pregnancy (Thilander and Rodriguez-Martinez, 1989b). The myometrial layers in the

parturient pig have the same thickness as in the pregnant myometrium (Thilander and

Rodriguez-Martinez, 1990). The thickness of both layers in the placental regions is less

than in the nonplacental ones (Thilander and Rodriguez-Martinez, 1989b and 1990).

The typical porcine myometrial cells are elongated spindle shaped, and

irregularly outlined with numerous cytoplasmic projections. The nucleus is centrally

located, elongated and oriented longitudinally to the cell. The intercellular space

between the muscle cells is chiefly occupied by collagen fibers and fibroblasts. The

cell membranes of different cells are in close proximity to each other. The cell size in

pregnancy is larger than in the non-pregnant state as pregnancy proceeds. Moreover,

the average cell diameter in the placental regions is greater than in the non-placental

regions (Thilander and Rodriguez-Martinez, 1989a, 1989b and 1990).

The ultrastructure of the porcine myometrium at well-defined stages of the

estrous cycle, pregnancy and parturition has been studied (Thilander and Rodriguez-

Martinez, 1989a, 1989b and 1990). In general, the basic ultrastructure of porcine

myometrial cells in pregnant sows resembles that of non-pregnant ones. However, the

density of gap junctions begins to increase and the size of gap junctions becomes

larger two days before parturition. In contrast, gap junctions are few and small

throughout the rest of gestation and the estrous cycle. Gap junctions are intercellular

channels that link cells to their neighbors and allow the passage of inorganic ions and

small molecules (Peracchia, 1980; Revel et al., 1985; Spray and Bennett, 1985). The

development of myometrial gap junction is physiologically regulated by steroid

hormones (Garfield *et al.*, 1980). Estrogens promote and progesterone suppresses the formation of gap junctions. Steroid hormones are thought to control genomic mechanisms and synthesis of connexin 43 proteins, the major components of the gap junction. Estrogens, particularly estradiol, stimulate the synthesis of gap junction by interacting with its receptors and stimulating the specific genome responsible for coding for the gap junction protein (Garfield, 1994). The increased gap junctions of the myometrium prior to and during parturition may provide low resistance pathways between muscle cells, allow a rapid and synchronized spread of action potentials leading to well-coordinated contractions (Verhoeff *et al.*, 1986).

The uterus receives its blood and nerve supply through the broad ligaments. The middle uterine artery provides the main blood supply to the uterus in the pig. In addition, there is a cranial supply from a branch of the ovarian artery and a caudal supply from a cranial branch of the vaginal artery (Del Campo and Ginther, 1973).

The medial uterine artery arises from the umbilical artery, which is given off from the ventral wall of the internal iliac artery, one of the terminal branches of the abdominal aorta (Nunez and Getty, 1969). The medial uterine artery has a tortuous cranioventral course in the medial side of the broad ligaments. It usually divides into two main branches, i. e., cranial and caudal branches, in the middle part of the cranial third of the ligament. The cranial branch of the uterine artery divides several times and supplies the cranial half of the uterine horns. The caudal branch forms an arch in the mesometrium several centimeters from and parallel to the uterine horn. The branches to the uterine horn are interconnected at the mesometrial attachment, forming a series of loops which extends the length of the horn. The arterial arch terminates in a prominent anastomosis with the uterine branch of the vaginal artery. A network of these anastomotic vessels supplies blood to the uterine body and the cervix.

The utero-ovarian artery, originating from the abdominal aorta, mainly supplies

blood to the ovary, ovarian bursa and oviduct. Some of its branches anastomose with the cranial branches of the medial uterine artery and supply blood to the tips of the uterus.

The main venous drainages of the uterus are the medial uterine vein and utero-ovarian vein (Nunez and Getty, 1970; Del Campo and Ginther, 1973). The medial uterine vein courses parallel to the satellite artery embedded in the broad ligament of the uterus. It drains into the common iliac vein of caudal vena cava. The utero-ovarian vein drains a plexus, near the ovary. It, enclosed with ovary artery, courses in the anterior border of the broad ligament. Then it drains into the common iliac artery or caudal vena cava.

The distribution of adrenergic and cholinergic nerves in the porcine myometrium during the estrous cycle, pregnancy and parturition has been studied using histochemical methods and electromicroscopy (Thilander, 1989; Thilander and Rodriguez-Martinez, 1989a, 1989b, 1989c and 1990). Both adrenergic and cholinergic nerves are present in longitudinal and circular layers of porcine myometrium. Adrenergic nerves are present both in vascular and in non-vascular smooth muscles, whereas the cholinergic nerves mostly accompany the blood vessels.

The distribution of uterine adrenergic nerves in cycling (Thilander and Rodriguez-Martinez, 1989c) and pregnant pigs (Thilander, 1989), but not in immature pigs (Lakomy et al., 1983), differs from the pattern reported in other species (Owman and Sjöberg, 1966 and 1972; Rosengren and Sjöberg; 1967; Garfield, 1986). In lagomorpha (guinea pigs and rabbits) and carnivores (cats and dogs), the adrenergic nerves are evenly distributed throughout the uterine horns, while the pigs present very scanty innervation, except the cervix. The cervix has a rich innervation. In the rat, the adrenergic nerves predominantly innervate blood vessels, whereas in the porcine myometrium these nerves are also seen in synapsis with groups or bundles of non-

vascular muscle cells (Thilander, 1989).

Histochemical microscopy in pigs (Thilander, 1989), guinea pigs (Bell and Malcolm, 1978; Thorbert, 1978), rabbits (Rosengren and Sjöberg, 1968), humans (Nakanishi et al., 1969; Thorbert et al., 1979) and sheep (Sigger et al., 1986; Renegar and Rexroad, 1990) revealed that the fluorescence intensity and the diameter of adrenergic nerves decreases as pregnancy proceeds, which is consistent with the ultrastructural investigations. This decrease is more pronounced in placental regions than in non-placental regions (Thilander, 1989).

Concerning the nerve-muscle relationship, there is a low density of nerves to myometrial smooth muscle cells as compared to richly innervated smooth muscle such as rat vas deferens and urinary bladder. In the latter, each muscle cell is closely related to a nerve axon whereas in the myometrium nerve fibers are associated with groups or bundles of muscle cells (Silva, 1967; Adham and Schenk, 1969).

As previously indicated the cholinergic innervation of the porcine myometrium is mostly associated with blood vessels (Thilander, 1989). The circular muscle layer has a more dense nerve network than the longitudinal one. The cervix has the richest innervation. This pattern is unaffected throughout the estrous cycle and pregnancy.

The estrous cycle and ovarian steroids in the pig

The information concerning this part is mainly reviewed from the following references: Anderson, 1987; Catchpole, 1991; Dziuk, 1991; Evans, 1989; Flood, 1991; Hafez, 1987; Jainudeen and Hafez, 1987; McDonald, 1989; Pineda, 1989; Stabenfeldt and Edqvist, 1993.

Puberty of the gilt is usually attained by the age of 6 to 7 months. The domestic female pig is polytocous and nonseasonally polyestrous with estrus occurring at intervals normally about 21 days (ranges: 19 to 23 days). The period of pregnancy

starts with fertilization and ends with parturition. The average length of gestation in sows is 114 days (range: 112 to 116 days).

Classically, the estrous cycle consists of four phases, including proestrus, estrus, metestrus and diestrus. Proestrus is the period of rapid follicles growth under gonadotropic stimulation that precedes the onset of estrus. It lasts about 2 days. Proestrus is associated with progressively declining levels of progesterone due to the regression of the corpus luteum (CL) from the preceding cycle. At this stage, the female pig is exposed and behaviorally (e.g., restlessness, mounting of other animals, lordosis response) responds to progressively increasing levels of estrogens secreted by the developing follicles.

Estrus, the period of sexual receptivity, lasts about 2 to 3 days (an average of 40 to 60 h). Ovulation occurs spontaneously and usually between 36 to 42 h after the onset of estrus or about 6 h before the end of estrus. It is conventional to designate the first day of estrus as day 0 of the estrous cycle. Proestrus and the portion of the estrual period prior to ovulation form the follicular phase of the estrous cycle.

Metestrus lasts 1 or 2 days, which is the period of early CL development. The onset of metestrus is usually defined as beginning with the end of estrus. During this phase, the reproductive system switches from estrogens to progesterone dominance.

Diestrus is the period of mature luteal activity. It begins about 4 days after ovulation and ends with regression of the CL. It is the phase that the reproductive organs are under the dominant influence of progesterone. This phase is the longest phase of the cycle for all of the domestic species, including pigs. Metestrus and diestrus form the luteal phase of the estrous cycle.

The physiologic and behavioral changes are cyclic and repeated over time, unless normally interrupted by pregnancy, or by a variety of pathologic conditions, such as the development of follicular cysts.

Anestrus is a stage of sexual quiescence characterized by the lack of estrous behavior. Anestrus is a normal stage of the reproductive function in the prepuberal and in aged animals. Anestrus is also normal for the pregnant animal of all species. In fact, pregnancy is the most common cause of anestrus in polyestrous species, such as pigs.

During the luteal and early follicular phases of the cycle, the follicles are small, i.e., 2 to 5 mm in diameter. About 10 to 20 follicles approach preovulatory size (8 to 11 mm), while the number of smaller follicles declines (< 5 mm) during the proestrous and estrous phases. During the luteal phase of the cycle, which occurs between days 5 and 16, the number of follicles with 2 to 5 mm in diameter increases. During days 7 to 12, the CL are fully formed, functional, meaty, distinct, encapsulated, and endowed with a good blood supply. Day 12 is critical in the life of the CL of the sow. If viable embryos are present in the uterus, then the CL will continue their function. If viable embryos are not present, these CL will initiate irreversible regression. Luteolysis becomes apparent macroscopically by days 15, in which progesterone production is falling. By day 16 the CL have lost most of their vascularity, shrinkage in size has begun. By day 18 (proestrous phase), an increase occurs primarily in the growth of preovulatory follicles ($\geq$ 8 mm in diameter). The waning CL are evident by their white color (corpora albicantia) and soft texture which signals the end of their function.

The major steroid hormones involved in female reproductive processes are progesterone and estrogens. In addition, they may modify the density of ARs in myometrium in the estrous cycle and during pregnancy. Therefore, the following information of endocrinology will be restricted to these two ovarian steroid hormones.

Ovarian steroid-secreting activity of the CL is indicated by concentrations of progesterone and estrogens, which change dramatically and quite predictably throughout the estrous cycle. The synthesis of progesterone in the CL is controlled by

luteinizing hormone (LH) in the nonpregnant animals. Estradiol-17β and estrone are the estrogens which are produced by the theca interna and granulosa cells of the ovary in the pig. In addition, the placenta is the main site producing estrone, a metabolite of estradiol-17β. Although the adrenal cortex also produces estrogens, under normal conditions, quantities are insufficient to replace the normal ovarian production of estrogens.

Progesterone levels are low at estrus (day 0). Following ovulation at mid-estrus, the follicular remnants luteinize resulting in the formation of progesterone-producing CL. Plasma progesterone levels begin to increase abruptly and are followed by a precipitous decline, which is coincidental with luteolysis, 15 to 18 days after estrus. These progesterone levels in peripheral venous blood correspond to those patterns in ovarian venous blood throughout the cycle, and follow a pattern similar to the morphologic development and decline of the CL as well as ultrastructural changes in luteal cells.

Estrogens, primarily estrone in peripheral plasma begin to increase coincidentally with the decline and disappearance of progesterone between day 15 and 20 of the estrous cycle. Peak values of circulating estrogens occur about 24 h before the onset of behavioral estrus. This reflects rapid growth and maturation of graafian follicles during the late proestrous phase of the cycle. Soon after ovulation, estrogen levels decline and remain low during the luteal phase of the cycle.

The growth and maturation of ovarian follicles are dependent on secretion of adenohypophyseal gonadotropins, follicular stimulating hormone (FSH) and LH, from the anterior pituitary. FSH promotes ovarian growth and follicular maturation. FSH in synergy with LH facilitates follicular growth and estrogen production. LH is also important for the ovulatory process and the luteinization of the granulosa cells, which results in the formation of the CL. Rising blood levels of estrogen suppress the

pituitary release of FSH and facilitate release of LH. As estrogen levels rise, a surge of LH occurs at the onset of estrus triggering ovulation. Ovulation usually occurs about 24 h after the LH surge. Then LH levels decline to low levels during the remainder of the cycle.

The release of FSH and LH is controlled by gonadotropin-releasing hormone (GnRH), which is secreted from the median eminence and transported to the anterior pituitary by a portal system of capillaries. The secretion of gonadotropin is influenced by GnRH in two ways. The first way is to vary the frequency or amplitude of the pulse release of GnRH which is essential for the maintenance of LH and FSH secretion by the anterior pituitary. The second way to influence FSH and LH secretion is to change the sensitivity of the anterior pituitary to the pulses of GnRH through the modulatory effects of estrogen and progesterone. In general, increasing concentrations of estrogen cause an increase in sensitivity to GnRH and result in an increased release of gonadotropins. However, progesterone has an opposite effect.

The placenta, like the CL, is a transient endocrine organ. It secretes steroid hormones, such as estrogens and progesterone, which are released into the fetal as well as the maternal circulation. The placenta of the sow is not capable of synthesizing sufficient amounts of progesterone using acetate and cholesterol derived from the maternal circulation to maintain pregnancy. Therefore, CL are essential for maintenance of pregnancy to term in the pig. In addition, the placenta relies on fetal cortisol to induce activity of the placental enzymes to synthesize estrogens from progesterone (Jainudeen and Hafez, 1987). Hence, during the latter half of gestation, a high rate of estrogen production occurs in the placenta of farm animals, including sows.

If the pig is pregnant, the CL will be maintained beyond day 15. Plasma progesterone levels associated with the first 14 days of the estrous cycle and

pregnancy are identical (Bazer and First, 1983). However, after that time plasma progesterone levels decrease from 30 - 40 ng/ml on day 12 to 14 of pregnancy to 10 25 ng/ml on day 25 of pregnancy (Guthrie *et al.*, 1974; Robertson and King, 1974; Knight *et al.*, 1977). Then the levels remain fairly constant until about day 100 of pregnancy. After that, the progesterone concentrations start a pre-parturition decline to an average level of 5 ng/ml on the day of parturition. A further sharp decline to less than 1 ng/ml was observed within 24 h after farrowing (Robertson and King, 1974).

The feto-placental unit is the major source of estrogen production during pregnancy. Starting at day 12 of the gestation the blastocysts undergo a rapid growth consistent with production of estrogens, mainly estradiol-17β. The maternal concentration of estrone sulfate is present as early as day 16 and reaches a peak near day 30, then declines at day 35 of pregnancy. The feto-placental unit is assumed to be the source of estrone sulfate because of the positive relationship between the number of fetuses and concentration of estrone sulfate in maternal plasma. This increase in estrogen concentrations from blastocysts may prevent uterine luteolytic action by suppressing $PGF_{2\alpha}$ secretion into uterine venous system, because estrogen administration to nonpregnant pigs during the luteal phase prolongs the CL life span. Therefore, the importance of estrogen secretion from the blastocysts is to maintain the luteal function during critical phases of early pregnancy.

The concentrations of estrone and estradiol-17β begin to rise again around days 80 of gestation and then fall to basal levels after delivery of conceptuses (Robertson and King, 1974). This indicates that the rise in estrogens is associated with fetal maturity and is primarily of placental origin.
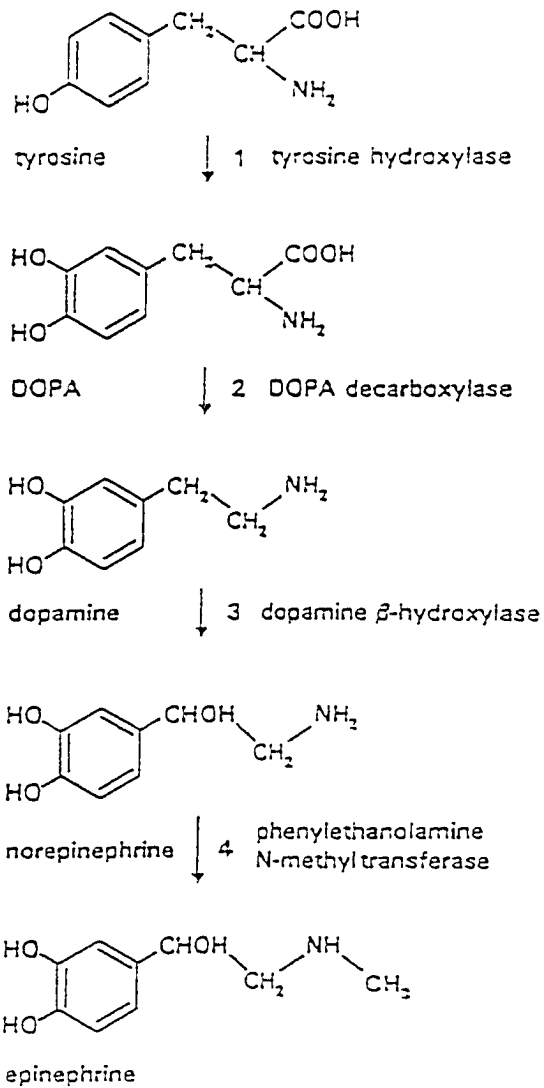
Catecholamines

The term catecholamine (CAT) designates the compounds which are derivatives of phenylethylamine, hydrooxylated in positions 3 and 4 of the aromatic ring (Euler, 1972) (Fig. 1). Dopamine is the biosynthetic precursor of epinephrine (EPI) and norepinephrine (NE). It acts essentially as a neurotransmitter in the central nervous system (CNS). NE is the neurotransmitter of sympathetic nerves. EPI is the only CAT in which the action is essentially hormonal which is mainly produced by the adrenal medulla, but is also found in the CNS.

Four steps are involved in the biosynthesis of CATs from tyrosine (Fig. 2). They include: 1) hydroxylation of the phenolic ring; 2) decarboxylation of the lateral chain; 3) hydroxylation of the lateral chain; and 4) N-methylation. All of the enzymes involved in the biosynthesis of CATs are soluble and are present in the cytosol of the chromaffin cells, with the exception of dopamine β-hydroxylase, which is found only in granules. Tyrosine is the normal precursor of CATs. Its source is essentially in dietary intake, but it is also derived from the hydroxylation of phenylalanine. Tyrosine is transformed into DOPA (3, 4-dihydroxyphenylalanine) by the action of tyrosine hydroxylase. The transformation of tyrosine into DOPA is the essential limiting step in the biosynthesis of CATs, since the activity of tyrosine hydroxylase in the adrenal medulla is 200 times less than DOPA-decarboxylase and dopamine β-hydroxylase (Hanoune, 1990). Because tyrosine hydroxylation is the key step in the synthesis of DOPA, two forms of the enzyme exist. One is not phosphorylated which is only slightly active, and the other, phosphorylated and very active. The phosphorylation step is under the control of a cAMP-dependent protein kinase. The activity of tyrosine hydroxylase is inhibited by a variety of compounds, particularly derivatives of tyrosine, such as α-methyltyrosine, and 3-iodotyrosine and its metabolites. Dopamine, NE and EPI also exert a negative feedback on the activity of this enzyme (Hanoune, 1990).

Fig. 1. The biosynthesis of catecholamines. The cofactors are: 1: Fe^{2+}-reduced pteridin; 2. pyridoxal phosphate; 3. ascorbic acid; and 4. S-adenosylmethionine.

tyrosine | 1 tyrosine hydroxylase

DOPA | 2 DOPA decarboxylase

dopamine | 3 dopamine β-hydroxylase

norepinephrine | 4 phenylethanolamine N-methyltransferase

epinephrine

Dopamine decarboxylase transforms DOPA into dopamine, which is competitively inhibited by several analogs, such as α-methyl DOPA. Hydroxylation of dopamine to NE is catalyzed by dopamine β-hydroxylase, in the presence of Ca^{2+}, ascorbic acid, and molecular oxygen. Dopamine β-hydroxylase is present in secretory granules associated with chromogranins, the function of which is unknown (Ganong, 1991).

The cytoplasmic enzyme, phenylethanolamine N-methyl transferase (PNMT), which is found in appreciable quantities only in the adrenal medullary cell and some neurons of the brain, catalyzes the conversion of NE to EPI. In these cells, NE apparently leaves the vesicles, is converted to EPI, and then enters other storage vesicles. NE is also stored in granules in adrenergic nerve terminals.

In the granulated vesicles, EPI and NE are bound to adenosine triphosphate (ATP) and associated with proteins chromogranins and essential ions, including Ca^{2+}, Mg^{2+} and ascorbates. The principal protein components are chromogranins (especially chromogranin A), dopamine β-hydroxylase and the precursors of Met- and Leu-enkephalin (Lewis et al., 1982; Viveros and Wilson, 1983). The CATs are held in the granulated vesicles by an active transport system, dependent on ATP and Mg^{2+}, and inhibited by reserpine.

The CATs are released from autonomic neurons and adrenal medullary cells by exocytosis. The process of exocytosis is initiated by acetylcholine released from the preganglionic neurons that innervate the secretory cells. Acetylcholine increases the permeability of the cells, and the Ca^{2+} that enters the cells from the extracellular fluid triggers exocytosis. The membrane of the granule becomes touching with the plasma membrane, and all of its contents, including CATs, ATP, chromogranins and dopamine β-hydroxylase, release into the blood because they are not membrane-bound. The storage vesicle is not reutilized, but rather is probably degraded. The synthesis of new

storage vesicles is necessary for an efficient synthesis.

The release of NE by the sympathetic neuronal terminals occurs in response to an action potential, along with an influx of Ca^{2+}. The secretion of EPI is increased by physiological or psychological stimuli, including hypoglycemia, physical exercise, hypoxia, anxiety, etc.

EPI and NE are degraded to biologically inactive products by oxidation and methylation (Fig. 2). Degradation essentially involves two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). COMT catalyzes the methylation of the hydroxyl group in position 3 and MAO catalyzes the oxidative deamination of the aliphatic chain. COMT is especially abundant in the liver and kidney, where the major portion of circulating CATs is degraded, but is not found in nerve endings. MAO is primarily located in the external membrane of mitochondria. It is distributed to all tissues, being particularly plentiful in the nerve endings where CATs are secreted.

Most circulating EPI and NE are O-methylated to metanephrine and normetanephrine (Fig. 2A). The O-methylated derivatives that are not excreted are largely oxidized to 3-methoxy 4-hydroxy-mandelic acid (VMA). Small amounts of O-methylated derivatives are conjugated to sulfates and glucuronides.

On the other hand, in the noradrenergic nerve endings (Fig. 2B), some of the NE, is being constantly converted by MAO to physiologically inactive deaminated derivatives, 3,4-dihydroxymandelic acid (DOMA) and its corresponding glycol, 3,4-dihydroxyphenylglycol (DOPEG). These compounds enter the circulation and may subsequently be converted to their corresponding O-methyl derivatives, VMA and 3-methyoxy 4-hydroxyphenylglycol (MOPEG), respectively.

After the release of NE from nerve terminals into the synaptic cleft, it is also inactivated by specialized transport systems, that mediate either neuronal uptake or

Fig. 2. A. Metabolism of circulating epinephrine and norepinephrine. Most of the conjugates are glucuronides and sulfates. B. Metabolism of norepinephrine in noradrenergic nerve endings. Epinephrine in nerve endings is presumably catabolized in the same pathway (From Ganong, 1991).
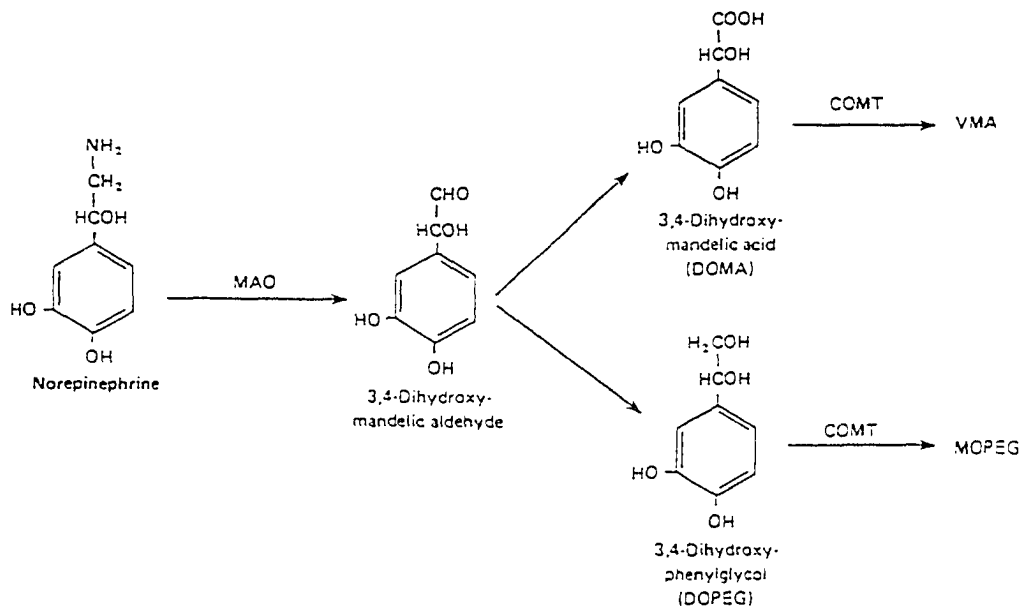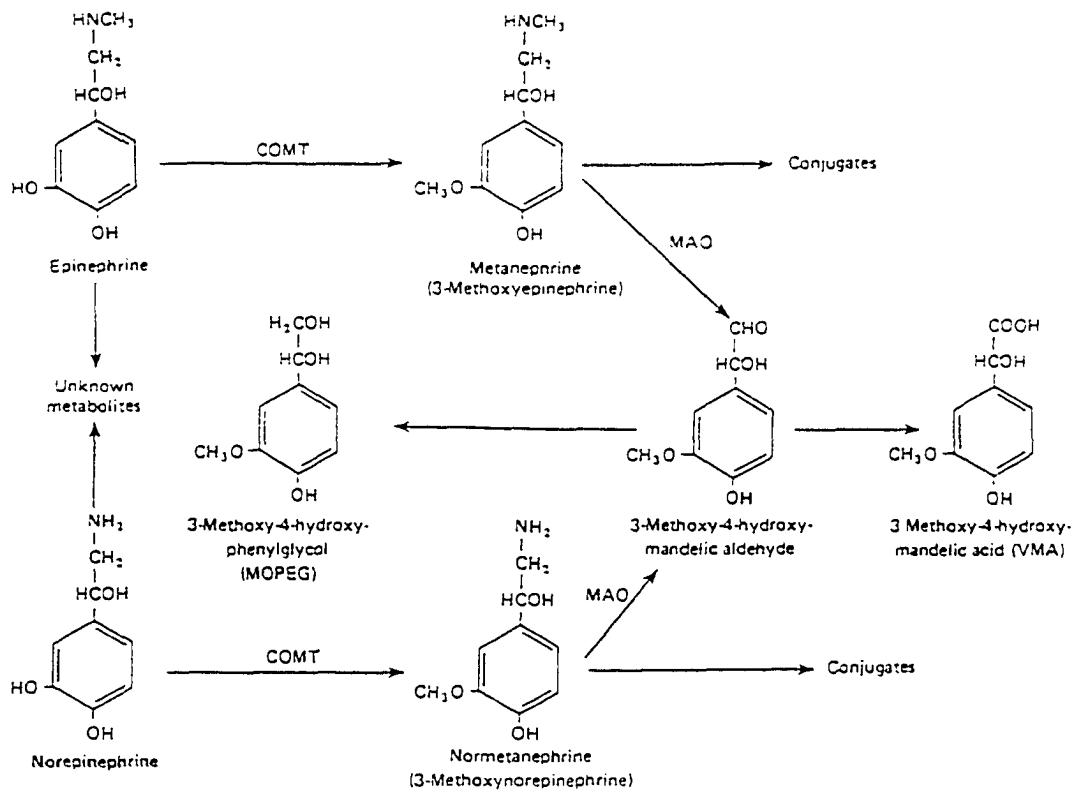
extranuronal uptake (Iversen, 1973). In neuronal uptake (uptake-1), the released NE is taken up into the adrenergic nerve terminals of the CNS and the peripheral autonomic nervous system, and involves an active transport system. Many drugs, including cocaine, desipramine, imipramine and amitryptiline, potentiate nerve stimulation by inhibiting this process. Extraneuronal uptake (uptake-2) occurs in various extraneural peripheral tissues (smooth muscles, heart, certain gland tissues). Physiologically, this uptake leads to a rapid intracellular degradation of CATs by COMT, in contrast with the "uptake-storage" of uptake-1. This process is inhibited by corticosterone, metepinephrine and normetepinephrine.

Classification of adrenoceptors

Adrenoceptors (ARs) are found in nearly all peripheral tissues and some neurons of CNS. They mediate the central and peripheral actions of the EPI and NE. Several types of neuronal varicosities have prejunctional (or presynaptic) ARs serving as auto- or heteroceptors that inhibit nerve evoked release of neurotransmitters.

The ARs were originally classified as α and β subtypes, based on the relative rank order of potency of six sympathomimetic amines, including EPI, NE and isoproterenol, on physiological response (Ahlquist, 1948). EPI was most potent in causing vasoconstriction, pupil dilation, uterine contraction, etc, and was designated as α-adrenergic. In contrast, isoproterenol was most potent in causing vasorelaxation, myocardial stimulation, bronchodilation and uterine relaxation, and was designated as β-adrenergic. It also was realized that both α- and β-ARs were present in the same tissue, such as uterus. This scheme, with modifications, still remains intact.

α-ARs were first subclassified based on their anatomical locations, as either α_1- ARs that were postsynaptic and mediated effector organ release (e.g. NE) or α_2-ARs that were presynaptic and regulated a negative feedback mechanism of

neurotransmitter release (Langer, 1974). The terms pre- and post-synaptic refer to the localization of the receptor sites with respect to the nerve terminal and effector organ. That is, postsynaptic receptors are located extraneuronally in the effector organ, whereas presynaptic receptors are situated on the membrane of the postganglionic neuron. However, this anatomical classification was confused by the existence of a_2-ARs in non-presynaptic locations, such as platelets and vascular smooth muscles (Berthelsen and Pettinger, 1977).

A functional classification of a-ARs was recommended on the basis of the type of function mediated by the receptor subtype (Berthelsen and Pettinger, 1977). a_1-ARs were proposed to mediate excitatory responses. Conversely, a_2-ARs were thought to mediate inhibitory responses in nature. However, NE-induced vasoconstriction could be inhibited by both a selective a_1-AR antagonist, prazosin (PRZ) and a selective a_2-AR antagonist, yohimbine (YOH) (Drew and Whiting, 1979). This indicated that the postsynaptic excitatory response was mediated not only by a_1-ARs but also a_2-ARs.

Later, a pharmacological subclassification is used to designate a_1- and a_2-ARs. This is based on the relative activity and affinity of specific agonists and antagonists, respectively (Starke, 1981). For instance, a-ARs that are activated by either methoxamine or phenylephrine, and that are antagonized by low concentrations of PRZ in a dose-dependent manner, are subclassified as a_1-ARs. In contrast, the ARs which are activated by either xylazine, clonidine or medetomidine, and the induced effect can be blocked competitively by either idazoxan, rauwolscine (RAU) or YOH, are designated as a_2-ARs.

It has been suggested that the ARs be divided into three families, the a_1-ARs, the a_2-ARs and β-ARs (Bylund, 1988). The rationale for this classification is based on three lines for evidence (Bylund, 1988; Bylund et al., 1994). First, the difference in affinity of selective drugs is 3 to 4 orders of magnitude between major subtypes (e.g.,

a_1, a_2, and β). However the affinity ratios among subtypes of each of these major groups are generally only 10 to 100. Second, second-messenger signalling pathways are different for each of these three major types. Finally, the predicted amino acid sequences of the ARs are more consistent with three rather than two major types (Bylund, 1992). Because the classification of a-ARs in this research did not use the method of molecular cloning, the foregoing brief review will not focus on that part.

Heterogeneity of a_1-Adrenoceptors   Although PRZ is a potent and highly selective a--AR antagonist, there is 100-fold range in the ability of PRZ to antagonize different a--AR-mediated responses (Agrawal *et al.*, 1984; Medgett and Langer, 1984; Flavahan and Vanhoutte, 1986). The wide range in PRZ affinity suggested the presence of two subtypes of a_1-AR with a high affinity for PRZ ($K_B < 0.4$ nM) and the other one with a lower affinity to PRZ ($K_B < 1.6$ nM) (Medgett and Langer, 1984).

Several a_1-AR antagonists, such as phentolamine, WB 4101, chlorethyl-clonidine (CEC), (+)-niguldipine and 5-methylurapidil were also used to distinguish a--AR subtypes. Phentolamine and WB 4101 produced biphasic displacement of specific [^{3}H]PRZ binding in rat cerebral cortex, while other a_1-AR antagonists, dihydroergocryptine and indoramin exhibited monophasic displacement. This suggested that phentolamine and WB 4101 could distinguish between high and low affinity of a_1-AR binding sites in rat cerebral cortex (Morrow *et al.*, 1985). Further studies confirmed these findings and designated the a_1-ARs with subnanomolar affinity for WB 4101 as a_{1A} and the a_1-ARs with low affinity as a_{1B} (Morrow and Creese, 1986).

Chlorethylclonidine (CEC), an irreversible alkylating derivative of clonidine, inactivated only approximately half of the a_1-ARs in the rat cerebral cortex, but did not inactivate the a_1-ARs in the rat hippocampus (Johnson and Minneman, 1987). However, all of the a_1-ARs in both brain regions were sensitive to another alkylating

drug, phenoxybenzamine. This indicated that CEC could differentiate between α_1-AR subtypes and that these subtypes had a differential distribution within different regions of the brain (Johnson and Minneman, 1987). Further studies showed that CEC inactivated nearly all of the α_1-ARs in rat liver and spleen, but very few of the α_1-ARs in the rat vas deferens (Han et al., 1987). Those α_1-ARs that were insensitive to CEC with high affinity sites for WB 4101, were designated as α_{1A} subtype. In contrast, those receptors which were sensitive to CEC with low affinity for WB 4101, were designated as α_{1B} subtype (Han et al., 1987).

Other α_1-AR antagonists, including benoxathian, 5-methyl-urapidil, (+)niguldipine and oxymetazoline also have a higher selectivity for the α_{1A} subtype than the α_{1B} subtype. In contrast, spiperone is a selective α_{1B}-AR antagonist.

A third distinct subtype of α_1-AR (α_{1C}) was cloned from a bovine brain cDNA library (Schwinn et al., 1990). The pharmacology of bovine α_{1C}-AR resembles that of the α_{1A}-AR. It possesses high affinity for PRZ. However, several ligands bind to α_{1C}-ARs with much higher affinities than α_{1A}-ARs. For instance, WB 4101 is 4-fold more selective for the α_{1C}-ARs than for the α_{1A}-ARs. Natural CATs, EPI and NE bind to the α_{1C}-AR with lower affinities relative to the α_{1A}-AR. Bovine α_{1C}-AR may also be differentiated by its sensitivity to CEC. CEC pretreatment only inactivated part of the α_{1C}-AR binding sites. Therefore, it is suggested that the sensitivity of CEC to the bovine α_{1C}-AR is between α_{1A}-ARs (insensitive) and the α_{1B}-ARs (sensitive).

Heterogeneity of α_2-Adrenoceptors   The α_2-ARs have been subclassified mainly by functional and radioligand binding studies, although several distinct α_2-AR proteins have been cloned and expressed.

Initially the subclassification of α_2-ARs was based on the ability of PRZ to inhibit the binding of [^{3}H]PRZ or [^{3}H]rauwolscine ([^{3}H]RAU) to tissue homogenates from a variety of isolated tissues or tumor cell lines (Bylund, 1985; Nahorski et al., 1985;

Petrash and Bylund, 1986). The α_2-ARs in the neonatal lung rat kidney and the NG108-15 cell (a neuroblastoma x glioma hybrid cell line) having a high affinity for PRZ and ARC 239 were designated as α_{2B} subtype (Bylund *et al.*, 1988). In contrast, the α_2-ARs in human platelets or in the HT29 cell (a human colonic adenocarcinoma cell line) having a low affinity for PRZ and ARC 239 were designated as α_{2A} subtype. Further studies showed that this subclassification scheme was not a result of species differences because both subtypes were detected in human brain cortex (Petrash and Bylund, 1986) and rat brain cortex (Kawahara and Bylund, 1985).

Although this subclassification is based mainly on data from radioligand binding studies, important functional studies on the inhibition of α_2-AR-mediated attenuation of cAMP production with PRZ have confirmed the existence of α_{2A}- and α_{2B}-subtypes in HT29 and NG108-15 cells, respectively (Bylund and Ray-Prenger, 1989).

Furthermore, several ligands have been shown to have selective actions for these two α_2-AR subtypes. For instances, benoxathian, BRL 44408 (Young *et al.*, 1989), oxymetazoline, RX 821001 (Niddam *et al.*, 1990), and WB 4101 are relatively selective for α_{2A}-ARs. In contrast, ARC 239, chlorpromazine, imiloxan, PRZ and spirocatrine are relatively selective for α_{2B}-ARs (Michel *et al.*, 1990). However, EPI and NE do not distinguish between α_{2A}- and α_{2B}-ARs (Bylund *et al.*, 1994).

Two additional subtypes, e.g. α_{2C} and α_{2D} have been identified by the correlation of the affinity for a variety of α-AR antagonists to compete for [^{3}H]RAU binding sites. The α_{2C}-AR has been found in a cell line derived from opossum kidney (Murphy and Bylund, 1988), in native opossum kidney (Blaxall et al., 1991) and in Y79 cells (a human retinoblastoma cell line) (Gleason and Hieble, 1992).

The α_{2C}-AR has similar characteristics to those of the α_{2B}-AR (e.g. a relative high affinity for PRZ), but the ratio of the affinities of PRZ and YOH is intermediate between those of the α_{2A}- and the α_{2B}-ARs (Murphy and Bylund, 1988). Moreover, the principal

characteristics of the α_{2C}-AR are a very high affinity for RAU and WB 4101, and a higher PRZ/oxymetazoline affinity ratio (Hieble and Ruffolo, 1995).

A fourth subtype, designated as α_{2D} has been found in bovine pineal gland (Simonneaux et al., 1991), rat submaxillary gland (Michel et al., 1989) and RINm5F cells (a rat pancreatic islet tumor cell line) (Remaury and Paris, 1992). This subtype has similar characteristics to α_{2A}-AR (e.g. a low affinity for PRZ, spiroxatrine and ARC 239), but has lower affinity for idazoxan, RAU and YOH than the other subtypes.

Several other tissues, including adipose tissues from rat, rabbit and hamster, and rabbit jejunal enterocytes, possess an α_2-AR having low affinity for RAU and YOH. It is likely that these tissues represent additional examples of the α_{2D}-subtype (Hieble and Ruffolo, 1995).

In the study of subclassification of presynaptic α_2-ARs, the radioligand binding techniques may not correspond precisely to any of the α_2-AR subtypes. The reason for this is not certain, but it is possible that the nerve terminals of sympathetic neurons are unable to provide sufficient amounts of plasma membrane proteins to perform radioligand binding assays. Therefore, functional studies, such as presynaptic α_2-AR-mediated inhibition of neurotransmitter release are used to subclassify presynaptic α_2-ARs (Ruffolo and Hieble, 1994). In general, the presynaptic α_2-ARs are considered as α_{2A}/α_{2D} subtype because of their low affinity to PRZ.

Heterogeneity of β-Adrenoceptors   All three β-AR subtypes (β_1, β_2, and β_3) have been identified by functional and pharmacological studies and molecular cloning, and can be activated by EPI and NE (Bylund et al., 1994). EPI and NE have differential affinities for β-AR subtypes. EPI and NE have equal potency at β_1-ARs. At β_2-ARs, however, EPI selectivity is up to 100-fold greater than NE (Lands et al., 1967a and 1967b). In contrast to β_1- and β_2-ARs, NE is more potent than EPI as a β_3-AR agonist to mediate biologic response (Bylund et al., 1994).

β_1-ARs are mainly present in heart and adipose tissues to increase heart rate and force of contraction, and lipolysis, respectively. β_2-ARs mediate smooth muscle relaxations, including airways, most blood vessels and uterus (Lands *et al.*, 1967a).

The avian β-AR of turkey erythrocyte has many similarities but also some significant differences, when compared to mammalian β_1-ARs (Neve *et al.*, 1986). This atypical β-AR has unusually low affinity to the β-AR antagonist propranolol. Based on the identification of selective agonists, such as BRL 37344, and the expression of a recombinant receptor, it is classified as a β_3-AR (Emorine *et al.*, 1989). The primary actions mediated by the β_3-ARs are lipolysis in white adipose tissue, thermogenesis in brown adipose tissue (Arch *et al.*, 1984), inhibition of glycogen synthesis in skeletal muscle (Challis *et al.*, 1988) and inhibition of contractile activity in gastrointestinal smooth muscle (Bond and Clark, 1988).

Adrenoceptors, both α and β, appear to belong to a superfamily of membrane receptors that transmit information into the interior of cells through coupling to guanine-nucleotide-binding, regulatory proteins (G-proteins) (Gilman, 1987; Lefkowitz and Caron, 1988; Birnbaumer, 1990; Summers and McMartin; 1993). However, the signaling mechanisms of the α- and β-AR classes are distinct. All subtypes of β-ARs are coupled by G, to link to the activation of adenylyl cyclase (AC) resulting in the generation of the second messenger cyclic 3',5' adenosine monophosphate (cAMP). α_1-ARs produce changes in cellular activity by increasing intracellular levels of free Ca^{2+}. They do this by coupling with phospholipase C through G_q, which initiates the hydrolysis of a membrane phospholipid, phosphatidylinositol bisphosphate, to produce two second messengers, diacylglycerol, which activates protein kinase C, and inositol 1,4,5,-trisphosphate (IP$_3$), which acts on a specific intracellular receptor to release sequestered Ca^{2+} (Berridage and Irvine, 1989). The α_2-ARs are coupled by pertussis-sensitive (PTX-sensitive) G-proteins to AC or alternatively to ion channels. Thus, they

alter cellular activity either by reducing intracellular levels of cAMP or by directly modifying activity of ion channels such as the Na^+/H^+ antiport, or Ca^{2+} channels, or K^- channels (Bylund, 1988).

The adrenoceptors in the myometrium and the influence of ovarian steroids   It has been demonstrated that all four main subtypes (a_1, a_2, β_1, β_2) of ARs are present in the myometrium of various species of mammals. It has also been suggested that ovarian steroids, estrogen and progesterone, modify the densities of myometrial a- and β-ARs in different stages of the estrous cycle and during pregnancy. Moreover, there are marked species differences with regard to the effect of ovarian steroids on the AR patterns in myometrium.

Using radioligand binding assays of [^{3}H]prazosin ([^{3}H]PRZ) and [^{3}H]rauwolscine ([^{3}H]RAU), it has been demonstrated that a_1- and a_2-ARs are present in the myometrium of different species, including humans (Bottari et al., 1983a and 1983b), rats (Maltier and Legrand, 1985; Legrand et al., 1993), guinea pigs (Arkinstall and Jones, 1988; Arkinstall et al., 1989; Haynes et al., 1993), rabbits (Falkay, 1990) and ewes (Vass-Lopez et al., 1990b).

Ovarian steroids, estrogens and progesterone modify the densities of myometrial a-ARs and the densities vary with stages in the estrous cycle and pregnancy. In general, the density of a_1-ARs is not affected by the ovarian steroids in rabbits (Hoffman et al., 1981) or ewes (Vass-Lopez et al., 1990b), and it does not change in the menstruous cycle or pregnancy in women (Bottari et al., 1983c and 1985). However, its density during pregnancy is 40% higher than that during an estrous cycle in guinea pigs (Arkinstall et al., 1989).

The changes of myometrial a_2-ARs under the influence of ovarian steroids are more variable than those of a_1-ARs in the different species. In rabbits (Hoffman et al., 1981; Jacobson et al., 1987; Riemer et al., 1987) and humans (Bottari et al., 1983c

and 1985) the myometrial a_2-AR density increases when the circulating estrogens are high. However, its density decreases in ewes (Vass-Lopez *et al.*, 1990a and 1990b) and sows (Rexroad and Guthrie, 1983) in the same endocrine environment. In ewes, the myometrial a_2-AR concentration is high (Rexroad, 1981; Vass-Lopez *et al.*, 1990a and 1990b) in the progesterone-treated ewe, and during pregnancy which is a high progesterone environment. In contrast, in humans (Bottari *et al.*, 1983c and 1985) and rabbits (Williams *et al.*, 1976) the a_2-AR density is decreased in the same endocrine environment. Moreover, the density of myometrial a_2-ARs increases greatly in mid-pregnancy, then decreases abruptly at the end of pregnancy in rats and guinea pigs (Kyozuka *et al.*, 1988; Legrand *et al.*, 1993), whereas the a_2-AR concentration increases in rabbits at term (Jacobson *et al.*, 1987).

Although a_2-ARs were reported to be present in the porcine myometrium, the studied used [^{3}H]dihydroergocryptine, a non-selective a-AR antagonist (Rexroad and Guthrie, 1983). [^{3}H]Dihydroergocryptine is unable to characterize the myometrial a_2-ARs as specifically as the selective a_2-ligand, such as [^{3}H]RAU.

Both β_1- and β_2-ARs have been identified in the myometrium of rats (Abrahamsson *et al.*, 1988; Maltier and Legrand, 1988), guinea pigs (Abrahamsson *et al.*, 1988), sheep (Crankshaw and Ruzycky, 1984) and humans (Abrahamsson *et al.*, 1988).

The density of β-ARs is also influenced by the ovarian steroids. The number of myometrial β_2-ARs is increased by estrogen and/or progesterone treatment in guinea pigs (Hatjis *et al.*, 1988). However, in the rabbit myometrium the β_2-AR does not change with the same treatments (Roberts *et al.*, 1977a and 1977b). At term, the density of β_2-ARs in myometrium is controversial. It has been reported that the number of β_2-AR does not change at parturition in humans (Dattel *et al.*, 1986), rats (Cohen-Tannoudji *et al.*, 1991), or in antiprogesterone-treated rat at term (El Alj *et al.*, 1989).

However, during the last weeks of human pregnancy (39 to 40 weeks) (Breuiller et al.,
1987) or in the rabbit at term (Vallieres et al., 1978), the density of β-AR decrease in
both the longitudinal and circular layers of the myometrium. This reduction in the
number of β_2-ARs correlates with the disappearance of β-AR-mediated AC response
(Litime et al., 1989) and occurs at the time when the progesterone-estrogen ratio is the
lowest in myometrial tissues (Férré et al., 1978).

Uterine Contraction

The contraction of smooth muscles, including myometrium can be stimulated by
membrane depolarization (e.g. High K^+ solution) or agonists (NE, oxytocin,
prostaglandins and acetylcholine). The primary result of the stimulation is an increase
in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) from 140 nM to 500 - 700 nM (Williams
and Fay, 1986). Ca^{2+} enters the sarcoplasm from the extracellular space via voltage
dependent Ca^{2+} channels (VDCCs) or receptor operated Ca^{2+} channels. Ca^{2+} also
enters the sarcoplasm from the sarcoplasmic reticulum (SR) via IP_3 receptors or
ryanodine receptors (Allen and Walsh, 1994). The VDCCs are the primary source of
activator Ca^{2+} ions in myometrium (Kosterin et al., 1994).

As a sequence of the elevated $[Ca^{2+}]_i$, Ca^{2+} binds to calmodulin to form a Ca^{2+}-
calmodulin complex (Fig. 3). The Ca^{2+}-calmodulin complex activates myosin light chain
kinase (MLCK) (Bárány and Bárány, 1990; Higashi et al., 1983). The active form of
MLCK leads to phosphorylation of MLC which results in an increase in the actin-
activated myosin Mg-ATPase. This phosphorylation triggers the cycling of myosin
crossbridges along the actin filaments. The development of force or contraction of the
muscle can occur with hydrolysis of ATP (Marston, 1989).

Relaxation of the muscle follows the restoration of $[Ca^{2+}]_i$ by extrusion of Ca^{2+}
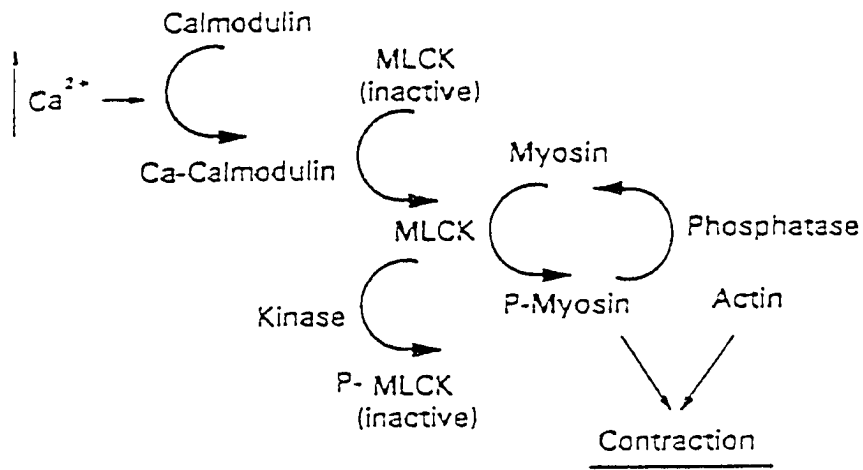from the cell by a sarcolemmal Ca^{2+}-ATPase pump (Carafoli, 1987, Strehler, 1991) or

Fig. 3. Scheme of contraction in uterine smooth muscle.

the Na^+-Ca^{2+} exchanger (Grover et al., 1981), or pumping Ca^{2+} into SR by Ca^{2+}-ATPase pump in the SR membrane (Wuytack et al, 1989). Phosphorylated MLCK showed a marked decrease in its affinity for Ca^{2+}-calmodulin, leading to a reduction in phosphorylation of MLC, causing uterine relaxation (Higashi et al., 1983). Relaxation also occurs as a result of the dephosphorylation of MLCs by myosin light chain phosphatase (Haeberle et al., 1985).

The uterus has spontaneous contractile activity. This means that the myometrium generates electrical activity spontaneously in vitro and in vivo without any hormonal or neural influence, but hormones can alter this activity (Marshall, 1980). For instance, estrogens promote the spontaneous contractions.

The basis of the myogenic mechanism is the spontaneous depolarization of pacemaker cells in the myometrium. However, unlike cardiac muscle, the pacemaker cells in the myometrium are not anatomically fixed or defined (Wray, 1993). The spontaneous activity is accompanied by cyclic phosphorylation and dephosphorylation of MLC (Janis et al., 1980). During spontaneous force development, phosphorylation increases from 0.35 mol phosphate/mol light chain to 0.8 mol/mol, while during spontaneous relaxation the phosphate content decreases to 0.35 mol/mol (Janis et al., 1981; Bárány et al., 1985).

It is generally believed that a decrease in $[Ca^{2+}]$, is coupled with relaxation (Bárány and Bárány, 1990). The decrease in $[Ca^{2+}]$, may be accomplished by inhibiting Ca^{2+} influx or by stimulating the Ca^{2+} efflux and sequestration. The VDCC blockers, such as verapamil cause a decrease in smooth muscle contraction by inhibiting specific Ca^{2+} channels. β-AR agonists are coupled to AC via G_s-protein, to increase cAMP levels. The increased cytoplasmic cAMP concentration activates the cAMP-dependent protein kinase (protein kinase A). The increased activity of protein kinase A leads to phosphorylation of membrane proteins resulting in a decreased Ca^{2+} entry into and

increased Ca^{2+} efflux and sequestration in myometrial cells (Do Khac, 1986; Anwer *et al.*, 1990). These events decrease intracellular Ca^{2+} and subsequently lower the activity of and calmodulin and MLCK.

Natural CATs, EPI and NE activate a-ARs to produce myometrial contraction, and stimulate β-AR to cause relaxations (Alquist, 1948). It is also well established that a relative preponderance of myometrial a- and β-ARs can be induced by changes in plasma concentrations of ovarian steroids, estrogens and progesterone, such as those which occur during pregnancy or different stages of the estrous cycle (Marshall, 1970).

In general, the excitatory, a-AR-mediated responses to CATs in myometrium are enhanced under conditions of estrogen dominance in rats (Diamond and Brody, 1966), rabbits (Miller and Marshall, 1965) and humans (Bottari *et al.*, 1985). In contrast, the inhibitory, β-AR-mediated responses are more prominent under the influence of progesterone.

The increased contractile activity of the estrogen-dominated uterus probably arises from an estrogen-induced increase in the membrane potential (changing from -35 mV in ovariectomized rat to -50 mV) which facilitates spontaneous depolarization by pacemaker cells (Marshall, 1980). Estrogens also induce hypertrophy of myometrial cells and stimulate the synthesis of the contractile proteins actin and myosin, metabolic enzymes and ATP through synthesis of specific RNA and protein (McKerns, 1977). Moreover, the increased contractility from estrogens may also result from the stimulation of gap junction formation, which enhances conduction and synchrony between cells (Sims *et al.*, 1982). Therefore, the influence of estrogens on the myometrium includes membrane, metabolic, and structural changes which promote excitation and contraction.

The mechanism by which progesterone decreases myometrial excitability is not completely clear (Riemer and Roberts, 1986). Progesterone produces hyperpolarization

in myometrium from -50 mV to about -65 mV (Marshall, 1980). The hyperpolarizing effect would be expected to reduce excitability and impulse conduction. Progesterone produces the formation of a high-affinity state β-ARs and increases the density of myometrial β-ARs, consistent with its role in decreasing uterine activity (Wray, 1993).

Although both α_1-ARs and α_2-ARs are present in the myometrium and the density of α_2-ARs is more than that of α_1-ARs in rabbits (Hoffman et al., 1981), rats (Maltier and Legrand, 1985), sheep (Rexroad, 1981; Vass-Lopez et al., 1990a and 1990b), humans (Bottari et al., 1985) and pigs (Rexroad and Guthrie, 1983), it is generally believed that α_1-ARs, but not α_2-ARs mediate increases in myometrial contractility (Hoffman et al., 1981; Digges, 1982; Wray, 1993). The general conclusion that α_1-ARs mediate an increase in myometrial contractility may be due to the fact that the majority of the data concerning the adrenergic influence on myometrial contractility has been collected from rodents (Digges, 1982). α-AR-mediated contraction on the myometrium is linked to phosphoinositide breakdown and IP_3 via G_q protein (Breuiller et al., 1991).

There is recent evidence that α_2-ARs can mediate myometrial contractility. Xylazine, an α_2-AR agonist, induces an increase in intrauterine pressure in cycling cows (LeBlanc et al., 1984a and 1984b; Rodriguez-Martinez et al., 1987), dogs (Wheaton et al., 1989), goats (Perez et al., 1994) and sheep (Marnet et al., 1987). Xylazine also increases electromyographic activity in the pregnant ewe (Jansen et al., 1984). Intravenous perfusion of the α_2-AR antagonist, YOH suppresses the spontaneous uterine electromyographic activity at the end of gestation or during labor in the ewe, but the α_1-AR antagonist, PRZ does not modify the uterine activity (Prud'Homme et al., 1988). In addition, xylazine causes a dose-dependent increase in myometrial contractility in vitro in tissues from cycling pigs (Ko et al., 1990a) and cows (Ko et al., 1990b). The effect of xylazine is antagonized by YOH, but not by PRZ. These findings

indicate that at least in ruminants and pigs the a_2-ARs may play a role in the regulation of myometrial contractility. Moreover, even a_1-ARs are present in the myometrium in pigs (Rexroad and Guthrie, 1983) and ewes (Vass-Lopez *et al.*, 1990b), its role in myometrial contractility in these species is still not clear.

The mechanism that explains how the a_2-AR mediates myometrial contractility is not known. a_2-AR activation can produce an inhibition of AC. This a_2-AR-mediated inhibition of AC is regulated by G proteins that act to couple a-AR activation to a reduction in the catalytic activity of AC. The inhibition of AC is abolished by pertussis toxin which inactivates G_i protein via ADP-ribosylation of the a-subunit. Inhibition of a_2-AR-mediated responses by pertussis toxin has been used as evidence for the critical role of an inhibition of AC (Nichols, 1991).

G proteins participate in the regulation of Ca^{2+} influx in vasoconstriction (Nichols *et al.*, 1988 and 1989). a_2-AR-mediated vasoconstriction is completely inhibited in rats pretreated with pertussis toxin. In addition, a_2-AR-mediated vasoconstriction results from Ca^{2+} influx through activation of VDCCs in the rat saphenous vein (Cheung, 1985). These results may indicate that a_2-AR-mediated vasoconstriction, which is dependent on Ca^{2+} influx via VDCC, involves a pertussis-toxin-sensitive G protein. However, the relationship between the inhibition of AC in vascular smooth muscle produced by a_2-AR agonists and the Ca^{2+} influx is still unknown.

RATIONALE

Natural catecholamines (CATs) epinephrine (EPI) and norepinephrine (NE) potentially modulate uterine contractions through adrenoceptors (ARs), in which α-action is excitatory and β-action is inhibitory. All four main types (α_1, α_2, β. & β_2) of ARs have been demonstrated in myometrium by using functional and radioligand binding studies in different species of animals. However, information is lacking on the effects of natural CATs on myometrial contractility in sows and the distribution of α-ARs in porcine myometrium.

Compared with *in vivo* studies, the use of isolated tissue preparations *in vitro* greatly reduces the problems related to the distribution and metabolism of agonists and eliminates complication, such as feedback effects. Isolated porcine longitudinal myometrium can be used to determine the response of specific drugs on myometrial contractility. In addition, specific agonists and antagonists can be used to classify and characterize the specific receptors in the myometrium. Radioligand probes can aid in identifying the receptor sites. With this technique, the receptors can be quantified, their specificity can be defined, and the kinetics of their interactions with radioligands can be examined. Furthermore, alterations in the density or characteristics of receptors in various physiological states, such as during the estrous cycle and during pregnancy can be directly examined. Because the density of receptors and the nature of the functions which translate external signals into cell responses determine the efficiency of the stimulus response mechanism (Kenakin, 1984), the results from radioligand binding assays can be used to compare and correlate the effect of specific α-ARs on porcine myometrial contractility.

Previous studies from our laboratory suggested that xylazine, an α_2-AR agonist, induced a dose-dependent increase in the amplitude of bovine and porcine myometrial

contractions (Ko *et al.*, 1990a and 1990b). These effects were blocked by yohimbine (YOH) and idazoxan, a_2-AR antagonists, but not by prazosin (PRZ), an a_1-AR antagonist. These results indicate that a_2-ARs in bovine and porcine myometrium mediate uterine contractions.

Although a_2-ARs are the dominant subtype in porcine myometrium and the longitudinal layer of porcine myometrium is primarily innervated by sympathetic nerves, the effects of natural CATs on a-ARs in porcine myometrial contractility are not well understood. Hence, in section one we examined whether natural CATs, EPI and NE, mediated myometrial contractions and whether the effect of EPI and NE was mediated by a_1- or a_2-ARs. Ca^{2+} is a major signal for triggering contraction of smooth muscles, including myometrium (Kosterin *et al.*, 1994), and the increase in $[Ca^{2+}]$ can be due to an increase in Ca^{2+} entry through Ca^{2+} channels and/or an increase in Ca^{2-} release from intracellular stores. Hence, we also studied if either or both components are involved in the CAT-induced contractions.

The effect of CATs on the uterus is closely related to the concentrations of ovarian steroids. Generally, it is believed that estrogens promote uterine contraction and progesterone increases relaxations. The plasma concentrations of ovarian steroids, estrogens and progesterone, in sows vary in the estrous cycle (Ford and Christenson, 1979; Thilander and Rodriguez-Martinez, 1989a) and during pregnancy (Ford *et al.*, 1984; Thilander and Rodriguez-Martinez, 1989b and 1990). In section two we studied and compared the effect of natural CATs and the role of extracellular Ca^{2-} on myometrial contractility in the different phases of the estrous cycle and during various stages of pregnancy. From the results of the above experiments we concluded that the effects of EPI and NE on myometrial contractility were mediated predominantly by a_2-ARs.

The use of $[^3H]$prazosin and $[^3H]$rauwolscine has identified myometrial a_1- and

a_2-ARs, respectively, in different species of animals. Ovarian steroids may modify the density of myometrial a-ARs. In section three of the research, we characterized and quantified the a_1- and a_2-ARs in porcine myometrium in the estrous cycle and during pregnancy. We correlated the relationship between the density of a_1- and a_2-ARs and the potency of CATs on myometrial contractility. Since a_2-ARs have four subtypes (a_{2A}, a_{2B}, a_{2C} & a_{2D}) in the cells or tissues, we also attempted to characterize the predominant a_2-AR subtype in the porcine myometrium.

From the results of section three we concluded that the a_2-AR in porcine myometrium is the a_{2A}-subtype. Therefore, in section four we tested if the a_{2A}-AR antagonist, WB 4101, blocked the effect of EPI-induced increase in myometrial contractility. We also correlated the relationship of the affinities of three a_2-AR antagonists, PRZ, WB 4101 and YOH, in porcine myometrium between functional experiments and radioligand binding assays.

Although a_1-ARs are also present in porcine myometrium in small amounts, the activity of a_1-AR on contractions is not clear. Therefore, in section five, we determined whether the a_1-AR agonist methoxamine has any stimulatory effect on myometrial contractility. It is likely that methoxamine induced myometrial contractions by activating a_2-, but not a_1-ARs.

The methodology and techniques described above provide an understanding of the effect of natural CATs on myometrial contractility and the distribution of specific a-ARs in cycling and pregnant sows.

α_2-ADRENOCEPTORS AND VOLTAGE-DEPENDENT CA^{2+} CHANNELS MEDIATE EPINEPHRINE- AND NOREPINEPHRINE-INDUCED INCREASE IN PORCINE MYOMETRIAL CONTRACTILITY *IN VITRO*

Chih-Huan Yang and Walter H. Hsu

Abstract

The adrenergic effect of epinephrine and norepinephrine on porcine myometrial contractility *in vitro* was investigated using longitudinally layered uterine strips from sows in the luteal phase of the estrous cycle. Epinephrine and norepinephrine alone (10^{-7} - 10^{-5} M) induced dose-dependent myometrial contractions and this effect was potentiated by pretreatment with propranolol. When uterine strips were pretreated with 10^{-6} M propranolol both epinephrine (10^{-9} - 3×10^{-7} M) and norepinephrine (10^{-3} - 10^{-6} M) caused a dose-dependent increase in myometrial contractility, with epinephrine being more potent than norepinephrine. In the presence of 10^{-6} M propranolol, higher doses of epinephrine (10^{-6} - 3×10^{-5} M) and norepinephrine (3×10^{-5} - 3×10^{-5} M) decreased the contractility progressively. This decreased contractility was reversed by a higher concentration of propranolol (3×10^{-6} M). The α_2-adrenoceptor antagonist, yohimbine (3×10^{-8}, 10^{-7}, 3×10^{-7} M), antagonized the effects of both epinephrine and norepinephrine in the same dose-dependent manner. In contrast, the α_1-adrenoceptor antagonist prazosin (10^{-5} M) did not block the epinephrine- or norepinephrine-induced increases in contractility. When uterine strips were pretreated with Ca^{2+}-free Tyrode's solution or 10^{-5} M verapamil, a voltage-dependent Ca^{2+} channel blocker, the

epinephrine- and norepinephrine-induced myometrial contractility was greatly

decreased. Moreover, this decreased contractility in Ca^{2+}-free medium was further

inhibited by 10^{-7} M yohimbine, and to a less extent by 10^{-7} M prazosin. These results

suggest that: 1) epinephrine- and norepinephrine-induced increase in myometrial

contractility in the luteal phase of the estrous cycle in the sow is mediated

predominantly by α_2-adrenoceptors and 2) this effect of epinephrine and norepinephrine

is attributed primarily to an increase in Ca^{2+} influx, through voltage-dependent Ca^{2-}

channels and at least in part due to calcium release from intracellular stores.

Introduction

Myometrium is under adrenergic influence, since all four subtypes (α_1, α_2, β_1, β_2)

of adrenoreceptors (ARs) are present in the myometrium of various species of

mammals (Bottari *et al.*, 1985). Although β_2-ARs are predominantly responsible for

myometrial relaxation (Bülbringn and Tomita, 1987), the α-AR subtype that mediates

myometrial contractility remains controversial. Despite the fact that in humans (Bottari

et al., 1985), pigs (Rexroad and Guthrie, 1983), rabbits (Hoffman *et al.*, 1981), rats

(Maltier and Legrand, 1985) and sheep (Rexroad, 1981; Vass-Lopez *et al.*, 1990), the

densities of myometrial α_2-ARs are greater than α_1-ARs, it is generally accepted that α_1-

ARs mediate increases in myometrial contractility (Hoffman *et al.*, 1981; Digges, 1982;

Wray, 1993). This conclusion may be biased because the majority of the the data

concerning adrenergic influence on myometrial contractility has been collected from

rodents. In rodents, α_1-, but not α_2-AR agonists, increase myometrial contractility

(Maltier and Legrand, 1985; Digges, 1982; Kyozuka *et al.*, 1988).

Evidence that α_2-ARs also mediate myometrial contractility is supported by

studies with xylazine, an α_2-AR agonist. Xylazine induced an increase in intrauterine

pressure in cycling cows (LeBlanc et al., 1984a; Rodriguez-Martinez et al., 1987), dogs

(Wheaton et al., 1989), goats (Perez et al., 1994) and sheep (Marnet et al., 1987).

This effect of xylazine is abolished by the a_2-AR antagonist, yohimbine (YOH), but not

by the a_1-AR antagonist, prazosin (PRZ) (Rodriguez-Martinez et al., 1987; Perez et al.,

1994). Xylazine also increases uterine electromyographic activity in the pregnant ewe

(Jansen et al., 1984). Intravenous perfusion of YOH suppresses the spontaneous

uterine electromyographic activity at the end of gestation or during labor in the ewe,

but PRZ does not modify uterine activity (Prud'Homme, 1988). In vitro xylazine causes

a dose-dependent increase in myometrial contractility in both cycling cows and sows

(Ko et al., 1990a; Ko et al., 1990b). This effect is antagonized by a_2-AR antagonists,

idazoxan and YOH in a dose-dependent manner, but not by PRZ (Ko et al., 1990a; Ko

et al., 1990b). These findings suggest that a_2-ARs play an important role in the

regulation of uterine contractility.

a_2-ARs are a dominant subtype over a_1-ARs in the porcine myometrium (Rexroad

and Guthrie, 1983), and the longitudinal myometrium is primarily innervated by

sympathetic, but not parasympathetic nerves (Taneike et al., 1994). The effect of

natural catecholamines (CATs) on a_1- and a_2-ARs in porcine myometrial contractility is

not well-understood. Therefore, the present study was designed to investigate the a-

adrenergic effect of the natural CATs epinephrine (EPI) and norepinephrine (NE) on

porcine myometrial contractility in vitro in the luteal phase of the estrous cycle. We

have used the myometrium of this phase as a model in other studies (Ko et al., 1990b;

Yu et al., 1995), and the specimens are readily available at abattoirs.

Ca^{2+} is a major signal for triggering smooth muscle contraction. Myometrial a-

and β-ARs may mediate an increase and a decrease in $[Ca^{2+}]$, respectively (Do Khac et

al., 1986; Nichols, 1991). In smooth muscle cells, activation of a_1-ARs mobilizes Ca^{2+}

from the sarcoplasmic reticulum and extracellular fluid in association with an increase

in the formation of 1,4,5-inositol triphosphate through activation of phospholipase C,
while activation of a_2-ARs increases cytosolic Ca^{2+} concentration ($[Ca^{2+}]$) through
opening voltage-dependent Ca^{2+} channels (VDCC) (Nichols, 1991). In this context, EPI
and NE may increase $[Ca^{2+}]$ via mobilization of Ca^{2+} from both extra- and intracellular
sources to induce myometrial contractions. Thus the experiments were also designed
to determine whether EPI- and NE-induced myometrial contractions are mediated
through an increase in Ca^{2+} release or influx.

Materials and Methods

Tissue preparation

The uterine specimens were collected from a local abattoir. Only the mid-
portion of the uterine horns was used in the experiments. Specimens were determined
to be in the luteal phase based on the presence of light red corpora lutea in the ovaries,
and the absence of embryos (Arthur et al., 1989). Tissues were stored in ice-cold
Tyrode's solution (137 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM
dextrose, and 12 mM $NaHCO_3$; pH 7.4) and transported to the laboratory. Upon
arrival, the endometrium was removed from the uterus; the myometrium was stored in
ice-cold Tyrode's solution aerated with 95% O_2-5% CO_2 and was used for experiments
within 30 h. There were no changes in responsiveness to contractants during this
period.

Longitudinal uterine strips (10 x 2 mm^2) were ligated with silk threads at both
ends and suspended vertically in a 10-ml double-jacketed glass bath containing
Tyrode's solution at 37°C and aerated with 95% O_2-5% CO_2. One thread was
attached to a fixed support while the other thread was connected to a Grass FT03
transducer (Grass Instrument Co., Quincy, MA) and myometrial contractions were

recorded isometrically with a 8-channel polygraph recorder (R411, Beckman Instruments Inc., Schiller Park, IL). The strips were equilibrated under a 2-g tension over 20 - 25 min before being exposed to 10^{-5} M carbachol (CARB) to determine their responsiveness to the contractant. Two three-minute exposures to CARB separated by a 15 min interval were performed with four 10-ml washes of Tyrode's solution used to remove CARB after the stimulation. The strips lost contractions within 15 min after the washout of CARB, and this quiescent state lasted > 25 min. The basal resting tension was readjusted to 2 g before the pretreatment drug was added. In NE experiments, no uptake blockers were used because neither the neuronal uptake-1 blocker desimipramine (10^{-7} M) (Furchgott, 1972) nor the extraneuronal uptake-2 blocker corticosterone acetate (10^{-5} M) (Iversen and Salt, 1970) affects the NE effect on myometrial contractility (Yang and Hsu, unpublished results; n = 6 uteri). In the following experiments, EPI or NE was added at 10-min intervals in cumulative doses to attain a dose-response relationship.

Experimental protocols

A. EPI- and NE-induced myometrial contractility and the influence of propranolol (PROP), PRZ and YOH

   In experiments designed to observe the β-AR-mediated effect from EPI or NE stimulation, a 10-min pretreatment with 10^{-5} M PROP was performed before each agonist was administered in cumulative doses. The control group did not receive PROP. The 10-min pretreatment was based on a preliminary experiment, in which β-AR antagonism by PROP reached a maximum in 10 min (n = 4 uteri).

   In another experiment, the α_1-AR antagonist PRZ (10^{-5} M) or the α_2-AR antagonist YOH (3 x 10^{-8}, 10^{-7}, or 3 x 10^{-7} M) was added with 10^{-5} M PROP to the organ bath for 10-min. The 10-min pretreatment was based on a preliminary experiment, in which α_2-AR antagonism by YOH reached a maximum in 10 min (n = 4

uteri). After 10-min of pretreatment with the antagonists, EPI or NE was given in cumulative doses. Controls received only EPI or NE without an α-AR antagonist.

Different strips from the same uterus were randomly assigned to all treatment groups in one trial, and each uterus was used for one trial only.

B. Effect of Ca^{2+}-free medium and verapamil on the EPI- and NE-induced myometrial contractility and the influence of PRZ and YOH

Ca^{2+}-free Tyrode's solution was prepared by excluding $CaCl_2$. Ca^{2+}-free groups were treated as follows: after the myometrial strips had been stimulated by CARB twice, and washed twice with 10 ml of Ca^{2+}-free Tyrode's solution at 5-min intervals, another 10 ml of Ca^{2+}-free medium was applied with PROP to block β-receptor-mediated uterine relaxation.

In a preliminary experiment, the Ca^{2+} chelating agent EGTA (10^{-5} M) did not change EPI-induced myometrial contraction in a Ca^{2+}-free medium (n = 6 uteri). Based on this result, EGTA was not used in the Ca^{2+}-free medium with the exception of one experiment. In this experiment, three treatments were assigned as follows: a. Ca^{2+}-free medium; b. 10^{-5} M verapamil in Ca^{2+}-containing medium; and c. Ca^{2+}-containing medium (control group). Verapamil, a VDCC blocker, was used to block Ca^{2+} influx. In addition, all groups had been pretreated with 10^{-6} M PROP before EPI or NE was adninistered.

In a separate experiment, we determined whether the EPI (10^{-5} M)-induced myometrial contractions in Ca^{2+}-free medium was mediated by α_1- or α_2-ARs by using 10^{-7} M PRZ and/or 10^{-7} M YOH.

Assessment of the contractile response

The contractile response was assessed by the area under the contraction curve and was determined with the use of a scanning program (SigmaScan, Jandel, Corte Madera, CA). These values were expressed as a percentage of the response to 10^{-5} M

CARB treatment for 10 min. In pilot studies many tissue strips lost contractions after a 3-min but not a 10-min stimulation by 10^{-6} M CARB after several washouts using Tyrode's solution. To transform data for 3-min CARB treatment to those for 10-min treatment, an independent experiment was performed to obtain a regression line to fit the tissue strips' responses to a 10-min 10^{-6} M CARB stimulation. The tissue strips were stimulated by 10^{-6} M CARB twice. After the initial 3 min CARB treatment and subsequent 4 - 5 washes with 10 ml of Tyrode's solution each for a total of 15 min, the strips were stimulated again by 10^{-6} M CARB for 10 min. By using the 3 min and 10 min areas that were produced by the second stimulation a regression line was calculated:

$$Y \ (10 \ min) = 2.95 * X \ (3 \ min) + 1.32, \ (n = 39).$$

In this study, the contractile area produced by the second 3-min 10^{-3} M CARB stimulation was transformed to a 10-min area using the above formula and this 10 min area was defined as the 100% 10^{-6} M CARB contractile response for each individual strip. The contractile response of the tissue strip was calculated from the contractile area produced by agonist EPI or NE over 10 min at each cumulative dose and was expressed as a percentage of the response to 10^{-6} M CARB.

Drugs

The following drugs were used: carbachol chloride, (-)epinephrine bitartrate, (-) norepinephrine bitartrate, propranolol HCl, yohimbine HCl, EGTA (Sigma Chemical Co., St. Louis, MO), prazosin HCl (Pfizer Inc., Groton, CT), and verapamil HCl (Knoll Pharmaceutical Co., Whippany, NJ). Drugs were dissolved in distilled water, except for epinephrine and norepinephrine, which were dissolved in 0.1% (W/V) ascorbic acid in 0.9% NaCl, and prazosin HCl, which was dissolved in 2% lactic acid to achieve a concentration of 1 mM. Drug-containing solutions were prepared by appropriate dilution of the stock solutions, which were stored at -20°C.

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Data analyses

The dose-response curves were produced by cumulative application of EPI and NE in approximately one-half log and one log increments in experiments A and B, respectively (van Rossum, 1963). The data were expressed as pD_2 (-log EC_{50}).

Dissociation constants (K_B) of YOH against the agonist were determined using the equation: K_B = [B]/(CR - 1), where B is the concentration of the antagonist (Furchgott, 1972). The response to YOH (3 x 10^{-8} M) was used for this calculation because YOH at this dose caused a consistent antagonism on contractility. The concentration ratio (CR) is calculated as EC_{50}'/EC_{50}, in which EC_{50} and EC_{50}' values are the values for the agonist in the absence and presence of the antagonist, respectively. The dissociation constant of the antagonist was expressed as pK_B (= -Log K_B). In the β-AR antagonism studies and experiment B, the contractile response was compared with the control group at the corresponding dose of the agonist.

Data were expressed as mean ± SE and analyzed by analysis of variance (ANOVA). The conservative F value was used to establish significance for the treatment effect. The least significant difference test as used to determine the difference between means of end points for which the ANOVA indicated a significant (P < 0.05) F ratio.

Results

### A. Effect of PROP, PRZ and YOH on EPI- and NE-induced increase in myometrial contractility

Both EPI (10^{-9} - 3 x 10^{-7} M) and NE (10^{-9} - 10^{-6} M) in the presence of 10^{-5} M PROP produced a dose-dependent increase in myometrial contractility (Figs. 1 and 2). The potency of EPI was significantly greater than that of NE (Table 1). Higher doses of

Fig. 1. Representative tracings of the uterine contractile response for 3×10^{-8} M epinephrine (A) and 3×10^{-8} M norepinephrine (B) in the presence of 10^{-6} M propranolol. Arrowheads show the administration of the agonist.

A.

Epinephrine
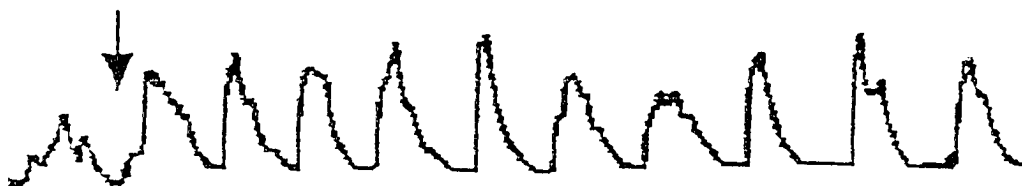

4 g
1 min

B.
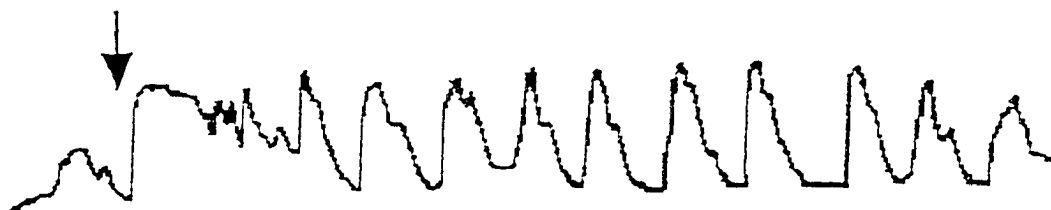
Norepinephrine

Fig. 2. Dose-response curves for epinephrine (EPI) and norepinephrine (NE) in the absence and presence of 10^{-6} M propranolol (PROP). Data are expressed as means ± SE (n = 6). Effects are shown in the presence of PROP (○: EPI; ▲: NE) and in the absence of PROP (●: EPI; ▼: NE).

*P < 0.05, compared with that in the absence of PROP at the corresponding concentration of the same agonist.

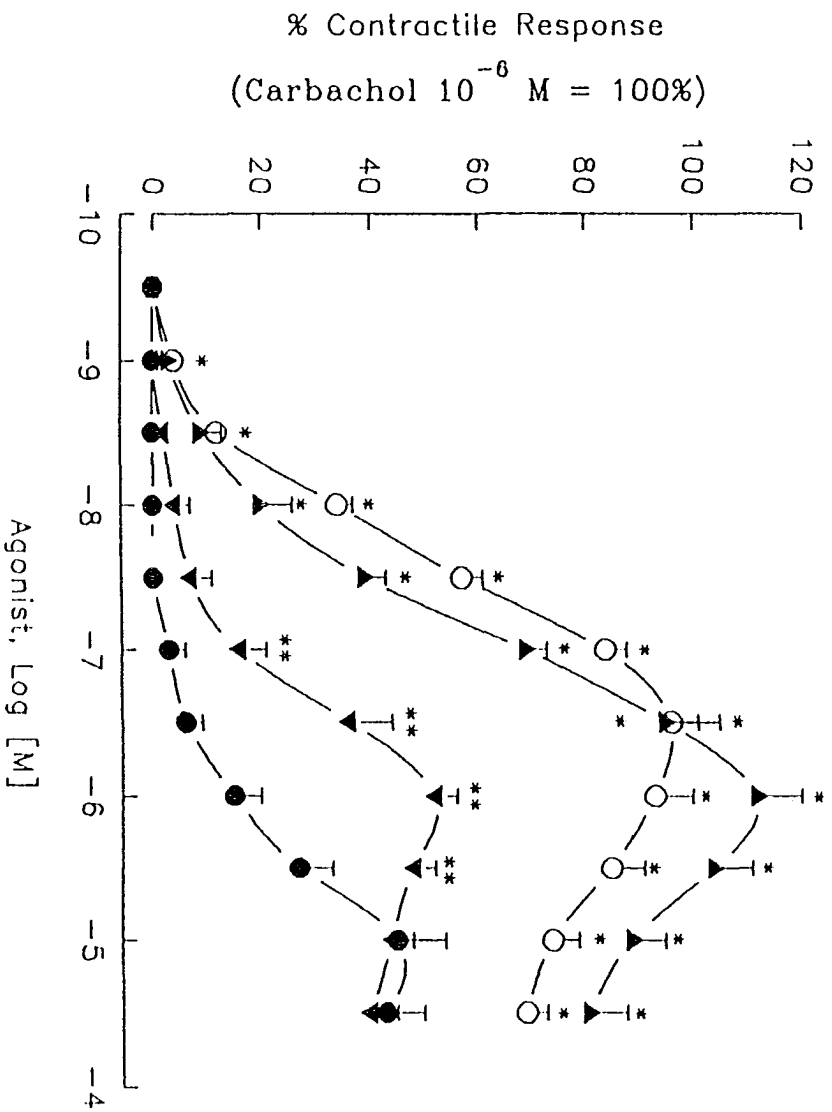**P < 0.05, compared with EPI in the absence of PROP at the corresponding agonist concentration.

Table 1. pD_2 values for epinephrine and norepinephrine in the control and 10^{-5} M

prazosin treatment groups in the presence of 10^{-6} M propranolol

Treatment	Epinephrine	Norepinephrine
Control	7.81 ± 0.07*	7.38 ± 0.08
Prazosin	7.59 ± 0.10*	7.11 ± 0.11

*P < 0.05, comparing with norepinephrine in the same treatment.

The data are expressed as mean ± SE (n = 6).

EPI (10^{-6} - 3×10^{-5} M) and NE (3×10^{-6} - 3×10^{-5} M) decreased the maximal contractility progressively. This decreased contractility was reversed by the addition of a higher concentration of PROP (3×10^{-6} M) (Fig. 3).

In the absence of 10^{-6} M PROP, EPI and NE, at higher concentrations than that in the presence of PROP, caused progressive increases in myometrial contractility (Fig. 2). The contractility reached maximum at 10^{-5} M of EPI or NE. However, the magnitude of these responses was much smaller than that in the PROP-pretreated groups (Fig. 2). The effect of NE alone on myometrial contractility was greater than that of EPI, since NE concentrations (10^{-8} - 10^{-7} M) that initiated the contractions were lower than those of EPI (10^{-7} - 3×10^{-6} M).

The a_2-AR antagonist, YOH, blocked the effects of both EPI and NE in a dose-dependent manner (Figs. 4A and 4B). The pK_B values for YOH against EPI and NE were not significantly different from each other (Table 2). In the presence of 3×10^{-7} M YOH, EPI ($\geq 3 \times 10^{-6}$ M) induced contractions with significantly smaller magnitude than other groups (Fig. 4A). This inhibition was reversed by 10^{-5} M PROP (Fig. 5). However, this phenomenon was not observed in the NE group. Norepinephrine (NE) still induced myometrial contraction to attain approximately the same maximum effect as the control and other YOH treatment groups (Fig. 4B).

The a_1-AR antagonist, PRZ, even at a high concentration of 10^{-6} M, failed to antagonize the effect of EPI or NE on myometrial contractility (Figs 6A and 6B). The pD_2 values of the PRZ group were not significantly different from those of control groups (Table 1).

B. Effects of Ca^{2+}-free medium and verapamil on EPI- and NE-Induced myometrial contractility

Both EPI and NE (10^{-9} - 10^{-6} M) caused a dose-dependent increase in myometrial contractility in the presence of 10^{-6} M PROP (Figs. 7A and 7B). This effect of EPI and

Fig. 3. Representative tracings of the uterine contractile response for epinephrine (EPI) in the presence of 10^{-6} M propranolol (PROP). The EPI-induced myometrial contractility was decreased progressively by the stimulation with high EPI concentrations (A, B and C), but was reversed by 3×10^{-5} M PROP (D). Cumulative doses of EPI were used.
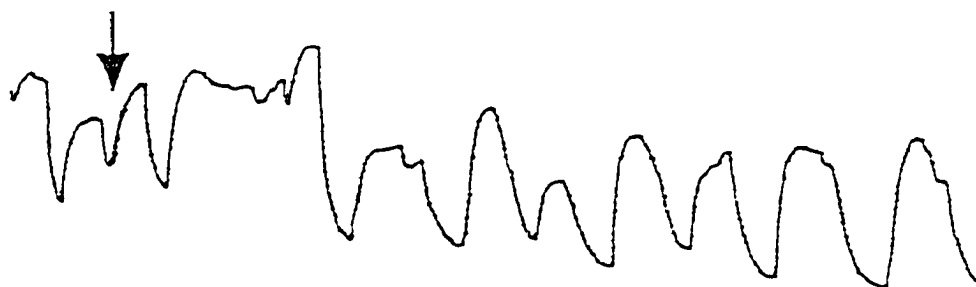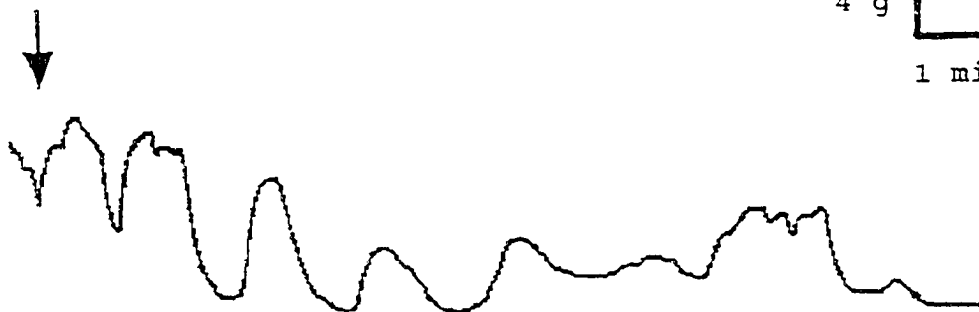
A.

EPI 3 x 10^{-5} M

C.

EPI 3 x 10^{-5} M

4 g

1 min

B.

EPI 10^{-5} M

D.
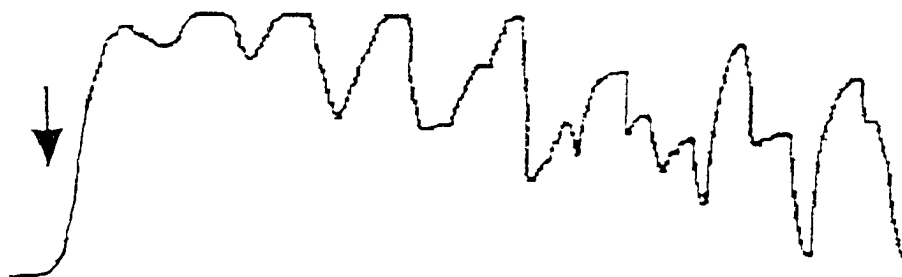
PROP 3 x 10^{-5} M

Fig. 4. Effect of yohimbine (YOH) on epinephrine (A)- and norepinephrine (B)-induced increases in myometrial contractility. All strips had been pretreated with 10^{-5} M propranolol for 10 min before the first dose of the agonist was applied. Data are expressed as means $\pm$ SE (n = 6). Effects are shown in the absence (O) and in the presence of YOH, 3×10^{-8} M, $\bullet$; 10^{-7} M, $\blacktriangle$; 3×10^{-7} M, $\blacktriangledown$.

A. Epinephrine

B. Norepinephrine

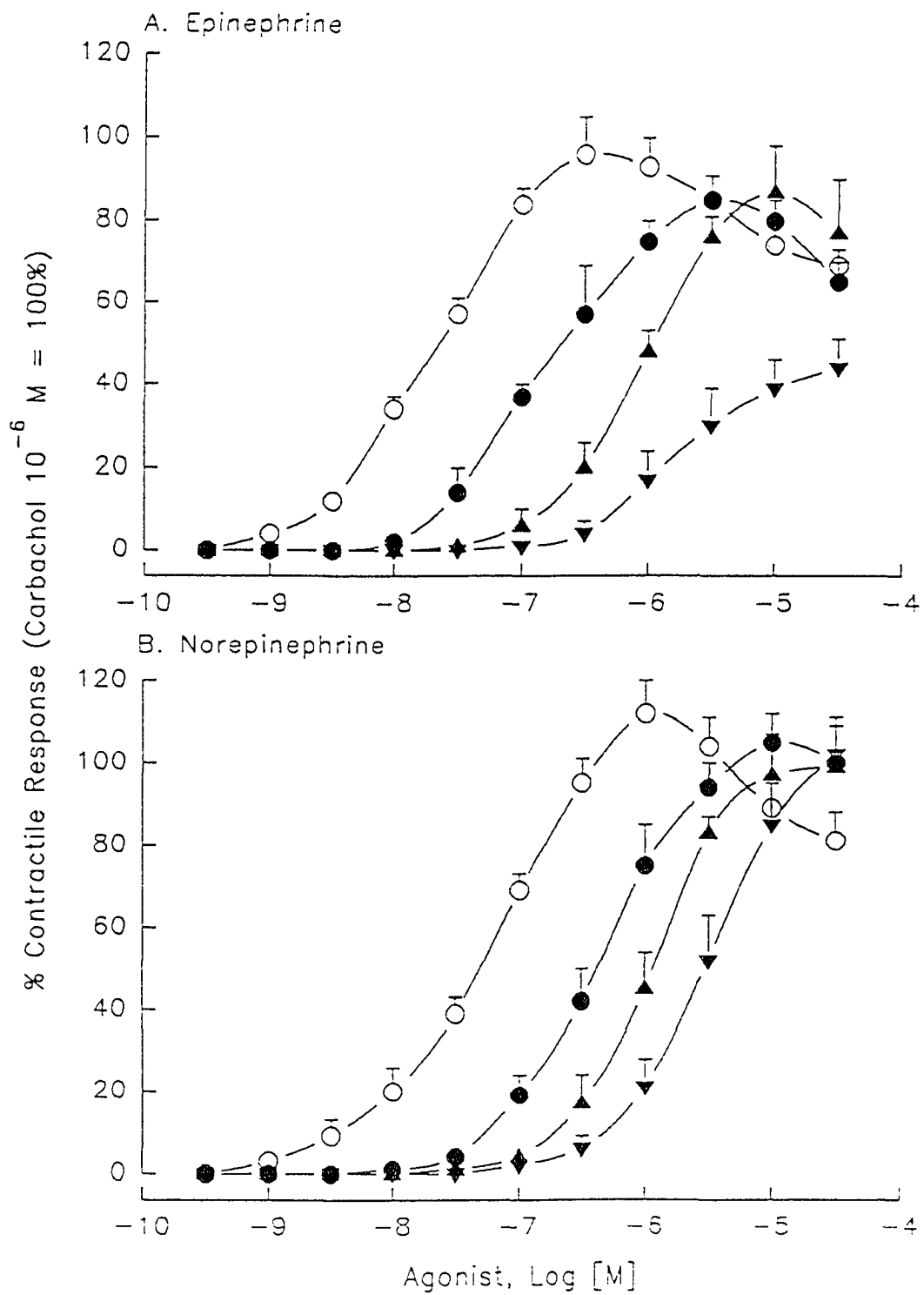% Contractile Response (Carbachol 10^{-6} M = 100%)

Agonist, Log [M]

Table 2. Dissociation constants (pK_B) for yohimbine against the agonists acting on the α_2-adrenoceptors in porcine myometrium in the luteal phase of the estrous cycle.

Agonist	pK_B [*]
	Yohimbine
Epinephrine	8.42 ± 0.14
Norepinephrine	8.26 ± 0.26

[*]The data are expressed as mean ± SE (n = 6).

The pK_B values for yohimbine against epinephrine and norepinephrine are not significantly different each other (P > 0.05).

Fig. 5. Representative tracings of the uterine contractile response for epinephrine (EPI) in the presence of 3×10^{-7} M yohimbine (YOH). The contractile responses were obtained by the cumulative doses (A - C). A higher concentration (10^{-5} M) of propranolol (PROP) further increased contractile response (D). Cumulative doses of EPI were used. The tissue strip had been pretreated with 10^{-6} M PROP at the beginning of the experiment which was before panel A was obtained. Data shown are the representative of three experiments.

A.

EPI 3×10^{-5} M

B.

EPI 10^{-5} M

C.

4 g

1 min

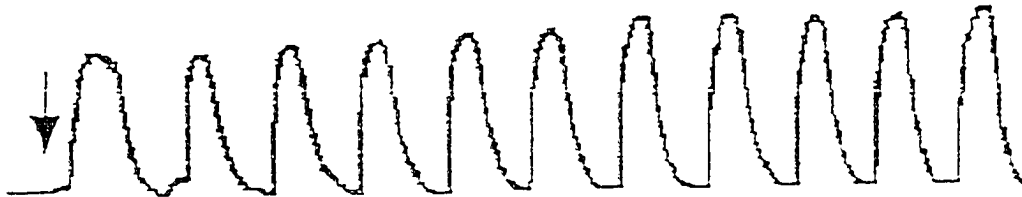EPI 3×10^{-5} M

D.

PROP 10^{-5} M

Fig. 6. Effect of prazosin (PRZ) on epinephrine (A)- and norepinephrine (B)-induced increase in myometrial contractility. All strips had been pretreated with 10^{-5} M PROP for 10 min before the first dose of the agonist was applied. Data are expressed as means ± SE (n = 6). Effects are shown in the absence (○) and in the presence of (●) of 10^{-6} M PRZ.

A. Epinephrine

B. Norepinephrine

% Contrractile Response (Carbachol 10^{-6} M = 100%)

Agonist, Log [M]
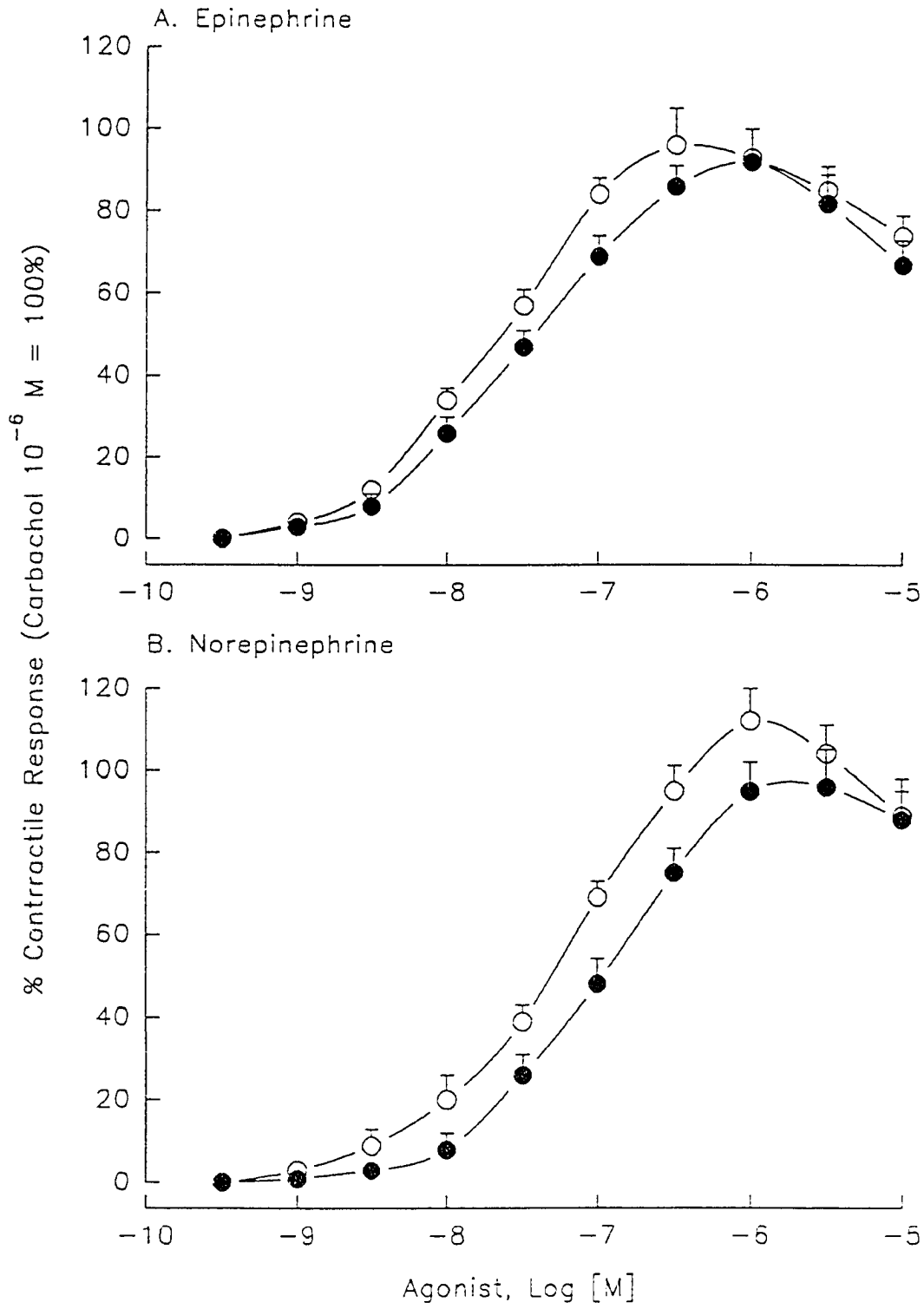
Fig. 7. Effects of Ca^{2+}-free medium and 10^{-5} M verapamil on epinephrine (A)- and

norepinephine (B)-induced increase in myometrial contractility. Data are

expressed as means ± SE (n = 6).

*P < 0.05, compared with the control group at the corresponding agonist

dose.

A. Epinephrine

B. Norepinephrine

Control
Ca^{2+}-free medium
10^{-5} M Verapamil

% Contractile Response (Carbachol 10^{-6} M = 100%)

Agonist, Log [M]
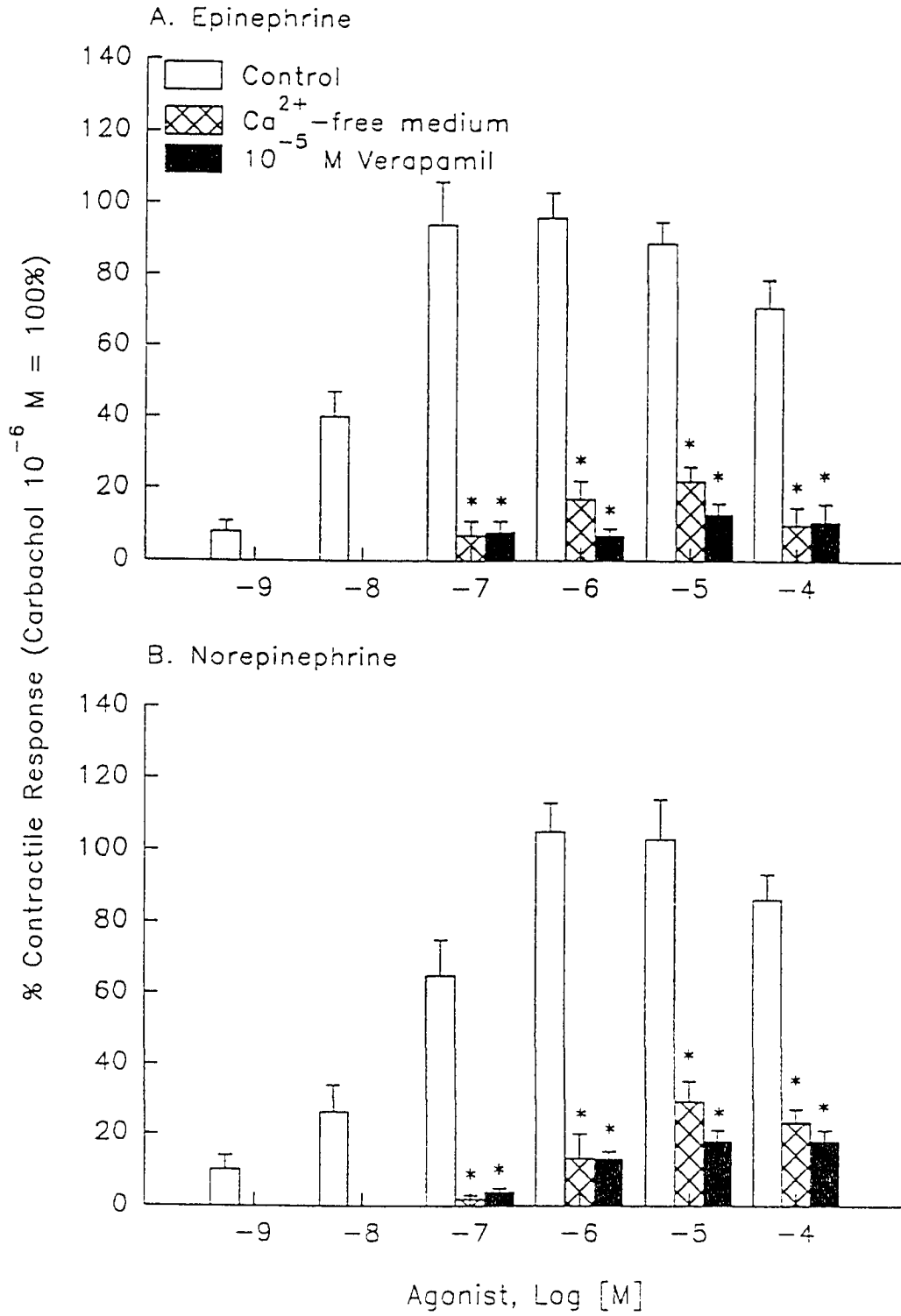
NE was significantly inhibited by Ca^{2+}-free Tyrode's solution or 10^{-5} M verapamil (Figs. 7A and 7B).

In the free-Ca^{2+} medium with 10^{-5} M EGTA, both 10^{-7} M PRZ and 10^{-7} M YOH significantly inhibited 10^{-6} M EPI-induced contractions (Fig. 8). The inhibitory effect of YOH was significantly greater than that of PRZ. The combination of 10^{-7} M PRZ and 10^{-7} M YOH failed to cause greater antagonism of EPI-induced myometrial contractions than YOH alone.

Discussion

The results of the present study suggest that EPI- and NE-induced contractility of porcine longitudinal myometrium is mediated predominately by a_2-ARs, and minimally by a_1-ARs. These findings agree with those of others that the a_2-ARs present in the porcine myometrium in the luteal phase of the estrous cycle (Rexroad and Guthrie, 1983) mediate contractions. In addition, these results are consistent with a previous report that a xylazine-induced increase in porcine myometrial contractility is mediated by a_2-ARs (Ko et al., 1990a). This a_2-AR function is mediated predominantly by Ca^{2+} entry through VDCC and to a much lesser extent through Ca^{2+} release from intracellular stores. In addition, results of the present study suggest that a_1-ARs also mediate EPI-induced Ca^{2+} release, but this effect is less potent than that mediated by a_2-ARs.

In the present study, the magnitude of myometrial contractility induced by EPI and NE was lower in the absence than that in presence of β-AR blockade. It is generally believed that myometrial relaxation is mediated by β_2-ARs (Digges, 1982; Bülbringn and Tomita, 1987). Unlike EPI, NE has little action on β_2-ARs (Weiner, 1985). Therefore, in the absence of PROP, EPI's action on myometrial contraction has

Fig. 8. Effect of 10^{-7} M prazosin (PRZ) or/and 10^{-7} M yohimbine (YOH) on 10^{-6} M

epinephrine-induced myometrial contraction in the Ca^{2+}-free medium with 10^{-5}

M EGTA. Data are expressed as mean $\pm$ SE (n = 6).

*P < 0.05, compared with Ca^{2+}-containing medium group.

**P < 0.05, compared with Ca^{2+}-free medium alone.

***P < 0.05, compared with Ca^{2+}-free medium with 10^{-7} M PRZ treatment.

% Contractile Response

(Carbachol 10^{-6} M = 100%)
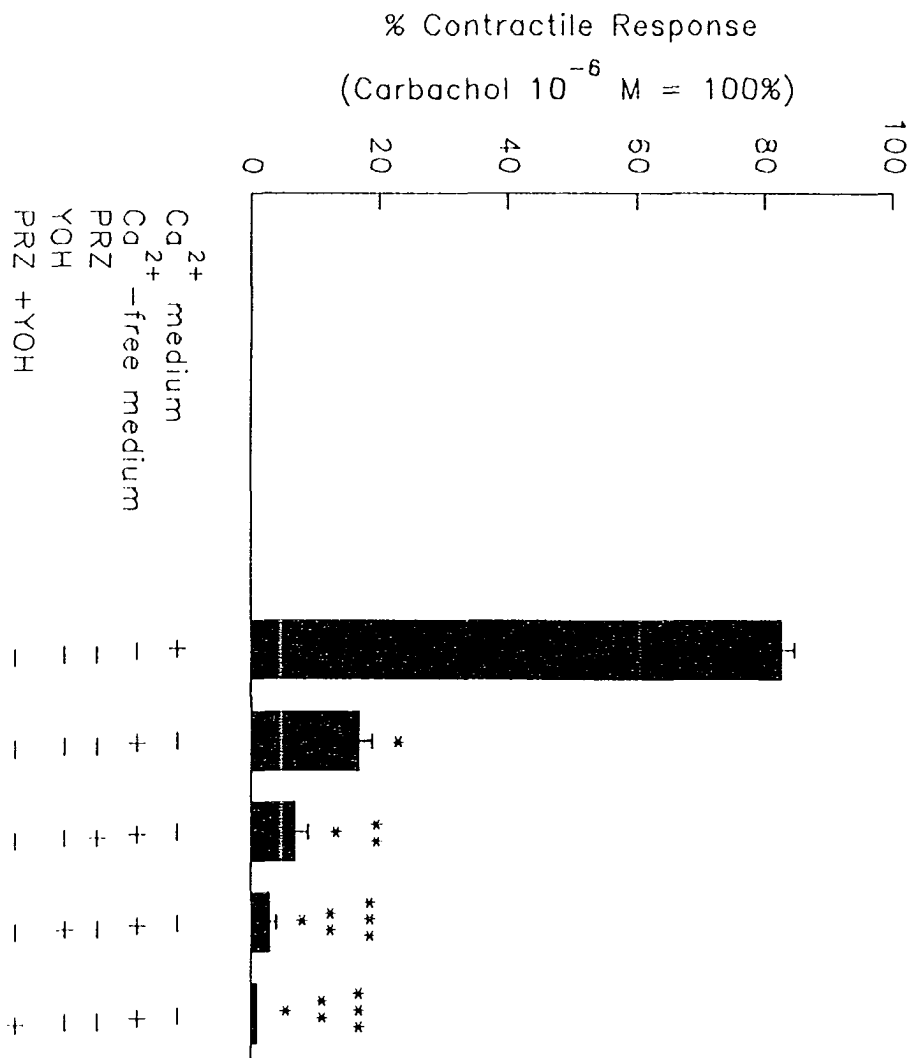
been reported to be less than that of NE. Since EPI is more potent than NE on the α-ARs of most organs (Weiner, 1985), in the presence of 10^{-6} M PROP, the EPI-induced myometrial contractions were greater than those induced by NE. However, the CAT-induced contractility decreased at higher concentrations ($> 10^{-6}$ M). This decreased contractility was reversed by a higher dose of PROP (3×10^{-5} M) indicating that it was caused by β_2-AR-mediated relaxation. Overall, with regard to porcine myometrial contractility the α-excitatory effect dominated over the β-inhibitory effect at higher concentrations of CAT ($> 10^{-6}$ M).

In the presence of PROP, the α_2-AR antagonist YOH competitively antagonized the EPI- and NE-induced increase in myometrial contractility in a dose-dependent manner. PRZ, the α_1-AR antagonist, even at a high concentration of 10^{-5} M, failed to do so. Since EPI and NE act on the same α_2-ARs, the pK_B values of YOH against EPI and NE were not significantly different (Furchgott, 1972). These results suggest that α_2-, but not α_1-ARs, mediated the EPI- and NE-induced increase in porcine myometrial contractility.

The antagonism by YOH at 3×10^{-7} M appeared to be a noncompetitive manner since high concentrations ($\geq 3 \times 10^{-5}$ M) of EPI did not overcome its inhibitory action (Bourne and Robert, 1995). However, the same effect did not occur in the NE-treated groups which was attributed to its lower potency in activating β_2-ARs. The EPI-decreased contractility at 3×10^{-7} M YOH was reversed by a higher dose (10^{-5} M) of PROP indicating that the noncompetetive antagonism was attributable to β_2-AR-mediated relaxation.

Our results in the sow were different from those in the rat (Acritopoulou-Fourcroy and Marcais-Collado, 1988) and rabbit (Hoffman et al., 1981). In these latter species α_1-AR antagonists abolish the myometrial contractility that is induced by EPI, NE or phenylephrine. Results of the present investigation, however, were consistent

with those reported in the cow (Ko *et al.*, 1990b; LeBlanc *et al.*, 1984a; Rodreguez-martinez *et al.*, 1987), sow (Ko *et al.*, 1990a), goat (Perez *et al.*, 1994) and sheep (Marnet *et al.*, 1987; Prud'Homme, 1988), suggesting that a_2-ARs play an important role in EPI- and NE-induced myometrial contractions in the sow.

The Ca^{2+}-free medium and VDCC blocker verapamil greatly inhibited the effect of EPI and NE on myometrial contractility. Hence, it is reasonable to suggest that a_2-AR-mediated myometrial contraction is largely attributable to an increase in Ca^{2+} influx through VDCC and to lesser extent to an increase in release from intracellular stores. The a_1-AR-mediated increase in $[Ca^{2+}]_i$ in smooth muscle is attributed to a release from intracellular stores followed by a Ca^{2+} influx through a capacitative mechanism (Nichols, 1991), suggesting that the EPI- and NE-induced increase in Ca^{2+} release in porcine myometrium may be due to activation of a_1-ARs.

In the present investigation, both PRZ and YOH blocked the contractile responses to a-adrenergic activation in Ca^{2+}-free medium, with the antagonism by YOH being greater than that by PRZ. These results suggest that the EPI-induced Ca^{2+} release from intracellular stores is mainly due to activation of a_2-ARs and to a lesser extent by a_1-ARs present in the porcine myometrium. In blood vessels, the a_2-AR-mediated Ca^{2+} release from intracellular stores evokes smooth muscle contractions (Daly *et al.*, 1990; Nielsen *et al.*, 1992). The mechanisms by which a_2-AR mediates contraction of smooth muscle in Ca^{2+}-free medium are not clear. We found that a_2-ARs in porcine myometrium mediated Ca^{2+} release from intracellular stores (ZhuGe and Hsu, unpublished results). In addition, the a_2-AR-mediated contraction in the rabbit saphenous vein in Ca^{2+}-free medium occurs without an increase in resting $[Ca^{2+}]$, indicating that the a_2-AR-mediated contraction may also involve an increase in the sensitivity of the contractile apparatus to Ca^{2+} (Aburto *et al.*, 1993).

The ovarian steroid hormones may influence the density of a-ARs in

myometrium. In guinea pig (Arkinstall and Jones, 1988), murine (Legrand et al., 1993) and ovine (Vass-Lopez et al., 1990) myometrium, a higher density of a_2-ARs is found when progesterone is the main circulating steroid. In contrast, the density of a_2-ARs in myometrium increases in a high estrogen environment in women (Bottari et al., 1985) and rabbits (Riemer et al., 1987). The density of a_1-ARs does not appear to be affected by ovarian steroid hormones (Germain et al., 1994). However, the influence of ovarian steroid hormone on a_1-AR density in the sow is unclear. Rexroad and Guthrie; 1983, used [^{3}H]dihydroergocryptine (DHE), a non-selective a-AR antagonist, and [^{3}H]PRZ to quantify myometrial a-ARs in cycling and early pregnant gilts. The results of that study suggested that the a_2-AR was the main receptor subtype because: 1) YOH had a much greater affinity to compete for [^{3}H]DHE binding sites than did PRZ, and 2) [^{3}H]PRZ binding sites for a_1-ARs were only present in small quantity. In addition, the density of a_2-ARs has been reported to be greater in the luteal phase than those at or near estrus (Rexroad and Guthrie, 1983). We have characterized and quantified myometrial a_1- and a_2-ARs using [^{3}H]PRZ and [^{3}H]rauwolscine binding assays in the luteal phase of the estrous cycle of the sow. The ratio between a_2- and a-AR numbers was greater than 65 (Yang and Hsu, unpublished results). These findings that the a_2-AR was the dominant subtype supported those of the present study that a_2-ARs are the primary mediators of EPI- and NE-induced myometrial contractions. The porcine myometrium in the luteal phase of the estrous cycle is exposed to low estrogens and high progesterone, and thus differs those of the follicular phase and the last trimester of the pregnancy, when exposure to estrogens is high (Thilander and Rodriguez-Martinez, 1989a, 1989b, 1990). Therefore further study is needed to determine whether the number of a_2-ARs changes in porcine myometrium of the cycling or pregnant sow and whether these changes in a_2-AR densities affect myometrial contractility.

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Although the physiological function of a_2-ARs in porcine myometrium is not clear, the results of the present study suggest that a_2-ARs mediate an increase in myometrial contractility and thus may play an important role in the regulation of porcine myometrial contractions. Considering this characteristic, activation of these receptors with a_2-AR agonists may induce abortion (LeBlanc *et al.*, 1984b) or parturition (Ko *et al.*, 1989).

In conclusion, the present work suggests that a_2-, but not a-ARs, mediate EPI- and NE-induced increases in porcine myometrial contractility in the luteal phase of the estrous cycle. The results also suggest that activation of a_2-ARs increases porcine myometrial contractility primarily by increasing Ca^{2+} entry through VDCC, and to a lesser extent by increasing Ca^{2+} release from intracellular stores.

Acknowledgements

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THE α_2-ADRENOCEPTOR-MEDIATED MYOMETRIAL CONTRACTILITY IN CYCLING AND PREGNANT SOWS

Abstract

The adrenergic effect of epinephrine and norepinephrine on porcine myometrial contractility *in vitro* was studied using the longitudinal layer of uterine strips from sows in the estrous cycle and various stages of pregnancy. The uterine strips in the follicular phase presented spontaneous contraction throughout the experiments, and the contractions were decreased by the action of epinephrine and norepinephrine in the absence of propranolol. In the presence of propranolol, neither epinephrine nor norepinephrine increased myometrial contractility. However, epinephrine and norepinephrine induced dose-dependent myometrial contractions in other reproductive stages using the same treatment. The contraction to catecholamines were potentiated by pretreatment with 10^{-6} M propranolol. In the presence of 10^{-6} M propranolol, epinephrine and norepinephrine induced dose-dependent increases in contractility in the luteal phase of the estrous cycle and during pregnancy. Comparing pD_2 values, the potency of epinephrine was greater than that of norepinephrine in all tested groups. The order of the potencies for epinephrine and norepinephrine was luteal phase $\geq$ late pregnancy (days of gestation = 73 -79) $\geq$ mid-pregnancy (days of gestation = 53 - 60) $\geq$ early pregnancy (days of gestation = 39 - 40) > prepartum period (days of gestation = 111 - 113). These induced myometrial contractions were inhibited by the α_2-adrenoceptor antagonist yohimbine (10^{-8} - 3×10^{-7} M) in a dose dependent manner, but not by prazosin (10^{-6} M). Contractions to epinephrine and norepinephrine on the

myometrium were greatly decreased in Ca^{2+}-free Tyrode's solution or by 10^{-5} M

verapamil, a voltage-dependent Ca^{2+} channel blocker, in all reproductive stages. The

inhibition in the follicular phase of the estrous cycle and in the prepartum period was

greater than in other tested reproductive stages. These results suggest that

epinephrine and norepinephrine-induced increase in myometrial contractility in the

cycling and pregnant sows is mediated predominantly by a_2-adrenoceptors and that this

effect of epinephrine and norepinephrine is attributed primarily to an increase in Ca^{2+}

influx, through voltage-dependent Ca^{2+} channels. The present findings also

demonstrated that the epinephrine- and norepinephrine-induced myometrial

contractions were less in the follicular phase and the prepartum period, a period

characterized by high estrogen exposure, than those in the luteal phase and other

stages of pregnancy, a period characterized by high progesterone exposure.

Introduction

It has been well documented that activation of a- and β_2-adrenoceptors (ARs)

causes myometrial contraction and relaxation, respectively (Ahlquist, 1962; Bülbringn

and Tomita, 1987). Although both a_1- and a_2-ARs are present in the myometrium, their

effect on contractility shows marked species differences. a_1-ARs mediate myometrial

contraction in humans (Bottari et al., 1985) and rodents (Hoffman et al., 1981; Digges,

1982; Maltier and Legrand, 1985; Kyozuka et al., 1988), despite a greater density of

a_2-ARs than a_1-ARs in these species. a_2-ARs mediate myometrial contractions in sows

(Ko et al., 1990a; Yang and Hsu, 1995) and a_2-AR agonists such as xylazine also

induce myometrial contractility in dogs (Wheaton et al., 1989), goats (Perez et al.,

1994), sheep (Jansen et al., 1984) and cows (LeBlanc et al., 1984; Ko et al., 1990b).

The contractile response of the uterus is modified by ovarian steroids, estrogens

and progesterone. It is generally believed that estrogens promote myometrial contractions whereas progesterone promotes relaxation (Miller and Marshall, 1965). Estrogen treatment in rabbits can increase myometrial contractility by increasing a_1-adrenergic sensitivity without altering receptor density (Riemer et al., 1987). However, this increased sensitivity is thought to be the result of an estrogen-mediated increase in the postreceptor effects of prostaglandins (Hurd et al., 1991). Estrogen treatment also causes a dramatic increase in the number of a_2-ARs in the rabbit myometrium but this increase does not influence myometrial contractions (Riemer et al., 1987). The plasma concentrations of estrogens and progesterone change during the estrous cycle and pregnancy in the sow (Ford and Christenson, 1979; Ford et al., 1984; Thilander and Rodriguez-Martinez, 1989a, 1989b and 1990). However, it is not clear whether the changes in the endogenous steroid concentrations influence AR-mediated myometrial contractility.

In the luteal phase of the estrous cycle, a phase in which progesterone is the dominant sex steroid, the a_2-subtype is the major AR influencing myometrial contractility (Yang and Hsu, 1995). However, the role of a-ARs on myometrial contractility in the follicular phase of the estrous cycle and during pregnancy is not well-understood. Therefore, the present study was undertaken to investigate the adrenergic effect of natural catecholamines on myometrial contractility and to determine which of the a-AR subtypes mediate myometrial contractility changes in the estrous cycle and during pregnancy. In addition, the study was also designed to investigate the role of extracellular Ca^{2+} on the myometrial contractility in cycling and pregnant sows.

Materials and Methods

Tissue preparation

Porcine uterine specimens were collected from a local abattoir and a surgical laboratory. Specimens from the follicular phase and luteal phase of the estrous cycle and various stages of pregnancy were used. The crown-rump measurement of the fetus was used to estimate the days of the gestation and specimens were classified as early pregnancy (EPG; days of gestation = 39 - 40), mid-pregnancy (MPG; days of gestation = 53 - 60) and late pregnancy (LPG; days of gestation = 73 - 79) (Evans and Sack, 1973). Prepartum specimens were obtained from sows undergoing a C-section at 111th - 113th day of the pregnancy according to the known breeding record. The uteri were visually inspected and classified as follicular phase if ovaries had follicles $\geq$ 6 mm in diameter and no corpora lutea. Luteal phase was identified by ovaries with light red corpora lutea and the absence of embryos (Arthur et al., 1989). Only the mid-portion of the uterine horns was used in the experiments. The tissues were stored in ice-cold Tyrode's solution and transported to the laboratory. Upon arrival, the endometrium and placenta were removed form the uterus; the myometrium was stored in ice-cold Tyrode's solution (137 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM dextrose, and 12 mM $NaHCHO_3$; pH 7.4) aerated with 95% O_2-5% CO_2 and was used for experiments within 30 h. There were no changes in responsiveness to contractants during this period.

The methods for studying porcine myometrial contractility were as previously described (Yang and Hsu, 1995). Longitudinal uterine strips (10 x 2 mm^2) were suspended vertically in a 10-ml organ bath containing Tyrode's solution maintained at 37°C and aerated with 95% O_2-5% CO_2. The contractions were recorded isometrically on a multiple-channel recorder (R411, Beckman Instruments Inc., Schiller Park, IL) by a

transducer (Grass FT03, Grass Instrument Co., Quincy, MA). The strips were equilibrated under a 2-g tension for 20-25 min before being exposed to two 10^{-6} M carbachol (CARB) treatments for 3 min each to determine their responsiveness to the contractant. Four washes with 10 ml of Tyrode's solution each were used to remove CARB after each 3-min stimulations. The interval between the two CARB stimulations was 15 min. With the exception of the follicular phase specimens, uterine strips lost contractions within 15 min after the washout of CARB. This quiescent state usually lasted > 25 min in the luteal phase specimens and > 45 min in the pregnancy specimens. The basal resting tension was readjusted to 2 g before the administration of the pretreatment drug. In the following experiments, epinephrine (EPI) or norepinephrine (NE) was added at a 10-min interval in cumulative doses to attain a dose-response relationship.

Experimental protocols

A. Effect of CARB on myometrial contractions during the estrous cycle and pregnancy

In pilot studies we noticed that many tissue strips lost contractions after a 3-min but not a 10-min stimulation by 10^{-6} M CARB and subsequent Tyrode's solution washouts. The contractile pattern and response to 10^{-6} M CARB as characterized by the area under the contraction curve (AUCC) varied and was dependent on the phase repreductive stage. In addition, the AUCC which was induced by the stimultion with EPI or NE was measured for 10 min because it reached maximal response in 10 min. Therefore, an independent experiment was performed to obtain a regression line for each reproductive stage to fit the tissue responses to a 10-min 10^{-6} M CARB stimulation from the responses to a 3-min CARB stimulation. The tissue strips were stimulated by 10^{-6} M CARB twice. After the initial 3 min CARB treatment and subsequent 4 - 5 washes with 10 ml of Tyrode's solution each for a total of 15 min, The strips were stimulated again by 10^{-6} M CARB for 10 min. By using the 3 min and

10 min areas that were produced by the second stimulation a regression line for each stage was calculated:

Follicular phase (F) $Y (10\ min) = 2.70 * (3\ min) + 1.10\ (n = 25)$

Luteal phase (L) $Y (10\ min) = 2.95 * (3\ min) + 1.32\ (n = 39)$

Early pregnancy (EPG) $Y (10\ min) = 3.42 * (3\ min) + 1.08\ (n = 30)$

Mid-pregnancy (MPG) $Y (10\ min) = 3.01 * (3\ min) + 4.02\ (n = 30)$

Late pregnancy (LPG) $Y (10\ min) = 3.42 * (3\ min) + 0.84\ (n = 30)$

Prepartum period (PPT) $Y (10\ min) = 2.26 * (3\ min) + 5.65\ (n = 11)$

Because the contractile responses to CARB among the different reproductive stages were different, the transformed 10-min AUCC to 10^{-6} M CARB stimulation in each stage was also different. Therefore, it was necessary to study the effect of 10^{-6} M CARB on myometrial contractility in different phases of the estrous cycle and various stages of pregnancy.

The myometial strips of different reproductive stages used in the following experiments B, C and D, and other related studies in our laboratory were used. The 10-min AUCC to 10^{-6} M CARB stimulation for each strip was transformed from data obtained in the second 3-min 10^{-6} M CARB stimulation using the above formula. The transformed 10-min AUCCs of different reproductive stages were compared with each other.

B. EPI- and NE-induced myometrial contractility in the follicular phase of the estrous cycle and the influence of propranolol (PROP)

The myometrial strips in the follicular phase did not lose contractions after the strips were subjected to CARB stimulation and subsequent washouts with Tyrode's solution. Therefore, PROP was given until the spontaneous contractile activity had stabilized which usually took approximately 60 min.

To observe the α- and β-AR-mediated effect from EPI or NE stimulation, a 10-

min pretreatment with 10^{-6} M PROP was applied before each agonist was administered in cumulative doses (3×10^{-9} - 3×10^{-5} M). The control group did not receive PROP. To observe the time effect on the myometrial contractions throughout the experiment, time control groups were also studied. These groups were treated in the same manner as the other groups except that no agonist was applied.

C. EPI- and NE-induced myometrial contractility in the luteal phase of the estrous cycle and pregnancy and the influence of PROP, PRZ and YOH

To exclude the β-AR-mediated effect from EPI or NE stimulation, the β-AR antagonist 10^{-6} M PROP was added for 10 min before each agonist was administered in cumulative doses (3×10^{-9} - 3×10^{-5} M) at 10 min intervals. The control group did not receive PROP.

To characterize the α-AR-mediated contractions from EPI and NE stimulation, the α_1-AR antagonist PRZ (10^{-6} M) or the α_2-AR antagonist YOH (10^{-8}, 3×10^{-8}, 10^{-7} or 3×10^{-7} M) was added with 10^{-6} M PROP. The control group did not receive the antagonist.

A 10-min pretreatment period for the antagonists was chosen because in the preliminary experiment (n = 4 animals) antagonism of ARs reached a maximum in 10 min. Different strips from the same uterus were randomly assigned to all treatment groups in one trial, and each uterus was used for one trial only.

D. Effect of Ca^{2+}-free medium and verapamil on EPI- and NE-induced myometrial contractility

Protocols for treatment with Ca^{2+}-free Tyrode's solution and Ca^{2+}-free groups were performed as previously described (Yang and Hsu, 1995).

The uterine strips were assigned to three treatments as follows: a. Ca^{2+}-free medium; b. verapamil (10^{-5} M) in Ca^{2+}-containing medium; and c. Ca^{2+}-containing medium (control group). Verapamil, a voltage-dependent Ca^{2+} channel (VDCC) blocker,

was used to block Ca^{2+} influx. All groups were pretreated with 10^{-5} M PROP for 10 min before EPI or NE was given. The uterine strips in the follicular phase lost their spontaneous contractions $\leq$ 30 min following treatment with Ca^{2+}-free medium or verapamil. Propranolol (PROP) was given only after the disappearance of spontaneous contractions had occurred.

Assessment of the contractile response

Protocal for the determination of the contractile response was as previously described (Yang and Hsu., 1995). Briefly, the contractile response was assessed by the AUCC and was determined with the use of a scanning program (SigmaScan, Jandel, Corte Madrera, CA). The contractile response of the tissue strip was calculated from the AUCC produced by EPI or NE over 10 min at each cumulative dose and was expressed as a percentage of the response to 10^{-5} M CARB.

Drugs

The following drugs were used: carbachol chloride, (-)epinephrine bitartrate, (-) norepinephrine bitartrate, propranolol HCl and yohimbine HCl (Sigma Chemical Co., St. Louis, MO); prazosin HCl (Pfizer Inc., Groton, CT); verapamil HCl (Knoll Pharmaceutical Co., Whippany, NJ). Drugs were dissolved in distilled water, except for epinephrine and norepinephrine, which were dissolved in 0.1% (W/V) ascorbic acid in 0.9% NaCl, and prazosin HCl, which was dissolved in 2% lactic acid to achieve a concentration of 1 mM. Drug-containing solutions were prepared before use by appropriate dilution of stock solutions and were stored at -20°C.

Data analyses

The purpose of the present study was to observe the changes in myometrial contractility in stages of the estrous cycle and pregnancy in response to EPI and NE. Hence, the data obtained from luteal phase specimens in the previous study (Yang and Hsu, 1995) were involved in this study for comparison.

Dose-response curves were produced by cumulative applications (van Rossum, 1963) of EPI and NE in approximately one-half log increment in experiments B and C, and one log increment in experiment D. The data from experiment C were expressed as pD_2 (-log EC_{50}).

Dissociation constants (K_B) of YOH against the agonist were determined using the equation: $K_B = [B]/(CR - 1)$, where B is the concentration of the antagonist (Furchgott, 1972). The response to YOH (3×10^{-8} M) was used to calculate K_B because YOH at this dose caused an obvious and consistent antagonism on contractility. The concentration ratio (CR) is defined as EC_{50}'/EC_{50}, in which EC_{50} and EC_{50}' values are the values for the agonist in the absence and presence of the antagonist, respectively. The dissociation constant of the antagonist was expressed as pK_B ($= -\log K_B$).

In experiment B, the contractile response of each treatment group in the follicular phase was compared at the corresponding dose of the agonist. In experiment D, the contractile response was compared with the control group at the corresponding dose of the agonist in the same phase. In addition, the contractile response at 10^{-5} M of the agonist was compared among the different phases because this dose caused maximal myometrial contractility (Yang and Hsu, 1995).

Data were expressed as mean $\pm$ SE and were analyzed by analysis of variance (ANOVA). The conservative F value was used to establish significance for the treatment effect. The least significant difference test was used to determine the difference between means of end points for which the ANOVA indicated a significant ($P < 0.05$) F ratio.

Results

A. Effect of CARB on myometrial contractions during the estrous cycle and pregnancy

The contractile responses of myometrial strips obtained during the estrous cycle and pregnancy to the 10-min CARB (10^{-6} M) stimulation were different. Their 10-min transformed AUCCs were significantly different ($P < 0.05$) among all groups except between luteal phase and late pregnancy (Table 1). The order of the transformed areas produced by the stimulation of 10^{-6} M CARB was EPG > MPG > L > LPG > PPT > F.

B. EPI- and NE-induced myometrial contractility in the follicular phase of the estrous cycle and the influence of PROP

The uterine strips showed spontaneous contractions in the time control groups in both absence and presence of 10^{-6} M PROP throughout the experiment (Figs. 1A and 1B).

In the absence of PROP, EPI ($\geq 3 \times 10^{-8}$ M) and NE ($\geq 3 \times 10^{-7}$ M) each progressively decreased myometrial contractility. The contractility was significantly different from those in PROP-pretreated groups (EPI, $\geq 3 \times 10^{-8}$ M; NE, $\geq 3 \times 10^{-7}$ M) and time control groups at the corresponding agonist concentrations (EPI, $\geq 3 \times 10^{-3}$ M; NE, $\geq 3 \times 10^{-6}$ M) ($P < 0.05$). In the presence of PROP, EPI and NE did not increase myometrial contractions when compared with the time controls.

C. EPI- and NE-induced myometrial contractility in the luteal phase and during pregnancy and the influence of PROP, PRZ and YOH

Both EPI and NE in the presence of 10^{-6} M PROP produced dose-dependent increases in myometrial contractility in all 5 reproductive stages studied (Figs. 2A and 3A). Higher doses of EPI and NE decreased the contractility progressively. The pD_2 of EPI was significantly greater than that of NE in the same stage ($P < 0.05$; Table 2). The rank order of the potencies (pD_2) of EPI and NE in different stages was L $\geq$ LPG $\geq$

Table 1. Effect of 10^{-6} M carbachol on the myometrial contractions in the estrous cycle and various stages of pregnancy

Stage	n[a]	Mean ± SE[b,c]
Follicular phase (F)	68	14.55 ± 0.64
Luteal phase (L)	250	18.20 ± 0.24
Early pregnancy (EPG)	196	21.39 ± 0.43
Mid-pregnancy (MPG)	204	19.40 ± 0.30
Late pregnancy (LPG)	162	17.50 ± 0.39
Prepartum (PPT)	145	16.05 ± 0.28

[a]n is the number of animals.

[b]The data are expressed as areas in cm^2. Data were transformed from areas of the second 3-min 10^{-6} M CARB stimulation to 10-min contractile areas using the formulas described in Materials and Methods.

[c]There are significantly (P < 0.05) different among all groups except for L vs. LPG, which was not significantly different.

Fig. 1. Effect of epinephrine (EPI) and norepinephrine (B) in the presence (○) and

absence (●) of propranolol (PROP) in the follicular phase of the estrous cycle.

The time control groups did not receive agonist in the presence (▲) and

absence (▼) of PROP. Data are expressed as means ± SE (n = 5). The data

of baseline contractile activity were obtained at 10 min before the agonist was

given.

*P < 0.05, comparing with that in the presence of PROP (○) at the

corresponding agonist concentration.

**P < 0.05, comparing with that in the time control in the absence of PROP

(▼) at the corresponding agonist concentration.

A. Epinephrine

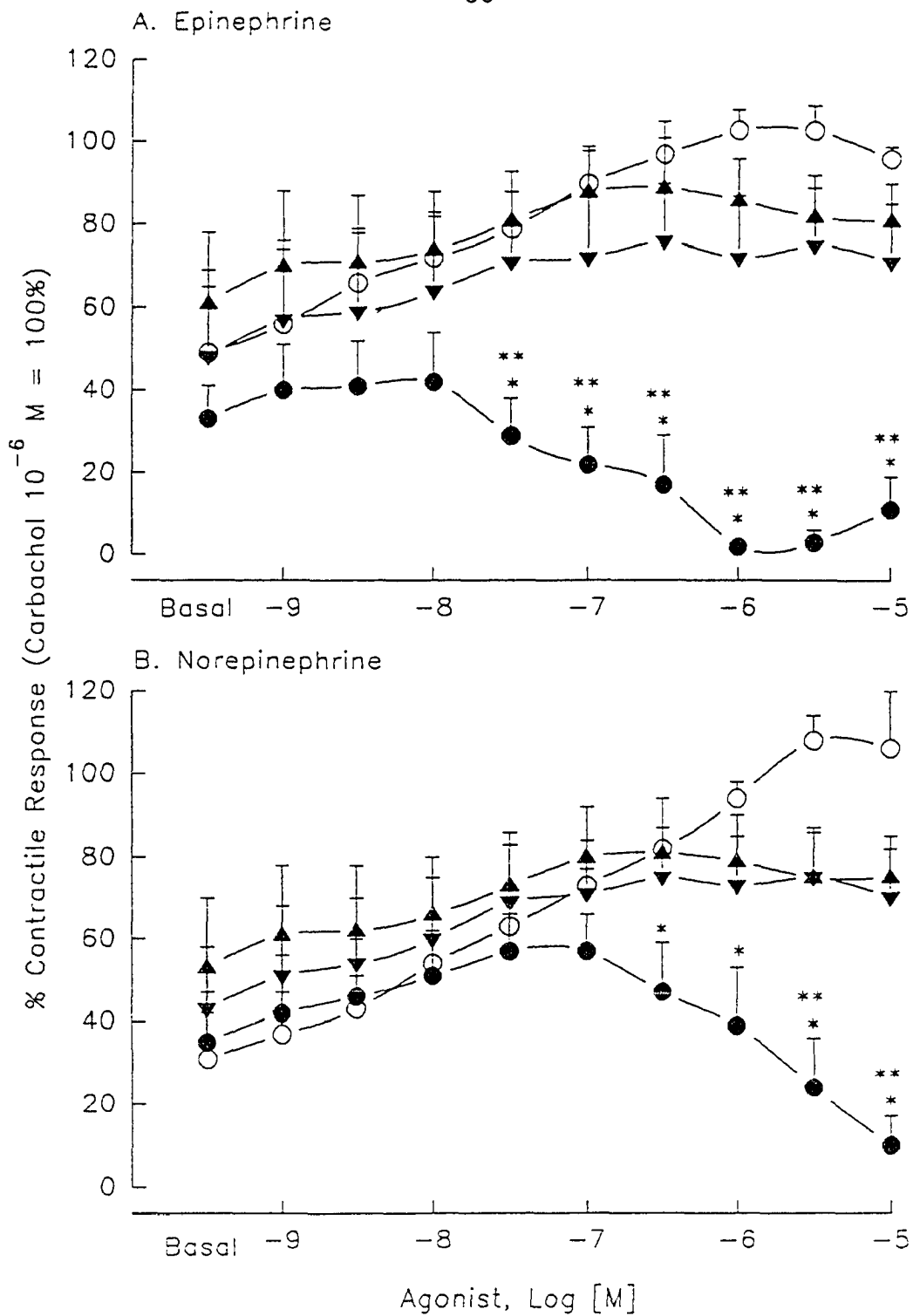

B. Norepinephrine

Agonist, Log [M]

Fig. 2. Dose-response curves for epinephrine in the luteal phase of the estrous cycle

and the pregnancy in the presence (A) and absence (B) of propranolol (PROP).

Uterine strips were obtained from sows in the luteal phase (○) of estrous

cycle, and in early pregnancy (●), mid-pregnancy (▲), late pregnancy (▼) and

prepartum period (■). Data are expressed as mean ± SE (n = 6).

A. PROP

B. w/o PROP

% Contractile Response (Carbachol 10^{-6} M = 100%)
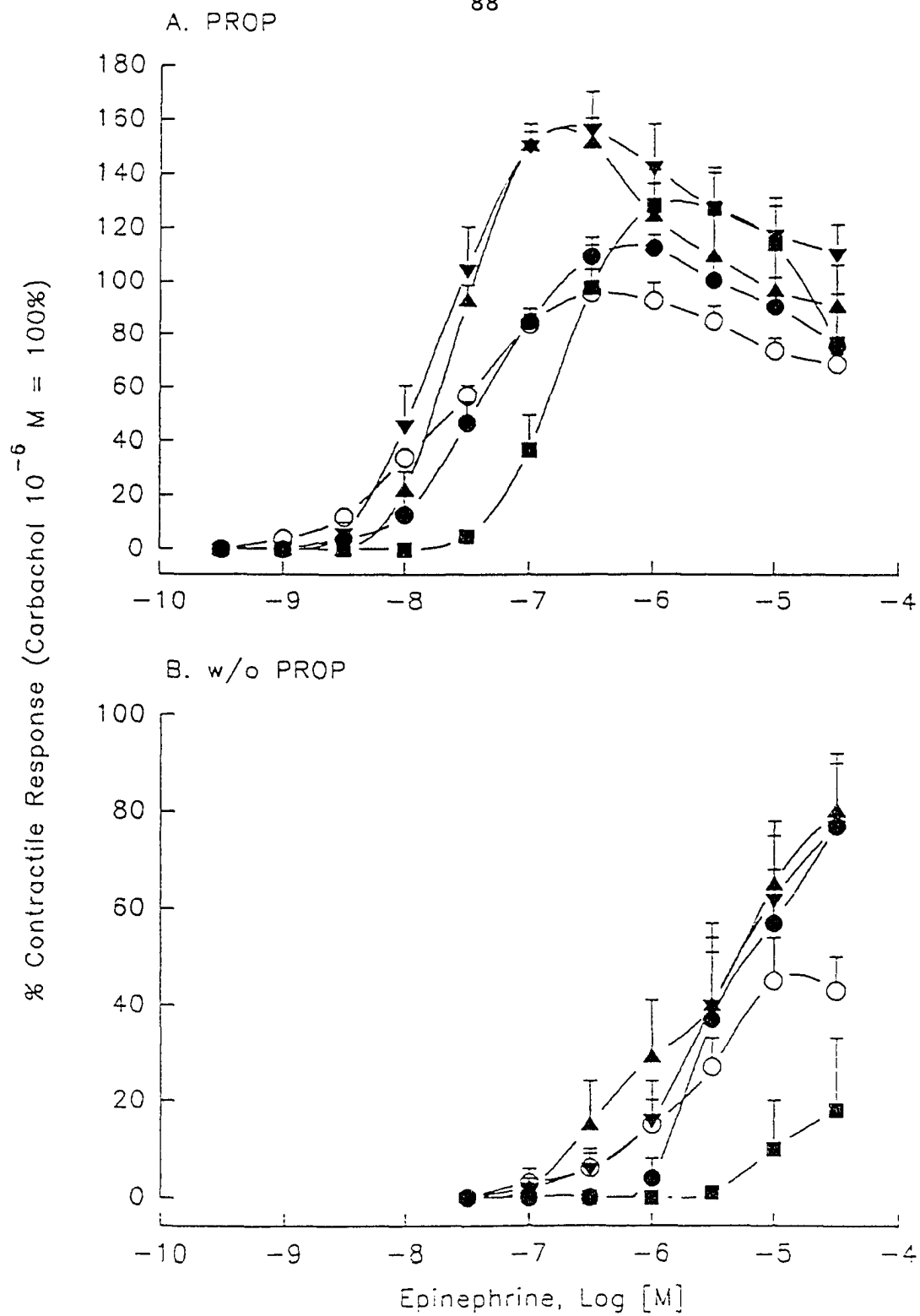
Epinephrine, Log [M]

Fig. 3. Dose-response curves for norepinephrine in the luteal phase of the estrous

cycle and the pregnancy in the presence (A) and absence (B) of propranolol

(PROP). Uterine strips were obtained from sows in the luteal phase (○) of

estrous cycle, and in early pregnancy (●), mid-pregnancy (▲), late pregnancy

(▼) and prepartum period (■). Data are expressed as mean ± SE (n = 6).

A. PROP

B. w/o PROP

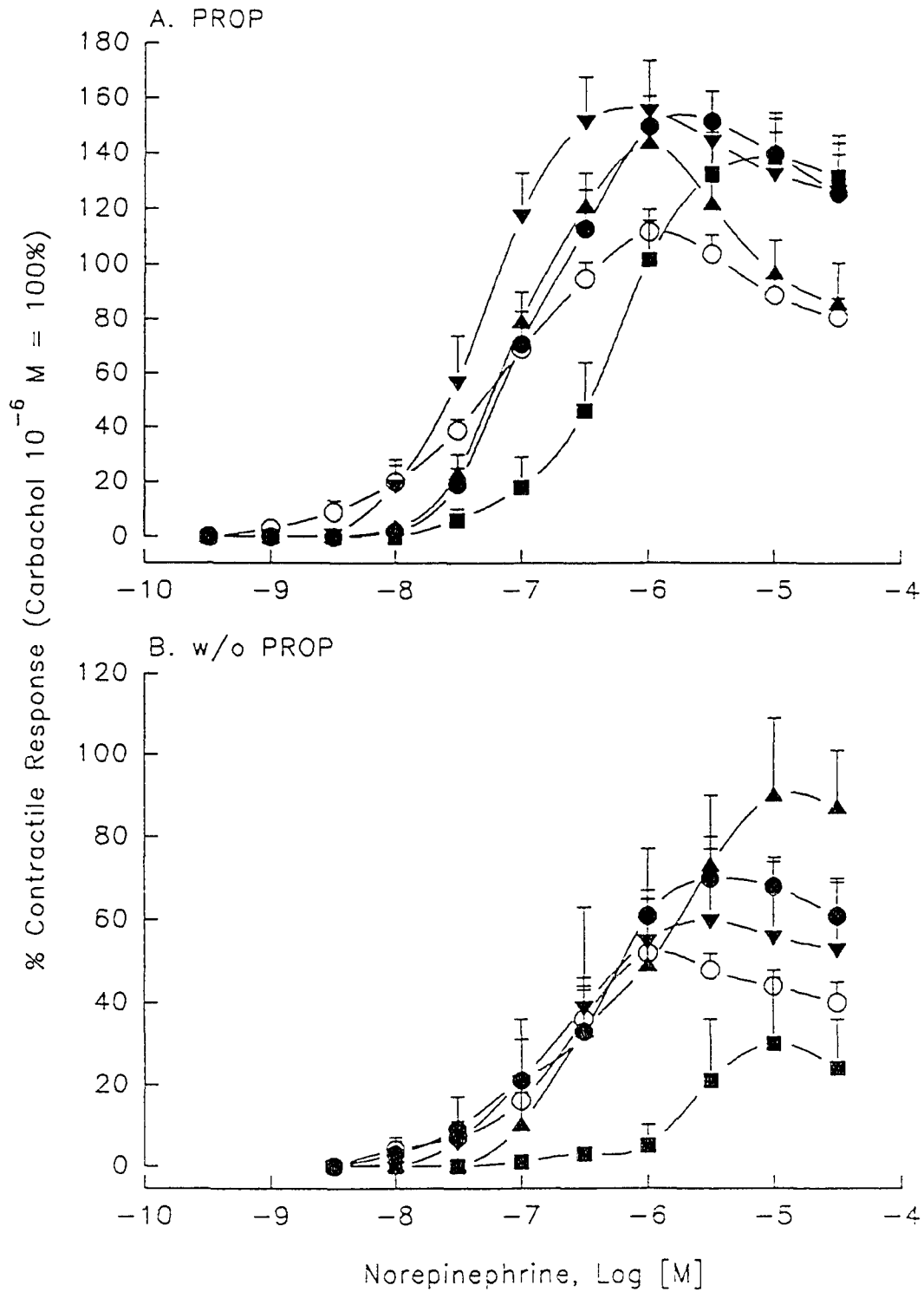% Contractile Response (Carbachol 10^{-6} M = 100%)

Norepinephrine, Log [M]

Table 2. pD_2 values for epinephrine and norepinephrine in the presence of 10^{-6} M propranolol in the control and prazosin (10^{-6} M)-treated groups in the luteal phase of estrous cycle and pregnancy

Treatment	Stage	Agonist	
		Epinephrine	Norepinephrine
Control			
	L	$7.81 \pm 0.07^{*,a}$	$7.38 \pm 0.08^{a,b,c}$
	EPG	$7.49 \pm 0.15^{*,a}$	6.95 ± 0.06^{a}
	MPG	$7.63 \pm 0.04^{*,a}$	7.06 ± 0.03^{a}
	LPG	$7.72 \pm 0.13^{*,a}$	$7.34 \pm 0.13^{a,b,c}$
	PPT	$6.80 \pm 0.10^{*}$	6.33 ± 0.16
Prazosin			
	L	$7.59 \pm 0.10^{*}$	7.11 ± 0.11
	EPG	$7.16 \pm 0.11^{*}$	6.59 ± 0.14
	MPG	$7.39 \pm 0.08^{*}$	6.99 ± 0.20
	LPG	$7.60 \pm 0.15^{*}$	7.17 ± 0.19
	PPT	$6.70 \pm 0.12^{*}$	5.86 ± 0.20

The data are expressed as mean $\pm$ SE (n = 6).

*P < 0.05, compared with norepinephrine in the same stage treatment.

[a]P < 0.05, compared with prepartum period (PPT) in the same agonist.

[b]P < 0.05, compared with mid-pregnancy (MPG) in the same agonist.

[c]P < 0.05, compared with early pregnancy (EPG) in the same agonist.

MPG $\geq$ EPG > PPT (Table 2).

In the absence of 10^{-6} M PROP, EPI and NE at concentrations of $\geq$ 10^{-5} M caused progressive increases in myometrial contractility (Figs. 2B and 3B). The prepartum specimens exhibited the smallest increase in contractility among the groups.

The α_2-AR antagonist, YOH, blocked the effects of both EPI and NE in a dose-dependent manner in all 5 stages studied. The pK_B values for YOH against EPI and NE were not significantly different among the five reproductive stages (Table 3).

The α_1-AR antagonist, PRZ, even at a high concentration of 10^{-5} M failed to antagonize the effect of EPI or NE on myometrial contractility. The pD_2 values from EPI or NE stimulation in the PRZ groups were not significantly different from those in control groups in all 5 stages (Table 2).

D. Effect of Ca^{2+}-free medium and verapamil on the EPI- and NE-induced myometrial contractility

Both EPI and NE ($\leq$ 10^{-5} M) caused a dose-dependent increase in myometrial contractility in the presence of 10^{-6} M PROP (Figs. 4A and 5A). This effect of EPI and NE was greatly decreased by 10^{-5} M verapamil (Figs. 4B and 5B) or in the Ca^{2+}-free Tyrode's solution (Figs. 4C and 5C). In the Ca^{2+}-free medium or with verapamil pretreatment the uterine strips from the follicular phase and prepartum period had a smaller contractile response to EPI and NE (10^{-5} M) than those from the luteal phase and other stages of pregnancy (P < 0.05).

Discussion

The results of the present study demonstrate that EPI- and NE-induced contractions of porcine longitudinal myometrium are mediated predominately by α_2-ARs, and minimally by α_1-ARs both in the luteal phase of the estrous cycle and during

Table 3. Dissociation constants (pK_a) for yohimbine against the agonists acting on the α_2-adrenoceptors in the estrous cycle and pregnancy

Agonist	Stage	n[a]	pK_a[b] Yohimbine
Epinephrine			
	L	6	8.42 ± 0.14
	EPG	6	8.04 ± 0.12
	MPG	6	8.11 ± 0.07
	LPG	6	7.90 ± 0.08
	PPT	5	8.09 ± 0.22
Norepinephrine			
	L	6	8.26 ± 0.26
	EPG	6	7.95 ± 0.07
	MPG	6	8.26 ± 0.10
	LPG	6	8.16 ± 0.12
	PPT	5	7.85 ± 0.09

[a]n is the number of animals.

[b]The pK_a values for yohimbine against epinephrine and norepinephrine are not significantly different among the reproductive stages.

Fig. 4. Effect of verapamil and Ca^{2+}-free medium on epinephrine (EPI)-induced

increase in myometrial contractility. Effects are shown in Ca^{2+}-containing

medium (control group) (A), 10^{-5} M verapamil (B) and Ca^{2+}-free medium (C).

Uteri w.. e obtained from sows in the follicular phase (F) and the luteal phase

(L) of the estrous cycle, and in early pregnancy (EPG), mid-pregnancy (MPG),

late pregnancy (LPG) and prepartum period (PPT). Uterine strips had been

pretreated with 10^{-6}M PROP. Data are expressed as mean $\pm$ SE (n = 6), and

those for the statistics was at 10^{-6} M EPI were compared.

[a]$P < 0.05$, compared with LPG group.

[b]$P < 0.05$, compared with L group.

[c]$P < 0.05$, compared with LPG group.

A. Control

F
L
EPG
MPG
LPG
PPT

B. Verapamil 10^{-5} M

C. Ca^{2+}-free Medium

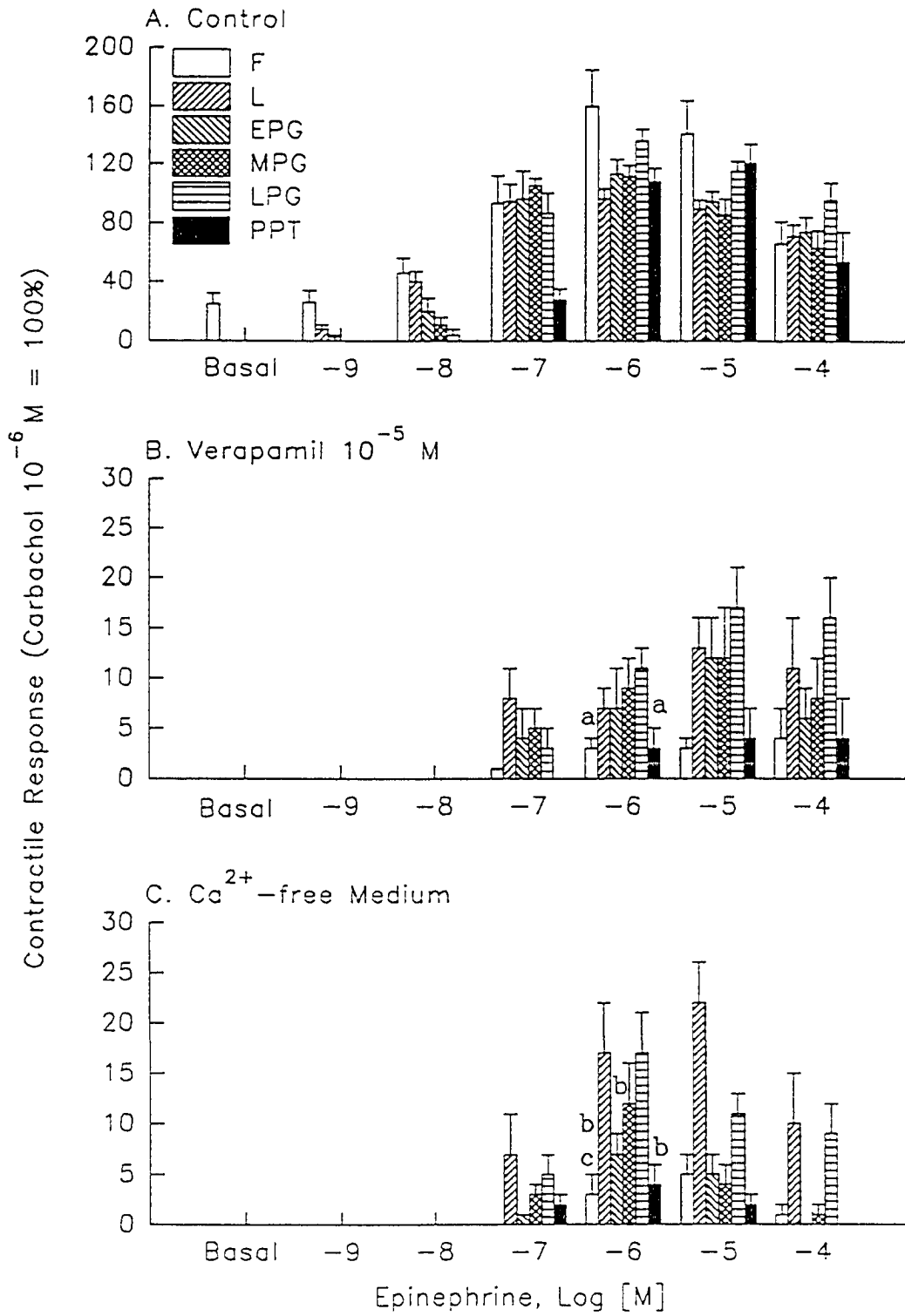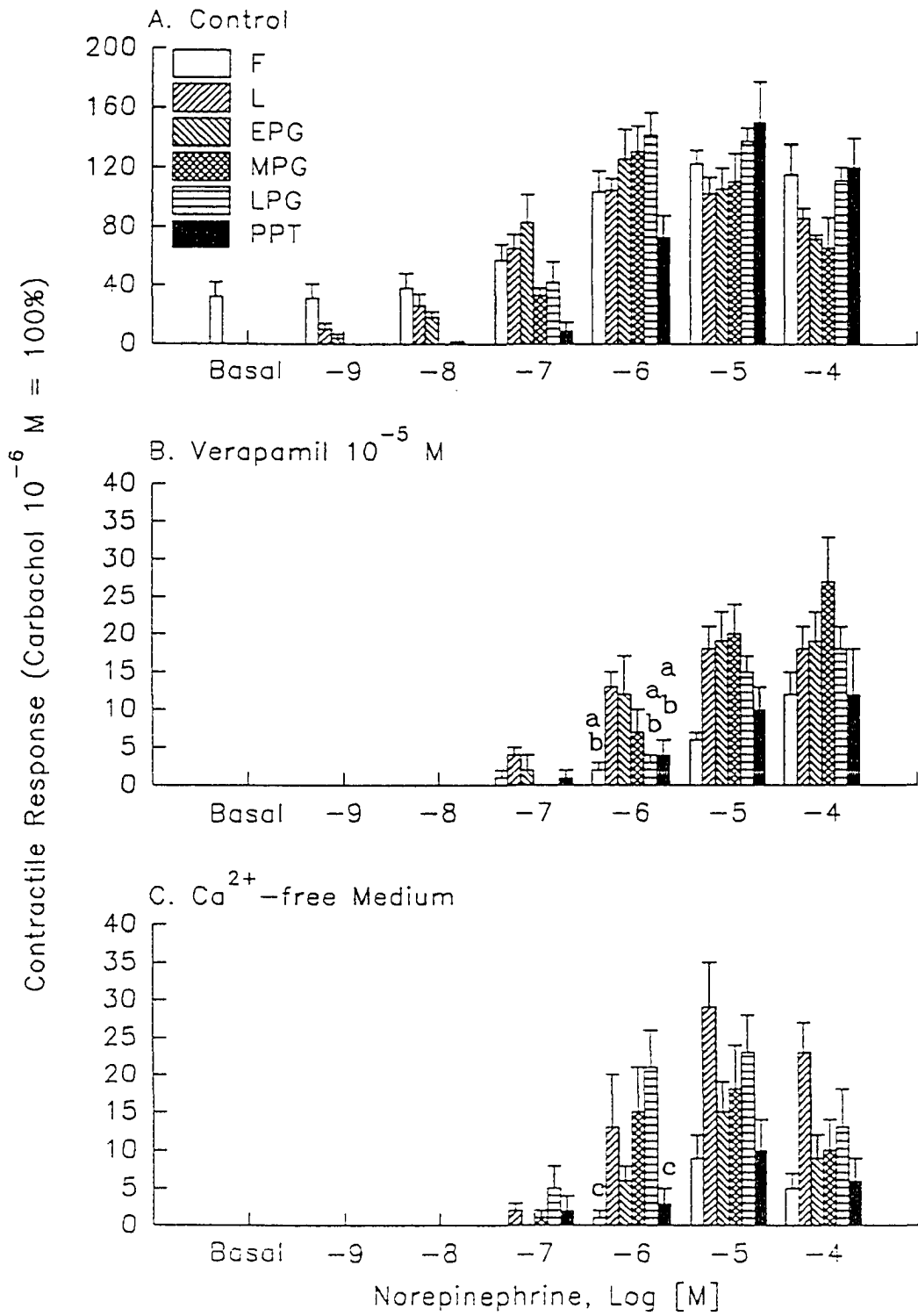Contractile Response (Carbachol 10^{-6} M = 100%)

Epinephrine, Log [M]

Fig. 5. Effect of verapamil and Ca^{2+}-free medium on norepinephrine (NE)-induced increase in myometrial contractility. Effects are shown in Ca^{2+}-containing medium (control group) (A), 10^{-5} M verapamil (B) and Ca^{2+}-free medium (C). Uteri were obtained from sows in the follicular phase (F) and the luteal phase (L) of the estrous cycle, and in early pregnancy (EPG), mid-pregnancy (MPG), late pregnancy (LPG) and prepartum period (PPT). Uterine strips had been pretreated with 10^{-6} M PROP. Data are expressed as mean $\pm$ SE (n = 6), and those for the statistics was at 10^{-6} M NE were compared.

[a]$P < 0.05$, compared with EPG group.

[b]$P < 0.05$, compared with LPG group.

[c]$P < 0.05$, compared with L group.

.

A. Control

B. Verapamil 10^{-5} M

C. Ca^{2+}-free Medium

Contractile Response (Carbachol 10^{-6} M = 100%)

Norepinephrine, Log [M]

pregnancy. These findings are consistent with those of the previous report that xylazine-induced increase in porcine myometrial contractility is mediated by a_2-ARs (Ko et al., 1990a). In addition, this a_2-AR mediated contractility is primarily dependent on Ca^{2+} influx through VDCC which is consistent with what has been reported for other smooth muscles (Wray, 1993).

In the presence of PROP, EPI and NE induced an increase in myometrial contractility in a dose-dependent manner in all reproductive stages except the follicular phase. In response to higher doses of both EPI and NE, myometrial contractility decreases because more β_2-ARs have been activated by EPI and NE (Yang and Hsu, 1995). The responses to EPI or NE on contractility were different among reproductive stages. In general, the responses to EPI and NE during all stages of the pregnancy were less than or equal to that in the luteal phase. Moreover, the least potency was observed in the prepartum period when compared with other pregnancy stages.

In the absence of PROP, the inhibitory effect of EPI ($\geq$ 3 x 10^{-8} M) and NE ($\geq$ 3 x 10^{-7} M), on myometrial contractions in the follicular phase was attributed to the activation of myometrial β_2-ARs by EPI or NE (Yang and Hsu, 1995). In the presence of PROP, EPI and NE did not cause a significnat dose-dependent increase in myometrial contractility in the follicular phase, in which the tissue strips were exposed to high estrogens and presented spontaneous contractions throughout the experiment.

The effect of estrogens on myometrial contractility is still controversial. In rat myometrium, estrogens exert an inhibitory effect on spontaneous contraction (Batra and Bengtsson, 1978), and on contractions that are evoked by the electrical stimulation (Osa and Ogasawara, 1984) or KCl-depolarization (Batra and Bengtsson, 1978). In addition, both estradiol and diethylstilbestrol inhibit myometrial Ca^{2+} channel activity in the cells isolated from pregnant rats (Yamamoto, 1995) and Ca^{2+} influx is decreased by diethylstilbestrol in the rat myometrium (Batra and Bengtsson, 1978).

However, Ca^{2+} influx in myometrial strips and myometrial Ca^{2+} channel density increase in estrogen-dominated rats (Batra, 1987). Furthermore, Ca^{2+} influx through VDCC does not significantly change between estrogen- and estrogen followed by progesterone-dominated murine myometrium (Ruzycky et al., 1987). The density of a_2-ARs in porcine myometrium in the follicular phase, in which the circulating estrogens are high, is less than that in the luteal phase in which the circulating progesterone is high (Yang and Hsu, unpublished). The lack of myometrial contractile response to catecholamines in the follicular phase may be due, at least in part, to the relative low concentration of a_2-ARs in the follicular phase when compared with the luteal phase (Yang and Hsu, unpublished).

CARB, a cholinergic agonist, mediates muscarinic M_2 and M_3 receptors via G and G_q protein signal transduction pathways, respectively, to cause myometrial contractions in guinea pigs (Eglen et al., 1989; Leiber et al., 1990). In the present study, the contractile responses to CARB in the myometrium of the estrous cycle and during pregnancy were variable. Generally, the myometrium which was exposed to a high progesterone environment such as those in the luteal phase and during pregnancy, except at peripartum period, had a higher response to CARB stimulation when compared with those in the follicular phase and in the prepartum period when the tissues were exposed to the high estrogen environment. It is unclear why CARB produces variable contractile activity at different stages of the reproductive cycle. However, maximal contractile activity in progesterone-dominated myometrium is greater than that in estrogen-dominated myometrium in the guinea pig (Ruzycky and Crankshaw, 1988). This contractile difference is positively correlated with the density of muscarinic receptors in the myometrium. It is not clear if the different myometrial contractile responses to CARB stimulation in reproductive stages in the present study can be attributed to the density of muscarinic receptors. In addition, CARB-mediated

[^{3}H]inositol phosphate accumulation and myometrial contractions in progesterone-dominated myometrium is greater than that in the estradiol-dominated myometrium in rats (Ruzycky and Triggle, 1987). It may be necessary to delineate the relationship between myometrial contractility and the stimulation of inositol phospholipid turnover by CARB in the porcine myometrium.

Nevertheless, the variable contractile responses to CARB in different reproductive stages does not influence the interpretation of the EPI- and NE-induced myometrial contractilities as each stage of the tissues had its own, independent transformation equation to derive the contractile response. Furthermore, in the present study, CARB-evoked myometrial contractile response was different from the EPI- or NE-evoked response through a_2-ARs.

Although the physiological role of a_2-ARs in porcine myometrium during pregnancy is not clear, a_2-ARs are known to mediate an increase in myometrial contractility. One of the major functions of the uterus during pregnancy is to provide a quiescent state to allow for fetal growth and development until parturition. The induced myometrial contractility may be influenced by other factors. For example, during pregnancy, the high circulating progesterone and the presence of myometrial β-ARs promote uterine relaxations (Wray, 1993). We have confirmed that porcine myometrial β-ARs reach a maximum mid-pregnancy (days of gestation = 56 - 60), at the same time that the circulating estrogens are low and progesterone concentrations are high (Thilander and Rodriguez-Martinez, 1989b). The maximum binding density (B_{max}) of β-ARs in porcine longitudinal layer in this stage is 359 ± 42 fmol/mg protein. Then the B_{max} of β-AR decreases progressively to 171 ± 19 fmol/mg protein in the prepartum period (Yang and Hsu, unpublished data), when the circulating estrogens are high (Ford et al., 1984; Thilander and Rodriguez-Martinez, 1990). Furthermore, estrogens reduce myometrial β-AR-mediated cAMP production leading to a decrease in

the myometrial relaxation in the rabbit (Riemer *et al.*, 1988). These findings suggest that β-ARs during pregnancy are influenced by the ovarian steroids and increasing or decreasing β-AR-mediated relaxations may affect a_2-AR-mediated porcine myometrial contractility during pregnancy.

In addition, the plasma estrogen concentrations increase prior to parturition in many species, including sows (Ford *et al.*, 1984; Thilander and Rodriguez-Martinez, 1990). The high estrogen levels stimulate the formation of gap junctions in myometrium, enhance uterine contractility through stimulation of prostaglandin production, and increase myometrial oxytocin receptors to facilitate labor (Garfield, 1994). Therefore, the activity of myometrial a_2-ARs may interact with other hormones, such as prostaglandin F_{2a} to cause myometrial contractions (Ko *et al.*, 1989) although the lower density of a_2-ARs in the prepartum period (Yang and Hsu, unpublished data) may be related to the decreased EPI- and NE-induced myometrial contractility.

The results of the present study confirmed and extended our previous findings that a Ca^{2+}-free medium and a VDCC blocker, verapamil, greatly reduced the effect of EPI and NE on porcine myometrial contractility during the estrous cycle and pregnancy. These results suggested that the a_2-AR-mediated myometrial contractility predominantly depends on the Ca^{2+} influx via VDCC and at least in part, due to calcium release from intracellular stores (Yang and Hsu, 1995). The contractile responses to 10^{-6} M of EPI and NE in the Ca^{2+}-free medium were different among the different reproductive stages which were consistent with contractile responses in the Ca^{2+}-containing medium. In general, the response was less in the tissues in the follicular phase and prepartum period which were exposed to the high estrogen environment than those in the luteal phase and other stages of pregnancy which were exposed to the high progesterone environment.

The density of a_2-ARs in the follicular phase is less than that in the luteal phase in the pig (Rexroad and Guthrie, 1983; Yang and Hsu, unpublished), and the density of a_2-ARs decreases during preparturient period in guinea pigs (Arkinstall and Jones, 1988) and rats (Legrand et al., 1993). In addition, as the pregnancy progresses in the sow, the thickness of the longitudinal layer decreases progressively (Thilander and Rodriguez-Martinez, 1989b). Therefore, it is also possible that the decreased density of a_2-ARs in the myometrium and the decreased thickness of myometrial strips reduces the contractile response in the Ca^{2+}-free medium. Future studies are needed to quantify the density of porcine myometrial a_2-ARs during the estrous cycle and pregnancy and to correlate the number of a_2-ARs with the myometrial contractility.

Ovarian hormones influence the activity of the VDCC in rat myometrial cells (Rendt et al., 1992; Yamamoto, 1995). Progesterone increases (Rendt et al., 1992) and estrogens decrease (Yamamoto, 1995) the Ca^{2+} channel activity. In contrast, the function of Ca^{2+} influx through myometrial VDCC in estrogen-dominated rats is not altered significantly compared to the progesterone followed by estrogen-dominated rats (Ruzycky et al., 1987), and estradiol does not inhibit myometrial VDCC activity in preparturient sows (ZhuGe and Hsu, unpublished). Hence, the mechanisms by which ovarian hormones influence Ca^{2+} channel and myometrial contractility in porcine myometrium need further investigation.

In conclusion, the results of the present study suggested that a_2-ARs mediate natural catecholamines EPI- and NE-induced increase in myometrial contractilities in the cycling and pregnant sows. The effect of EPI and NE is attributed primarily to an increase in Ca^{2+} influx, through VDCC. This study also demonstrates that the EPI- and NE-induced myometrial contractions in the follicular phase and the prepartum period, which are exposed to high estrogens levels, were less than those in the luteal phase and other stages of pregnancy, which are exposed to high progesterone levels.

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Acknowledgements

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CHARACTERIZATION OF α_1- AND α_2-ADRENOCEPTORS IN PORCINE MYOMETRIUM

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Abstract

Binding of [^{3}H]prazosin and [^{3}H]rauwolscine was used to identify porcine myometrial α_1- and α_2-adrenoceptors, respectively, during the estrous cycle and pregnancy. Binding of [^{3}H]prazosin and [^{3}H]rauwolscine to membrane proteins from the porcine myometrium was saturable with high affinities and was rapidly reversed by 10^{-5} M phentolamine. Saturation binding studies with [^{3}H]prazosin showed that the density of α_1-adrenoceptors was low throughout the reproductive stages tested; the order of the maximum density of binding sites (B_{max}) in fmol/mg protein was luteal phase (23.6 $\pm$ 2.1) $\approx$ early pregnancy (days of gestation = 37 - 40) (22.0 $\pm$ 1.3) $\geq$ late pregnancy (days of gestation = 77 - 79) (20.0 $\pm$ 3.9) $\geq$ mid-pregnancy (days of gestation = 57 - 60) (15.6 $\pm$ 3.4) > prepartum period (days of gestation = 111 - 113) (11.3 $\pm$ 1.1) > follicular phase (7.5 $\pm$ 1.6). The density of α_1-adrenoceptors accounted for 1 - 3% of total α-adrenoceptors, and the equilibrium dissociation constants (K_D) being 21.5 - 33.5 pM, which were not significantly different among the tested groups. The density of α_2-adrenoceptors varied in the different reproductive stages although the K_D values (4.6 - 6.9 nM) were not changed significantly. The order of B_{max} values in fmol/mg protein was early pregnancy (2,426 $\pm$ 430) $\approx$ very late pregnancy (days of gestation $\geq$ 100) (2,392 $\pm$ 341) $\geq$ late pregnancy (2,049 $\pm$ 131) $\approx$ mid-pregnancy (1,999 $\pm$ 318) $\geq$ luteal phase (1,568 $\pm$ 135) $\approx$ prepartum period

(1,507 ± 236) > follicular phase (265 ± 50). Therefore, the density of myometrial α_2-adrenoceptors was higher in the tissues exposed to a high progesterone environment than the tissues exposed to a low progesterone environment. From the competition binding studies in myometrial membranes from luteal phase, the drug affinities, including idazoxan, oxymetazoline, prazosin, RX 821002, WB 4101 and yohimbine, were highly correlated when porcine myometrium was compared with known α_{2A}-subtype tissues or cells. In contrast, comparison of porcine myometrium with other known α_2-subtype, i.e., α_{2B}, α_{2C}, and α_{2D}, tissues and cells resulted in poor correlation. The present findings suggested that α_1- and α_2-ARs are present in the porcine longitudinal myometrium during the estrous cycle and pregnancy. The α_2-AR is the dominant α-AR in porcine myometrium and its density varies among the reproductive stages. Its density is high in progesterone-dominated stages, such as the luteal phase and pregnancy, and is low in estrogen-dominated stages, such as the follicular phase. In contrast, α_1-ARs are in low concentrations throughout the reproductive stages. Furthermore, porcine myometrial α_2-AR may be the α_{2A} subtype based on the results of competition experiments.

Introduction

Using radioligand binding assays of [^{3}H]prazosin (PRZ) and [^{3}H]rauwolscine (RAU), it has been demonstrated that α_1- and α_2-adrenoceptors (ARs) are present in the myometrium of different species, including humans (Bottari et al., 1983a and 1983b), rats (Maltier and Legrand, 1985; Legrand et al., 1993), guinea pigs (Arkinstall and Jones, 1988; Arkinstall et al., 1989; Haynes et al., 1993), rabbits (Falkay, 1990), ewes (Vass-Lopez et al., 1990b) and pigs (Taneike et al., 1995). The functions of α_1- and α_2-ARs to mediate myometrial contractions are species different. α_1-ARs mediate

myometrial contractility in rats (Kyozuka *et al.*, 1988), rabbits (Hoffman *et al.*, 1981), guinea pigs (Hartley *et al.*, 1983) and humans (Bottari *et al.*, 1985) even through the density of myometrial a_2-ARs in these species may exceed that of a_1-ARs. In contrast, a_2-ARs mediate myometrial contractility in pigs (Ko *et al.*, 1990a; Yang and Hsu, 1995a and 1995b), cows (LeBlanc *et al.*, 1984a and 1984b; and Ko *et al.*, 1990b), goats (Perez *et al.*, 1994) and sheep (Prud'Homme, 1988).

The ovarian steroids, estrogens and progesterone modify the densities of myometrial a-ARs and these densities vary in the estrous cycle and pregnancy. The density of a_1-ARs is not affected by the ovarian steroids in rabbits (Hoffman *et al.*, 1981) or ewes (Vass-Lopez *et al.*, 1990b), and does not change in the menstruous cycle or pregnancy in women (Bottari *et al.*, 1983c and 1985). However, the a-AR density is 40% higher during pregnancy when compared to that during the estrous cycle of guinea pigs (Arkinstall and Jones, 1989).

The findings pertaining to myometrial a_2-ARs are more variable than those of a-ARs in the different species. In rabbits (Hoffman *et al.*, 1981; Jacobson *et al.*, 1987; Riemer *et al.*, 1987) and humans (Bottari *et al.*, 1983c and 1985) the myometrial a_2-AR density increases when plasma estrogen concentrations are high. However, the density decreases in ewes (Vass-Lopez *et al.*, 1990a and 1990b) and sows (Rexroad and Guthrie, 1983) in the same endocrine environment. In ewes, the myometrial a_2-AR concentration is high (Rexroad, 1981; Vass-Lopez *et al.*, 1990a and 1990b) in the progesterone-treated ewe and during pregnancy which is in a high progesterone environment. In contrast, in humans (Bottari *et al.*, 1983c and 1985) and rabbits (Williams *et al.*, 1977) the a_2-AR density is decreased in the same endocrine environment. Moreover, the density of myometrial a_2-ARs increases greatly in mid-pregnancy, then decreases abruptly at the end of pregnancy in rats and guinea pigs (Kyozuka *et al.*, 1988; Legrand *et al.*, 1993), whereas the a_2-AR concentration

increases in rabbits at term (Jacobson *et al.*, 1987).

The presence of α_2-ARs in porcine myometrium has been reported using [^{3}H]dihydroergocryptine (DHE), a non-selective α-AR antagonist (Rexroad and Guthrie, 1983). The use of [^{3}H]DHE is unable to quantify the α_1-ARs in the myometrium, nor does it characterize the myometrial α_2-ARs specifically as the selective α_2-AR ligand, such as [^{3}H]RAU. Recently, although [^{3}H]PRZ and [^{3}H]RAU have demonstrated the presence of α_1- and α_2-ARs in porcine myometrium, respectively, the study is limited to gilts in the follicular phase of the estrous cycle (Taneike *et al.*, 1995).

The present study was undertaken to characterize and quantify α_1- and α_2-ARs in the porcine myometrium in the estrous cycle and pregnancy using the same radioligands. The α_2-ARs in myometrium may be heterogenous because both α_{2A}- and α_{2B}-subtypes are present in rat myometrium (Legrand *et al.*, 1993). However, from the functional studies we have demonstrated that natural catecholamine-induced increase in porcine longitudinal myometrial contractility in the estrous cycle and during pregnancy is mediated predominantly by α_2-ARs (Yang and Hsu, 1995a and 1995b), because this increased contractility is not inhibited by α_1- and α_{2B}-AR antagonist, PRZ ($\leq 10^{-6}$ M). Therefore, the present study was also to determine the α_2-AR subtype(s) in porcine myometrium.

Materials and Methods

Tissue preparation

The uteri used in the study were obtained from a local packing plant and a surgical laboratory. The specimens from the follicular phase and luteal phase in the estrous cycle and various stages of pregnancy were used. The crown-rump measurement of the fetus was used to estimate the days of the gestation in the early

pregnancy (EPG; days of gestation = 37 - 40), mid-pregnancy (MPG; days of gestation = 57 - 60), late pregnancy (LPG; days of gestation = 77 - 79) and very late pregnancy (VLPG; days of gestation ≥ 100 days) (Evans and Sack, 1973). The prepartum specimens (PPT) were obtained from sows undergoing the C-section at 111th - 113th day of the pregnancy according to the known breeding record. The uteri were determined to be in the follicular phase through visual inspection of ovaries containing follicles ≥ 6 mm in diameter and without corpora lutea. In the luteal phase the ovaries had light red corpora lutea and the absence of embryos (Arthur *et al.*, 1989). Only the mid-portion of the uterine horns was used in the experiments. The tissues were stored in ice-cold Tyrode's solution (137 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM dextrose, and 12 mM $NaHCO_3$; pH 7.4) and transported to the laboratory. Upon arrival, the endometrium and placenta were removed from the uterus; the myometrium was maintained in ice-cold Tyrode's solution aerated with 95% O_2-5% CO_2. Only the longitudinal layer of myometrial tissues with serosa were minced into approximately 2 mm x 10 mm at 4°C. They were immersed in the hypotonic solution (10 mM Tris, 0.5 mM dithiothreitol, 1 mM Na_2 EDTA; pH 7.4) and stored at -80°C until assayed.

Membrane preparation

The minced, frozen myometrial tissues were thawed at room temperature, they were homogenized in ice-cold hypotonic solution for three times of 15 sec each with a Tissumizer® (SDT-1810, Tekmar Co., Cincinnati, OH) at 55 setting. The homogenate was centrifuged at 1,000 x g (J-6B Centrifuge, Beckman Instruments Inc., Palo Alto, CA) for 10 min at 4°C. The supernatant was collected and recentrifuged at 50,000 x g (J-21B Centrifuge, Beckman Instruments Inc., Palo Alto, CA) for 30 min at 4°C. The pellet was collected and resupended in 25 mM glycylglycine (pH 7.6) at 4°C. The protein concentration in this membrane suspension was determined as previously

described (Lowry *et al.*, 1951).

<u>Binding assays</u>

In saturation experiments of a_1- and a_2-ARs, one tissue sample from each six or seven reproductive stages was studied simultaneously with others using a latin square block design. In kinetic experiments for a_1- and a_2-ARs and competition experiments for a_2-ARs, only the tissue samples in the luteal phase of the estrous cycle were used.

For a_1- and a_2-AR saturation experiments, the total incubation volume was 400 μl. The total binding was determined with duplicate tubes containing 200 μl membrane protein suspension/tube, in which 1 mg/ml and 60 μg/ml of protein suspensions were used for a_1- and a_2-AR bindings based on the results of preliminary experiments, respectively. In these preliminary experiments, we found that there were only small amounts of a_1-ARs in myometrial membrane protein. In contrast, the a_2-AR density were high in protein suspension. Therefore, in order to decrease non-specific bindings we used 1 mg/ml of membrane protein suspension for a_1-ARs to increase their bindings; and used 60 μg/ml for a_2-AR binding (Bylund and Toews, 1993). The protein suspension was incubated with 100 μl [^{3}H]PRZ at a final concentration of 0.02 - 1.0 nM for 40 min at 30°C or with 100 μl [^{3}H]RAU at a final concentration of 0.05 - 2.0 nM for 30 min at 22°C in 25 mM glycylglycine. Non-specific bindings were determined by incubation of membrane protein and respective radioligand with a parallel set containing 10^{-5} M phentolamine. The specific binding was determined by subtracting non-specific binding from total binding. Approximate 85% of total binding at 0.1 nM [^{3}H]PRZ and 90% at 0.5 nM [^{3}H]RAU were obtained.

In kinetic studies, 0.1 nM [^{3}H]PRZ and 0.5 nM [^{3}H]RAU were used in both association and dissociation experiments for a_1- and a_2-ARs, respectively. For association experiments, 200 μl membrane suspension and the fixed concentration of radioligand were incubated with or without 10^{-5} M phentolamine (100 μl) for various

times from 15 sec to 70 min. The nonspecific and specific bindings were proceeded as the same as in the saturation experiments. For dissociation experiments, 200 μl membrane suspension was preincubated with the fixed radioligand (100 μl) for 40 min and 30 min for a_1- and a_2-ARs, respectively. Then, a final concentration of 10^{-5} M phentolamine (100 μl) was added and incubated for various additional times from 1 to 40 min.

For competition experiments on a_2-ARs, duplicate tubes containing 200 μl membrane suspension, 100 μl [^{3}H]RAU at a final concentration of 0.5 nM, and 100 μl of increasing concentrations of various unlabelled a-adrenergic agents including idazoxan and yohimbine (a_2-AR antagonists) (Timmermans, 1989), oxymetazoline (a_{2A}-AR agonist) (Bylund et al., 1988), prazosin (a_1- and a_{2B}-AR antagonists) (Bylund et al., 1988), RX 821002 (a_{2A}-AR antagonist) (Uhlén and Wikberg, 1991) and WB 4101 (a_1- and a_{2A}-AR antagonist) (Ruffolo and Heible, 1994) were incubated for 30 min at 22°C. Other procedures were performed as for the a_2-AR saturation experiments.

The reaction of all experiments was terminated by adding 3.5 ml ice-cold buffer (50 mM Tris HCl, 0.5 mM MgCl$_2$; pH 7.5) rapidly into each tube. The bound radioactivity was separated from free radioligand by filtration through buffer-presoaked glass fiber filter (GF/B or GF/C, Whatman Inc., Clifton, NJ) using the 12-sample cell harvester (Millipore Corp., Bedford, MA). Filters and tubes were washed rapidly three times with 3.5 ml ice-cold buffer each. Air-dried filter was placed into a 20-ml plastic scintillation vial with 10 ml scintillation cocktail (ScintiVerse®, Fisher Scientific, Fair Lawn, NJ). The scintillation vials containing the filter were shaken overnight and the radioactivity was measured with a liquid scintillation analyzer (1600TR, PACKARD Instrument Co., Meriden, CT).

Drugs and Chemicals

The following chemicals and drugs were used: [7-methoxy-^{3}H]prazosin (72.2 Ci/mmol) and [methyl-^{3}H]rauwolscine (86.2 Ci/mmol) (DuPont, NEN research product, Boston, MA); oxymetazoline HCl and yohimbine HCl (Sigma Chemical Co., St. Louis, MO); RX 821002 and WB 4101 HCl (Research Biochemicals Inc., Natick, MA); idazoxan (Reckitt & Colman Pharmaceutical Div., Kingston Upon Hull, England); phentolamine HCl (Ciba-Geigy Pharmaceutical Co., Summit, NJ); prazosin HCl (Pfizer Inc., Groton, CT). The radioligands were diluted with 25 mM glycylglycine in appropriate concentrations immediately before use. Drugs were prepared as 1 mM stock solutions by dissolving them in distilled water, except for prazosin HCl, which was dissolved in 2% lactic acid to achieve a concentration of 1 mM, and stored at -20°C. The stock solutions were diluted with 25 mM glycylglycine to appropriate concentrations before use.

Data analyses

In kinetic studies, the data of association experiments were linearized by plotting the natural logarithm of the amount specifically bound at equilibrium state (B_{eq}) divided by that amount minus the amount bound (B) at discrete times less than the equilibrium state, ln $[B_{eq}/(B_{eq}-B)]$, versus time (Limbird, 1986). The slope of this line is the pseudo-first order association rate constant or observed rate constant, k_{ob} (min^{-1}). The first order dissociation rate constant, k_{-1} (min^{-1}), was determined by a plot of ln (B/B_{eq}) versus time, where B_{eq} is the amount of radioligand bound just before the addition of 10^{-5} M phentolamine and B is the amount bound at any time thereafter, which yields a regression line with the slope of -k_{-1}. The association rate constant, k_{+1} (M $\cdot$ min^{-1}), is determined from k_{+1} = (k_{ob}-k_{-1})/L, where L is the radioligand concentration. The equilibrium dissociation constant, K_D, equals k_{-1}/k_{-1} (Limbird, 1986).

For saturation experiments, the maximal number of binding sites/mg of membrane protein (B_{max}) and equilibrium dissociation constant (K_D) were estimated by Rosenthal plot analysis (Rosenthal, 1949) from a computer-assisted linear regression of the data plotted as bound versus free ligand (Tallarida and Murray, 1986). The Hill coefficient (n_H) was obtained from the slope of log logit plot of saturation-binding curves (Bennett, 1978).

For competition studies, the data were analyzed by a computer-assisted curve fitting to obtain IC_{50} (the concentration of competing adrenergic compound which inhibits 50% of specific binding) and Hill coefficient (n_H). The inhibition constant (K) of the competing adrenergic agent was calculated from IC_{50}, $K_i = IC_{50}/(1 + L/K_D)$ (Cheng and Prusoff, 1973), where L is [^{3}H]RAU concentration, which is 0.5 nM, and K_D is the mean equilibrium dissociation constant, which is obtained from saturation experiments in the luteal phase of the estrous cycle, of 5.6 nM (Bennett, 1978). The Hill coefficient (n_H) was obtained from the slope of the log logit plot (Hill plot), which was plotted as log [$\%B_{max}/(100\%-\%B_{max})$] versus log (concentration of competing adrenergic compound). B_{max} is the specific radioligand binding occurring in the absence of any competing agent. Binding occurring in the presence of a competing agent was expressed as percentage of B_{max} (Bennett, 1978).

Data were expressed as mean ± SE and analyzed by analysis of variance (ANOVA). The data for B_{max} in different reproductive stages were analyzed using a randomized block factorial design. The conservative F value was used to establish significance for the treatment effect. The least significant difference test was used to determine the difference between means of end points for which the ANOVA indicated a significant ($P < 0.05$) F ratio.

Results

Kinetic studies of [^{3}H]PRZ and [^{3}H]RAU bindings

Specific binding of [^{3}H]PRZ and [^{3}H]RAU to porcine myometrial membrane of 50,000 x g fraction in the luteal phase of the estrous cycle was rapid. The association half life ($t_{1/2}$) were 4 and 3 min, respectively (Fig. 1A and 2A). It reached equilibrium in 30 min and maintained at a steady state up to 70 min under our conditions. Both specific bindings of [^{3}H]PRZ and [^{3}H]RAU were reversed rapidly by 10^{-5} M phentolamine. The dissociation $t_{1/2}$ were 8.9 ± 0.9 and 12.3 ± 0.8 min, respectively (Figs. 1A and 2A). Using pseudo-first-order association and first-order dissociation kinetics, linear transformations of kinetic binding data yielded straight regression lines (Figs 1B and 1C, and Figs. 2B and 2C), indicating each that radioligand binds to a single class of binding sites (Murphy and Bylund, 1988).

Saturation studies of [^{3}H]PRZ and [^{3}H]RAU bindings

The results of saturation binding experiments in porcine myometrial membrane suspensions using [^{3}H]PRZ and [^{3}H]RAU were summarized in Tables 1 and 2, respectively. [^{3}H]PRZ and [^{3}H]RAU bound to porcine myometrial membranes with high affinity and in a saturable manner (Figs. 3A and 4A). The K_D values for both α-ARs were not changed significantly among all the tested groups (Tables 1 and 2). The densities of α_1-ARs in all groups were low and were 1 - 3% of total α-ARs. The B_{max} was between 7.5 ± 1.6 and 23.6 ± 2.1 fmol/mg membrane protein. The rank order of the B_{max} was L ≈ EPG ≥ LPG ≥ MPG > PPT > F.

In contrast, saturation binding experiments with [^{3}H]RAU revealed the presence of a high density of α_2-ARs as their B_{max} values were greater than those of [^{3}H]PRZ in all groups (Table 2). Generally, the B_{max} values of [^{3}H]RAU during pregnancy, except in prepartum period, were greater than that in the luteal phase. The B_{max} value of

Fig. 1. A. Time course for association (●) and dissociation (■) of specific [^{3}H]PRZ binding to porcine longitudinal myometrium in the luteal phase of the estrous cycle. 200 μl of membrane protein suspensions (1 mg/ml) were incubated with the final concentration of 0.1 nM [^{3}H]PRZ at 30°C for the indicated times. After equilibrium was reached, unlabelled phentolamine (10^{-5} M) was added (arrow) to initiate radioligand dissociation. The specific binding, which was calculated as total binding minus nonspecific binding determining with 10^{-5} M phentolamine. Data points are expressed as mean ± SE (n = 4).

B. Natural log pseudo first-order kinetic plot of association data where B and B_{eq} are specific [^{3}H]PRZ binding at time point shown and at equilibrium, respectively. The slope, was determined by the linear regression analysis, equals to the observed association rate constant (k_{ob}), which was 0.138 ± 0.016 min^{-1} (correlation coefficient, r, = 97.9 ± 0.9%). Each point is the mean of four experiments each performed in duplicate.

C. Natural log first-order kinetic plot of [^{3}H] PRZ dissociation curve. The slope, was determined by linear regression analysis, equals to the dissociation rate constant (k_{-1}), which was 0.080 ± 0.008 min^{-1} (r = 99.4 ± 0.2%). The second-order association rate constant (k_{+1}), was calculated from k_{+1} = (k_{ob} - k_{-1})/[L], where L is the concentration of [^{3}H]PRZ in the assay (0.1 nM), was 0.558 ± 0.009 nM * min^{-1}. The kinetic K_D equals to k_{-1}/k_{+1}, which was 162 ± 36 pM. Points are means of four experiments.
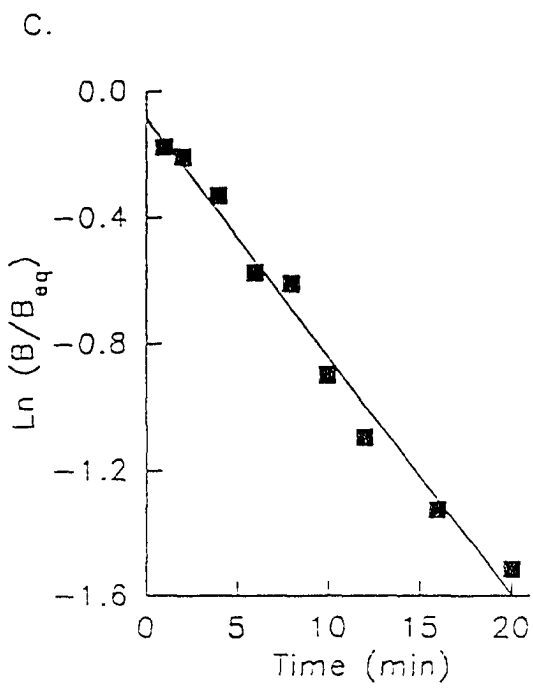
A. 10^{-5} M phentolamine

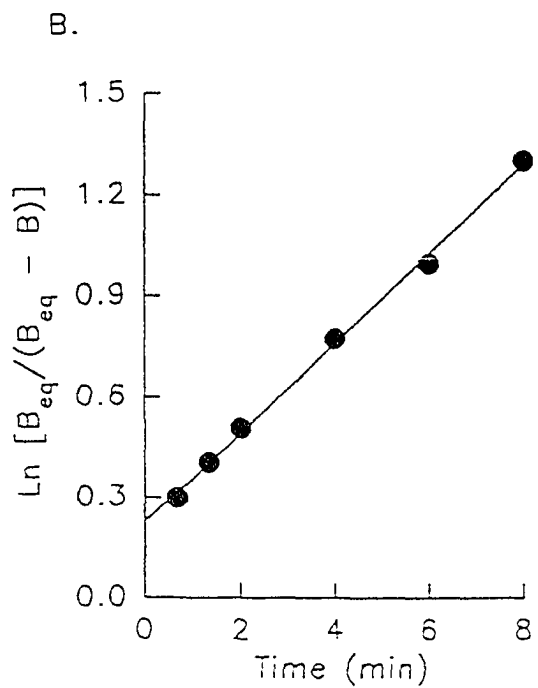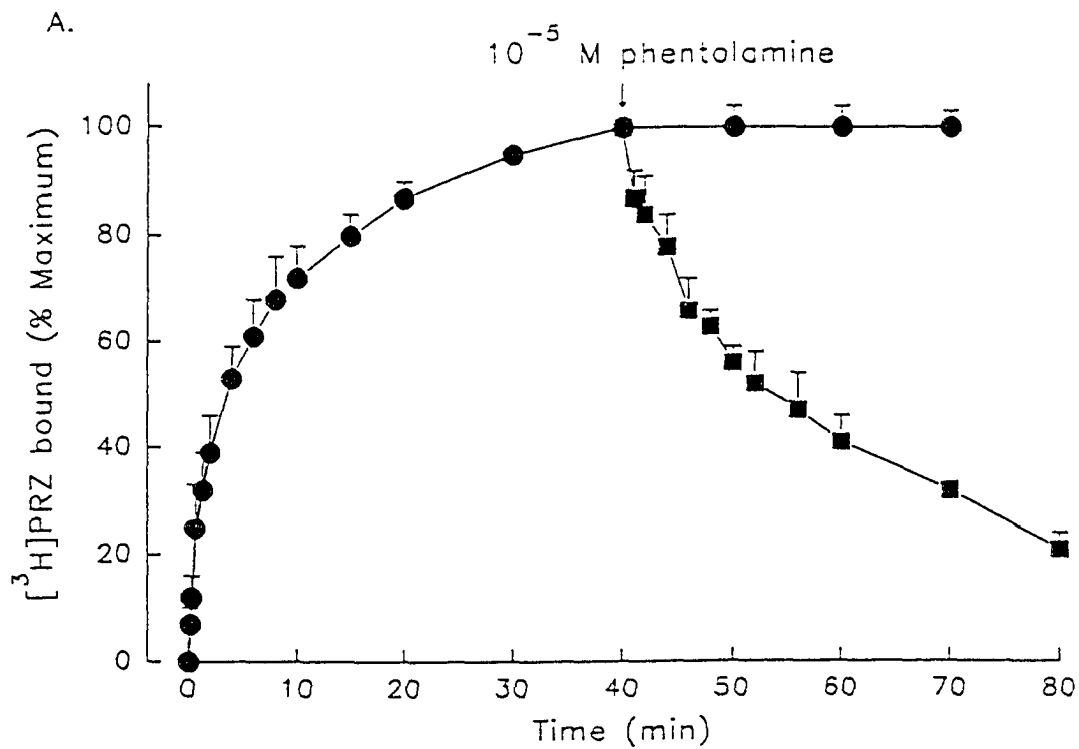Fig. 2. A. Time course for association (●) and dissociation (■) of specific [³H]RAU

binding to porcine longitudinal myometrium in the luteal phase of the estrous

cycle. 200 μl of membrane protein suspensions (60 μg/ml) were incubated

with the final concentration of 0.5 nM [³H]RAU at 22°C for the indicated

times. After equilibrium was reached, unlabelled phentolamine (10^{-5} M) was

added (arrow) to initiate radioligand dissociation. The specific binding, which

was calculated as total binding minus nonspecific binding determining with

10^{-5} M phentolamine. Data points are expressed as mean ± SE (n = 4).

B. Natural log pseudo first-order kinetic plot of association data where B and

B_{eq} are specific [³H]RAU binding at time point shown and at equilibrium,

respectively. The slope, was determined by the linear regression analysis,

equals to the observed association rate constant (k_{ob}), which was 0.192 ±

0.012 min^{-1} (correlation coefficient, r, = 99.8 ± 0.1%). Each point is the

mean of four experiments.

C. Natural log first-order kinetic plot of [³H]RAU dissociation curve. The

slope, was determined by linear regression analysis, equals to dissociation rate

constant (k_{-1}), which was 0.057 ± 0.004 min^{-1} (r = 98.2 ± 0.8%). The

second-order association rate constant (k_{+1}), was calculated from k_{-1} = (k_{ob} -

k_{-1})/[L], where L is the concentration of [³H]RAU in the assay (0.5 nM), was

0.269 ± 0.031 nM * min^{-1}. The kinetic K_D equals to k_{-1}/k_{+1}, which was 228

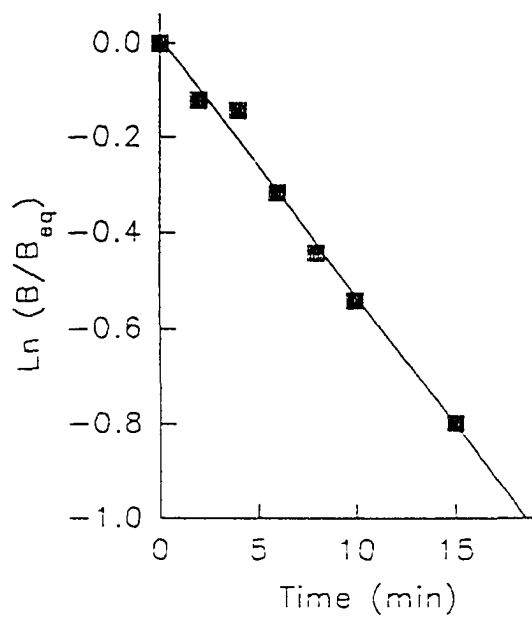± 42 pM. Points are means of four experiments with each performed in

duplicate.

A.

10^{-5} M phentolamine
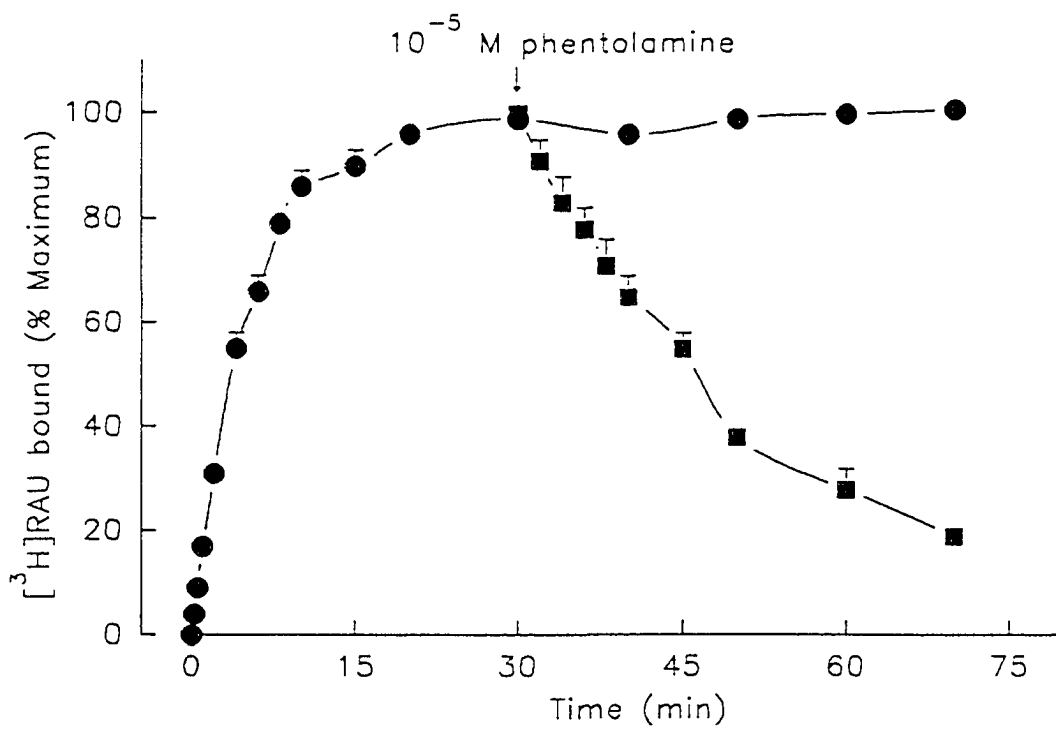
B.
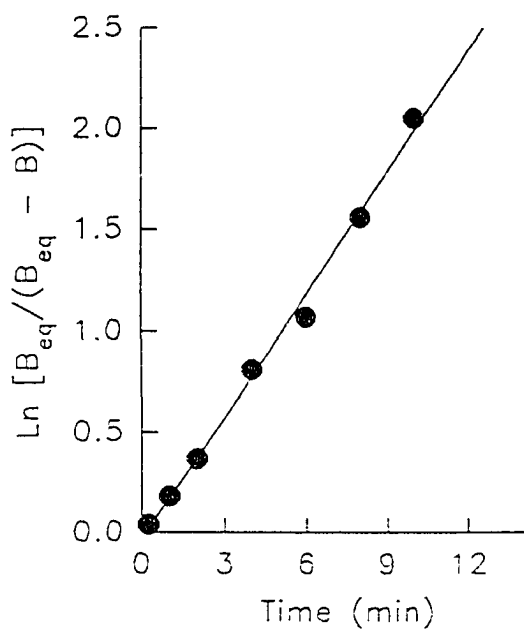
C.

Table 1. Specific binding of [^{3}H]PRZ binding to porcine longitudinal myometrium in the estrous cycle and during pregnancy

		[^{3}H]PRZ*			
Stage	B_{max} fmol/mg protein	K_D pM	r** %	n_H	r*** %
F	7.5 ± 1.6	33.5 ± 11.1	95.0 ± 3.0	1.07 ± 0.03	95.1 ± 2.3
L	23.6 ± 2.1[a,b,c]	30.9 ± 2.3	99.0 ± 0.3	1.13 ± 0.05	99.4 ± 0.3
EPG	22.0 ± 1.3[a,b]	21.5 ± 0.8	98.5 ± 1.0	1.00 ± 0.08	98.6 ± 0.7
MPG	15.6 ± 3.4[a]	23.2 ± 3.2	99.5 ± 0.1	1.13 ± 0.03	99.5 ± 0.2
LPG	20.0 ± 3.9[a]	24.6 ± 7.1	99.5 ± 0.5	1.11 ± 0.06	99.5 ± 0.5
PPT	11.3 ± 1.1	31.1 ± 7.4	98.8 ± 0.7	0.99 ± 0.05	98.6 ± 1.0

*Values shown were obtained from Rosenthal plot analysis and are mean ± SE (n = 4).

**Correlation coefficient for the slope of Rosenthal plot.

***Correlation coefficient for the slope (Hill coefficient = n_H) of Hill plot.

[a]P < 0.05, comparing with the follicular phase of the estrous cycle (F).

[b]P < 0.05, comparing with prepartum period (PPT).

[c]P < 0.05, comparing with mid-pregnancy (MPG).

Table 2. Specific binding of [^{3}H]RAU binding to porcine longitudinal myometrium in the estrous cycle and during pregnancy

			[^{3}H]RAU*		
Stage	B_{max} fmol/mg protein	K_D nM	r^{**} %	n_H	r^{***} %
F	265 ± 50	4.6 ± 1.0	97.5 ± 1.4	1.01 ± 0.02	98.9 ± 0.6
L	1,568 ± 135[a]	5.6 ± 0.7	99.0 ± 0.8	1.00 ± 0.03	98.8 ± 0.5
EPG	2,426 ± 430[a,b,c]	6.9 ± 0.5	97.7 ± 1.0	1.14 ± 0.04	99.6 ± 0.2
MPG	1,999 ± 318[a]	5.6 ± 1.2	98.1 ± 0.7	1.06 ± 0.01	99.3 ± 0.3
LPG	2,049 ± 131[a]	5.6 ± 0.7	98.0 ± 0.7	1.08 ± 0.05	99.0 ± 0.4
VLPG	2,392 ± 341[a,b,c]	6.8 ± 1.2	98.9 ± 0.6	1.07 ± 0.05	99.5 ± 0.3
PPT	1,507 ± 236[a]	6.6 ± 1.7	97.9 ± 1.1	1.05 ± 0.02	99.4 ± 0.2

*Values shown were obtained from Rosenthal plot analysis and are mean ± SE (n = 4).

**Correlation coefficient for the slope of Rosenthal plot.

***Correlation coefficient for the slope (Hill coefficient = n_H) of Hill plot.

[a]$P < 0.05$, comparing with the follicular phase (F) of the estrous cycle.
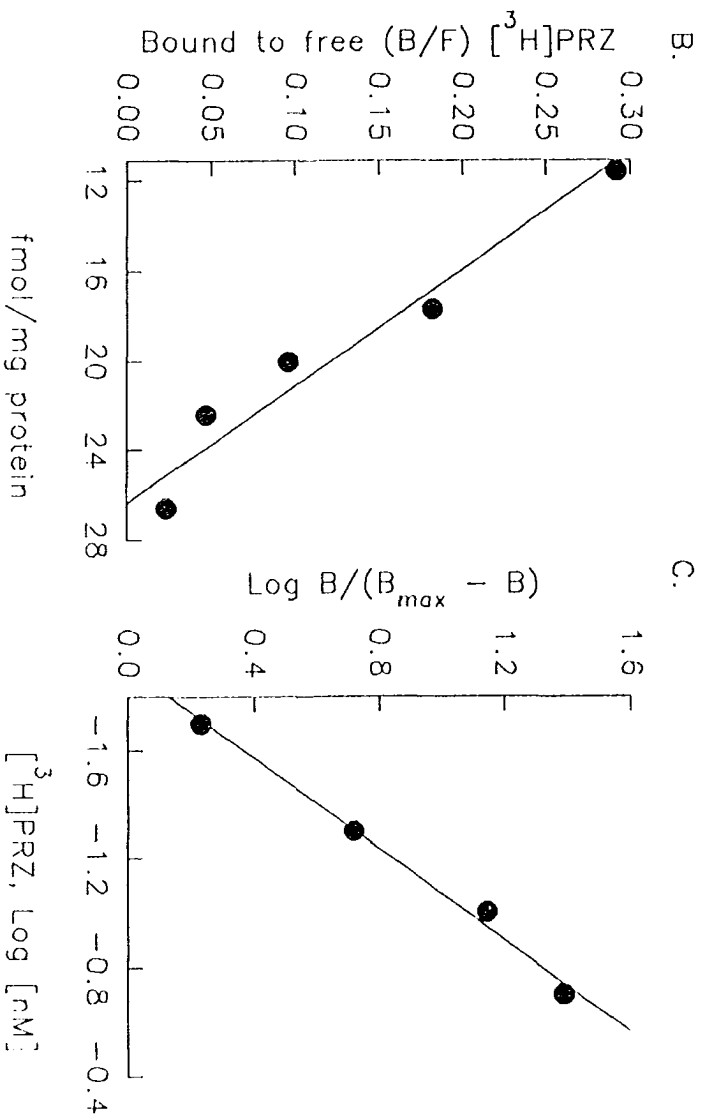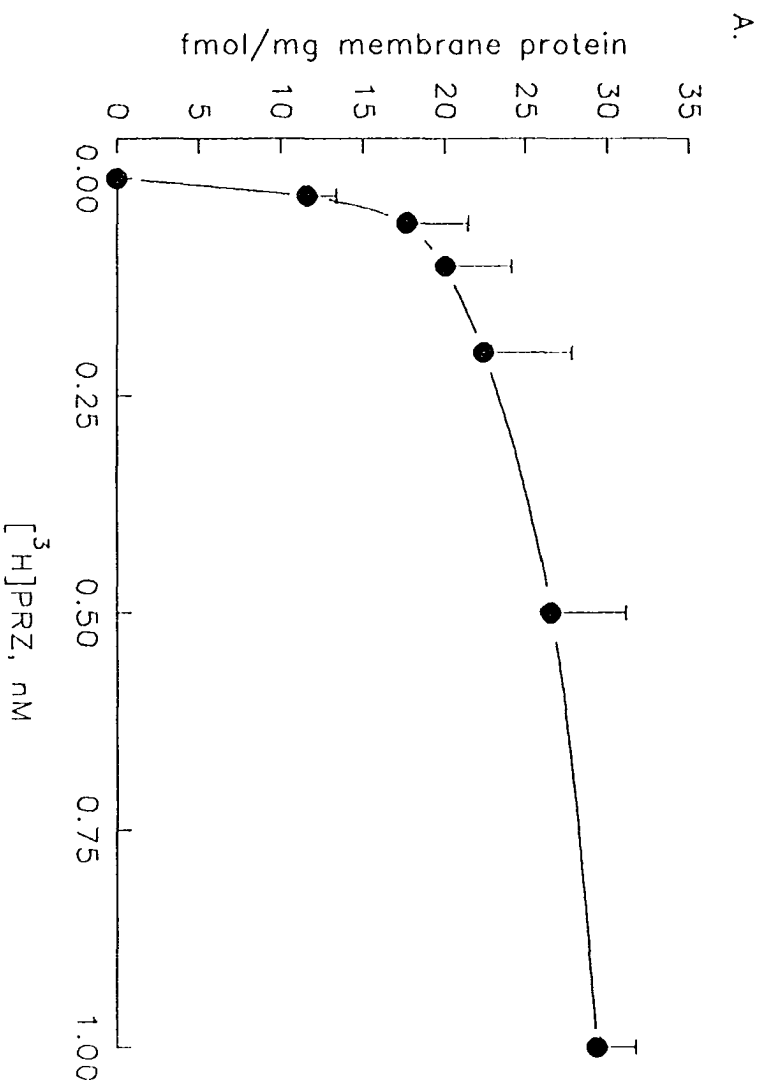
[b]$P < 0.05$, comparing with the luteal phase (L) of the estrous cycle.

[c]$P < 0.05$, comparing with prepartum period (PPT).

Fig. 3. A. Saturation binding curve of [^{3}H]PRZ in porcine myometrium in the luteal phase of the estrous cycle. 200 μl of membrane protein suspensions (1 mg/ml) were incubated with [^{3}H]PRZ at a final concentration of 0.02 - 1.0 nM at 30°C for 40 min and then filtered to separate bound from free radioligand. The values given are those of specific binding, which were calculated as total binding minus nonspecific binding determining with 10^{-5} M phentolamine. The points are expressed as mean ± SE (n = 4).

B. The Rosenthal plot of specific [^{3}H]PRZ binding data from A. Line of best fit was calculated by linear regression and given values for B_{max} and K_D of 23.6 ± 2.1 fmol/mg protein and 30.9 ± 2.3 pM (correlation coefficient, r, = 99.0 ± 0.3%), respectively. This transformation yields a straight line, indicating the existence of a single class of binding sites. Each point is the mean of four experiments each performed in duplicate. The B_{max} and K_D values of all experiments are shown in Table 1.

C. Hill plot of specific [^{3}H]PRZ binding to porcine myometrium. The slope of the plot (or Hill coefficient, n_H), which was determined by linear regression, equaled to 1.00 (correlation coefficient, r, = 99.9%). Each point represents the mean of duplicate determinations from a representative experiment in the luteal phase of the estrous cycle. The n_H and r values of all experiments are shown in Table 1.
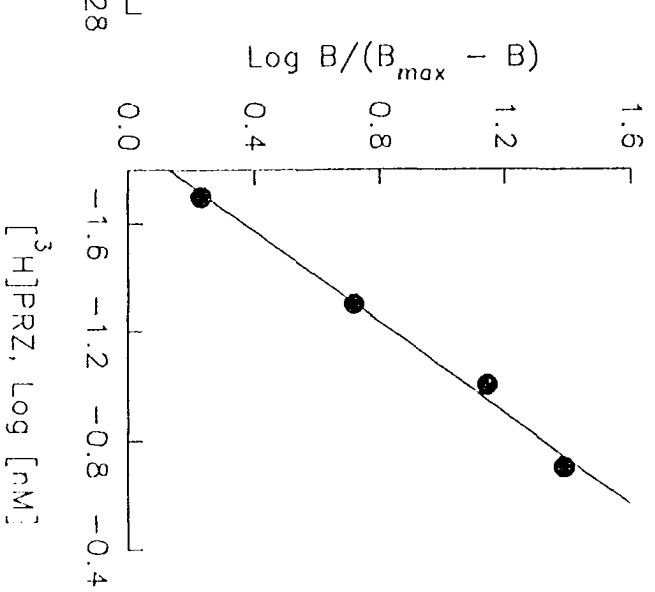
A.

fmol/mg membrane protein

[³H]PRZ, nM

B.

Bound to free (B/F) [³H]PRZ

fmol/mg protein

C.

Log B/(B$_{max}$ − B)

[³H]PRZ, Log [nM]

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Fig. 4. A. Saturation binding curve of [^{3}H]RAU in porcine myometrium in the luteal

cycle of the estrous cycle. 200 μl of membrane protein suspensions (60

μg/ml) were incubated with [^{3}H]RAU at a final concentration of 0.05 - 2.0 nM

at 22°C for 30 min and then filtered to separate bound from free radioligand.

The values given are those specific binding, which were calculated as total

binding minus nonspecific binding determining with 10^{-5} M phentolamine. The

points are expressed as mean ± SE (n = 4).

B. The Rosenthal plot of specific [^{3}H]RAU binding data from A. Line of best

fit was calculated by linear regression and given values for B_{max} and K_D of

1,568 ± 135 fmol/mg protein and 5.6 ± 0.7 nM (correlation coefficient, r, =

99.0 ± 0.8%), respectively. This transformation yields a straight line,

indicating the existence of a single class of binding sites. Each point is the

mean of four experiments each performed in duplicate. The B_{max} and K_D values

of all experiments are shown in Table 2.

C. Hill plot of specific [^{3}H]RAU binding to porcine myometrium. The slope of

the plot (or Hill coefficient, n_H), which was determined by linear regression,

equaled to 0.93 (correlation coefficient, r, = 99.0%). Each point represents

the mean of duplicate determinations from a representative experiment in the

luteal phase of the estrous cycle. The n_H and r values of all experiments are

shown in Table 2.
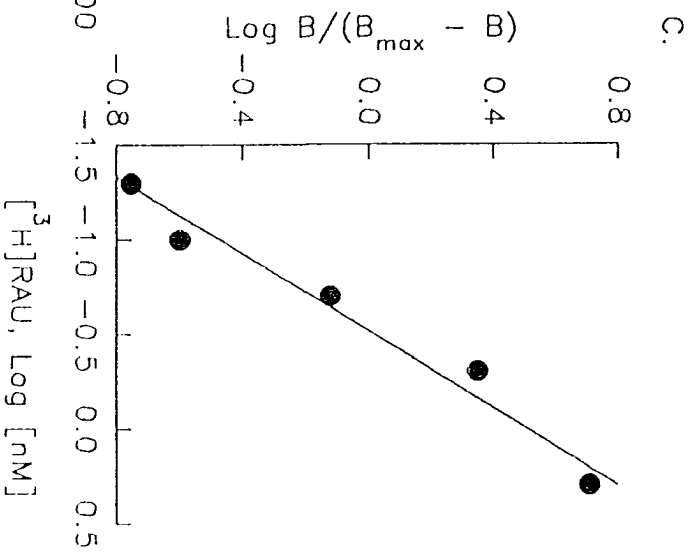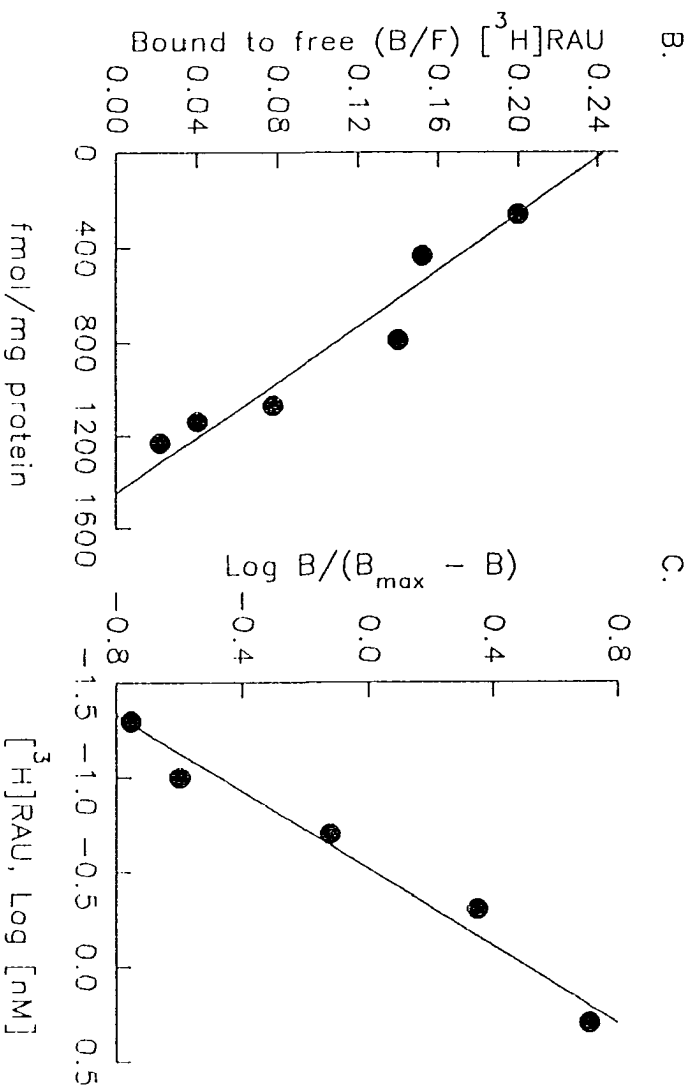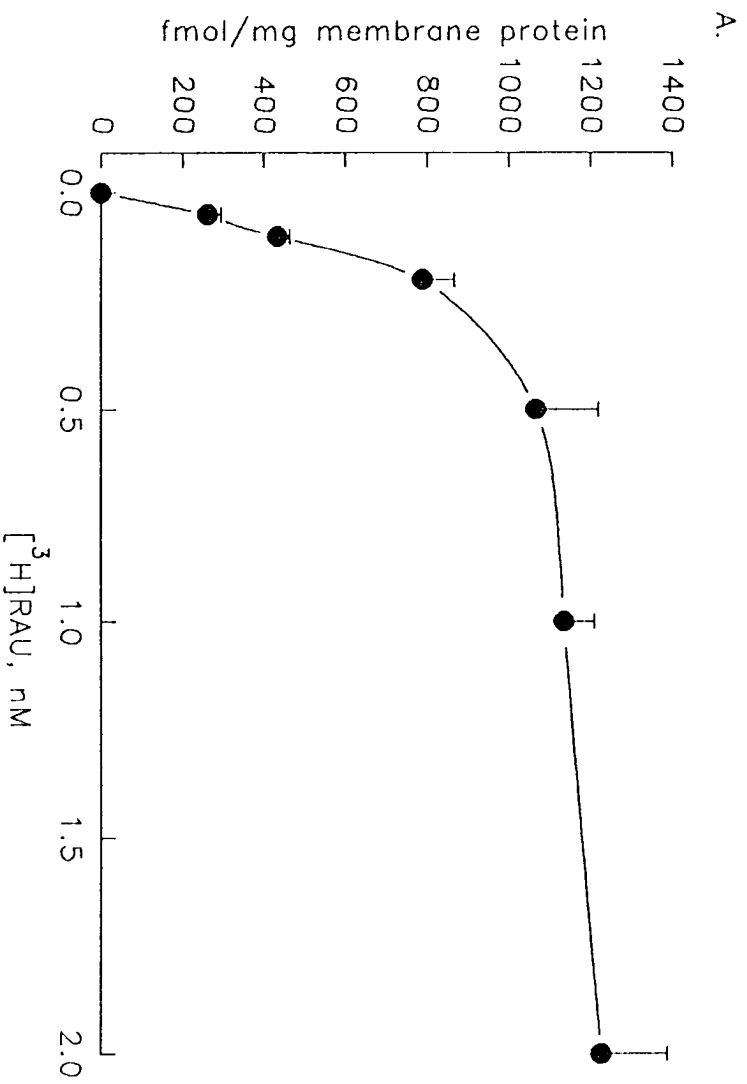
[³H]RAU in prepartum period decreased to 60% comparing with the samples collected from very late gestation period ($\geq$ 100 days) (Table 2). The follicular phase had the smallest B_{max} value of [³H]RAU among all groups studied.

Linear transformation of the saturation data according to Rosenthal plot analysis yields a straight line (Figs. 3B and 4B), indicating that [³H]PRZ or [³H]RAU bound to a single class of receptors over the concentration range used (corresponding coefficient, r, = 99%). Furthermore, Hill plots of the saturation data with [³H]PRZ and [³H]RAU were linear with the average Hill coefficient (n_H) of 0.99 - 1.13 (r = 95.1 - 99.5%) and 1.00 - 1.14 (r = 98.8 - 99.6%), respectively, for both radioligands, indicating no evidence of cooperativity or multiple receptors of different affinities in all tested groups (Legrand *et al.*, 1993) (Figs. 3C and 4C and Tables 1 and 2).

Competition studies of [³H]RAU bindings

The specificity of [³H]RAU binding was determined by competition binding experiments with selective agonist or antagonists. The relative rank order of affinity for competing agents as expressed by K_i values was: RX 821002 > yohimbine > oxymetazoline > idazoxan > WB 4101 > PRZ (Table 3). From the α-AR antagonists tested, the α_2-selective drug, such as yohimbine has an approximatively 630-fold higher affinity than the α_1-selective drug PRZ. These results clearly demonstrated the α_2-AR specificity of the [³H]RAU binding sites in porcine myometrial membrane. All adrenergic compounds used in this study interacted at the [³H]RAU binding site in a homogenous manner, with competition curves fitting best the one-site model (Figs. 5 and 6) and Hill coefficients (n_H) close to 1.0 (Table 3).

To provide a more definitive characterization of α_2-AR subtypes in porcine myometrium, the affinities of these adrenergic drugs were compared with their affinities (K_i) for the tissues of known α_2-AR subtypes (Table 4). The data of porcine myometrium were close to those of the cells in which only α_{2A}-ARs are found, e.g.,

Fig. 5. Inhibition of specific [^{3}H]RAU binding by a_2-AR compounds. Porcine longitudinal myometrial membrane suspensions in the luteal phase of estrous cycle were incubated with [^{3}H]RAU at a final concentration of 0.5 nM and various concentrations, as indicated, of yohimbine (■), RX 821002 (●), WB 4101 (▽), idazoxan (▼), oxymetazoline (○) and prazosin (■) at 22°C for 30 min. For each concentration of adrenergic agent, [^{3}H]RAU binding is expressed as a percentage of specific binding in the absence of any agent (maximal binding). The data points are expressed as mean ± SE (n = 4).
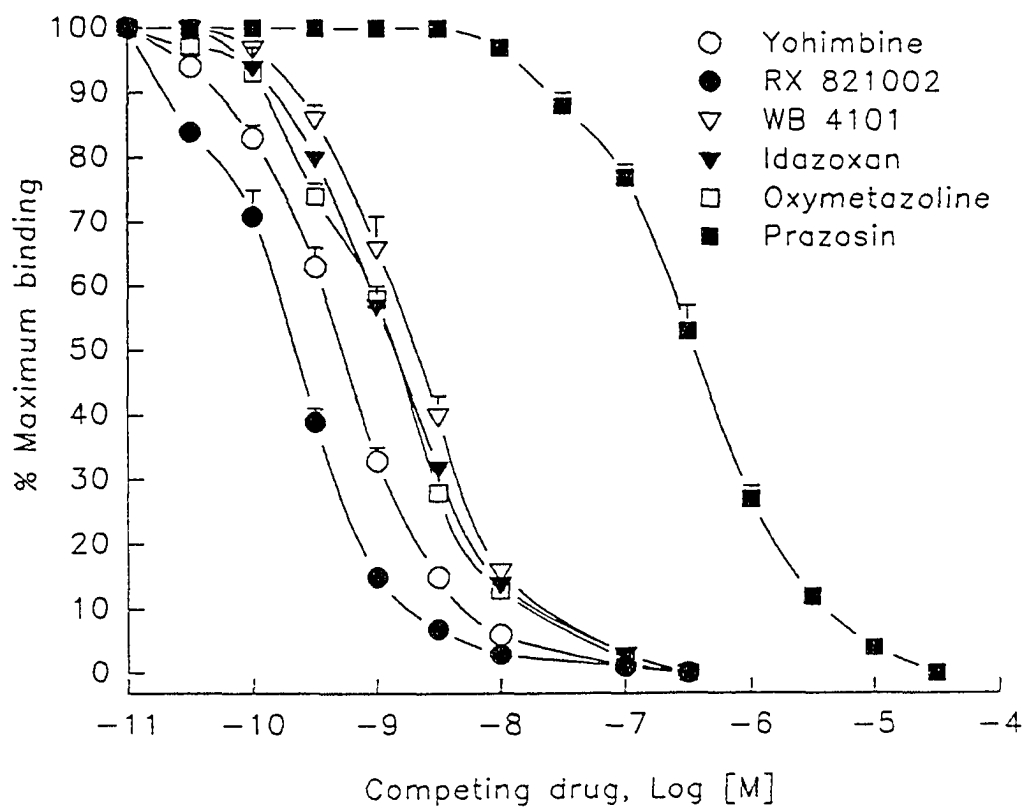
Fig. 6. Hill plots of the inhibition of specific [^{3}H]RAU binding by a_2-AR compounds, including yohimbine (○), RX 821002 (●), WB 4101 (▽), idazoxan (▼), oxymetazoline (□) and prazosin (■). Points are means of four experiments each performed in duplicate. The slopes of the plot, were determined by the linear regression, equal to Hill coefficients (n_H). The n_H and r (correlation coefficient) for all compounds are shown in Table 3.
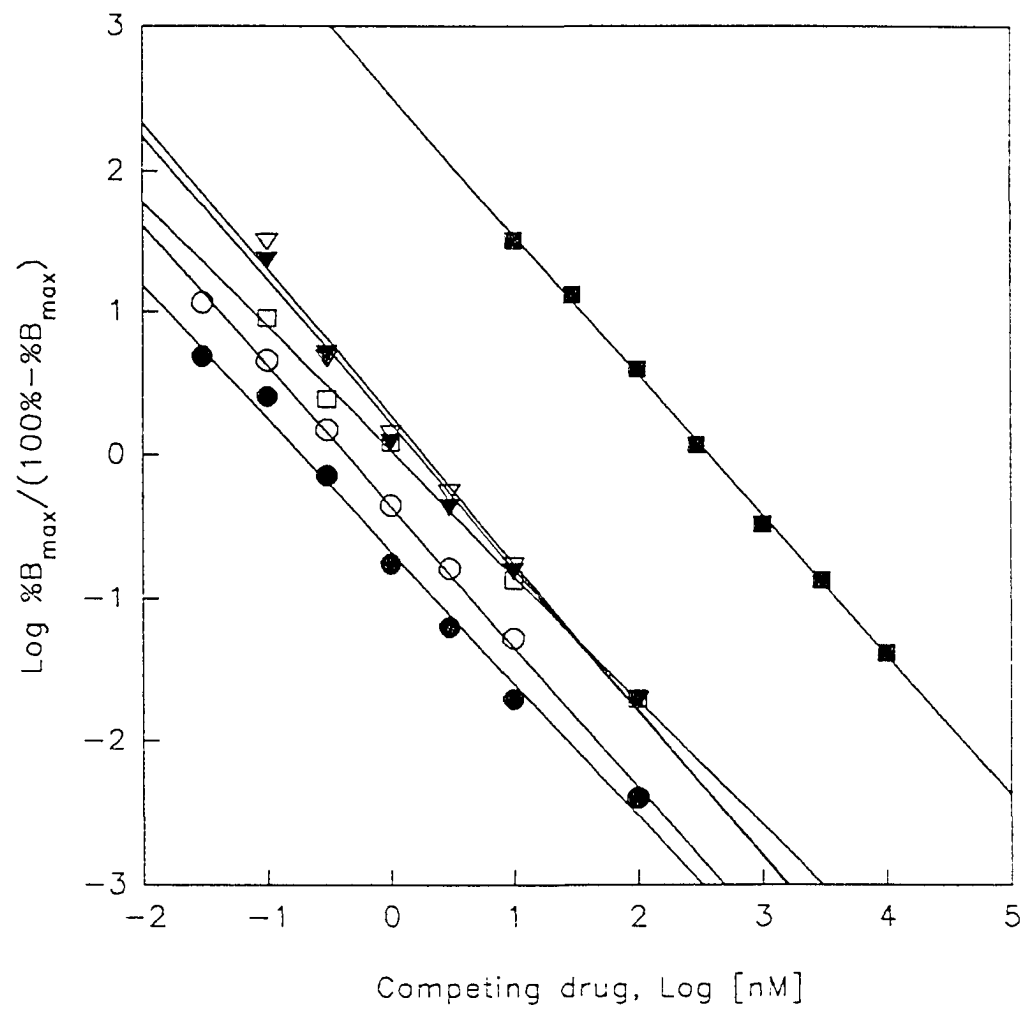
Table 3. Inhibition of specific [^{3}H]RAU binding to porcine myometrial membranes by adrenergic agonist or antagonists[a]

	K_i (nM)	n_H	r^b (%)
Agonist			
Oxymetazoline (α_{2A})	1.15 ± 0.01	0.91 ± 0.02	99.5 ± 0.2
Antagonist			
RX 821002 (α_{2A})	0.20 ± 0.02	1.00 ± 0.02	99.5 ± 0.3
Yohimbine (α_2)	0.48 ± 0.05	0.99 ± 0.02	99.9 ± 0.0
Idazoxan (α_2)	1.49 ± 0.10	0.91 ± 0.04	99.8 ± 0.1
WB 4101 (α_{2A})	2.14 ± 0.23	1.02 ± 0.04	99.6 ± 0.2
Prazosin (α_{2B})	302 ± 25	0.93 ± 0.03	99.8 ± 0.1

[a]The values of inhibition constants (K_i), Hill coefficients (n_H) and correlation coefficients (r) are expressed as mean ± SE (n = 4).

[b]Correlation coefficient for the slope of Hill plot (n_H).

Table 4. Inhibition constant (K_i) of [^{3}H]RAU on various tissues by a_2-Adrenergic compounds

Tissue/Cell line	Type	Yohimbine a_2	Oxymetazoline a_{2A}	Idazoxan a_2	WB 4101 a_{2A}	Prazosin a_{2B}
Porcine myometrium	a_{2A}	0.48 ± 0.05	1.15 ± 0.01	1.49 ± 0.10	2.14 ± 0.23	302 ± 25
HT29 cells[a,b]	a_{2A}	0.65 ± 0.18	0.79 ± 0.15	1.90 ± 0.03	1.20 ± 0.39	316 ± 19
Human platelet[c,d]	a_{2A}	0.85 ± 0.09	0.63	n. d.	3.52 ± 0.20	339
NG108-15 cells[a]	a_{2B}	0.67 ± 0.04	38 ± 9	3.90 ± 0.20	6.40 ± 0.40	3.7 ± 0.7
Neonatal rat lung[a,b]	a_{2B}	1.00 ± 0.10	71 ± 8	4.70 ± 0.20	8.70 ± 2.60	5.4 ± 0.5
OK cells[a]	a_{2C}	0.19 ± 0.04	31 ± 3	0.56 ± 0.02	0.27 ± 0.01	15 ± 3
Opossum kidney[a]	a_{2C}	0.40 ± 0.15	73 ± 12	2.40 ± 0.10	1.60 ± 0.50	36 ± 3
Bovine pineal gland[e]	a_{2D}	3.60 ± 0.60	1.45 ± 0.32	n. d.	7.60 ± 0.80	106 ± 10
Rat submaxillary gland[f]	a_{2D}	n. d.	8.71	5.62	60.26	457

Values are expressed as mean ± SE if possible. The data of porcine myometrium are shown in mean ± SE (n = 4). n. d. not determined. Data were obtained from [a], Blaxall et al., 1991; [b], Bylund et al., 1988; [c], Cheung et al., 1982; [d], MacKinnon et al., 1994; [e], Simonneaux et al., 1991; [f], Michel et al., 1989.

HT29 cells (a human colonic adenocarcinoma cell line) and human platelets. When comparing the K_i ratios of various drugs in porcine myometrium with known α_2-AR subtype tissues and cell lines, these ratios were close to the α_{2A} subtype expressed by HT29 cells and human platelets, and distinct from the α_{2B} subtype expressed by NG108-15 cells (a neuroblastoma-glioma cell line) and the neonatal rat lung, the α_{2C} subtype expressed by OK cells (an opossum kidney cell line) and the opossum kidney, and the α_{2D} subtype expressed in the bovine pineal gland and rat submaxillary gland (Table 5). Moreover, the excellent correlations of drug potencies, which were expressed as pK_i ($= -\log K_i$) values, were seen between porcine myometrium and the tissues (i.e., HT cells and human platelet) containing α_{2A} subtypes (Fig. 7 and Table 6). In contrast, the correlations were poor between porcine myometrium and either α_{2B}, α_{2C} or α_{2D} subtype tissues (Fig. 8 and Table 6).

Discussion

The results of the present study identified and characterized porcine longitudinal myometrial α_1- and α_2-ARs using [^{3}H]PRZ and [^{3}H]RAU in the estrous cycle and during pregnancy. The results also provided evidence that the α-AR-mediated myometrial contractility in sows was correlated with the quantities of myometrial α-ARs, in which α_2-ARs were the major α-ARs and predominantly mediated catecholamine-induced myometrial contractility (Ko et al., 1990a; Yang and Hsu, 1995a and 1995b). In addition, the density of α_2-ARs in porcine myometrium changed in the estrous cycle and pregnancy, while the α_1-ARs remained at a low concentration. Moreover, from the competition binding experiments we suggested that the α_2-AR in the porcine myometrium is the α_{2A}-subtype.

Our results of kinetic, saturation and competition experiments clearly indicated

Table 5. Comparison of K_i value ratios for various α_2-AR subtypes

Tissue/Cell lines	Type	Prazosin/ Oxymetazoline	Prazosin/ WB 4101	Oxymetazoline/ WB 4101
Porcine myometrium	α_{2A}	263	150	0.54
HT29 cells[a]	α_{2A}	400	260	0.66
Human platelet[a]	α_{2A}	330	330	1.01
NG108-15 cells[a]	α_{2B}	0.10	0.58	5.90
Neonatal rat lung[a]	α_{2B}	0.08	0.62	8.20
OK cells[a]	α_{2C}	0.50	56	260
Opossum kidney[a]	α_{2C}	0.49	22	46
Bovine pineal gland[b]	α_{2D}	73	13.9	0.19
Rat submaxillary gland[b]	α_{2D}	52.5	7.58	0.14

[a]K_i value ratios were obtained from Blaxall *et al.*, 1991.

[b]K_i value ratios were calculated from the data in Simonneaux *et al.*, 1991.

Fig. 7. Correlation between adrenergic drug affinity estimates (pK_i = -log K_i, in which K_i values from Tables 3 and 4) obtained for the a_2-ARs of porcine myometrium and a_{2A}-subtype tissues (A, HT29 cells; B, human platelets) labelled with [^{3}H]RAU. The drugs used were yohimbine (O), oxymetazoline (●), idazoxan (▲), WB 4101 (△) and prazosin (□). Correlation coefficients and slopes of the correlation lines are summarized in Table 6.
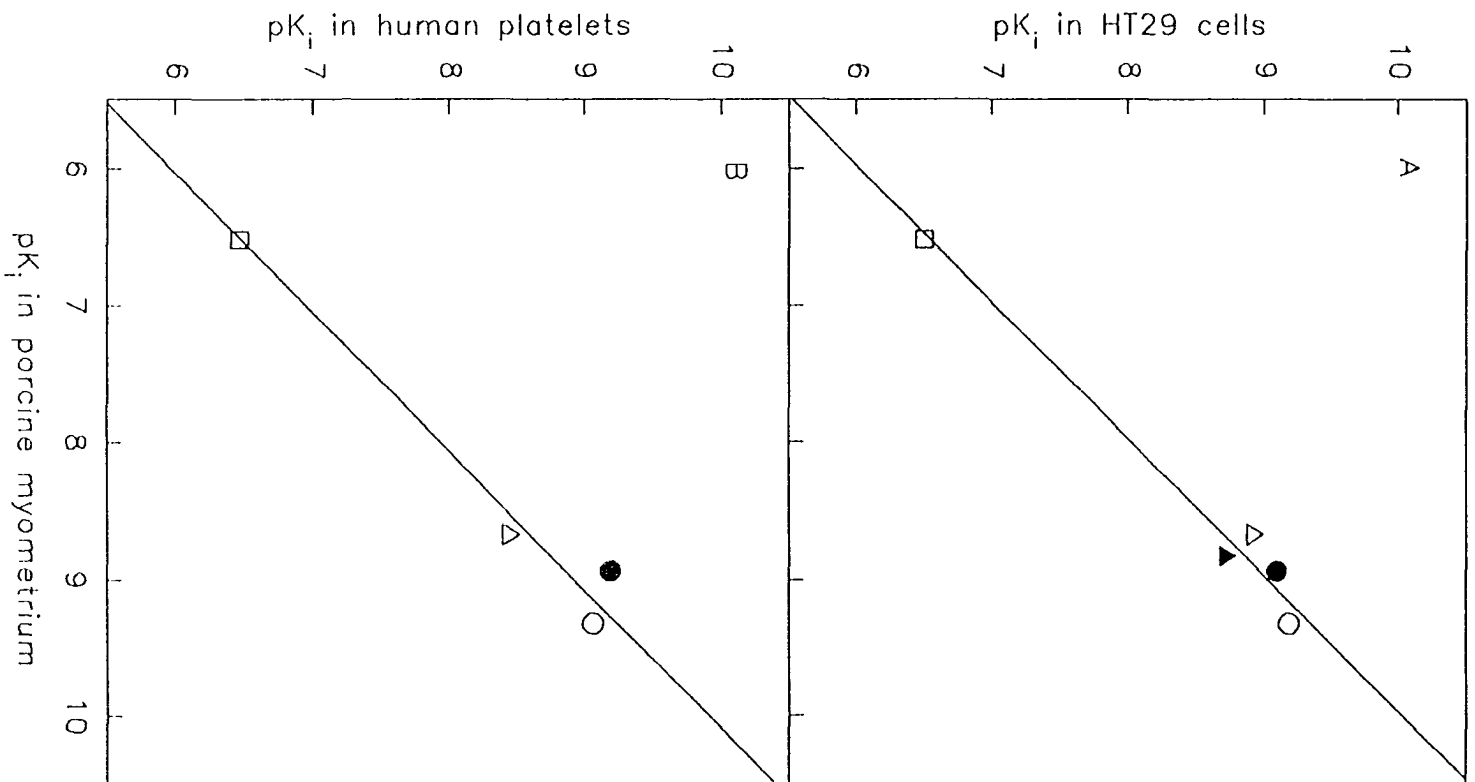
Table 6. Correlations between a_{2A}-AR subtype in porcine myometrium and other known a_2-AR subtype tissues and cell lines

	Slope	r (%)
Porcine myometrium		
vs. HT29 cell (a_{2A})	0.98	98.4
vs. human platelet (a_{2A})	0.99	98.3
vs. NG108-15 cell (a_{2B})	0.02	3.3
vs. rat neonatal lung (a_{2B})	0.04	6.6
vs. OK cell (a_{2C})	0.46	44.4
vs. opossum kidney (a_{2C})	0.46	53.7
vs. bovine pineal gland (a_{2D})	0.60	93.6
vs. rat submaxillary gland (a_{2D})	0.70	90.1

Values are correlation coefficients (r) and slopes from regression analysis of the pK_i (= -log K_i) values for the 4-5 drugs in Table 4. The correlation plots between porcine myometrium and individual known a_2-AR subtype cell lines and tissues are shown in Figs. 7 and 8.

Fig. 8. Correlation between adrenergic drug affinity estimates (pK_i = -log K_i, in which K_i values from Tables 3 and 4) obtained for the α_2-ARs of porcine myometrium and of various tissues labelled with [^{3}H]RAU, including rat neonatal lung (A; α_{2B} subtype), NG108-15 cells (B; α_{2B} subtype), OK cells (C; α_{2C} subtype), opossum kidney (D; α_{2C} subtype), bovine pineal gland (E; α_{2D} subtype) and rat submaxillary gland (F; α_{2D} subtype). The drugs used were yohimbine (○), oxymetazoline (●), idazoxan (▲), WB 4101 (△) and prazosin (□). Correlation coefficients and slopes of the correlation lines are summarized in Table 6.

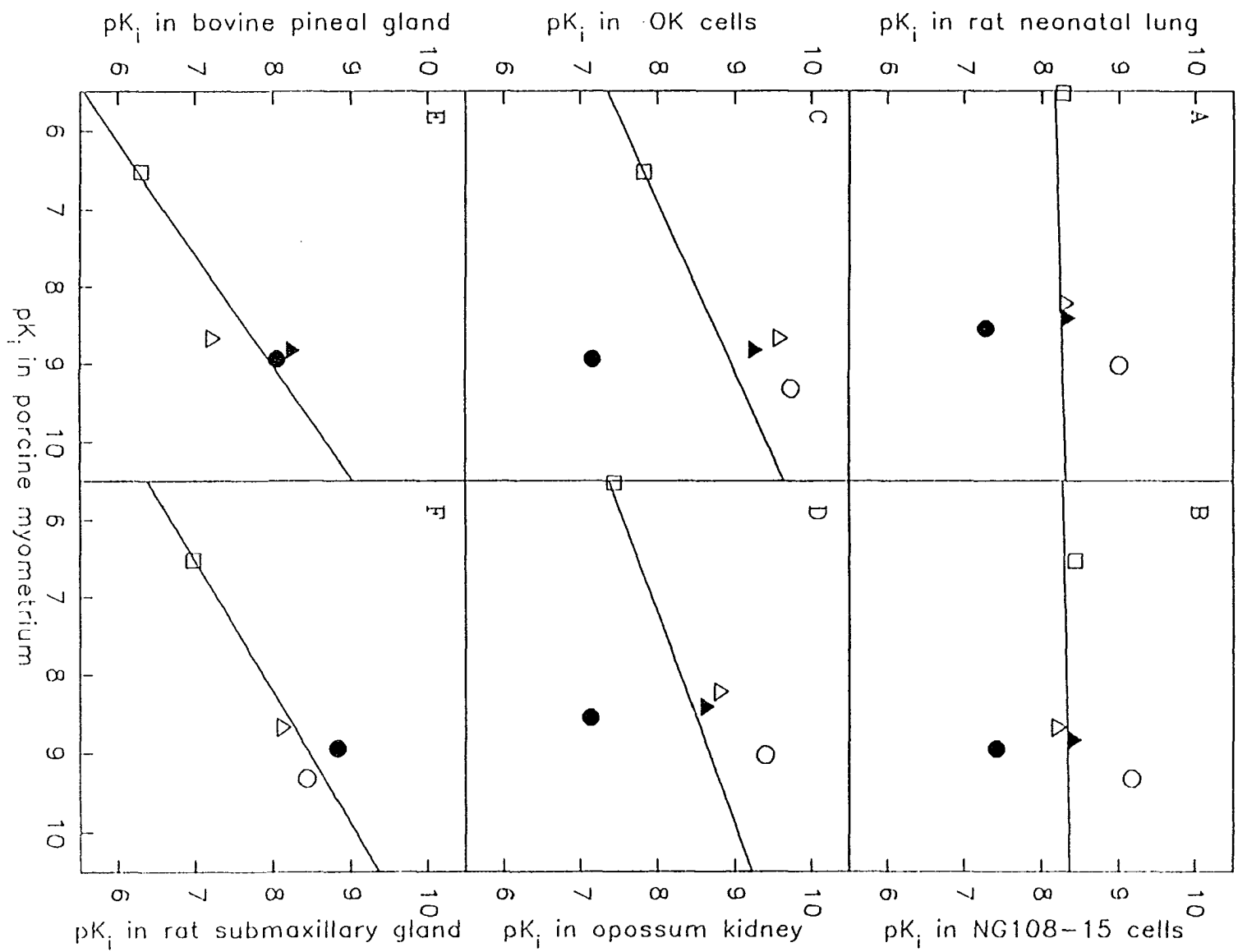the a_1- and a_2-AR specificity of the [^{3}H]PRZ and [^{3}H]RAU binding sites, respectively, in porcine myometrial membrane. The presence of these a-ARs has been previously suggested in porcine myometrium using [^{3}H]dihydroergocryptine (DHE) (Rexroad and Guthrie, 1983). However, this ligand has been shown to bind with almost equal affinity to both a_1- and a_2-ARs, which does not distinguish these two subtypes (Williams et al., 1976). As shown in our results, a_1- and a_2-ARs were directly characterized in porcine myometrium with [^{3}H]PRZ and [^{3}H]RAU, the specific a_1- and a_2-AR radioligands, respectively, which are superior to [^{3}H]DHE. In this context, it is suggested that both [^{3}H]PRZ and [^{3}H]RAU are useful in the study on porcine myometrial a_1- and a_2-ARs, respectively.

The concentration of a_1-ARs in porcine myometrium was quite low, and only accounted for 1 - 3% of total a-ARs in all reproductive stages tested. The low percentage of a_1-ARs was in agreement with the data in ewes (Vass-Lopez et al., 1990b), but was remarkably less than that in humans (Bottari et al., 1985), rats (Maltier and Legrand, 1985), rabbits (Riemer et al., 1987), guinea pigs (Arkinstall and Jones, 1988 and 1989) and pigs (Taneike et al., 1995). The results were not consistent with the data that a_1-AR density did not change in the reproductive stages or following ovarian steroid treatments in humans (Bottari et al., 1985), rabbits (Riemer et al., 1987) and ewes (Vass-Lopez et al., 1990b), nor with guinea pigs (Arkinstall and Jones, 1989) in which the concentrations increase at term. The porcine myometrial a_1-AR concentrations in the luteal phase and in the reproductive stages during pregnancy, except in the prepartum period, were similar, and were greater than those in the follicular phase and prepartum period, but the percentages in total myometrial a-ARs remained low. These findings of extremely low concentrations of a_1-ARs in porcine myometrium provide the evidence to support our previous findings (Yang and Hsu, 1995a and 1995b) that the epinephrine- and norepinephrine-induced increase in

myometrial contractility is minimally mediated by a_1-ARs. In our results, the density of myometrial a_1-ARs in the follicular phase is lower than that in other report (Taneike *et al.*, 1995). The reason for the discrepancy of the results is not clear.

Although previous study (Taneike *et al.*, 1995) has found that a_2-ARs are present in porcine myometrium using [^{3}H]RAU, the selective a_2-AR radioligand, the results are limited to the follicular phase in which the density of a_2-AR is the lowest in the reproductive stages we studied. In the present study, we demonstrated that the densities of porcine myometrial a_2-ARs changed in reproductive stages, which were in agreement with the results obtained from ewes (Vass-Lopez *et al.*, 1990a and 1990b), humans (Bottari *et al.*, 1985) and guinea pigs (Arkinstall and Jones, 1988). Generally, the a_2-AR concentration in porcine myometrium in the luteal phase and pregnancy, in which plasma progesterone concentration is high (Thilander and Rodriguez, 1989a and 1989b), is greater than that in the follicular phase, in which the myometrium is exposed to a low progesterone environment (Thilander and Rodriguez, 1989a and 1990). These results were consistent with the results in ewes (Vass-Lopez *et al.*, 1990a and 1990b), guinea pigs (Arkinstall and Jones, 1988) and pigs (Taneike *et al.*, 1995), but were in contrast to humans (Bottari *et al.*, 1983c and 1985) and rabbits (Hoffman *et al.*, 1981; Jacobson *et al.*, 1987; Riemer *et al.*, 1987), in which a_2-AR concentrations increase in the estrogen-dominated environments.

The present study confirmed that the dominant a_2-ARs in porcine myometrium mediate catecholamine-induced myometrial contractility (Yang and Hsu, 1995a and 1995b). The concentration of myometrial a_2-ARs in the luteal phase is lower than that in the pregnancy, except in the prepartum period in which a_2-AR density was higher than that in luteal phase. However, the potency of natural catecholamines to induce myometrial contractility in the presence of propranolol *in vitro*, which is mediated by a_2-ARs, is not significantly different among these reproductive stages (Yang and Hsu,

1995b). The myometrial a_2-AR concentrations in pregnancy, which are greater than those in the estrous cycle, are also seen in guinea pigs (Arkinstall and Jones, 1988) and ewes (Vass-Lopez et al., 1990b). The physiological significance regarding high myometrial a_2-AR population during pregnancy is not clear; however, at least, a_2-ARs mediate an increase in porcine longitudinal myometrial contractility during pregnancy (Yang and Hsu, 1995b). The uterus during pregnancy is to provide a quiescent state to allow the fetus to grow and develop until the delivery at term, the stability of the uterus is related to the inhibitory β-AR action on myometrium (Wray. 1993). Therefore, the increased myometrial a_2-ARs in pregnancy may have additional physiological roles. It is possible that the a_2-ARs regulate some aspects of cellular metabolism important for uterine function, and that the ovarian steroid-induced changes in a_2-AR density may mediate changes in the metabolic activity of the uterus during pregnancy (Ruffolo and Hieble, 1994). However, no studies have yet been performed to investigate the effect of a_2-AR activation on uterine metabolic function. It is also likely that a_2-ARs are to counterbalance the β_2-AR-mediated myometrial relaxation. This could be important at terms, because without the participation of a_2-ARs, there could be excessive myometrial relaxation, which may interfere with parturition process.

The concentration of myometrial a_2-ARs in prepartum period was similar to that in the luteal phase. However, its catecholamine-induced myometrial contractility is less than that in the luteal phase (Yang and Hsu, 1995b). Therefore, it is possible that changes in signal transduction system during prepartum period lead to a lower response to a_2-AR stimulations than luteal phase.

The myometrial a_2-ARs in the follicular phase, in which the tissues are exposed to a low progesterone environment, are only 11 - 17% those of other reproductive stages. This reduction in myometrial a_2-ARs is consistently accompanied by a smaller

increase in a_2-AR-mediated porcine myometrial contractility than other reproductive stages (Yang and Hsu, 1995b). From our findings, it is suggested that progesterone may play a role in regulation of the a_2-AR density in porcine myometrium.

Estrogens may influence the density of a_2-ARs because they decrease the a_2-ARs in rabbit platelets (Mishra et al., 1985) and female rat hypothalamus (Karkanias and Etgen, 1994). On the contrary, the concentration of a_2-ARs increases in human and rabbit myometrium (Jacobson et al., 1987), but does not change in ovine myometrium (Vass-Lopez et al., 1990a and 1990b) nor in female rabbit brain, spleen and kidney (Mishra et al., 1985). In porcine myometrium estrogens may not influence the density of a_2-ARs, because we found that the a_2-AR concentration in prepartum period was still 5-fold as that in the follicular phase even the plasma concentrations of estrogens in the prepartum period were supposed to be 7 fold as high as in the follicular phase (Thilander and rodriguez, 1989a and 1990). Therefore, further studies using ovariectomized animals with or without supplementation of steroids will provide answers to this question.

During the pregnancy, the density of a_2-ARs in the prepartum period was lower than that in other stages of pregnancy, in which the plasma concentrations of progesterone have started to decrease (Thilander and Rodriguez, 1990). The diminished myometrial a_2-AR receptor density also occurs in guinea pigs at term (Arkinstall and Jones, 1988), and is associated with pregnancy-related myometrial denervation (Thorbert, 1978; Arkinstall and Jones, 1988). In sows, the adrenergic innervation of the uterus in very late pregnancy and at parturition is scanty, compared to cycling animals and early to mid-pregnancy (Thilander, 1989). However, it has not been demonstrated that myometrial a_2-AR receptor density correlates with the structural changes of the adrenergic nerves in this species. Nevertheless, based on our results, at least in part, the decreased density of a_2-ARs in this stage is attributed to

the tissues that are exposed to a lower progesterone environment (Thilander and Rodriguez, 1990).

The pharmacological characteristics, as expressed in K values of a_2-AR competing drugs, of porcine myometrial a_2-ARs are identical to those of the a_{2A}-AR subtype as defined by HT29 cells (Bylund et al., 1988) and human platelets (Cheung et al., 1982). It is different from the a_{2B} subtype expressed in NG108-15 cells (Bylund et al., 1988) and neonatal rat lungs (Bylund et al., 1988), the a_{2C} subtype expressed in OK cells (Blaxall et al., 1991) and opossum kidney (Blaxall et al., 1991), and the a_{2D} subtype expressed in the bovine pineal gland (Simonneaux et al., 1991) and rat submaxillary gland (Limberger et al., 1992).

The ratios of K_i values for PRZ, oxymetazoline and WB 4101 in competition with [^{3}H]RAU can be used as an indicator for classification of a_2-AR subtypes (Blaxall et al., 1991). The ratio of K_i values in porcine myometrium was in the same range as in HT29 cells and human platelets, which express only a_{2A}-AR subtype, and were remarkably different from those in other tissues which have expressed only a_{2B}, a_{2C}, or a_{2D} subtype. Furthermore, the correlation of pK_i values between the porcine myometrial a_2-ARs and a_{2A}-AR prototype tissues, HT29 cells and human platelets, was high with correlation coefficients being 98.4 and 98.3%, respectively. In contrast, the correlations between porcine myometrial a_2-ARs and a_{2B}-, or a_{2C}-prototype tissues were poor, and correlations of porcine myometrial a_2-ARs with a_{2D}-AR prototype tissues were lower than that with a_{2A}-AR. However, considering the pharmacological characteristics with the corresponding K_i ratios, the a_2-ARs in the porcine myometrium were distinct from a_{2D} subtype. For instance, the K_i ratio of PRZ and oxymetazoline for porcine myometrial a_2-ARs is 4 - 5 fold of known a_{2D}-prototype tissues, bovine pineal gland and rat submaxillary gland.

Our results a_{2A} is the predominant a_2 subtype in porcine myometrium were

different from that in the rat (Legrand *et al.*, 1993). From the competition studies, both oxymetazoline, the a_{2A}-AR agonist and PRZ, the a_{2B}-AR antagonist have high affinities for myometrial a_2-AR of the rat. Therefore, it is suggest that both a_{2A}- and a_{2B}-ARs are present in the murine myometrium, However, porcine myometrial a_2-ARs had high affinities with a_{2A}-AR agents, e. g. oxymetazoline, RX 821002 and WB 4101, but had low affinities with PRZ, the a_{2B}-AR antagonist.

Based on our findings, porcine longitudinal myometrium had a higher density of a_2-ARs than that in humans (Bottari *et al.*, 1985), ewes (Vass-Lopez *et al.*, 1990b), guinea pigs (Arkinstall and Jones, 1988), rats (Maltier and Legrand, 1985) and rabbits (Riemer *et al.*, 1987). Hence, porcine longitudinal myometrium is a suitable model for the mechanistic studies of a_{2A}-ARs, particularly pertaining to those in smooth muscles.

In conclusion, the present study suggested that a_1- and a_2-ARs are present in the porcine longitudinal myometrium in the estrous cycle and pregnancy. a_2-AR is the major a-AR in porcine myometrium and its concentrations vary among the reproductive stages. Its density is high in progesterone-dominated stages, such as the luteal phase and pregnancy, and is low in estrogen-dominated stages, such as the follicular phase. In contrast, a_1-ARs are in low concentrations throughout the reproductive stages and account for only 1 - 3% of total porcine myometrial a-ARs. Furthermore, based on the competition binding studies we suggest that porcine myometrial a_2-AR is predominantly the a_{2A} subtype.

Acknowledgements

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EFFECTS OF WB 4101 AND PRAZOSIN ON EPINEPHRINE-INDUCED PORCINE MYOMETRIAL CONTRACTILITY: EVIDENCE FOR PARTICIPATION OF α_2-ADRENOCEPTORS

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Abstract

The effect of epinephrine on myometrial contractility *in vitro* was studied using porcine uterine strips of the longitudinal layer in the luteal phase of the estrous cycle. In the presence of 10^{-6} M propranolol, epinephrine (10^{-9} - 3×10^{-7} M) caused a dose-dependent increase in myometrial contractility. The α_{2A}-adrenoceptor antagonist, WB 4101 (3×10^{-8}, 10^{-7}, 3×10^{-7} M), antagonized the effect of epinephrine in a dose-dependent manner. In contrast, the α_1- and α_{2B}-adrenoceptor antagonist, prazosin (10^{-6} and 3×10^{-6} M) did not block the epinephrine-induced increases in contractility. Comparing the affinity of α_2-adrenergic antagonists in porcine myometrium, there was an excellent correlation between K_B values from function studies and K values from the radioligand binding studies (correlation coefficient = 100%, slope = 1.01). Thus, the functional data were consistent with the radioligand binding data and supported the existence and definition of α_{2A}-adrenoceptor subtype in porcine myometrium. These results suggested that epinephrine-induced increase in myometrial contractility in the luteal phase of the estrous cycle in the sow is mediated by α_{2A}-adrenoceptors.

Introduction

Natural catecholamine-induced porcine myometrial contractility in
vitro is predominantly mediated by a_2-adrenoceptors (ARs) (Yang and Hsu, 1995a and
1995b). From [^{3}H]prazosin and [^{3}H]rauwolscine binding studies, both a_1- and a_2-ARs
are present in the porcine myometrium and most of the ARs are a_2-ARs (Yang and Hsu,
1995c). We also have suggested that porcine myometrial a_2-AR is the a_{2A} subtype
because from the competition binding studies the drug potencies in porcine
myometrium are correlated highly with the known a_{2A}-prototype tissues, the human
platelet (Bylund, 1985) and the HT29 cell, a human colonic adenocarcinoma cell line
(Bylund et al., 1988), but poorly with the known a_{2B}-, a_{2C}-, and a_{2D}-prototype tissues
(Yang and Hsu, 1995c).

However, receptors can not be identified solely on the basis of binding studies
(Bylund and Ray-Prenger, 1989). For instances, although WB 4101 is a potent a_{2A}-AR
antagonist (Ruffolo and Heible, 1994), in which it has high affinity for a_2-ARs of
porcine myometrium (Yang and Hsu, 1995c), it also has high affinity for a_{2C}-AR
tissues, such as the OK cell, an opossum kidney cell line (Murphy and Bylund, 1988)
and opossum kidney (Blaxall et al., 1991). Therefore, the present study was
undertaken to determine if the pharmacological characteristics observed in binding
studies were also observed in functional studies using myometrial contractility in the
porcine longitudinal myometrium. If so, how close they were correlated with each
other.

Materials and Methods

Tissue preparation

The uterine specimens were collected from a local abattoir. Only the mid-portion of the uterine horns was used in the experiment. The specimens were determined to be in the luteal phase based on the presence of light red corpora lutea on the ovaries, and the absence of embryos (Arthur *et al.*, 1989). Tissues were stored in ice-cold Tyrode's solution (137 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM dextrose, and 12 mM $NaHCO_3$; pH 7.4) and transported to the laboratory. Upon arrival, the endometrium was removed from the uterus; the myometrium was stored in ice-cold Tyrode's solution aerated with 95% O_2-5% CO_2 and was used for experiments within 30 h. There were no changes in responsiveness to contractants during this period.

The methods for studying porcine myometrial contractility were as previously described (Yang and Hsu, 1995a and 1995b). In brief, the longitudinal uterine strips (10 x 2 mm^2) were suspended vertically in a 10-ml organ bath containing Tyrode's solution at 37°C and aerated with 95% O_2-5% CO_2. The contractions were recorded isometrically on a multiple-channel recorder (R411, Beckman Instruments Inc., Schiller Park, IL) through a transducer (Grass FT03, Grass Instrument Co., Quincy, MA). The strips were equilibrated under a 2-g tension for 20-25 min before being exposed to 10^{-5} M carbachol (CARB) twice for 3 min each to determine their responsiveness to the contractant. Two of three-minute exposures to CARB separated by a 15 min interval were performed with four of 10-ml washes with Tyrode's solution used to remove CARB after the stimulation. The quiescent state usually lasted > 25 min. The basal resting tension was readjusted to 2 g before the pretreatment drug was added. In the following experiments, epinephrine (EPI) was added at a 10-min interval in cumulative

doses to attain a dose-response relationship.

Effect of WB 4101 and prazosin on EPI-induced myometrial contractility

The α_1- and α_{2A}-AR antagonist WB 4101 (3×10^{-8}, 10^{-7} or 3×10^{-7} M) or the α- and α_{2B}-AR antagonist prazosin (PRZ) (10^{-6}, 3×10^{-6} or 10^{-5} M) was added with 10^{-6} M propranolol (PROP) which inhibits β-AR-mediated relaxation (Yang and Hsu, 1995a and 1995b), to the organ bath for 10 min. After pretreatment with the antagonists for 10 min, EPI was given in cumulative doses (3×10^{-9} - 10^{-5} M). The control group did not receive an antagonist.

The 10-min pretreatment for α- and β-AR antagonists was chosen, because in the preliminary experiment, α- and β-AR antagonisms by yohimbine and PROP reached maximum in 10 min, respectively (n = 4 uteri) (Yang and Hsu, 1995a).

Different strips from the same uterus were randomly assigned to all treatment groups in one trial and each uterus was used for one trial only.

Assessment of the contractile response

The determinations of the contractile response were as previously described (Yang and Hsu, 1995a). Briefly, the contractile response of epinephrine was assessed by the area under the contraction curve (AUCC) and was determined with the use of a scanning program (SigmaScan, Jandel, Corte Madrera, CA). The values were expressed as a percentage of response to a 10^{-6} M CARB treatment for 10 min. In pilot studies we noticed that many tissue strips lost contractions after a 3-min but not a 10-min stimulation by 10^{-6} M CARB after several washouts with Tyrode's solution. To transform the data for the 3-min CARB treatment to those for the 10-min treatment, an independent study was performed to obtain a regression line (Yang and Hsu, 1995a):

Y (10 min) = 2.95 * X (3 min) + 1.32 (n = 39).

In this study, the AUCC produced by the second 3-min 10^{-5} M CARB

stimulation, was transformed to a 10-min area using the above formula and this 10-min area was defined as the 100% 10^{-6} M CARB contractile response. The contractile response of the tissue strip was calculated from the AUCC produced by the agonist EPI 10 min over each cumulative dose and was expressed as a percentage of the response to 10^{-6} M CARB.

Drugs

The following drugs were used: carbachol chloride, (-)epinephrine bitartrate, and propranolol HCl (Sigma Chemical Co., St. Louis, MO); prazosin HCl (Pfizer Inc., Groton, CT), and WB 4101 HCl (Research Biochemicals Inc., Natick, MA). Drugs were dissolved in distilled water, except for epinephrine, which was dissolved in 0.1% (W/V) ascorbic acid in 0.9% NaCl, and prazosin HCl, which was dissolved in 2% lactic acid to achieve a concentration of 1 mM. Drug-containing solutions were prepared by appropriate dilutions of the stock solutions, which were stored at -20°C.

Data analyses

The dose-response curves were produced by a cumulative application of EPI one-half log increments (van Rossum, 1963). The data were expressed as pD_2 (-log EC_{50}).

Dissociation constants (K_B) of prazosin and WB 4101 against EPI were determined using the equation: $K_B = [B]/(CR - 1)$, where B is the concentration of the antagonist (Furchgott, 1972). The responses to 3×10^{-5} M PRZ and 3×10^{-8} M WB 4101 were used for these calculations, respectively. The concentration ratio (CR) equals to EC_{50}'/EC_{50}, in which EC_{50} and EC_{50}' values are the values for the agonist in the absence and presence of the antagonist, respectively. The dissociation constant of the antagonist was expressed as pK_B. In PRZ antagonism experiments, the contractile response was compared with the control group at the corresponding dose of the agonist.

Data were expressed as mean $\pm$ SE and analyzed by analysis of variance

(ANOVA). The conservative F value was used to establish significance for the

treatment effect. The least-significant difference test as used to determine the

difference between means of end points for which the ANOVA indicated a significant

($P < 0.05$) F ratio.

Results

Epinephrine (EPI) (10^{-9} - 3×10^{-7} M) in the presence of 10^{-5} M PROP produced a

dose-dependent increase in myometrial contractility in the luteal phase of the estrous

cycle (Fig. 1). Higher doses of EPI decreased the contractility progressively. The pD_2

value was 7.95 $\pm$ 0.23 (n = 5), which was not significantly different from that of the

previous study (7.81 $\pm$ 0.07, n = 6) (Yang and Hsu, 1995a).

The a_{2A}-AR antagonist, WB 4101, blocked the effects of EPI in a dose

dependent manner (Fig. 1A). The pK_B value for WB 4101 against EPI (7.76 $\pm$ 0.14, n

= 5) was not significantly different from the previous study for yohimbine against the

same agonist (8.42 $\pm$ 0.14, n = 6), but was significantly different from PRZ against

EPI (5.65 $\pm$ 0.06, n = 5) in this study.

The a_1- and a_{2B}-AR antagonist, PRZ, at 10^{-6} or 3×10^{-5} M, failed to antagonize

the effect of EPI on myometrial contractility (Fig. 1B). However, at 10^{-5} M, it inhibited

the effect of EPI (10^{-7} and 3×10^{-7} M) (Fig. 1B).

In order to assess the agreement between the functional studies and radioligand

binding studies in a more quantitative manner (Table 1), correlation analysis was

performed between the K_B values from myometrial contractility studies (Yang and Hsu,

1995a and present study) and the K values from [^{3}H]rauwolscine binding studies (Yang

and Hsu, 1995c). As shown in Fig. 2 the correlation coefficient (r) of the K_B values

Fig. 1. Effect of WB 4101 (A) and prazosin (B) on epinephrine-induced increases in myometrial contractility. Data are expressed as mean ± SE (n = 5). All strips had been pretreated with 10^{-6} M propranolol for 10 min before the first dose of the agonist was applied. Effects are shown in the absence (○) and (A) in the presence of WB 4101, 3 × 10^{-8} M, ●; 10^{-7} M, △; 3 × 10^{-7} M, ▲, or (B) in the presence of prazosin, 10^{-6} M, ●; 3 × 10^{-6} M, △; 10^{-5} M, ▲. *$P < 0.05$, compared with the control group at the corresponding agonist dose.

A. WB 4101

B. Prazosin

% Contractile Response (Carbachol 10^{-6} M = 100%)

Epinephrine, Log [M]
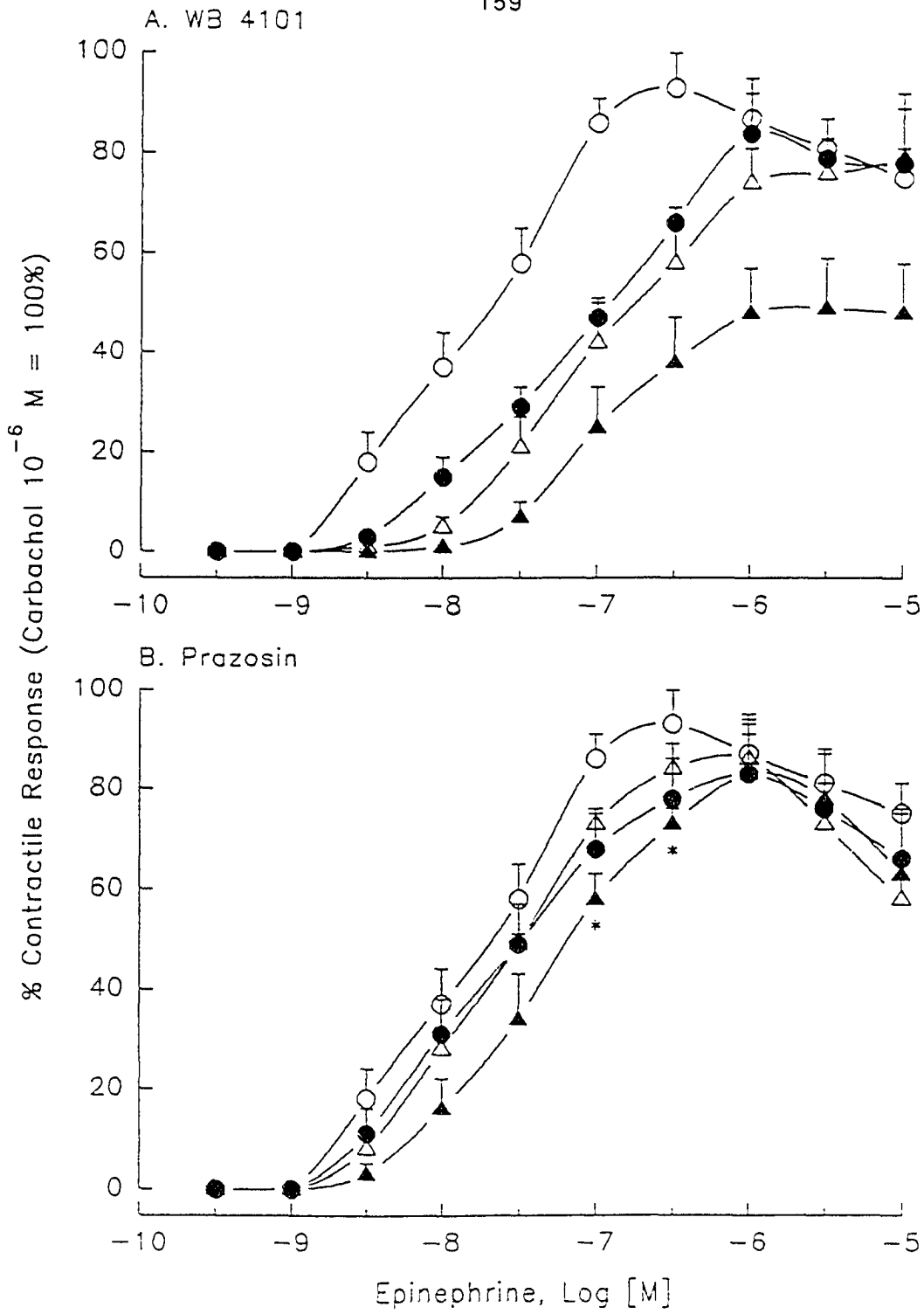
Table 1. Comparison of K_B values from functional studies with K_i values from receptor binding studies

Antagonist	Porcine myometrium	
	$K_B{}^a$ (nM)	$K_i{}^b$ (nM)
Yohimbine	3.80 ± 0.06 (6)	0.48 ± 0.05 (4)
WB 4101	17.38 ± 0.31 (5)	2.14 ± 0.26 (4)
Prazosin	2,239 ± 24 (5)	302 ± 25 (4)

[a] K_B values of yohimbine were transferred from pK_B values in Yang and Hsu (1995a).

[b] K_i values of binding data were taken from Yang and Hsu (1995c).

Parentheses indicate the number of observations.

Fig. 2. Correlation of antagonist affinities from functional (K_B) and binding (K_i) studies for porcine myometrium. Data for correlations were taken from Table 1. The data were best described by the expression $Y = 1.0125 * X + (-9.1461)$ (correlation coefficient, r, = 100% and slope = 1.01).
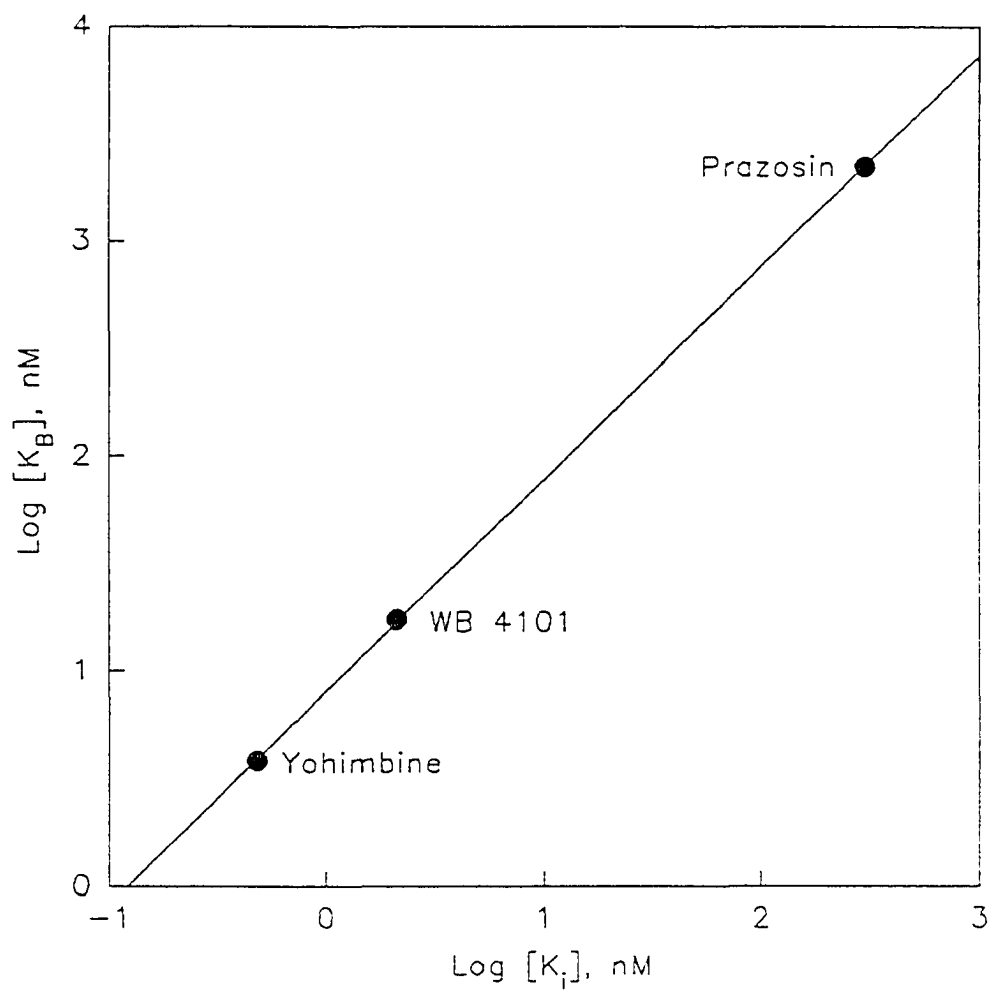
with the K_i values was 1.00 (slope = 1.01), indicating an excellent agreement between the functional and binding assays.

Discussion

The results of the present study suggest that the EPI-induced contractility of porcine longitudinal myometrium is mediated predominately by a_{2A}-ARs, and minimally by a_1- and a_{2B}-ARs in the luteal phase of the estrous cycle. These findings are consistent with those of the radioligand binding studies that the a_2-AR in the porcine myometrium is the a_{2A}-AR subtype (Yang and Hsu, 1995c). These results confirm and extend the previous report that the a_{2A}-ARs present in the porcine myometrium mediate excitatory contractions (Yang and Hsu, 1995a).

In the presence of PROP, EPI induced an increase in myometrial contractility in a dose-dependent manner. Its pD_2 value was consistent with the results from the same treatment of the previous study (Yang and Hsu, 1995a). In response to higher doses of EPI the myometrial contractility decreased because more β_2-ARs had been activated by the agonist (Yang and Hsu, 1995a).

In the presence of PROP, the a_{2A}-AR antagonist WB 4101 competitively antagonized the EPI-induced increase in myometrial contractility in a dose-dependent manner. However, the a_1- and a_{2B}-AR antagonist, PRZ, failed to do so, except that at an excessively high concentration (10^{-5} M), it antagonized the effect of EPI (10^{-7} and 3 x 10^{-7} M) ($P < 0.05$).

Since the pK_B value of WB 4101 against EPI was not significantly different from the value of yohimbine against EPI (Yang and Hsu, 1995a), it indicated that both have high affinities for the porcine myometrial a_2-ARs, which mediated myometrial contractions, whereas PRZ has a lower affinity. These results indicated a high

correlation between the functional and binding assays (Yang and Hsu, 1995a, 1995b and 1995c).

The antagonism by WB 4101 at 3×10^{-7} M appeared to be a noncompetitive manner when high concentrations of EPI ($\geq 10^{-6}$ M) did not overcome its inhibitory action (Bourne and Robert, 1995). The similar noncompetitive antagonism was seen when the uterine strip was pretreated with 3×10^{-7} M yohimbine, which was attributed to the β_2-AR mediated relaxation because the higher dose of PROP (10^{-5} M) further reversed the decreased contractility (Yang and Hsu, 1995a).

Prazosin (PRZ) is a selective α_1-and α_{2B}-AR antagonist (Bylund and U'Prichard, 1983; Bylund et al., 1988). Although PRZ at 10^{-5} M blocked the contractile effect of EPI in porcine myometrial strips, it would not suggest that α_1-ARs mediate the effect of EPI since the lower doses (10^{-6} and 3×10^{-6} M) used in this study failed to antagonize the effect of EPI. PRZ at 10^{-5} M may have α_2-AR antagonistic effects (Van Zwieten and Timmermans, 1983; Ko et al., 1990). In addition, we have observed high concentrations of the α_1-AR agonist methoxamine (10^{-6} - 10^{-4} M) caused porcine myometrial contractions. PRZ at much lower concentrations (10^{-8} - 10^{-7} M) partially antagonized methoxamine's actions (Yang and Hsu, unpublished observation). Moreover, PRZ at a lower concentration (10^{-8} M) blocks α_1-AR in other tissues, such as ovine umbilical veins (Zhang and Dyer, 1991). Because PRZ inhibited EPI-induced myometrial contractions much less than WB 4101, these results suggested that the EPI-induced increase in myometrial contractility was mediated predominantly by α_{2A}-, and minimally by α_{2B}-ARs. These results were in agreement with those of the radioligand binding studies in which PRZ does not displace [^{3}H]rauwolscine in porcine myometrial membrane until 10^{-8} M, whereas WB 4101 starts to displace at 10^{-10} M (Yang and Hsu, 1995c).

WB 4101 is a selective α_{2A}-AR antagonist (Niddam et al., 1990), and it is also

selective for a_{2C}-ARs in OK cells and opossum kidneys (Bylund, 1992). Although the high affinity of WB 4101 for porcine myometrial a_2-ARs (Yang and Hsu, 1995c) is close to the data for a_{2C}-AR prototype tissues (Murphy and Bylund, 1988; Blaxall et al., 1991), the high affinity of oxymetazoline, an a_{2A}-AR agonist (Bylund et al., 1988), only occurred in porcine myometrium (Yang and Hsu, 1995c), but not in a_{2C}-AR prototype tissues (Blaxall et al., 1991). In addition to the comparison of selective a_2-AR subtype agents, good correlations of porcine myometrium are only obtained with a_{2A}-AR prototype tissues, but not with a_{2B}-, a_{2C}-, or a_{2D}- prototype tissues (Yang and Hsu, 1995c). Therefore, our findings provided evidence that WB 4101 expresses the a_{2A}-AR selectivity in porcine myometrium.

We also compared the affinities of three a_2-AR antagonists, including PRZ, WB 4101 and yohimbine, in porcine myometrium using both the function (K_B value) (Yang and Hsu, 1995a and present study) and the radioligand binding (K_i value) techniques (Yang and Hsu, 1995c). There was an excellent correlation between K_B values and K_i values. Hence, the functional data are consistent with the radioligand binding data which further support the existence and definition of a_{2A}-AR subtype in porcine myometrium.

Although the K_B and K_i values were highly correlated, the K_B values were significantly higher than the K_i values. For examples, the K_B value for WB 4101 is 8-fold greater than the K_i value. The affinity difference of antagonists was probably caused by different assay conditions in functional and radioligand binding studies (Bylund and Ray-Prenger, 1989). The assay conditions used for the radioligand binding studies in myometrial membrane proteins are the results of efforts to optimize the binding in terms of high affinity and low nonspecific binding of [^{3}H]rauwolscine (Yang and Hsu, 1995c). In contrast, the studies on myometrial contractility in vitro were performed under conditions that were comparable to the isolated organ bath system.

In addition, the drug penetration through the tissue mass could be a factor in the isolated organ system.

The density of α_{2A}-ARs in porcine longitudinal myometrium is higher than that in other α_{2A}-AR prototype tissues, e.g. HT cells and human platelets (Bylund *et al.*, 1988). Moreover, this specimen is readily available and provides a large amount of α_{2A}-ARs. Therefore, porcine myometrium should be a good tissue model for the study of α_{2A}-ARs.

In summary, the present work suggested that α_{2A}-, but not α_{2B}-ARs, mediate EPI-induced increases in porcine myometrial contractility in the luteal phase of the estrous cycle. Furthermore, there is an excellent agreement between K_B values for α_2-AR antagonists determined by functional assays in porcine myometrial strips and the K_i values determined from radioligand binding assays in porcine myometrial membrane.

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EFFECTS OF YOHIMBINE AND PRAZOSIN ON METHOXAMINE-INDUCED INCREASE IN PORCINE MYOMETRIAL CONTRACTILITY *IN VITRO*

A paper submitted to Life Science, Pharmacological Letters.

Chih-Huan Yang and Walter H. Hsu

Abstract

a_2-Adrenoceptors are major a-adrenoceptors in porcine myometrium and mediate catecholamine-induced increase in myometrial contractility. However, the activity of porcine myometrial a_1-adrenoceptors on contractions is not clear. Hence, the objective of the study was to investigate if there is a_1-adrenergic effect on porcine myometrial contractility *in vitro* using uterine strips of the longitudinal layer in the luteal phase of the estrous cycle. Methoxamine, the a_1-adrenoceptor agonist, at high concentrations of 10^{-5}, 3×10^{-5} M and 10^{-4} M, caused a dose-dependent increase in myometrial contractility. Both the a_1-adrenoceptor antagonist, prazosin (10^{-8}, 3×10^{-8}, 10^{-7} M) and the a_2-adrenoceptor antagonist, yohimbine (10^{-8}, 3×10^{-8} M) inhibited the methoxamine-induced increases in myometrial contractility. However, after the application of 10^{-4} M methoxamine, when myometrium was greatly contracted, yohimbine (3×10^{-7} M) abolished the contractions, but prazosin (10^{-5} M) only slightly reduced the contractions. These results suggest that both a_1- and a_2-adrenoceptors mediate the methoxamine-induced increase in porcine myometrial contractility, with a_2-adrenoceptors mediating greater of this effect than a_1-adrenoceptors. These findings are attributed to the fact that with regards to a-adrenoceptor subtypes, porcine myometrium contains predominantly a_2-adrenoceptors.

Introduction

Our previous reports have demonstrated that natural catecholamine-induced porcine myometrial contractility is mediated by α_{2A}-adrenoceptors (ARs), but not by α_1-ARs (Yang and Hsu, 1995a, 1995b & 1995c), despite the fact that α_1- and α_2-AR subtypes account for 1 - 3 and > 97% of α-ARs, respectively (Yang and Hsu, 1995c).

The present study was undertaken to determine if the α_1-AR agonist methoxamine has any stimulatory effect on myometrial contractility. We hypothesized that if methoxamine increased myometrial contractions, this effect should be blocked by α_2-, but not α_1-AR antagonists.

Materials and Methods

Tissue preparation

Porcine uteri were collected from a local packing plant. Only the mid-portion of the uterine horns was used in the experiment. The uteri were determined as in the luteal phase based on the presence of light red corpora lutea on the ovaries, and the absence of embryos (Arthur et al., 1989). The tissues were stored in ice-cold Tyrode's solution (137 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 11 mM dextrose, and 12 mM $NaHCO_3$; pH 7.4) and transported to the laboratory. Upon arrival, the endometrium was removed from the uterus; the myometrium was stored in ice-cold Tyrode's solution aerated with 95% O_2-5% CO_2 and was used for experiments within 30 h. There were no changes in responsiveness to contractants during this period.

The methods for studying porcine myometrial contractility were as previously described (Yang and Hsu, 1995a and 1995b). In brief, the longitudinal uterine strips (10 x 2 mm^2) were suspended vertically in a 10-ml organ bath containing Tyrode's

solution maintained at 37°C and were aerated with 95% O_2-5% CO_2. The

contractions were recorded isometrically on a multiple-channel recorder (R411,

Beckman Instruments Inc., Schiller Park, IL) through a transducer (Grass FT03, Grass

Instrument Co., Quincy, MA). The strips were equilibrated under a 2-g tension for 20-

25 min before being exposed to 10^{-6} M carbachol (CARB) twice for 3 min each to

determine their responsiveness to the contractant. Four washes with 10 ml of

Tyrode's solution each were used to remove CARB after its 3-min stimulations. The

interval between the two CARB stimulations was 15 min. Usually the strips lost

contractions within 15 min after the washout of CARB, and this quiescent state usually

lasted > 25 min. The basal resting tension was readjusted to 2 g before the

pretreatment drug was added. In the following experiments, methoxamine was added

at a 10-min interval in cumulative doses to attain a dose-response relationship.

Effect of prazosin and yohimbine on methoxamine-induced myometrial contractility

The α_1-AR antagonist prazosin (PRZ) (10^{-8}, 3×10^{-8} or 10^{-7} M) or the α_2-AR

antagonist yohimbine (YOH) (3×10^{-9}, 10^{-8} or 3×10^{-8} M) was added to the organ bath

for 10 min. After 10-min of pretreatment with the antagonists, methoxamine was

given in cumulative doses (10^{-6} - 10^{-4} M). Control received only methoxamine without

an α-AR antagonist.

The 10-min pretreatment for α-AR antagonist was chosen, because in the

preliminary experiment, α-AR antagonism by YOH reached maximum in 10 min,

respectively (Yang and Hsu, 1995a).

Different strips from the same uterus were randomly assigned to all treatment

groups in one trial of experiment, and each uterus was used for one trial only.

Assessment of the contractile response

The determinations of the contractile response were as previously described

(Yang and Hsu, 1995a). Briefly, the contractile response of methoxamine was

assessed by the area under the contraction vurve (AUCC) and was determined with the use of a scanning program (SigmaScan, Jandel, Corte Madrera, CA). The values were expressed as a percentage of response to a 10^{-6} M CARB treatment for 10 min. In pilot studies we noticed that many tissue strips lost contractions after a 3-min but not a 10-min stimulation by 10^{-6} M CARB after several Tyrode's solution washouts. To transform the data for the 3-min CARB treatment to those for the 10-min treatment, an independent study was performed to attain a regression line (Yang and Hsu, 1995a):

Y (10 min) = 2.95 * X (3 min) + 1.32 (n = 39).

In this study, its area under the AUCC, produced by the second 3-min 10^{-6} M CARB stimulation, was transformed to a 10-min area using the above formula and this 10-min area was defined as the 100% 10^{-6} M CARB contractile response. The contractile response of the tissue strip was calculated from the AUCC produced by agonist methoxamine over 10 min at each cumulative dose and was expressed as a percentage of the response to 10^{-6} M CARB.

Drug

The following drugs were used: carbachol chloride and yohimbine HCl (Sigma Chemical Co., St. Louis, MO); methoxamine HCl (Burroughs Wellcome Co., Research Triangle Park, NJ), and prazosin HCl (Pfizer Inc., Groton, CT). Drugs were dissolved in distilled water, except for prazosin HCl, which was dissolved in 2% lactic acid to achieve a concentration of 1 mM. Drug-containing solutions were prepared by appropriate dilutions of the stock solutions, which were stored at -20°C.

Data analyses

The dose-response curves were produced by cumulative application of methoxamine in approximately one-half log increments (van Rossum, 1963). The data were expressed as pD_2 (-log EC_{50}) and were expressed as mean ± SE. In PRZ and YOH antagonism experiments, the contractile response was compared with the control

group at the correponding dose of the agonist. Data were expressed as mean $\pm$ SE and analyzed by analysis of variance (ANOVA). the conservative F value was used to establish significance for the treatment effect. The least-significant difference test as used to determine the difference between means of end points for which the ANOVA indicated a significant ($P < 0.05$) F ratio.

Results

Methoxamine, the selective α_1-adrenoceptor agonist, at high concentrations of 10^{-5} - 10^{-4} M, caused a dose-dependent increase in myometrial contractility (Fig. 1). The pD_2 value of methoxamine was 4.95 $\pm$ 0.07 (n = 5). Both the α_1-AR antagonist, PRZ (10^{-8}, 3×10^{-8}, 10^{-7} M) (Fig. 1A) and the α_2-AR antagonist, YOH (3×10^{-9}, 10^{-9}, 3×10^{-9} M) (Fig. 1B) inhibited significantly the methoxamine-induced increases in myometrial contractility. However, after the administration of methoxamine while the muscle was contracted, the addition of 3×10^{-7} M YOH (Fig. 2A) greatly reversed, but 10^{-6} M prazosin (Fig. 2B) only slightly reduced the effect of methoxamine.

Discussion

The results of the present study suggested that methoxamine-induced contractility of porcine longitudinal myometrium is mediated by both α_1- and α_2-ARs in the luteal phase of the estrous cycle because both PRZ and YOH inhibit the effect of methoxamine. These findings provided the evidence and extended the results of our previous studies in which α_1-ARs in porcine myometrium mediate minimal contractile activity (Yang and Hsu, 1995a, 1995b, 1995c & 1995d). Furthermore, the results also showed that methoxamine, the selective α_1-AR agonist had agonistic activity at

Fig. 1. Effect of prazosin (A) and yohimbine (B) on methoxamine-induced increases in myometrial contractility. Data are expressed as mean ± SE (n = 5). Effects are shown in the absence (O) and (A) in the presence of prazosin, 10^{-3} M, ●; 3×10^{-8} M, ▵; 10^{-7} M, ▲; or (B) in the presence of yohimbine, 3×10^{-3} M, ●; 10^{-8} M, ▵; 3×10^{-8} M, ▲.

*$P < 0.05$, compared with the control group at the corresponding agonist dose.

A. Prazosin

B. Yohimbine

% Contractile Response (Carbachol 10^{-6} M = 100%)
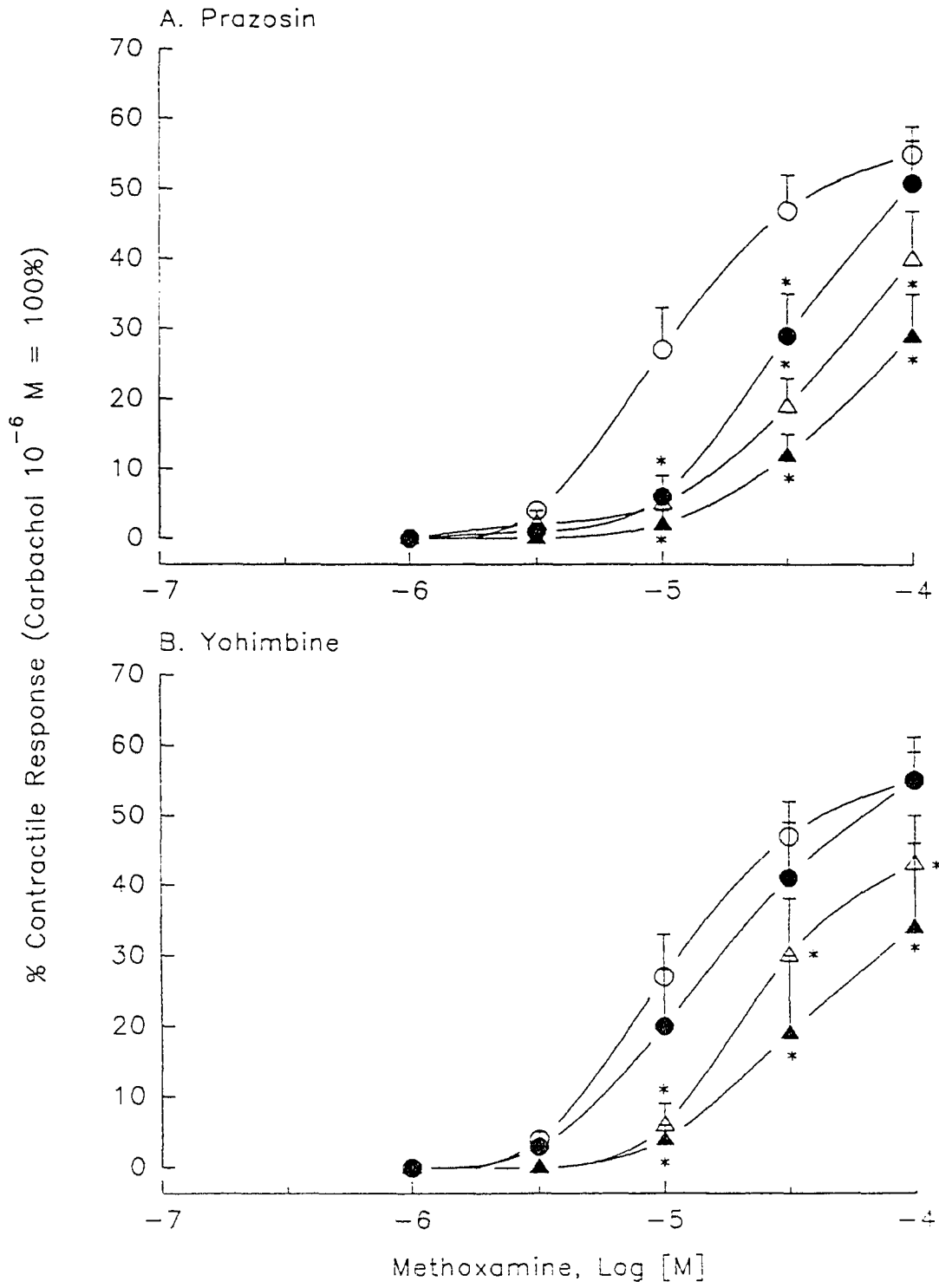
Methoxamine, Log [M]

Fig. 2. Representative tracings of the uterine contractile response for methoxamine. The contractile responses at 10^{-4} M methoxamine (A and C) were obtained from cumulative doses (3×10^{-6} - 10^{-4} M) in the absence of α-adrenoceptor antagonist. The methoxamine-induced myometrial contractility was greatly reversed by 3×10^{-7} M yohimbine (B), but was slightly reduced by 10^{-5} M prazosin (D). Data shown are the representative of three experiments.

A.

10^{-4} M Methoxamine

B.

3×10^{-7} M Yohimbine

4 g

1 min
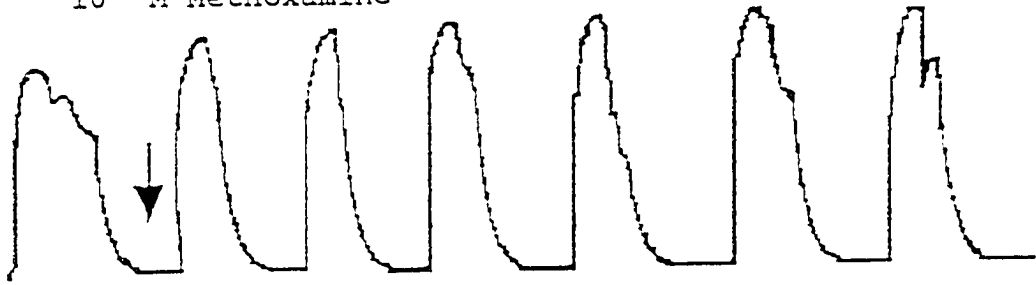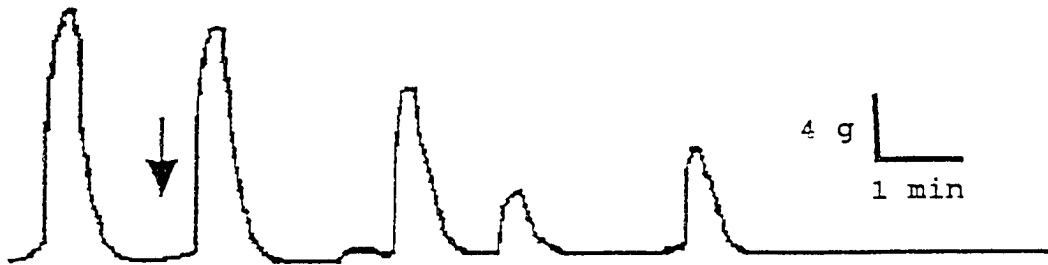
C.

10^{-4} M Methoxamine

D.

10^{-5} M Prazosin

porcine myometrial α_2-ARs.

In our previous studies PRZ, the α_1-AR antagonist, at 10^{-5} M in the presence of PROP failed to antagonize the effect of epinephrine and norepinephrine on the porcine myometrial contractility. The potency of natural catecholamines on myometrial contractility is not changed by the effect of an α_1-AR antagonist PRZ. We suggest that α_1-ARs in porcine myometrium have minimal function to mediate contractility (Yang and Hsu, 1995a). Results from th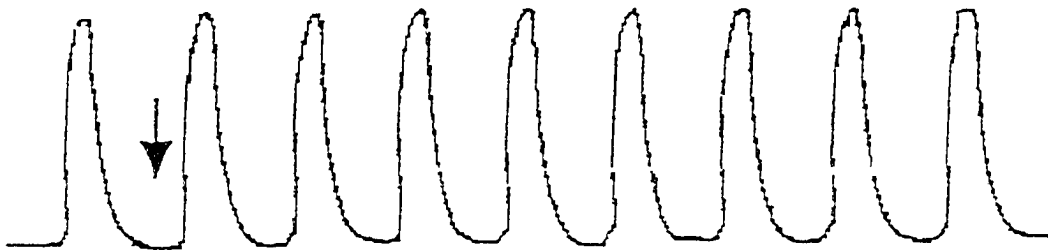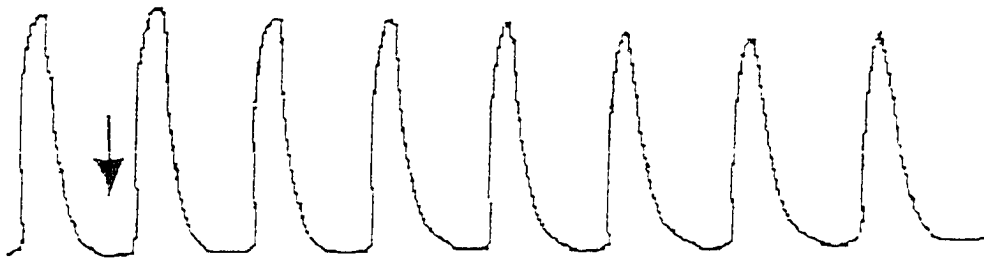e studies of radioligand binding assays indicated that α_1-AR density is only 1 - 3% of total α-ARs in porcine myometrium, and PRZ has very low affinity to displace [^{3}H]rauwolscine, a specific α_2-AR antagonist, from binding sites (Yang and Hsu, 1995c).

The present findings that the low potency of methoxamine, which was compared with that of epinephrine and norepinephrine to induce myometrial contractility (Yang and Hsu, 1995a), at least in part, supported that α_1-ARs mediate minimal response on myometrial contractility because the contractility was inhibited by PRZ.

However, methoxamine has activity at α_2-ARs, in addition to potent activity at α_1-ARs (Nichols and Ruffolo, 1991). It was reasonable to expect that it activated both α_1- and α_2-ARs in high concentrations which induced myometrial contractions in this study. Our results that both PRZ and YOH at lower concentrations inhibited the effect of methoxamine supported this contention. Under the stimulation with a highest concentration (10^{-4} M) of methoxamine in this study, the myometrial contractions were mediated predominantly by α_2-ARs because it was abolished by YOH, but not by PRZ. The inability of PRZ at 10^{-6} M to reverse the effect of methoxamine was probably due to the extremely low α_1-AR density in porcine myometrium which might have been fully activated at $< 10^{-4}$ M methoxamine. In conclusion, our results suggest that both α_1- and α_2-ARs mediate the methoxamine-induced myometrial contractions, with α_2-ARs

mediating greater of this effect than a_1-ARs did. These findings are attributed to the fact that with regards to a-AR subtypes, porcine myometrium contains predominantly a_2-adrenoceptors.

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GENERAL DISCUSSION

The classification of α-ARs in this study was undertaken by both functional and radioligand binding studies. The functional study is based on the receptor occupation theory where it is assumed that the occupation of a receptor by a drug leads to a stimulus and a subsequent response (Kenakin, 1984). The radioligand binding study identifies the specific receptors, determines the density of the receptors in the tissues, and characterizes the subtype of the receptors (William and Lefknowitz, 1978a).

In the functional study, myometrial contractility *in vitro* was used to test the potency of catecholamines (CATs) on α-AR subtype-mediated activity and to distinguish the affinity of specific α-AR antagonists against α-AR agonists on porcine myometrium. In this species, α_2-ARs, specifically α_{2A}-ARs, predominantly mediated the natural CAT-induced increase in myometrial contractility. In addition, the natural CAT-induced relaxation through β-AR-mediated action was also detected. In the radioligand binding study, α_{2A}-AR was the dominant α-AR in the porcine myometrium throughout the estrous cycle and during pregnancy. α_1-ARs were present in small amounts (1-3% of α-ARs), which minimally mediated myometrial contractility.

The presence of a heterogenous population of receptors serving antagonistic responses with respect to each other can subtract the effects mediated by the respective receptors (Kenakin, 1984). In this study the effect of the β-AR-mediated relaxation on α_2-AR-mediated contractions in porcine myometrium provided a good example. The most common method used to detect and eliminate this problem is by using selective antagonists of the interfering receptor population (Kenakin, 1984). In our results, the α_2-AR responses on porcine myometrial contractility were strikingly potentiated when the relaxant β-ARs were blocked by PROP in all reproductive stages tested, except in the follicular phase. In the follicular phase, the spontaneous

contractions were present throughout the experiment. In the absence of PROP, the spontaneous contractions were decreased progressively by the natural CATs, and this was attributed to the activation of myometrial β-ARs. In the presence of PROP, neither EPI nor NE caused a dose-dependent increase in myometrial contractility in this phase. The lack of agonistic activity of the natural CATs is due partially to the low concentration of a_2-ARs in the porcine myometrium because oxytocin induced a dose-dependent myometrial contractility in this phase (Yu and Hsu, 1993).

In the presence of PROP, EPI was more potent than NE in inducing an increase in myometrial contractility in the luteal phase of the estrous cycle and various stages of pregnancy. Differences in relative potency between agonists may result from differences in their relative affinity for receptors and/or their relative efficacy (Kenakin, 1984).

The efficiency of the stimulus response mechanisms in different tissues may vary. Two factors can affect the efficiency of the stimulus response mechanism in a tissue: 1) the number of receptors in the tissue, 2) the second messenger system which translates receptor stimulus into a cellular response (Kenakin, 1984). The second messenger system often performs as an amplifier in biological system (Ariens and Simonis, 1976; Goldberg, 1975). In general, the density of porcine myometrial a_2-ARs in pregnancy was greater than that in the luteal phase. However its CAT-induced myometrial contractility was less than that in the luteal phase. Therefore, it is possible that changes in the signal transduction system during pregnancy may lead to a lower response to a_2-AR stimulation than in the luteal phase. In addition, as the pregnancy progresses in the sow, the thickness of the longitudinal myometrium decreases progressively (Thilander and Rodriguez, 1989b and 1990). It is possible that the decreased thickness of myometrial strips provides fewer a_2-ARs, then lowers the contractile response to CATs *in vitro* study.

Selective agonism can provide useful information about the presence or absence of certain receptors in a given tissue. If a selective agonist produces a response in a tissue, a distinction should be made between selectivity and specificity (Kenakin, 1984). In the present study, both EPI and NE caused a dose-dependent increase in myometrial contractility. The effect of the natural catecholamine was effectively antagonized by YOH and WB 4101, the α_2-/α_{2A}-AR antagonist, but not by PRZ, an α_1- and α_{2B}-AR antagonist, indicating that the contraction was mediated by the α_2-ARs, specifically α_{2A}-ARs. This finding was consistent with that in radioligand binding assays in which the α_2-subtype was found to be the dominant α-ARs in the porcine myometrium and the affinity of the α_2-/α_{2A}-AR drugs to compete [^{3}H]rauwolscine binding was greater than that of PRZ, the α_1-/α_{2B}-AR drug.

On the other hand, if a tissue does not respond to a selective agonist, it could mean either that the receptor is not present or that the stimulus-response mechanism of the cell produces insufficient amplification of the receptor stimulus to generate a response. In this study, the weak agonist activity of methoxamine, the α_1-AR agonist in the porcine myometrium could be due to the low concentration of α_1-ARs.

For the most part, the definitive classification of the major drug receptor types and subtypes has been accomplished by using selective competitive antagonists. In general, antagonists are more selective for receptor subtypes than the agonists (Kenakin, 1984). The potency of a competitive antagonist depends on its equilibrium dissociation constant (K_B) for the drug receptor. Since competitive antagonists possess no intrinsic efficacy, the interaction of a competitive antagonist with a drug receptor is a strictly chemical process. The rate of onset and offset of the antagonist with the drug receptor is controlled only by the molecular forces. Therefore, the K_B values are independent of receptor function, location, and animal species. A similar K_B value for a specific competitive antagonist against different agonists provides strong evidence that

these agonists act on the same type of receptors.

In this study, either the K_B values of YOH *vs.* EPI and NE in the tissues of the same reproductive stage, or the K_B values of YOH *vs.* EPI or NE in the various reproductive stages were not significantly different, indicating that the natural CATs, EPI and NE, acted on the same type of α-AR, i.e., α_2-AR. On the other hand, if an agonist-induced response is not antagonized by a specific competitive antagonist, it can be concluded that the respective receptor is not present. In this study, the low concentration of α_1-ARs in porcine myometrium provides evidence that explains why potent and selective α_1-AR antagonist, PRZ, even at high concentrations failed to block the natural CATs-induced porcine myometrial contractility.

Since the ultimate goal of α-AR binding studies is to gain insight into the molecular mechanism by which adrenergic agonists elicit physiological response, it is imperative that binding data be related to data from physiological response measurements (Williams and Lefkowitz, 1978b). In these studies, the affinities of three α_2-AR antagonists, including PRZ, WB 4101 and YOH, in porcine myometrium were compared between the function (K_B values) and radioligand binding (K values) experiments. The excellent correlation between the results of these two studies supports the contention that the binding sites are indeed the physiologically active α_2-ARs through which CATs and antagonists act.

From these *in vitro* studies we found that the α_2-ARs in porcine myometrium mediated natural CAT-induced myometrial contractions but their physiological function in the uterus is still not clear. Considering the contraction which is mediated by α_2-ARs *in vivo*, the action of β-ARs, especially β_2-ARs, on myometrial contractility can not be neglected. β-ARs are present in the porcine myometrium (Yang and Hsu, unpublished results) and mediate uterine relaxation in this study.

In the absence of PROP, both EPI and NE decreased myometrial contractility

progressively in the follicular phase but induced contractions when high concentrations ($\geq$ 3 x 10^{-7} M) of EPI and NE were applied in all reproductive stages. These results implicate that at low concentrations of natural CATs β-inhibitory action is greater than that of α-excitatory action in controlling myometrial contractility.

In physiological condition, the action of natural CATs on uterine motility may be similar to that in the *in vitro* study without β-AR antagonism, i.e., natural CATs activate α- and β-ARs simultaneously. Therefore, if natural CATs in the body can induce myometrial contractions, their concentrations should be at least as high as $\geq$ 3 x 10^{-7} M to overcome the β-AR-mediated relaxations. Plasma EPI and NE concentrations increase during labor in women (Lederman *et al.*, 1977 and 1978) and sheep (Eliot, *et al.*, 1981), but the concentrations may not increase so high as to cause uterine contraction (NE: 1 ng/ml plasma $\approx$ 3 x 10^{-9} M in sheep) (Eliot, *et al.*, 1981). However, the potency of natural CATs to induce myometrial contraction *in vitro* may not reflect the same physiological response as *in vivo* because the assay conditions used for myometrial contractility *in vitro* were the results of efforts to optimize the isolated tissues in organ bath system.

The plasma estrogen concentrations increase prior to parturition in many species, including sows (Ford *et al.*, 1984; Thilander and Rodriguez-Martinez, 1990). The high estrogen levels stimulate the formation of gap junctions in myometrium, enhance uterine contractility through stimulating prostaglandin production and increase myometrial oxytocin receptors to facilitate labor (Garfield, 1994). Therefore, the activity of myometrial α_2-ARs may interact with other hormones and autacoids, such as prostaglandin $F_{2\alpha}$ to increase myometrial contractions. Furthermore, since our results suggested that the action of α_2-ARs in porcine myometrium is excitatory, it is feasible to use an α_2-AR agonist in combination with prostaglandin $F_{2\alpha}$ to facilitate delivery of the fetus or synchronize farrowing in preparturient sows (Ko *et al.*, 1989).

Moreover, it is likely that a_2-ARs counterbalance the β_2-AR-mediated myometrial relaxation. This could be important at term, because without the participation of a_2-ARs, there could be excessive myometrial relaxation when animals are stressed, which may interfere with parturition process. Although it is postulated that a_2-ARs regulate some aspects of cellular metabolism important for uterine function, and that the ovarian steroid-induced changes in a_2-AR density may involve the control of the metabolism of the uterus during the estrous cycle and pregnancy (Ruffolo and Hieble, 1994), it is yet to be investigated.

From the results of the present study, we suggest that the porcine myometrial a_2-AR appears to be under the control of progesterone since its density is high in a progesterone-dominant environment, such as the luteal phase or pregnancy. In contrast, the density of a_2-ARs was low when myometrium was exposed to a low progesterone environment, such as the follicular phase. However, estrogens might not influence the density of porcine myometrial a_2-ARs because the a_2-AR concentration in prepartum period was still 5-fold greater than that in the follicular phase even through the plasma concentrations of estrogens in prepartum period was reported to be 7 fold higher than in the follicular phase (Thilander and Rodriguez, 1989a and 1990). Therefore, further research using ovariectomized pigs supplemented with steroids is needed to determine which sex steroids, or combinations, is responsible for the changes in a_2-AR density.

We do not know why physiological changes in progesterone concentration would produce prominent changes in a_2-AR density in porcine myometrium. Steroid hormones are known to regulate the expression of various proteins through activation of gene transcription (Beato, 1989). The testosterone-enhanced a_2-AR expression in hamster fat cells is a result of the regulation of the synthesis and/or turnover of the a_2-ARs (Saulnier-Blache et al., 1992; Bouloumie et al., 1994). Therefore, we hypothesize

that progesterone may induce an increase in the density of porcine myometrial α_2-ARs via enhanced transcription. Further studies are also needed to define the mechanisms involved in the regulation of α_2-AR expression by sex steroids in porcine myometrium.

GENERAL SUMMARY

The adrenergic effect of natural catecholamines (CATs) epinephrine (EPI) and norepinephrine (NE) on contractility *in vitro*, and identification and characterization of α-adrenoceptors (ARs) were studied in longitudinal myometrium of sows during the estrous cycle and pregnancy. The uterine strips in the follicular phase presented spontaneous contraction throughout the experiments, and the contractions were decreased by the action of EPI and NE in the absence of propranolol (PROP), the β-AR antagonist. In the presence of 10^{-6} M PROP, neither EPI nor NE increased myometrial contractility in this phase. However, EPI or NE alone induced a dose-dependent increase in myometrial contractility in other reproductive stages. This effect was potentiated by pretreatment with 10^{-6} M PROP and the potency of EPI was greater than that of NE. The order of the potencies of EPI and NE was luteal phase (L) $\geq$ late pregnancy (LPG) (days of gestation = 73 - 79) $\geq$ mid-pregnancy (MPG) (days of gestation = 53 - 60) $\geq$ early pregnancy (EPG) (days of gestation = 39 - 40) > prepartum period (PPT) (days of gestation = 111 - 113). These induced myometrial contractions were inhibited by both the α_2-AR antagonist yohimbine (YOH) (10^{-8} - 3 x 10^{-7} M) and the α_{2A}-AR antagonist WB 4101 (3 x 10^{-8} - 3 x 10^{-7} M) in a dose-dependent manner, but not by prazosin (PRZ), the α_1-AR antagonist even at the high concentrations up to 3 x 10^{-6} M. Although methoxamine, the α_1-AR agonist, at high concentrations of 10^{-5} - 10^{-4} M, also caused a dose-dependent increase in myometrial contractility, the induced increase was inhibited by both PRZ (10^{-3}, 3 x 10^{-3}, 10^{-7} M) and YOH (10^{-8}, 3 x 10^{-8} M). Moreover, the effect of methoxamine at 10^{-4} M, when the myometrium had been greatly contracted, was abolished by YOH (3 x 10^{-7} M), but was only slightly reduced by PRZ (10^{-6} M).

When uterine strips were pretreated with Ca^{2+}-free Tyrode's solution or 10^{-5} M

verapamil, a voltage-dependent Ca^{2+} channel (VDCC) blocker, the EPI- and NE- induced

myometrial contractility was greatly decreased. This decreased contractility in Ca^{2+}-

free medium was further inhibited by 10^{-7} M YOH, and to a lesser extent by 10^{-7} M

PRZ. Therefore, the results in functional studies suggested that a_2-, specifically a_{2A}-

ARs, mediated EPI- and NE-induced increase in myometrial contractility in sows, which

was attributed primarily to an increase in Ca^{2+} influx through VDCC and at least in

part, due to calcium release from intracellular stores.

In radioligand binding studies, we used [^{3}H]prazosin ([^{3}H]PRZ) and

[^{3}H]rauwolscine ([^{3}H]RAU) as specific ligands to identify a_1- and a_2-ARs, respectively, in

porcine myometrium. Both ligands were saturable with high affinities to a_1- and a_2-

ARs. They were rapidly reversed by 10^{-5} M phentolamine, an a-AR antagonist.

Saturation binding studies with [^{3}H]RAU showed that the density of a_2-ARs was high

comparing to the a_1-ARs, and a_2-ARs accounted for at least 97% of total a-ARs in all

reproductive stages. The equilibrium dissociation constants (K_D) being 4.6 - 6.9 nM

were not significantly changed among reproductive stages. The order of the maximum

binding density (B_{max}) in fmol/mg protein of a_2-ARs was EPG (2,426 ± 430) ≥ very

late pregnancy (VLPG) (days of gestation ≥ 100) (2,392 ± 341) ≥ LPG (2,049 ±

131) ≈ MPG (1,999 ± 318) ≥ L (1,568 ± 135) ≈ PPT (1,507 ± 236) > F (265 ±

50). However, the density of a_1-ARs remained low in all reproductive stages although

the K_D values (21.5 - 33.5 pM) were not significantly different among the tested

groups. The order of B_{max} in fmol/mg protein of a_1-ARs was L (23.6 ± 2.1) ≈ EPG

(22.0 ± 1.3) ≥ LPG (20.0 ± 3.9) ≥ MPG (15.6 ± 3.4) > ≈ PPT (11.3 ± 1.1) > F

(7.5 ± 1.6). From the competition binding studies in myometrial membranes from the

luteal phase, the drug affinities, including idazoxan, oxymetazoline, PRZ, RX 821002,

WB 4101 and YOH, were highly correlated between porcine myometrium and known

a_{2A}-subtype cells, such as human platelets and HT29 cells. In contrast, correlations

were poor between porcine myometrium and other known a_2-subtype tissues or cells,
i.e., a_{2B} (neonatal rat lung and NG18-105 cells), a_{2C} (opossum kidney and OK cells),
and a_{2D} (bovine pineal gland and rat submaxillary gland). Moreover, when comparing
the affinity of a_2-AR antagonists, including WB 4101, PRZ and YOH, in porcine
myometrium, there was an excellent correlation (r = 100%, slope = 1.01) between
dissociation constants from the contractility study and inhibition constants from the
radioligand binding study.

Therefore, from the above results we suggested that the a_{2A}-AR is the major a-
AR in porcine myometrium. It mediates natural CAT-induced increase in myometrial
contractility *in vitro* in cycling and pregnant sows. Our data also suggested that
ovarian steroids, especially progesterone, play an important role in the regulation of
porcine myometrial a-ARs, i.e., in a high-progesterone environment (in the luteal phase
or during pregnancy), the density of a-ARs is increased. However, in a low-
progesterone environment (in the follicular phase) the density of a-ARs is decreased.
a_1-ARs are present in porcine myometrium in small amounts and mediate minimal
response on myometrial contractions. We also suggested that the effect of natural
CATs on myometrial contractility is primarily mediated by an increase in Ca^{2+} influx
through VDCC, and in part, through Ca^{2+} release from intracellular stores.

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