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Involvement of cyclic nucleotides and prostaglandins during contraction and relaxation of isolated human umbilical arteries

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Involvement of cyclic nucleotides and prostaglandins during contraction and relaxation of isolated human umbilical arteries

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACh	Acetylcholine
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
8BcAMP	8-Bromo-cyclic AMP
8BcGMP	8-Bromo-cyclic GMP
BSA	Bovine serum albumin
cAMP	Cyclic AMP (cyclic adenosine-3',5'-monophosphate)
CDR	Calcium dependent regulator
cGMP	Cyclic GMP (cyclic guanosine-3',5'-monophosphate)
CN-PDE	Cyclic nucleotide phosphodiesterase
cpm	Counts per minute
dBcAMP	Dibutyryl-cyclic AMP
dBcGMP	Dibutyryl-cyclic GMP
ddH_2O	Double-distilled water
DHA	Dehydroascorbic acid
DTT	Dithiothreitol
EDTA	(Ethylenedinitrilo)tetraacetic acid
EGTA	Ethylenebis(oxyethylenenitrilo)-tetraacetic acid
ETA	Eicosa-5,8,11,14-tetraynoic acid
GTP	Guanosine triphosphate
5-HT	5-Hydroxytryptamine (serotonin)
HUA	Human umbilical artery

Indo	Indomethacin
ITP	Inosine triphosphate
MIX	1-Methyl-3-isobutyl xanthine
PDE	Phosphodiesterase
PG	Prostaglandin
POPOP	1,4-Bis-(2-(5-phenyloxazolyl))-benzene
PPO	2,5-Diphenyloxazole
PPP	Polyphoretin phosphate
RCS	Rabbit aorta contracting substance
RIA	Radioimmunoassay
SHR	Spontaneously hypertensive rat
SUA	Sheep umbilical artery
Tx	Thromboxane
W-7	N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide

INTRODUCTION

Literature Review

Cyclic nucleotides

Since the discovery of cyclic AMP (cAMP) by Sutherland and coworkers (Sutherland and Rall, 1958; Rall and Sutherland, 1958), there have appeared numerous publications that implicate cAMP as a mediator or modulator of various cellular functions in many different types of tissues. More recently, another endogenous cyclic nucleotide, cyclic GMP (cGMP), has received increasing attention as a possible cellular mediator or regulator (Goldberg et al., 1973a,b, 1975, 1978; Böhme et al., 1978; Murad et al., 1978a,b).

Due to the huge volume of literature dealing with the subject of cyclic nucleotide involvement in cell functions, this review will refer only to those articles and reviews that are of particular relevance to the function of cyclic nucleotides in vascular smooth muscle contractility. Research in the area of cyclic nucleotide function in smooth muscle in general (Andersson, 1972; Andersson et al., 1972, 1975; Bär, 1974; Andersson and Nilsson, 1977; Diamond, 1977; Schultz and Hardman, 1976; Diamond, 1978) and in vascular smooth muscle in particular (Pöch and Kukovetz, 1972; Somlyo et al., 1972; Namm and Leader, 1976; Hardman et al., 1977) has been extensively reviewed.

Adenylate cyclase Adenylate cyclase was first demonstrated in vascular tissue by Sutherland et al. (1962) and they suggested that beta-adrenergic agonists may stimulate the production of cAMP in vascular

tissue by a mechanism analogous to that previously proposed for the liver (Sutherland and Rall, 1958; Rall and Sutherland, 1958). However, Klainer et al. (1962) were unable to detect a stimulation of adenylate cyclase activity by epinephrine in broken cell preparations from vascular and intestinal smooth muscle. Likewise, Schonhofer et al. (1971) were unable to demonstrate an activation of adenylate cyclase by isoproterenol, norepinephrine, 5-hydroxytryptamine (5-HT) or histamine in homogenates of thoracic and abdominal aorta of rabbits. Furthermore, Triner et al. (1971) and Triner et al. (1972a) were also unable to demonstrate activation of adenylate cyclase in homogenates of arteries following addition of various catecholamines. However, in the studies by Schonhofer et al. (1971), Triner et al. (1971) and Triner et al. (1972a), it was observed that NaF could stimulate the adenylate cyclase in these preparations of homogenized vascular tissue. NaF-induced stimulation of adenylate cyclase activity has been observed in homogenate preparations of many other types of tissues (Sutherland et al., 1962; Perkins, 1973).

Small increases in adenylate cyclase activity in vascular homogenate were observed by Volicer and Hynie (1971) following the addition of catecholamines. In contrast, Amer (1973) and Ramanathan and Shibata (1974) reported that catecholamines could cause a 2- to 4-fold increase in adenylate cyclase activity in homogenates of rat aorta. Hardman et al. (1977) suggested that the differences in demonstrating adenylate cyclase activation by beta-adrenergic agonist (catecholamines) may be due in part to the lability of the enzyme under the assay conditions. However, they emphasized that the status of the tissue prior to homogenization is also important. For example, they found that prolonged preincubation of intact

pig coronary arteries under conditions favorable for contractile responses (i.e., bathed in oxygenated, glucose-containing, balanced salt solutions) enhanced the responsiveness of adenylate cyclase to isoproterenol after homogenization. They reported that isoproterenol produced, at most, a 15-20% increase in adenylate cyclase activity in vessels homogenized immediately after dissection, but greater than a 200% increase in adenylate cyclase activity in vessels homogenized after incubation for several hours in an oxygenated, glucose-containing, balanced salt solution.

Another experimental condition that must be considered when showing hormone responsiveness of adenylate cyclase was demonstrated by Rodbell et al. (1971). They reported that a guanyl nucleotide was essential for the expression of glucagon-sensitivity of adenylate cyclase in liver homogenates. This finding was confirmed by the work of Leray et al. (1972), who found that the presence of GTP was necessary for the activation of adenylate cyclase by either glucagon or epinephrine in "purified" liver plasma membrane from adrenalectomized rats. The NaF-induced stimulation of adenylate cyclase activity in this preparation, on the other hand, was not affected by GTP. The obligatory role of the purine nucleotide was further supported by the findings of Ross et al. (1978), who found that isoproterenol- and prostaglandin E_1 (PGE_1)-induced activation of adenylate cyclase in disrupted, fractionated S49 lymphoma cells was absolutely dependent on the presence of a regulatory purine nucleotide. They observed that GTP, ITP, or the nonhydrolyzable analogs, Gpp(NH)p or $GPP(CH_2)p$, could act as the regulatory nucleotide. Isoproterenol was found to bind to the beta-adrenergic receptors, but adenylate cyclase

activity was not increased unless the purine (e.g., 50 μM GTP) was added. Thus, in the absence of the regulatory nucleotide, there appeared to be an uncoupling between the receptor occupation and the enzyme activation (Ross et al., 1978). There is evidence that the regulatory nucleotide interacts with a specific regulatory subunit which is distinct from the hormone receptor (Pfeuffer and Helmreich, 1975).

A model that describes the involvement of the regulatory nucleotide in the activation of adenylate cyclase by beta-adrenergic agonists has been presented by Lefkowitz and Williams (1978). The model also affords an explanation for the commonly reported phenomena of desensitization of the beta-adrenergic receptor and of the adenylate cyclase activity (Su et al., 1976a,b; Lefkowitz and Williams, 1978; Lin et al., 1977). Furthermore, this model emphasizes the concept of separate subunits which are associated with either beta-adrenergic agonist binding or with adenylate cyclase activity (Lefkowitz and Williams, 1978). Indeed, it has been recently shown that hormone receptors, such as beta-adrenergic receptors and PGE_1 receptors, can be transferred from one adenylate cyclase system to another (Schramm et al., 1977). By using a cell fusion technique, Schramm et al. (1977) demonstrated that adenylate cyclase systems that had previously been unresponsive to either beta-adrenergic agonist or to PGE_1 could be made responsive by the transfer of the appropriate receptor. They concluded that the hormone receptor is a unit independent of the enzyme. Furthermore, they concluded that the coupling between receptors and adenylate cyclase may be universal for all eukoaryotic cells, since

they were able to show that receptors and enzymes from vastly different cell types and species were compatible with each other.

The possible involvement of calcium in regulating adenylate cyclase activity was proposed by Bockaert et al. (1972). They reported that "minute amounts" of calcium were necessary for oxytocin-induced activation of adenylate cyclase in frog bladder epithelial cells. The addition of EGTA, a calcium chelator, was observed to inhibit the response. Further addition of Ca^{++} to bring the free Ca^{++} concentration to 10^{-6} M was found to restore the response. Concentrations of Ca^{++} higher than 5×10^{-5} M, however, were associated with inhibition of the oxytocin-induced activation of adenylate cyclase (Bockaert et al., 1972).

A low molecular weight protein, endogenous to many tissues, has been found to confer calcium-dependent regulatory properties to adenylate cyclase in brain homogenates (Cheung et al., 1978). This protein was shown to be identical to the Ca^{++}-dependent regulator (CDR) or modulator protein that regulates cyclic nucleotide phosphodiesterase (Peak I) activity (see section on Cyclic Nucleotide Phosphodiesterases in Literature Review). The CDR protein appears to either stimulate or inhibit adenylate cyclase activity depending on the concentration of Ca^{++} or on the tissue analyzed (Cheung et al., 1978; Brostrom et al., 1978).

Adenylate cyclase activity in arterial vessels of rats was found to vary depending on the source of the artery (Triner et al., 1971). For example, Triner et al. (1971) found the highest activity of adenylate cyclase in the coronary artery, whereas the lowest activity was associated with the lumbar aorta. These observations led the authors to conclude

that basal adenylate cyclase activity in the vasculature tended to increase toward the periphery and this may, in turn, reflect the proportion of smooth muscle in these vessels.

Volicer et al. (1973) observed that the basal and fluoride-stimulated activities of adenylate cyclase were distributed in the three layers of blood vessels similar to that of cAMP itself; i.e., the lowest activity was associated with the tunica adventitia, whereas the highest activity was associated with the intima and media--layers that contain smooth muscle.

In general, adenylate cyclase activity for a variety of tissues has been shown to be located predominantly in the particulate fraction of the tissue homogenates (Perkins, 1973).

Guanylate cyclase Hardman and Sutherland (1969) demonstrated the presence of guanylate cyclase in several tissues, including rat uterus. Early investigations of the subcellular localization of guanylate cyclase indicated that this enzyme was located primarily in the soluble fractions of cell homogenates; this was in direct contrast to the particulate localization of mammalian adenylate cyclase (Goldberg et al., 1973b). However, the proportion of particulate to soluble guanylate cyclase differed considerably between various tissues. For example, 80-90% of enzyme activity was associated with the soluble fraction in rat lung, spleen, and liver, whereas most of the guanylate cyclase activity in rat small intestine was found to reside in the particulate fraction. However, latent guanylate cyclase activity in the particulate fractions of rat intestine was observed following treatment with Triton X-500, a nonionic (solubiliz-

ing) detergent, which caused a 2.5 fold increase in particulate guanylate cyclase activity (Ishikawa et al., 1969). Similarly, Hardman et al. (1971) reported that nonionic detergents could induce a 7- to 10-fold increase in particulate guanylate cyclase activity from rat lung, liver, and spleen, but only a 2- to 3-fold increase in enzyme activity in the soluble fraction.

In a recent report, Murad et al. (1978a) stated that guanylate cyclase in most mammalian tissues is predominantly located in the particulate fraction when the latent but expressible activity in the presence of detergents is considered. Guanylate cyclase activity has also been found in fractions of endoplasmic reticulum of heart and in fractions of nuclear material from liver and uterus (Murad et al., 1978a).

Kimura and Murad (1974) identified two forms of guanylate cyclase activity in rat heart. They found that almost all of the cellular guanylate cyclase activity resided in either the 1000 X g particulate fraction or the 100,000 X g supernatant fraction, each fraction having about equal enzyme activity. Triton X-100 was found to increase the particulate guanylate cyclase activity by 4- to 5-fold, whereas the soluble enzyme activity was increased by only 50-60%. They were able to differentiate between the particulate and soluble enzymes based upon physical and chemical properties. For example, the two enzymes separated into two peaks during gel filtration chromatography. Ca^{++} stimulated the activity of the soluble guanylate cyclase, but inhibited the particulate enzyme. Both enzymes were inhibited by ATP, but the half-maximal inhibition of the soluble and particulate guanylate cyclases were with 0.4 mM ATP and >1 mM

ATP, respectively. Furthermore, Mn^{++}, at a concentration greater than 2 mM, markedly inhibited the particulate guanylate cyclase but had little effect on the soluble enzyme. Based upon this study and upon subsequent research, Murad et al. (1978a) concluded that there is both a calcium-dependent and a calcium-independent mechanism for tissue production of cGMP; this, in turn, may be related to the multiple forms of guanylate cyclase.

Although cholinergic and alpha-adrenergic agonists can cause an elevation of cGMP levels in certain intact cells, this response was observed to be lost when the tissues were placed in a Ca^{++}-free medium or when the cells were homogenized (Hardman and Sutherland, 1969; Schultz and Hardman, 1975). Since one of the cellular guanylate cyclases appeared to be sensitive to Ca^{++} concentrations, it has been hypothesized that calcium may act as an intracellular (second) messenger to transfer the signal from an exogenous stimulus (agonist-receptor interaction, membrane depolarization, etc.) to the intracellular Ca^{++}-sensitive guanylate cyclase (Rasmussen and Goodman, 1977). Further support for this hypothesis was obtained when Clyman et al. (1975a) showed that the elevation of cGMP levels in vascular tissue (human umbilical arteries) induced by one of several vasoactive agents (histamine, bradykinin, and K^{+}) depended on the presence of extracellular Ca^{++}.

In contrast to the above mentioned vasoactive agents, sodium azide (NaN_3) was found to stimulate cGMP accumulation in rat brain and liver by a mechanism that was independent of extracellular Ca^{++} concentrations (Kimura et al., 1975). Furthermore, NaN_3 was observed to stimulate the

activity of a Ca^{++}-independent guanylate cyclase in a particulate fraction of homogenized rat cerebral cortex. Guanylate cyclase in the soluble fraction was not stimulated by NaN_3 and appeared to have properties similar to the soluble guanylate cyclase in rat heart (Kimura and Murad, 1974). The particulate guanylate cyclase was found to be stimulated by hydroxylamine (NH_2OH) as well, but was not stimulated by cyanide, a compound that has metabolic inhibitory properties similar to NaN_3 (Kimura et al., 1975).

DeRubertis and Craven (1976a) found that a number of chemical carcinogenic and mutagenic agents of the nitrosamine family as well as $NaNO_2$ and NH_2OH could stimulate the accumulation of cGMP in the liver, renal cortex, lung, and colonic mucosa of rats and in the human colonic mucosa. These agents were also found to stimulate guanylate cyclase activity in tissue homogenates of rat liver and renal cortex. All responses induced by these agents were found to be independent of Ca^{++} (DeRubertis and Craven, 1976a). The authors noted that the agents with the greatest propensity toward formation of free radicals (e.g., N-methyl-N'-nitro-N-nitrosoguanidine) were also the most potent stimulators of cGMP accumulation in these tissues and it was suggested that these two characteristics may be related.

In a later study by Murad and coworkers, vasoactive agents with pharmacological actions similar to $NaNO_2$ were found to stimulate guanylate cyclase from several tissues, including bovine tracheal smooth muscle (Katsuki et al., 1977). Sodium niroprusside, hydroxylamine, and nitroglycerin (each at 1 mM) caused a calcium-independent stimulation of soluble but not particulate guanylate cyclase activity. However, the effects

of two of these agents (hydroxylamine and nitroglycerin) appeared to differ depending on the tissue analyzed. It was found that a protein activator, which they named azide-activator protein (Murad et al., 1978a), was needed to demonstrate the stimulatory effects of hydroxylamine, nitroglycerin and azide on guanylate cyclase activity. Catalase and peroxidase were found to substitute for the azide-activator protein and it was determined that these enzymes catalyzed the conversion of azide and hydroxylamine into nitric oxide (NO), which was responsible for the activation of guanylate cyclase. $NaNO_2$ and presumable nitroglycerin were also converted to the reactive NO before activation of guanylate cyclase (Murad et al., 1978a). In support of this idea, pure NO was found to stimulate crude guanylate cyclase from tracheal muscle, lung and liver and the carcinogenic nitrosamines and nitroprusside, which possess the NO group in their chemical structure, appeared to stimulate guanylate cyclase without prior conversion to NO (i.e., without the presence of the azide-activator protein) (Katzuki et al., 1977).

Arnold et al. (1977) reported that cigarette smoke, which contains NO, stimulated the guanylate cyclase activity in both particulate and soluble fractions of various tissues, including heart, lung, liver, kidney, cerebral cortex, and cerebellum from rats and bovine tracheal mucosa. They suggested that the presence of NO in cigarette smoke and its potential ability to stimulate guanylate cyclase and elevate cGMP levels may be related to its suspected carcinogenicity.

Goldberg et al. (1978) demonstrated that guanylate cyclase activity in splenic cells could be regulated by an oxidative-reductive-related mechanism. They found that dehydroascorbic acid (DHA), an oxidizing

agent, stimulated both particulate and soluble guanylate cyclases, whereas dithiothreitol (DTT), a reducing agent, suppressed guanylate cyclase activity in both fractions. It was further observed that ascorbic acid, the reduced form of DHA, had no effect on guanylate cyclase (or cyclic nucleotide phosphodiesterase) activity in splenic cell homogenates. Yet ascorbic acid has been shown to elevate intracellular levels of cGMP in various (intact cell) preparations, including human umbilical artery (Clyman et al., 1975b), platelets (Goldberg et al., 1975; Schoepflin et al., 1977) and splenic cells (Goldberg et al., 1978). Based upon these data and upon further experiments, Goldberg et al. (1978) concluded that ascorbic acid must undergo a metabolic transformation to either the monoanionic free radical or the dianionic form (i.e., DHA) for expression of its effect on guanylate cyclase in intact cells.

Fatty acid hydroperoxides and prostaglandin endoperoxides (PGG_2 and PGH_2) have also been shown to activate the soluble (but not particulate) forms of splenic cell guanylate cyclase (Goldberg et al., 1978). Prostaglandins E_2 and $F_{2\alpha}$, the fatty acids (arachidonic, linoleic, oleic, and stearic), and the hydroxy analogs of these fatty acids were found to have no effects. The stimulatory effects of PGG_2 and DHA appeared to be additive with respect to the rate of activation and the maximally attained catalytic activity of guanylate cyclase. Goldberg et al. (1978) stated that their results indicate the existence of two regulatory sites--one site for hydrophobic oxidants (e.g., PGG_2) and another site for hydrophilic oxidants (e.g., DHA)--which regulate the activity of guanylate cyclase in spenic cells. They further proposed that these oxidants act by

promoting sulfhydryl-disulfide interconversions at the cyclase regulatory sites.

Recent studies by Murad's group have added further evidence for the involvement of free radicals in the regulation of guanylate cyclase activity. Murad et al. (1978a,b) demonstrated that the guanylate cyclase in a partially purified soluble fraction of rat liver could be stimulated by the addition of superoxide dismutase. They proposed that the active agent in this reaction was the hydroxyl radical which is formed from superoxide ion and H_2O_2. They suggested that this proposed mechanism explains the numerous reports of stimulated guanylate cyclase activity and the increased levels of cellular cGMP induced by various redox agents (cf. Goldberg et al., 1978). They further proposed that this mechanism may be involved in the actions of various stimulant (physiological, hormonal, and autocoids (prostaglandins, histamine, 5-HT, bradykinin, etc.)) on guanylate cyclase.

Cyclic nucleotide phosphodiesterases Hydrolysis of cyclic nucleotides by the enzyme cyclic nucleotide phosphodiesterase (CN-PDE) represents the only known catabolic route for cyclic nucleotide metabolism (Butcher and Sutherland, 1962; Strada and Thompson, 1978). Butcher and Sutherland (1962) reported the occurrence of CN-PDE activity in a variety of rat tissues: brain, kidney, intestine, liver, heart, skeletal muscle, and two vascular tissues (aorta and femoral artery). The enzyme activity was found in both soluble and particulate fractions. They stated that the CN-PDE activity was of sufficient concentrations to play an important role in the inactivation of the amounts of cyclic nucleotides found in these

tissues. Furthermore, they reported that the CN-PDE activity required Mg^{2+} for activity, was stimulated by imidazole and was inhibited competitively by the methylxanthines--theophylline (50% inhibition at ~0.5 mM), caffeine (50% inhibition at ~3 mM), and threobromine (50% inhibition at ~3 mM). Later, CN-PDE activity was demonstrated in bovine carotid artery by Volicer et al. (1973) and in pig coronary artery by Wells et al. (1975a). Both authors reported that the enzyme activity was associated primarily with the intima and media layers of these blood vessels.

Based upon kinetic studies, Vulliemoz et al. (1974) suggested that CN-PDE in rat uterus, human myometrium, rat aorta and dog bronchus existed in at least two forms (isoenzymes). They found that the high affinity forms of the enzyme have a very similar apparent K_m for each of the tissues: 4 μM in rat uterus, 3 μM in human myometrium, 3 μM in rat aorta, and 3 μM in dog bronchus. The high K_m forms of the enzyme, in contrast, were observed to have different K_m's for each tissue: 150 μM in rat uterus, 200 μM in human myometrium, 30 μM in rat aorta, and 10 μM in dog bronchus. They suggested that the differences in K_m (and in V_{max}) of the CN-PDE in these tissues may be the cause of the differences in relaxing effects of certain CN-PDE inhibitors in these tissues.

In support of the findings of Vulliemoz et al. (1974), Wells et al. (1974) found that CN-PDE activity in pig coronary arteries could be resolved into two peaks by DEAE-cellulose chromatography. They found that peak I had affinity for cGMP (apparent K_m = 2 - 4 μM), had a relatively low affinity for cAMP (apparent K_m = 40 - 100 μM), and showed classical kinetic behavior (i.e., linear Lineweaver-Burk plots). Peak II, in contrast, specifically hydrolyzed cAMP in preference to cGMP and displayed an

apparent negatively cooperative kinetic behavior with cAMP as substrate. The activity of peak I was found to be stimulated by 3- to 8-fold by an endogenous heat-stable, nondialyzable protein that separated from the two peaks of CN-PDE during DEAE-cellulose chromatography (Wells et al., 1975a). The protein was demonstrated to be dependent on calcium for its stimulatory effect and had properties indistinguishable from those of the calcium-dependent regulator (CDR) or modulator protein first found in brain tissue (Cheung et al., 1978; Kakiuchi et al., 1978). Peak II activity was found to be unaffected by CDR (Hardman et al., 1977). CDR has also been found to be identical to the Ca^{2+}-dependent protein modulator that activates adenylate cyclase (Cheung et al., 1978; Brostrom et al., 1978), Ca^{2+}-Mg^{2+} ATPase (Gopinath and Vincenzi, 1977; Jarrett and Penniston, 1977), and myosin light chain kinase (Dabrowska et al., 1978) activities.

Amer and McKinney (1970, 1972) reported that cholecystokinin, gastrin, and gastrin pentapeptide stimulate partially purified preparations of CN-PDE from dog aorta and rabbit gall bladder. They reported that these peptides appeared to convert the high-K_m form (peak I) of CN-PDE to the low-K_m form (peak II) with cAMP as substrate.

Early studies by Kukovetz, Pöch, and coworkers (Kukovetz et al., 1969, 1971; Kukovetz and Pöch, 1970, 1972; Pöch and Kukovetz, 1971a,b, 1972) have demonstrated that a number of vasodilators are able to inhibit the CN-PDE activity in bovine coronary artery. Based upon their data, they suggested that drug-induced relaxation of vascular smooth muscle may be the result of the inhibition of CN-PDE activity and the subsequent elevation of intracellular levels of cAMP in the tissue. In support of this concept, Lugnier et al. (1972) found that theophylline, papaverine,

and two isoquinoline derivatives cause an inhibition of barium-induced contractions of rat aorta that was quantitatively correlated with inhibition of CN-PDE activity.

Wells et al. (1975b) found that the vasodilators, 1-methyl-3-isobutylxanthine (MIX), papaverine, and theophylline, inhibited both peak I and II activities from pig coronary artery. Fifty-percent inhibition of peak I activity with either 1 μM cAMP or 1 μM cGMP as substrate was found to be produced by MIX (2.6-4.2 μM), papaverine (13 μM), or theophylline (58-100 μM). The presence or absence of CDR was found to have no influence on the inhibitor potencies. Peak II activity, in contrast, was found to be more sensitive to the inhibitory effects of papaverine than to MIX or theophylline. Concentrations required to produce 50% inhibition of the hydrolysis of 1 μM cAMP were 11 μM for MIX, 2.8 μM for papaverine, and 190 μM for theophylline. The ability of each agent to inhibit vascular CN-PDE activity appeared to be roughly parallel to their ability to induce relaxation of vascular smooth muscle (Wells et al., 1975b).

Other tissues have been found to contain more than two forms of CN-PDE. For example, Weiss and Levin (1978), using polyacrylamide gel electrophoresis, separated the CN-PDE activity of the soluble fraction of homogenized rat cerebral cortex into four peaks, of which only one (peak II) was stimulated by CDR. Peaks III and IV were found to be especially sensitive to the inhibitory effects of papaverine, whereas peak II (in the presence of CDR) was very sensitive to inhibition by trifluoperizine and other phenothiazine antipsychotic drugs. Peak I was relatively insensitive to inhibition by any of these agents. Weiss and Levin (1978) stated that the mechanism by which the antipsychotic drugs inhibit the Ca^{2+}-CDR-

dependent activation of CN-PDE is through a selective Ca^{2+}-dependent binding to the CDR protein which in turn interferes with the interaction between Ca^{2+}-CDR and CN-PDE.

In a recent study of the CN-PDE activity in the soluble fraction of sonicated human aorta, Hidaka et al. (1978) found that the catalytic activity could be separated into five fractions (FI-FV) by DEAE-cellulose chromatography. Fraction FV was found to contain more than 90% of the cAMP-hydrolytic activity--an activity that was unaffected by Ca^{2+} and CDR. Fractions FI and FII were observed to hydrolyze cGMP with higher activity in the presence of EGTA than in the presence of Ca^{2+}. Fractions FIII and FIV, on the other hand, were observed to have cGMP-hydrolytic activity that was activated in the presence of Ca^{2+} and CDR. Hidaka et al. (1978) demonstrated that a vasodilator, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), inhibited cGMP-PDE activity in fractions FIII and FIV in the presence of Ca^{2+} and CDR. Based upon their results, they suggested that W-7 inhibited the Ca^{2+}-dependent cGMP-PDE activity by competitively inhibiting the Ca^{2+}-CDR interaction with CN-PDE, a mechanism that appears analogous to that of the phenothiazine drugs. The cGMP-hydrolytic activity of fractions FIII and FIV, in the presence of Ca^{2+} and CDR, were inhibited by 50% with W-7 at concentrations of 25 μM and 30 μM, respectively. Interestingly, W-7 (1 μM-100 μM) was observed to produce a dose-dependent relaxation of isolated strips of rabbit renal artery that had been previously contracted with KCl or $PGF_{2\alpha}$. However, Hidaka et al. (1978) also observed that W-7 inhibited superprecipitation and ATPase activity of mouse myosin B and they suggested that these mechanisms, rather than PDE inhibition, may be the cause of the W-7-induced vasodilation.

In general, the multiple forms of CN-PDE have been shown to have differences in their substrate affinities and specificities, heat and cation sensitivities, chromatographic and electrophoretic mobilities, isoelectric points, molecular shape and size, ontogeny, subcellular localization, and sensitivities to pharmacological agents (Strada and Thompson, 1978).

Cellular levels of cyclic nucleotides Volicer and Hynie (1971) reported that isoproterenol, at a concentration (1 μM) that produced maximal relaxation, elevated cAMP levels in isolated rat aorta. This response was partially antagonized by the beta-adrenergic receptor blocker, propranolol (0.1 mM). However, they found that norepinephrine, at a concentration (1 μM) that produced contraction, also elevated cAMP levels in this preparation. In the presence of propranolol (0.1 mM), norepinephrine was observed to still produce contraction but lowered cAMP levels. They further observed that angiotensin (0.1 μM) lowered cAMP levels in association with contraction in the rat tail artery. In contrast, angiotensin (0.1 μM) was unable to change cAMP levels in the rat aorta. Volicer and Hynie (1971) concluded that their results were consistent with the concept that vasodilation is associated with increases in cAMP and vasoconstriction is associated with decreases in cAMP.

Similar results were obtained by Andersson (1973a), who found that isoproterenol (2.4 μM) relaxed the histamine-contracted bovine mesenteric artery and elevated cAMP levels in this tissue. Contractions induced by histamine (9 μM) or phenylephrine (9.8 μM), however, were associated with an initial reduction in cAMP levels followed by a more sustained elevation in cAMP levels. Since the initial decrease in cAMP levels preceded the contractions of the arteries, Andersson (1973a) proposed that reduction in

cAMP levels initiates vascular smooth muscle contraction. He hypothesized that a lowering of intracellular levels of cAMP in smooth muscle could lead to a release of Ca^{2+} from the intracellular Ca^{2+} stores. This would be consistent, he pointed out, to the proposed role of cAMP in stimulating the accumulation of Ca^{2+} in microsomal fractions from smooth muscle (Andersson, 1972).

In contrast to the findings of Andersson (1973a), Seidel and Addison (1974) found no changes in cAMP levels in dog coronary arteries during contractions induced by phenylephrine (0.48 μM). They observed that epinephrine-induced contractions, on the other hand, were associated with increases in cAMP levels. In the presence of the alpha-adrenergic blocking drug, phentolamine, epinephrine (6.3 μM) caused relaxation and still elevated the cAMP levels. Aminophylline (0.54 mM) was also observed to cause relaxation and to elevate cAMP levels in dog coronary arteries. It should be emphasized, however, that the levels of cAMP measured in this study represent the levels in arteries that had been exposed to the various agents for 3 to 6 minutes (Seidel and Addison, 1974).

Triner et al. (1972a) found that norepinephrine, epinephrine, and isoproterenol (each at concentrations of 5 μM and 10 μM) elevated cAMP levels in isolated dog aorta. They observed that the degree of cAMP elevation depended on the particular catecholamine used; i.e., the greatest elevation of cAMP levels was observed with isoproterenol and the smallest elevation of cAMP levels was observed with norepinephrine. They further noted that, at the concentrations used, norepinephrine and epinephrine produced contractions of the aorta, whereas isoproterenol produced relaxation. However, they found that the isoproterenol-induced relaxation of

5-HT-contracted aorta occurred at concentrations between 0.01 and 0.1 μM, which were considerably lower than concentrations of isoproterenol needed to show elevated levels of cAMP (Triner et al., 1972a). Thus, evidence was presented for a dissociation (based upon dose-reponse relationships) between increases in cAMP levels and relaxation in vascular smooth muscle.

Sheperd et al. (1973) reported an increase in cAMP levels in dog mesenteric arteries after a 20 minute exposure to isoproterenol (1 mM). They did not explain in the report their reasons for using such a high concentration of isoproterenol or for the long exposure time. They reported that two other vasodilators, papaverine (0.3 mM) and PGE_1 (10 μM) elevated cAMP levels in the same preparation. They concluded that their results were consistent with the concept that increases in intracellular levels of cAMP mediate relaxation.

Sutherland et al. (1973) found that histamine (100 μM) caused contractions of pig coronary arteries and a 2-fold rise in both cAMP and cGMP levels. They observed, however, that the increase in cGMP levels was demonstrated only in the presence of the CN-PDE inhibitor, MIX (20 μM), whereas the cAMP elevations occurred in the absence or presence of MIX (20 μM). MIX (100 μM), by itself, was found to reduce histamine-induced contractions and to elevate both cAMP and cGMP levels by 3- to 4-fold. They also noted that isoproterenol (20 μM) and MIX (20 μM), in combination, acted synergistically in elevating cAMP levels.

Andersson (1973b) reported that four vasodilators (papaverine, nitroglycerin, diazoxide, and hydralazine) all caused an elevation of cAMP levels in rabbit colon. He found that the elevation in cAMP preceded

relaxation induced by these drugs and that the degree of cAMP increase was correlated with the degree of relaxation. He further reported that papaverine and nitroglycerin inhibited the CN-PDE activity which was associated with the microsomal fraction possessing a cAMP-stimulated Ca^{2+} binding capacity. Papaverine was observed to stimulate the binding of Ca^{2+} by this microsomal preparation. Andersson (1973a) suggested that these vasodilators may be acting by raising the intracellular levels of cAMP, which in turn stimulated intracellular Ca^{2+} binding and leads to a reduction in Ca^{2+} available for the activation of the contractile mechanism. He pointed out, however, that drugs such as papaverine, may be acting by a mechanism other than inhibition of CN-PDE; for example, papaverine apparently has local anesthetic properties on various tissues (Andersson, 1973b).

In contrast to the findings of Andersson (1973b), Collins and Sutter (1975) were unable to detect an elevation of cAMP in the rabbit anterior mesenteric portal vein following the addition of diazoxide. They observed that, like papaverine and isoproterenol, diazoxide inhibited the spontaneous contractions of this blood vessel. They concluded that an increase in cAMP levels was not necessary for relaxation of vascular smooth muscle.

Further evidence of a dissociation between increases in cAMP levels and relaxation of vascular smooth muscle was provided by the work of Daniel and Crankshaw (1974). They reported that isoproterenol, at a concentration (8.1 μM) that produced maximal relaxation, did not change cAMP levels in rabbit pulmonary arteries. Nitroglycerin (0.11 mM), on the other hand, was observed to cause a small but significant elevation in

cAMP levels. They further demonstrated that the degree of relaxation produced by three CN-PDE inhibitors (theophylline, papaverine, and Ro-7-2956) was not correlated with the degree of cAMP elevation elicited by each agent. They concluded that an increase in cAMP does not appear to be sufficient or necessary for vascular smooth muscle relaxation.

Ljung et al. (1975) found that relaxation of the myogenically active rat portal vein induced by either isoproterenol (10 μM) or papaverine (0.1 mM) was associated with increases in cAMP levels. However, they observed that the isoproterenol-induced relaxation was temporally correlated with the elevated cAMP levels whereas the papaverine-induced relaxation was not. They further showed that relaxations unrelated to cAMP levels could be induced by longitudinal vibration or by increasing the osmolality of the perfusing solution. Ljung et al. (1975) concluded that an increase in cAMP was not an obligatory requirement for relaxation in vascular smooth muscle.

Lau and Lum (1977, 1978) have recently shown that relaxation induced by a β-adrenergic agonist can be dissociated from an elevation in cAMP. For example, they found that salbutamol, a selective β_2-agonist, could relax isolated strips of bovine tracheal smooth muscle without elevating cAMP levels.

The first evidence of a link between cholinergic stimulation and elevations in cellular cGMP levels was provided by the work of George et al. (1970), who found elevated levels of cGMP in rat heart after perfusing that organ with acetylcholine (ACh). They demonstrated that the increase in cGMP levels was associated with a depression of cardiac function. Later, Schultz et al. (1972) showed that cholinergic agonists increased

cGMP levels in rat vas deferens. They observed that atropine, a selective muscarinic antagonist, blocked the increase in cGMP induced by cholinergic agonists. In later studies, Schultz et al. (1973a,b,c) and Schultz and Hardman (1975, 1976) demonstrated that the basal levels of cGMP as well as the increases in cGMP levels induced by cholinergic agonist were dependent on extracellular Ca^{2+}. For example, Schultz et al. (1973b) found that incubation for 30 minutes in Ca^{2+}-free medium lowered the cGMP levels in rat vas deferens by 80% and in guinea pig small intestine by 30%. Re-addition of Ca^{2+} (1.8 mM) to the medium for 3 minutes restored cGMP levels to near normal in both preparations. Methacholine, a selective muscarinic agonist, and ACh were observed to elevate cGMP levels in these tissues by 2- to 3-fold within 2 to 3 minutes. No response was noted, however, in the absence of extracellular Ca^{2+}. Likewise, histamine, norepinephrine, and K^{+} were observed to increase cGMP levels in the presence, but not in the absence, of Ca^{2+} (Schultz and Hardman, 1975). In an independent study, Lee et al. (1972) found that several muscarinic agonist elevated the cGMP levels in rabbit cerebral cortex, rat heart, and guinea pig ileum. The increase in cGMP was blocked by atropine, but not by hexamethonium (a nicotinic blocker). Tetramethylammonia, a nicotinic agonist, at concentrations up to 100 μM, had no effect on cGMP levels in these tissues. Interestingly, isoproterenol (1 μM) was observed to partially inhibit and isoproterenol (10 μM) to completely inhibit the elevation of cGMP induced by bethanechol (1 μM) in guinea pig ileum. Isoproterenol (1 or 10 μM), by itself, had no effect on cGMP levels. Furthermore, bethanechol (10 μM) was observed to partially inhibit the elevation of cAMP levels induced by isoproterenol (10 μM) in guinea pig ileum. This

effect of bethanechol was inhibited by pretreatment of the preparation with atropine (1 μM). The authors suggested that cholinergic effects could be mediated through a high cGMP/cAMP ratio and adrenergic effects could be mediated through a high cAMP/cGMP ratio. They concluded from their data that the interactions of ACh at muscarinic receptors (but not nicotinic receptors) led to an elevation of cGMP levels which in turn mediates the muscarinic actions of ACh. As further support for this hypothesis, Lee et al. (1972) pointed out the differences in responses associated with nicotinic versus muscarinic stimulations. For example, ACh activation of nicotinic receptors is associated with explosive physiological responses that take only a few milliseconds to develop (e.g., membrane potential changes and skeletal muscle contractions). Activation of muscarinic receptors, on the other hand, is associated with relatively slow physiological responses that take at least a few hundred milliseconds to several minutes to develop fully (e.g., contraction of smooth muscle, negative inotropism and chonotropism in heart, and increases in exocrine gland secretion). They stated that it is unlikely that the fast nicotinic responses would involve a complex chain of biochemical reactions--although this mechanism may be involved in the slower muscarinic responses. Lee et al. (1972) concluded that the physiological antagonism between cholinergic and adrenergic agonists on the contractility of both cardiac and smooth muscle appear to be reflected in, and may result from, antagonistic actions at the levels of cAMP and cGMP in these tissues.

These findings as well as numerous other reports of antagonistic actions of agonists that elevate the levels of either cAMP or cGMP led Goldberg et al. (1973a,b, 1975) to propose the Yin Yang (dualism) hypothe-

sis. Goldberg et al. (1975) postulated that there are two basic types of bidirectionally controlled systems: an A-type, which is facilitated by cAMP and suppressed by cGMP; and a B-type, which is promoted by cGMP and inhibited by cAMP. They also proposed a monodirectional system in which the two cyclic nucleotides could act cooperatively.

The argument that cAMP and cGMP mediated opposing functions in vascular smooth muscle was strengthened when Dunhan et al. (1974) reported that the $PGF_{2\alpha}$-induced contraction of strips of bovine and canine vein were associated with elevated levels of cGMP. No change in cAMP levels was observed with $PGF_{2\alpha}$. The vasodilator, PGE_2, on the other hand, was observed to elevate cAMP levels and to have no effect or to lower cGMP levels. Thus, they observed that contractions of vein strips were associated with increases in cGMP levels (or increases in the cGMP/cAMP ratio) and that relaxations were associated with increases in cAMP levels and/or decreases in the cGMP/cAMP ratio (Dunham et al. (1974).

In agreement with the report by Dunham et al. (1974), Kadowitz et al. (1975) reported that $PGF_{2\alpha}$ (10 μM) induced contractions of dog lobar veins that were associated with increases in cGMP levels. No changes in cAMP levels were noted. In the canine lobar arteries, in contrast, they observed that $PGF_{2\alpha}$ had no effects of contractility and had no significant effects on cyclic nucleotide levels. They further observed that PGE_1 (10 μM) caused relaxation of both the artery and vein and elevated cAMP levels in both preparations. In the veins, PGE_1 was also observed to lower cGMP levels.

Still further support for the hypothetical role of cGMP as a mediator in vascular smooth muscle contraction was presented by Clyman et al.

(1975c). They found that a variety of vasoconstrictors (bradykinin, histamine, 5-HT, and K^+) stimulated the accumulation of cGMP in isolated segments of full term human umbilical arteries. These agents were found to have no effect on the cAMP levels. PGE_1, but not isoproterenol, was observed to elevate cAMP levels without affecting the cGMP levels in these arteries. The authors pointed out that the lack of response to isoproterenol was consistent with the reported lack of beta-adrenergic receptors in this preparation. Based upon their data, Clyman et al. (1975c) concluded that cyclic nucleotides in human umbilical arteries are independently controlled and that cGMP is involved in contraction of the artery at birth. However, contractile activity of the arteries were apparently not measured in this study.

In a later study of the same tissue, Clyman et al. (1975a) demonstrated that the PGE_1-induced elevation of cAMP was independent of extracellular Ca^{2+}. However, when arteries were incubated in Ca^{2+}-free medium, a 50% reduction in cGMP levels was observed. Furthermore, they observed that the elevation of cGMP induced by histamine, ACh, bradykinin, and K^+ did not occur in Ca^{2+}-free medium. Ionophores A23187 and X537A, which facilitate the movement of Ca^{2+} into the cells, was found to mimic the effects of the calcium-dependent agents on the cGMP levels. In contrast to the other vasoconstrictive agents, 5-HT was found to elevate cGMP levels in a manner that was independent (or even inhibited by) extracellular Ca^{2+}. Clyman et al. (1975a) suggested that the identification of two apparently different mechanisms for the accumulation of cGMP in the human umbilical arteries may reflect the existence in this tissue of two separate guanylate cyclase systems--one that is dependent on Ca^{2+} and

another that is inhibited by Ca^{2+}. Clyman et al. (1975a) referred to the findings of Kimura and Murad (1974), who reported finding two distinct guanylate cyclases with such characteristics in heart muscle (see Guanylate Cyclase section of Literature Review).

In another publication, Clyman et al. (1975b) reported that the elevations in cGMP levels in the human umbilical artery induced by bradykinin, histamine, ionophore A23187, and MIX were dependent on the presence of O_2. They also found that methylene blue, sodium ascorbate, and 5-HT elevated cGMP levels in this preparation by a mechanism which was partially inhibited by O_2 and Ca^{2+}.

Within the last few years, there has been an increasing number of reports that demonstrate a dissociation between increases in cGMP levels and contractions in smooth muscle. For example, Diamond and Hartle (1974) showed that the spontaneous contractions of the rat uterus were not associated with changes in cyclic nucleotide levels. In a later study, Diamond and Holmes (1975) found that K^+ (127 mM) produced a sustained contracture of the rat myometrium that was associated with elevated cAMP levels and with decreased cGMP levels. Still later, Diamond and Blisard (1976) reported that phenylephrine (5 μM) produced sustained contractures of canine femoral arteries, but did not affect cGMP levels. Carbachol (100 μM), on the other hand, elevated cGMP, but had no effect on arterial tension. Two smooth muscle relaxants, papaverine and nitroglycerin, were observed to elevate cGMP but not to alter cAMP levels in both rat myometrium and canine femoral artery (Diamond and Holmes, 1975; Diamond and Blisard, 1976). Thus, Diamond and coworkers were able to show that elevations of cGMP in smooth muscle can be associated with either relaxations,

with no change in contractility, or with contractions, depending upon the tissue or the drug used. Furthermore, they were able to show that contractions of smooth muscle can be associated with either increases, decreases, or no changes in cGMP levels.

Using the bovine tracheal smooth muscle, Katsuki and Murad (1977) demonstrated a dissociation, based upon the time course and dose-response relationship, between increases in cGMP levels and contractions induced by carbachol. They further showed that the NO_2-like vasodilators, such as nitroglycerin, $NaNO_2$, NaN_3, and NH_2OH, caused calcium-independent elevations of cGMP levels without changing the cAMP levels in bovine trachealis muscle. In a recent study using the rat vas deferens, Schultz et al. (1977a) found that after the addition of 2 mM Mn^{2+} to the bathing solution, ACh (100 μM) and norepinephrine (100 μM) were unable to induce muscle contraction, yet were still able to elevate cGMP levels.

Schultz et al. (1977b) recently reported that a number of vasodilators were able to elevate cGMP levels in the rat vas deferens. These vasodilators included: nitroglycerin, diazoxide, hydralazine, minoxidil, dipyridamole, prenylamine, cinnarizine, lidoflozine, perhexiline, D-600 (the methoxy derivative of verapamil), SKF-525A, and chlorpromazine. Each drug was given at a concentration of 0.1 mM and caused a 2- to 3-fold increase in cGMP levels after 2 to 3 minutes of exposure. However, it should be noted that in this study the vas deferens were incubated in a medium free of calcium, presumably to potentiate the effects of the NO_2-like drugs. Based upon their data, Schultz et al. (1977b) hypothesized that these drugs caused relaxation of smooth muscle by elevating cGMP levels which in turn reduced or prevented calcium influx into the cyto-

plasm of the muscle. This hypothesis, however, was recently challenged by Diamond and Janis (1978), who found that, in contrast to the findings of Schultz et al. (1977b), sodium nitroprusside did not cause relaxation of the contracted rat vas deferens. In agreement with Schultz et al. (1977b), they did find elevated levels of cGMP following exposure of the vas deferens to nitroprusside. Since an increase in cGMP levels occurred without a concomitant relaxation, Diamond and Janis (1978) reasoned that nitroprusside-induced relaxation must not be mediated by cGMP. Also in contrast to the report by Schultz et al. (1977b), Diamond and Janis (1978) showed that both hydralazine (1 mM) and verapamil (20 μM) were without effect on cGMP and cAMP levels of the rat vas deferens. At these concentrations, both agents were observed to cause relaxations of 16% and 37%, respectively.

In two recent reviews of his research, including studies of intestinal, uterine, vascular, and vas deferens smooth muscle, Diamond (1977, 1978) concluded that total tissue levels of cAMP and cGMP do not correlate well with changes in smooth muscle tension. He further stated that, unless cyclic nucleotides are compartmentalized within the cell, the cyclic nucleotides do not play a prominent role in the control of smooth muscle tension.

It has been proposed that cyclic nucleotides are involved in the development of hypertension (Amer, 1975). Essential hypertension is characterized by increased peripheral vascular resistance, which has been attributed to changes in the structure, tone and sensitivity of vascular smooth muscle (Amer, 1977). These changes include: (a) increased cellular proliferation resulting in an increased wall/lumen ratio, (b) in-

creased vascular smooth muscle tone, (c) increased sensitivity of the smooth muscle to vasoconstrictive agents, and (d) decreased sensitivity to vasodilatory agents (Amer, 1977). All of these conditions have been associated with abberations in the cyclic nucleotide systems of blood vessels (Amer, 1977).

Triner et al. (1972b) reported that the aortas of spontaneously hypertensive rats (SHR) were less sensitive to the relaxant effects of isoproterenol, dibutyryl cAMP, and theophylline as compared to aortas from normotensive rats. They further reported that 5-10 times higher concentrations of isoproterenol were needed to maximally elevate cAMP levels in SHR versus normal aortas. However, they found that basal activities of adenylate cyclase and CN-PDE appeared to be similar in SHR and normal aortas. Triner et al. (1972b) concluded that a decrease in cAMP response in the tissue may be the underlying factor of altered vascular reactivity in SHR rats.

While studying the cAMP system in aortas of SHR and stress-hypertensive rats, Amer (1973) also found that basal adenylate cyclase activity was normal but that the ability of adenylate cyclase to be stimulated was impaired. Unlike the study by Triner et al. (1972b), Amer (1973) found that the CN-PDE activity (especially the low K_m form [peak II]) was elevated and was associated with reduced levels of cAMP in aortas from hypertensive rats as compared to normotensive controls. He also found lower levels of cAMP and higher proportions of peak II CN-PDE activity in the kidney and heart of SHR rats; thus showing that the biochemical abberations were not specific for vascular tissue. In a later study on aortas from SHR, stress-hypertensive and desoxycorticosterone-acetate-hyperten-

sive rats, Amer et al. (1974) consistently found elevated cGMP/cAMP ratios and increased activity of CN-PDE (low K_m form) in hypertensive rats versus normal rats. They also observed that all hypertensive rats had elevated guanylate cyclase activity and reduced sensitivity of adenylate cyclase to stimulation by isoproterenol. Furthermore, similar abnormalities were noted in the cyclic nucleotide systems of aortas from rats made acutely hypertensive by bilateral lesions of the Nucleus tractus solitarii (Amer, 1975). Amer (1975) concluded that aortas from four different types of hypertensive rats with widely different etiologies seem to exhibit similar defects in their cyclic nucleotide metabolism.

Ramanathan and Shibata (1974) also found decreased levels of cAMP in vascular smooth muscle (aorta, portal vein and renal artery) from SHR rats as compared to normotensive controls. It is interesting that they were able to demonstrate decreased cAMP levels in the blood vessels of young SHR rats that had not yet developed hypertension. Contrary to the report by Amer (1973, 1975), however, Ramanathan and Shibata (1974) found that the activities of adenylate cyclase and CN-PDE were lower in SHR blood vessels. More recently, Taylor and Shirachi (1977) found significantly lower CN-PDE activity (using either cAMP or cGMP as substrate) in the SHR versus normotensive rats.

In addition to their antihypertensive action in hypertensive rats, diazoxide, minoxidil and guanethidine were observed to reverse the elevated cGMP/cAMP ratios (Amer, 1977). However, Taylor and Shirachi (1977) found that two antihypertensives, reserpine and chlorothiazide, had no influence on the reduced CN-PDE activity associated with hypertension in rats.

In a review of his work, Amer (1975) concluded that the increased cGMP/cAMP ratios in the vessels from four types of hypertensive animals provide a possible biochemical basis for the increased vascular smooth muscle tone and the elevated vascular resistance that is characteristic of these animals. In the human condition of essential hypertension, Amer (1977) suggested that a sustained increase in sympathetic nerve activity or renin-angiotensin levels early in the development of hypertension may lead to an irreversible loss of adenylate cyclase sensitivity to stimulation and to increased cAMP-PDE activity. This, he claimed, leads to an increased cGMP/cAMP ratio which mediates the increased vascular smooth muscle tone.

Prostaglandins

Literature in the areas of prostaglandin biosynthesis, metabolism, receptors, and interactions with the cyclic nucleotide systems has been comprehensively reviewed by Samuelsson et al. (1975, 1978a,b). In addition, the subjects of the pharmacology of prostaglandins (PGs) (Jones, 1977), PG-synthesis inhibition (Vane, 1978), the PG-endoperoxides and the newly discovered thromboxanes (Samelsson, 1977) and prostacyclin (PGI_2) (Moncada and Vane, 1977) have been recently reviewed as well. In the present review, only those articles and reviews that are of particular relevance to the subject of PG involvement in the regulation of HUA contractility will be cited. A brief description of the historical development in the area of PG research will be given.

Kurzrok and Lieb (1930) first reported that human semen caused isolated strips of human uterus to either contract or relax, depending on the source of the semen or of the uterus. Later, Goldblatt (1935) found that

human seminal fluid caused a fall in blood pressure after intravenous injection into cats and caused contraction of several isolated smooth muscle preparations (rabbit small intestine, guinea pig uterus, and guinea pig seminal vesicles). In an independent study, von Euler (1934, 1935a,b, 1936) found that substances, which he named "prostaglandin" and "vesiglandin," could be isolated from extracts of accessory reproductive glands and that these substances could contract uterine and intestinal smooth muscle and cause vasodepressant effects. Von Euler (1935b) found that these substances were dialyzable through cellophane, were soluble in absolute ethanol, and were destroyed by heating at 100°C for 1 min in normal alkaline solution. Although these newly discovered substances were pharmacologically similar to other low molecular weight, endogenous substances such as ACh, histamine and substance P, they were distinguishable because of their acidic lipid nature (Jones, 1977).

Utilizing the technical advances that had been made in lipid chemistry at the time, Bergström and Sjövall (1957, 1960a) was able to isolate in crystalline form a component of "prostaglandin" which they named PGF (prostaglandin factor). They found that PGF contracted isolated smooth muscles, but lacked the vasodepressant effects of "prostaglandin." Bergström and Sjövall (1960b) reported the isolation in crystalline form of a second component of "prostaglandin" (named PGE) which lowered blood pressure. The proposed chemical structures of PGE and PGF were reported by Bergström et al. (1962). Using fractionation techniques, Bergström and coworkers found that PGE could be separated into PGE_1, PGE_2, PGE_3 and PGF into $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (von Euler and Eliasson, 1967).

From the structures it was predicted that the PGs were formed from essential fatty acids. The validity of this concept was demonstrated when Bergström et al. (1964) reported that homogenates of sheep vesicular glands could synthesize PGE_2 from arachidonic acid. Since then many other active products of arachidonic acid metabolism have been identified in various biological tissues. These products include $PGF_{2\alpha}$, PGG_2, PGH_2, PGI_2, and thromboxane A_2 (TxA_2) (Samuelsson et al., 1978a).

Other investigators during the 1950s and 1960s identified acidic lipids from various biological sources that had pharmacological properties similar to those of the PGs (Ambache, 1963; von Euler and Eliasson, 1967). For example, Ambache (1957) reported finding a smooth muscle stimulating agent, which he called irin, in extracts of rabbit iris. Vogt (1957) reported that a phosphatidic acid (darmstoff) with smooth muscle stimulating properties could be isolated from the intestines of frogs and horses. The actions of these substances (as well as vesiglandin) were later attributed, at least in part, to the effects of PGs within the extracts (von Euler and Eliasson, 1967).

The responses of mammalian cardiovascular systems to the PGs vary greatly depending upon the species and the location and type of blood vessel (Nakano, 1973). In general, the E-type PGs lower arterial blood pressure through a direct dilation of peripheral resistance vessels (Jones, 1977). These depressor effects have been found to be unaltered by drugs that block muscarinic, histamine, and β-adrenergic receptors (Jones, 1977), thus illustrating the specific action of the PGEs. $PGF_{2\alpha}$ causes pressor effects in rats, sheep, and dogs, but depressor effects in cats

and rabbits (Jones, 1977). The pressor effects of $PGF_{2\alpha}$ are not abolished by pretreatment with hexamethonium (a nicotinic cholinergic receptor blocker), phenoxybenzamine (an α-adrenergic receptor blocker) or reserpine (a depletor of endogenous norepinephrine and epinephrine stores) (Jones, 1977).

It has been proposed that the vasodilation caused by the E-type PGs is mediated by an elevation in cAMP levels, whereas the vasoconstrictive action of $PGF_{2\alpha}$ is mediated by an elevation in cGMP levels (Dunham et al., 1974; Kadowitz et al., 1975). For a review of the interactions of prostaglandins with cyclic nucleotides in various tissue, see Kuehl et al. (1972) and Kuehl (1974).

The possibility that PGs may act as endogenous regulators of HUA contractility was first raised when Karim (1967) reported finding PGs (PGE_1, PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$) in extracts of HUA. Karim (1967) and Hillier and Karim (1968) further reported that PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ caused contractions whereas PGE_1 caused relaxations of isolated strips of HUA. When all four PGs were added as a mixture, in the proportions found in umbilical cord vessels, a contraction of HUA occurred (Karim, 1967). In agreement with the findings of Karim (1967) and Hillier and Karim (1968), Park et al. (1972) found that PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ produced dose-dependent contractions in isolated strips of HUA. However, unlike the results of Karim and coworkers, PGE_1 was found to cause dose-dependent contractions of HUA; although PGE_1 was somewhat less potent than PGE_2 or $PGF_{2\alpha}$ in this respect (Park et al., 1972).

Tuvemo and Wide (1973) reported that isolated strips of HUA release a $PGF_{2\alpha}$-immunoreactive substance into the surrounding medium during a time

when the strips were exhibiting spontaneous muscle tone. They reported that the PG-synthetase inhibitor, indomethacin (40 μg/ml), blocked the PG release and caused the strips to relax. Strandberg and Tuvemo (1975) further studied the effects of PG-synthetase inhibitor, eicosa-5,8,11,14-tetraynoic acid (ETA), and PG antagonist, polyphloretin phosphate (PPP), as well as lower concentrations of indomethacin on the spontaneous tone of the isolated strips of HUA. They reported that ETA (5 μg/ml), PPP (40 μg/ml) or indomethacin (8 μg/ml) reduced the tone of HUA, but had no influence on contractions produced by 5-HT, an agonist that they assumed was acting by a mechanism independent of PG release. They concluded that intramural synthesis of PGs contributed to the maintenance of the tone of isolated HUA strips.

In a later study, Tuvemo et al. (1976a,b) found that the PG-endoperoxides (PGG_2 and PGH_2) were approximately 100 times more potent than PGE_2 or $PGF_{2\alpha}$ in contracting HUA. They further demonstrated that thromboxane B_2 (TxB_2), the stable metabolite of TxA_2, appeared in the medium surrounding the HUA strips; thus indicating that in addition to PGs, TxA_2 is also synthesized in HUA. The formation of TxB_2 was found to be inhibited by indomethacin (8-40 μg/ml) or by ETA (25 μg/ml) (Strandberg and Tuvemo, 1975). Based upon these data, Tuvemo et al. (1976a) hypothesized that local generation of PG and/or TxA_2 may be involved in the closure of the HUA at birth.

Svensson et al. (1977) have recently found that TxA_2 is 9-60 times more potent than PGH_2 in contracting HUA strips and they proposed that the actions of PG-endoperoxides on HUA may be mediated by the subsequent conversion to TxA_2. However, using a stable analogue of PG-endoperoxide, 15(s)-

hydroxy-9α,11α(epoxymethano)prosta,5,13-dienoic acid (EPA), Tuvemo et al. (1978) demonstrated that much of the contractile activity of the PG-endoperoxides can be attributed to the endoperoxides themselves.

In addition to isolated strips of HUA, individual components of these or related vessels have been studied for their ability to synthesize PGs. For example, Joyner and Strand (1978) found that cultured endothelial cells isolated from HUA or from human umbilical veins were able to spontaneously synthesize PGs. They found that an unidentified substance in fetal calf serum could selectively stimulate the synthesis of PGF-like material in these cultures. Furthermore, it was found that angiotensin II stimulated and indomethacin inhibited the release of PGs from cultured endothelial cells from human umbilical veins (Gimbrone and Alexander, 1975). It was later found that cultured smooth muscle cells from human umbilical veins could also generate PGs and with a rate 20 times greater than the endothelial cells (Alexander and Gimbrone, 1976). It was further demonstrated that the PG release from the smooth muscle cells could be inhibited by indomethacin (ID50 = 1.8 nM) and stimulated by bradykinin (10-fold increase), angiotensin (3.5-fold increase), histamine (2.3-fold increase), and 5-HT (50% increase) (Alexander and Gimbrone, 1976). They concluded that endogenous production of PGs in vascular smooth muscle cells may represent an intrinsic control mechanism affecting basal tone and modulating the responsiveness of blood vessels to vasoactive agents.

Since the discovery of prostacyclin (PGI_2, previously called PGX), much of the emphasis in PG research in blood vessels has shifted to the study of the synthesis and actions of this compound (Moncada and Vane, 1977). PGI_2 is a PG-like substance that is produced by blood vessels in

the presence of arachidonic acid or more effectively by the presence of PG-endoperoxides. It is a potent inhibitor of platelet aggregation and relaxes vascular smooth muscle (Bunting et al., 1976; Moncada et al., 1976a). It was suggested that the generation of PGI_2 by blood vessel walls could be the biochemical mechanism underlying the unique ability of blood vessels to resist platelet adhesion. Moncada et al. (1976b) further reported that the highest capacity for PGI_2 generation resided at the intimal surface of blood vessels and that this capacity progressively diminished toward the adventitial surface. They hypothesized that platelet aggregation that occurs upon exposure of platelets to subintimal surfaces is caused by the insufficient generation of PGI_2 by those layers (Moncada et al., 1977).

6 Keto-$PGF_{1\alpha}$, the stable hydrolytic product of PGI_2, has been identified as the major product of arachidonic acid metabolism in various blood vessels from fetal, maternal and nonpregnant cows (Terragno et al., 1978). In the cases where a direct comparison could be made between adult and fetal vessels (aorta and pulmonary artery), the fetal vessels appeared to have a much greater capacity for synthesizing PGI_2 (Terragno et al., 1978). Other laboratories have also found that 6 keto-$PGF_{1\alpha}$ is the major metabolite of arachidonic acid in blood vessels of fetal lambs (Pace-Asciak and Rangaraj, 1978) and fetal calves (Powell and Solomon, 1977). These authors have suggested that local generation of PGI_2 may be important in the maintenance of patency of the ductus arteriosus during fetal life (Terragno et al., 1978; Powell and Solomon, 1977; Pace-Asciak and Rangaraj, 1978). In support of this hypothesis, Clyman et al. (1978)

recently reported that the PGI_2 released from rings of lamb ductus arteriosus was significantly higher in tissues taken from animals earlier in gestation (98-103 days) than in animals near term (136-146 days).

Pomerantz et al. (1978) recently reported that PGI_2 at low concentrations (1 nM-1 μM) caused relaxation whereas at higher concentrations (1-25 μM) it caused contractions of spirally cut strips of HUA. This response of PGI_2 is reminiscent of the effects of PGE_1 on HUA reported by Tuvemo (1978). Pomerantz et al. (1978) suggested that the increases in O_2 tension that occurs in the circulation immediately after birth may result in augmented arachidonic acid metabolism, in increased synthesis of PGs and in elevation of PG concentrations to the contractile range; thus, HUA constricts. They alternatively suggested that the post-partum metabolism of arachidonic acid may be "redirected from the prostacyclin pathway to other vasoactive compounds." The latter hypothesis is indeed a viable possibility in light of the discovery by Vane and coworkers that prostacyclin synthesis is inhibited by lipid peroxides (Bunting et al., 1976; Moncada et al., 1976b; Vane, 1978)--agents that are formed in elevated O_2 tension (Haugaard, 1968).

It has been proposed that the nonsteroidal anti-inflammatory drugs (aspirin, indomethacin, etc.) are acting via the mechanism of PG synthesis inhibition (Vane, 1971; Smith and Willis, 1971; Ferreira et al., 1971). The impetus for this proposal was initiated by the findings of Piper and Vane (1969), who reported that aspirin, indomethacin, or mefenamate antagonized the release of rabbit aorta contracting substance (RCS) from guinea pig lung during anaphylaxis. At the time, it was believed that RCS

was a PG intermediate, and this concept was later confirmed when RCS was identified as a mixture of PGG_2, PGH_2, and TxA_2 (Samuelsson, 1977).

Many other mechanisms of action have been proposed for the aspirin-like drugs. These mechanisms include: (1) uncoupling of oxidative phosphorylation, (2) displacement of an anti-inflammatory peptide from plasma protein, (3) interference with leucocyte migration, (4) inhibition of leucocytic-phagocytosis, (5) stabilization of lysosomal membranes, (6) inhibition of the synthesis of lipoperoxides, and (7) hyperpolarization of neuronal membranes (Ferreira and Vane, 1974). In addition, Northover (1977) has recently reviewed the evidence that links the action of indomethacin with its ability to antagonize Ca^{2+}. A strong case is presented for the calcium antagonistic actions of indomethacin in altering vascular contractility. However, it appears that all of the proposed mechanisms presented above--with the exception of PG synthesis inhibition--are associated with concentrations of indomethacin (and aspirin) considerably higher than their therapeutic concentration (Ferreira and Vane, 1974; Flower, 1974; Northover, 1971, 1973).

Indomethacin has been used extensively as a research tool to elucidate the involvement of PGs in various physiological, pharmacological, and pathological condition (Flower, 1974). For example, indomethacin has been used to illustrate the influence of endogenously generated PGs in the maintenance of smooth muscle tone in isolated preparations (Eckenfels and Vane, 1972; Tuvemo and Wide, 1973; Strandberg and Tuvemo, 1975; Coceani et al., 1978; Clyman et al., 1978). In addition, indomethacin has been used in experiments designed to test the hypothesis that PGs mediate the

responses of various smooth muscle contracting agents (Chong and Downing, 1973; Manku and Horrobin, 1976; Famaey et al., 1977a,b).

Statement of the Problem

From the review of the literature it is apparent that cyclic nucleotides and prostaglandins may be involved as mediators or modulators in regulating the contractility of vascular smooth muscle. However, the evidence for such a role is inconsistent and incomplete. Many of the inconsistencies of these findings may be related to differences in experimental conditions and/or to differences in the smooth muscle studied.

The present study was initiated to ascertain whether cAMP and cGMP act as mediators in contractions and relaxations of isolated strips of human umbilical arteries (HUA) induced by a variety of vasoactive agents. One of the criteria for establishing a mediator role for cAMP (and cGMP) in a physiological response is to demonstrate that cAMP (or cGMP) levels change prior to or concurrent with the response (Sutherland et al., 1968; Namm and Leader, 1976). To determine if this criterion could be met in HUA contractile responses, levels of cAMP and cGMP were measured in isolated strips of HUA which had been clamp-frozen at selected times following exposure to a vasoactive agent. Changes in cyclic nucleotide levels were correlated with changes in the contractile state of the vascular strips. A second criterion for establishing a mediator role for cAMP is that the addition of cAMP or its derivative should induce responses that are proposed to be mediated by cAMP (Sutherland et al., 1968; Namm and Leader, 1976). Therefore, in the present study, dibutyryl and 8-bromo derivatives of cAMP and

cGMP were tested for their effects on HUA contractility. To ascertain whether prostaglandins mediate the actions of a number of agonists, contractile responses to these agonists in HUA strips preincubated in indomethacin (an inhibitor of prostaglandin synthesis) were compared to responses of untreated strips.

METHODS AND MATERIALS

Preparation of Isolated Strips of Umbilical Artery

1. Human umbilical cords were obtained at full term following normal vaginal delivery (Mary Greeley Hospital, Ames, Iowa). Sheep umbilical cords were obtained by Cesarean section on day 120-125 of gestation (full gestation $\cong$ 147 days) (Dyer, 1970b).
2. Arteries were dissected free of other vessels and of the surrounding Wharton's jelly and were helically-cut into strips (2-3 cm long and ~3 mm wide) (Dyer, 1970b). The weight of the strips ranged from 40-150 mg. Strips of human umbilical artery (HUA) were used the same day as delivery and strips of sheep umbilical artery (SUA) were used within 24 hours after storage at 4°C.

3a. For the study of cyclic nucleotide levels, strips were suspended under 1 g resting tension in 50 ml organ baths containing a modified Krebs-bicarbonate (Krebs) solution at 37°C aerated with O_2-CO_2 (95:5). The strips were allowed to relax for 3-4 hours before addition of drugs. Spontaneous tone of the vessels (especially HUA) occurred during the first few hours of incubation with 1 g resting tension. The Krebs solution used for the first part of the cyclic nucleotide study, which included the effects of 5-HT on cyclic nucleotide levels in HUA and SUA, contained: 115.3 mM NaCl, 4.7 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 1.82 $CaCl_2$, 7.88 mM glucose, 22.14 mM $NaHCO_3$, and 0.0269 mM Na_2EDTA. The EDTA was routinely added to all Krebs solution to prevent autoxidation of agonists such as

catecholamines (Furchgott, 1955). In later experiments, which included the effects of histamine, PGs, and nitroglycerin on cyclic nucleotide levels in HUA, the Krebs solution was modified to include 1 mM pyruvic acid and the bicarbonate concentration was adjusted to 24.9 mM to maintain the pH at 7.4. The addition of pyruvic acid was suggested by Dr. Ferid Murad, Professor of Clinical Pharmacology, University of Virginia, School of Medicine. He found that the addition of 1 mM pyruvic acid to the medium reduced the large variation in cGMP levels that are frequently seen in isolated biological preparations. In all experiments of the present study, the medium was replaced with fresh Krebs solution every 20-30 min.

At predetermined times following the addition of the vasoactive agents, strips were instantaneously frozen between large Wollenberger clamps precooled to -190°C in liquid nitrogen (Wollenberger et al., 1960). The need for such rapid cooling of the tissue has been emphasized by Mayer et al. (1974) and by Bär (1975). Samples were stored in liquid nitrogen until assayed. Care was taken to be sure that the samples were kept very cold from the time of freezing until homogenization, since it has been reported that cyclic nucleotide phosphodiesterase activity is substantial in biological preparations even at temperatures as low as 0°C (Goldberg and O'Toole, 1971).

For comparative purposes, some strips of HUA were incubated in 50 ml of Krebs solution at 37°C aerated with O_2-CO_2 (95:5) but without any resting tension.

3b. For the study of the contractile effects of purine nucleotides, phosphodiesterase inhibitors, and indomethacin, isolated strips of

HUA were suspended under 1 g resting tension in 10 ml of Krebs solution (same as for 5-HT study above) at 37°C and aerated with O_2-CO_2 (95:5). The strips were allowed to relax for 3-4 hours before the addition of a vasoactive agent.

4. With the exception of the prostaglandins (PGs) and indomethacin, which were dissolved in 100% ethanol, drugs were dissolved in saline (0.9% NaCl) prior to addition. The volume of drug additions ranged from 10-500 µl into the 10 ml or 50 ml organ baths.

Determination of Cyclic Nucleotide Levels

Preparation of strips for radioimmunoassay (RIA)

Two procedures were used for the preparation of umbilical arteries for assaying cyclic nucleotides. Procedure A, which was a modification of the method of Clyman et al. (1975c), included partial purification of cAMP and cGMP using alumina and anion exchange chromatography. Procedure A was used for the first experiments that included the 5-HT effects in HUA and SUA. In the other experiments (histamine, PG, and nitroglycerin effects) procedure B was used.

Procedure A

1. Attached threads were removed from the arterial strips in a cold box (packed in dry ice) using a scalpel and forceps precooled in liquid nitrogen. The strips were immediately transferred to the extraction solution (4 ml of 0.2 M HCl-98.3% ethanol (Binder et al., 1975)) which had been precooled to -80°C in a dry ice-ethanol bath. Preliminary experiments had been conducted to determine the most suitable extraction solution. Aqueous solutions of 6% trichloroacetic acid (see

method of Steiner, 1974) or 2.4% perchloric acid (see method of Clyman et al., 1975c) were found to be unacceptable because of the great difficulty in homogenizing the arterial strips with the equipment available (Tekmar Tissumizer [SDT-100N]) and because of the excessive frothing of the perchloric acid solution. The ethanol-HCl solution was found to be most suitable for the following reasons: (a) the homogenization of the samples could be performed at a very low temperature (-80°C) thereby assuring that there was no enzyme activity during the homogenization process, (b) the low temperature appeared to facilitate the break-up and homogenization of the arterial strips (probably because of the increased shearing force), and (c) the ethanol-HCl solution did not froth during homogenization.

2. Tubes containing the extraction mixture were partially submersed in the dry ice-ethanol bath (-80°C) throughout the homogenization. At no time did the extraction solution temperature rise above -40°C as measured by a thermocouple. Goldberg and O'Toole (1971) have stated that, ideally, the extraction procedure which involves denaturation of interfering enzymes should be carried out at temperatures below 0°C. The strips were homogenized with a Tekmar Tissumizer (SDT-100N with a 10 mm shaft) for approximately 2 min (until all tissue had been homogenized by visual observation).
3. The extracts were centrifuged at 10,000 X g for 30 min at 4°C.
4. A 0.5 ml aliquot of supernatant was transferred to another tube (labeled "A") for cAMP determination.
5. The remaining 3.5 ml of extract supernatant was transferred to another tube (labeled "B") for cGMP determination.

6. ^{3}H-cAMP, 4000 counts per minute (cpm), was added to each "A" tube and ^{3}H-cGMP, 2000 cpm, was added to each "B" tube for the determination of recovery.
7. The contents of the "A" and "B" tubes were evaporated to dryness under a stream of air at room temperature.
8. The residues in both the "A" and "B" tubes were taken up in 1.0 ml tris-HCl buffer (25 mM tris, pH 7.4). The side and bottom of the tubes were rubbed down thoroughly with a glass stirring rod and the solution mixed to be sure that the cyclic nucleotides were in solution.
9. The entire 1.0 ml of the samples was applied to neutral alumina columns (0.5 X 2.5 cm) which had been prewashed with 10 ml tris buffer (same as above). The eluates of the alumina columns were allowed to drip directly into columns (0.5 X 2.5 cm) of Bio-Rad AG1-X8 (anion exchange resin). The cyclic nucleotides were eluted from the alumina with 6 ml tris buffer (same as above).
10. The AG1-X8 columns containing the samples for cAMP were washed with 10 ml double-distilled H_2O (ddH_2O). The cAMP was eluted with 7 ml of 1 N formic acid.
11. The AG1-X8 columns containing the samples for cGMP determination were washed with 10 ml of 1 N formic acid. The cGMP was eluted with 9 ml of 4 N formic acid (the first 2 ml of eluate contained very little of the cGMP and therefore was discarded).
12. The eluates were evaporated to dryness at 80°C under a stream of air. The samples were stored in a freezer until assayed.

13. Just prior to assaying, the residues were taken up in 1.0 ml acetate buffer (50 mM Na acetate, pH 6.2). 100 µl aliquots were used for the RIA (in duplicate or triplicate) and 100 µl aliquots were used for recovery determinations (in duplicate). The remaining portion of the samples were stored in the freezer for future analysis (if needed).

Procedure B Samples were homogenized, centrifuged, and evaporated the same as in Procedure A; however, instead of taking up the residue (step 8 of Procedure A) in tris buffer, the samples were taken up in 1.0 ml of acetate buffer (50 mM Na acetate, pH 6.2) and used directly in the RIA (0.1 ml aliquots) and for recovery determination (0.1 ml aliquots). In other words, steps 8-12 of Procedure A were eliminated in Procedure B.

The reasons for changing from Procedure A to Procedure B were threefold. First, a considerable savings of time could be accomplished by using Procedure B in place of Procedure A. Second, during a consultation with Dr. Gary Brooker, Professor of Pharmacology, University of Virginia, School of Medicine, it was pointed out that prior purification of cAMP and cGMP was unnecessary when using RIA because of the high specificity of the antibodies. In agreement with this concept, Bär (1975) has stated that purification of cAMP is unnecessary and that aliquots of raw, neutralized tissue extracts can be used directly in RIA. He further stated that the only potentially interfering substance would be cGMP, but because of the low concentrations of cGMP in smooth muscle, this should not be a problem. The results of the present study, however, illustrate that pharmacologically elevated levels of cGMP may interfere with RIA determinations of cGMP (see nitroglycerin effects in Results).

Radioimmunoassay of cyclic AMP and cyclic GMP

All cGMP concentrations were determined by using RIA kits obtained from New England Nuclear. Cyclic AMP levels in HUA and SUA in the 5-HT study were determined by using RIA kits from New England Nuclear. In later experiments, which determined the effects of histamine, PGs, and nitroglycerin, cAMP levels were determined by RIA using antibody provided by Dr. Gary Brooker and a procedure that was a modification of the method of Harper and Brooker (1975) was followed.

New England Nuclear kits The RIA kits from New England Nuclear are based upon the procedure of Steiner et al. (1972a,b) as modified by Harper and Brooker (1975). The kits utilize a succinyl tyrosine-(^{125}I)-methyl ester of cAMP (or cGMP) as the labeled antigen and a pre-reacted primary and secondary antibody complex to achieve a separation of bound and free antigen. The primary antibody was prepared in rabbits against a succinyl cAMP- (or cGMP-) albumin conjugate and the secondary antibody was prepared in sheep against rabbit globulin. Based upon the observation first made by Steiner et al. (1972a) that cyclic nucleotides substituted at the 2'-O-position had a higher affinity for the antibody and thus displaced the (^{125}I)-labeled antigen at a lower concentration, Harper and Brooker (1975) developed an assay in which the cyclic nucleotides in standards and samples were acetylated at the 2'-O-position with acetic anhydride. Acetylation of cyclic nucleotides increased the sensitivity of the RIA by about 40-fold for both cAMP and cGMP and therefore amounts as low as a few femtomoles (10^{-15} mole) could be detected (Harper and Brooker, 1975).

The RIA procedure was as follows:

1. Standards of cAMP or cGMP (2.5, 5, 10, 25, 50, 100, 250, 500 fmole/assay tube) were prepared in acetate buffer (50 mM Na acetate, pH 6.2).
2. All additions of solutions (prior to incubation) were made at room temperature. Beckman Bio-vials were used as the assay tubes.
3. 100 µl of each standard solution was added (in triplicate) to the standard tubes.
4. 200 µl of acetate buffer (same as above) was added (in triplicate) to the blank tubes and 100 µl of acetate buffer was added (in triplicate) to the "0" standard tubes.
5. 100 µl of sample (in acetate buffer) was added (in duplicate or triplicate) to the sample tubes.
6. 7 µl of acetylating reagent (1 volume of acetic anhydride to 2 volumes of triethylamine) was added to each tube containing standards (including "0" standard) and samples. The contents of the tubes were immediately mixed on a vortex mixer.
7. 100 µl of (^{125}I)-labeled antigen was added to all tubes (including two additional tubes for total counts).
8. 100 µl of antiserum complex (preconjugated primary-secondary antibodies) was added to the sample, standard and "0" standard tubes.
9. All tubes (except the total count tubes) were mixed on a vortex mixer.
10. All tubes were covered with Parafilm and incubated in a refrigerator (4°C) for 16-18 hours.
11. 1 ml of cold acetate buffer was added to all tubes (except the total count tubes) and the contents were mixed on a vortex mixer.

12. All tubes (except total count tubes) were centrifuged at 5000 X g at 4°C for 15 min in a Beckman J-21B centrifuge using the JA-21 fixed-angle rotor modified to hold RIA tubes. The RIA tubes were found to make a snug fit when they were inserted into polycarbonate centrifuge tubes (16.1 X 79.5 mm).
13. The supernatant (containing the free antigen) was removed by aspiration and discarded.
14. All tubes, including the total count tubes, were measured for gamma radioactivity for 5 min in a Beckman Biogamma II (solid scintillation) counter.

Assay using antibody from Dr. Brooker The antibody for these experiments was kindly donated by Dr. Gary Brooker. Following the suggestion of Dr. Brooker, the procedure used was a modification of the method of Harper and Brooker (1975). The labeled antigen was obtained from New England Nuclear separate from the kit and proper dilutions were made in acetate buffer such that 100 µl of labeled antigen solution would contain ~10,000 cpm of activity. The samples and standards were prepared as described above and the procedure was the same as the New England Nuclear kit procedure up to step 7. The following steps were different from the previous assay.

8. The antibody was dissolved in acetate buffer (50 mM Na acetate, pH 6.2) containing 1 mg/ml bovine serum albumin (BSA) (crystallized and lyophilized, No. A-4378, Sigma Chem. Co.) to give a dilution of 1:100. Since this concentration of antibody is stable when stored in the freezer, many small aliquots (50 µl) of antibody solution (1:100 dilu-

tion) were stored in this way for future use. Thawing and refreezing of antibody solution was avoided to prevent loss of activity. Final dilution of the antibody for RIA was made immediately prior to assaying. To determine the optimal dilution of the antibody, a preliminary experiment was set up to determine the percent of labeled antigen that binds (in the absence of unlabeled antigen) to various dilutions of antibody. Final dilutions of antibody were made in acetate buffer with 30 mg/ml BSA (as described in the method of Harper and Brooker, 1975). The results were as follows: at 1:10,000 dilution, 77.7% bound; at 1:30,000 dilution, 61.1% bound; and at 1:100,000 dilution, 29.7% bound. Since approximately 50% binding of the labeled antigen is desired for the "0" standard (i.e., in the absence of unlabeled antigen), because it gives the optimal sensitivy with a workable concentration range (Walker and Keane, 1977), a dilution of 1:40,000 was selected for the rest of the experiments.

9. 100 µl of antibody (1:40,000 dilution in acetate buffer with 30mg/ml BSA) was added to all tubes except the total count tubes and the blank tubes.
10. All tubes were mixed on a vortex mixer, covered with Parafilm and incubated in the refrigerator (4°C) for 10-20 hours.
11. 1.0 ml of cold charcoal-albumin solution (2 mg/ml Norit A (Fisher No. C-176) plus 2.5 mg/ml BSA (Fraction V, Sigma) in 100 mM potassium phosphate buffer, pH 6.3) was added to all tubes, except the total count tubes. The free antigen, labeled and unlabeled, is adsorbed to the surface of the charcoal, leaving the antigen-antibody complex in solution.

12. The resulting mixture was mixed on a vortex mixer and allower to incubate for 20 min in the refrigerator.
13. All tubes, except the total count tubes, were centrifuged as described above (New England Nuclear kits).
14. The supernatants were decanted into glass test tubes (12 X 75 mm) and these tubes, plus the total count tubes, were measured for gamma-radioactivity (as above).

Determination of recovery

The percent recovery of ^{3}H-cAMP and ^{3}H-cGMP was determined so that the loss of cyclic nucleotide during the preparation procedure could be adjusted for in the final calculations. The percent recoveries of both cyclic nucleotides ranged between 60-80%. The procedure for the determination of recoveries was as follows:

1. 100 µl of sample (in acetate buffer) was added in duplicate to scintillation vials (glass 20 ml).
2. 100 µl of acetate buffer was added to the blank vials.
3. 100 µl of ^{3}H-cyclic nucleotide (same radioactivity as was added to the samples) in acetate buffer was added to the total count vials.
4. 10 ml of scintillation cocktail[1] was added to all tubes.
5. The tubes were tightly capped and thoroughly mixed in a vortex mixer.
6. The beta-radioactivity of the tubes was measured in a Packard Tri-carb (Model 2425) liquid scintillation counter.

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[1]Scintillation cocktail was prepared at least 1 day in advance of its use and contained 1 liter toluene, 500 ml Triton X-100, 6 g PPO, and 75 mg POPOP.

7. Percent recovery of ^{3}H-cyclic nucleotide was calculated as follows:

$$\% \text{ recovery} = \frac{1000 \text{ X (cpm of sample - cpm of blank)}}{\text{cpm of total counts - cpm of blank}}$$

Determination of protein content

The method used for the determination of protein was a modification of the method used by Lowry et al. (1951). All additions of reagents and incubations were done at room temperature.

1. The precipitated protein (from the acidified ethanol extraction of cyclic nucleotides) was dissolved in 3 ml of 1 N NaOH for 24-48 hours with occasional mixing.
2. 20 µl of the 1N NaOH-protein solution of the samples (in duplicate) was diluted to a volume of 0.5 ml with 1 N NaOH.
3. Standards were prepared from BSA (crystalline and lyophilized, No. A-4378, Sigma Chem. Co.) dissolved in 1 N NaOH. 0.5 ml of standard solutions (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml) was added (in duplicate) to the standard tubes.
4. 5 ml of Reagent D[1] was added to the 0.5 ml of samples and standards and the resulting solutions were mixed on a vortex mixer.
5. After 10 minutes of incubation, 0.5 ml of Folin Reagent (diluted 1:1 with ddH_2O) was added to samples and standards and the solutions were immediately mixed.

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[1]Reagent D was prepared fresh each day and contained 50 ml of 2% Na_2CO_3, 1 ml of 1% Na,K tartrate, and 1 ml of 0.5% $CuSO_4 \cdot 5H_2O$.

6. After 30-60 min of incubation, the optical density of the solutions (samples and standards) were measured at 660 nm on a Beckman DB-GT Spectrophotometer.

Calculations

The RIA data were calculated as the "normalized percent bound" ($\%B/B_0$) with B = cpm of standard or sample - cpm of blank and B_0 = cpm of "0" standard - cpm of blank. The blank cpm included the instrument background and the average radioactivity left in the tubes (in the absence of antibody) after aspiration. A typical standard curve for cGMP (and for cAMP as well) is shown in Figure 1. The average $\%B/B_0$ of the standards are plotted on the ordinate (linear scale) and the tube contents of standard cGMP are plotted on the abscissa (log scale). A typical sigmoidal curve results. Although this curve could have been used to interpolate the cGMP contents of the sample tubes, it was decided that a computer data analysis package should be used, since it would provide a more rapid and accurate method for calculating cGMP contents in a large number of samples. This computer package was designed and programmed by Brad Smith (presently at the Department of Veterinary Physiology, University of Illinois, Urbana) and was based on the mathematics developed by Dr. D. Rodbard (NIH, Bethesda, Maryland). In this package, the $\%B/B_0$ values are converted into logit values according to the equation.

$$\text{logit value} = \ln \frac{\%B/B_0}{100 - \%B/B_0}$$

A typical plot of the logit values versus cGMP content is shown in Figure 2. Note that the sigmoidal standard curve has been converted into a straight line. The computer program calculates a weighted linear regres-

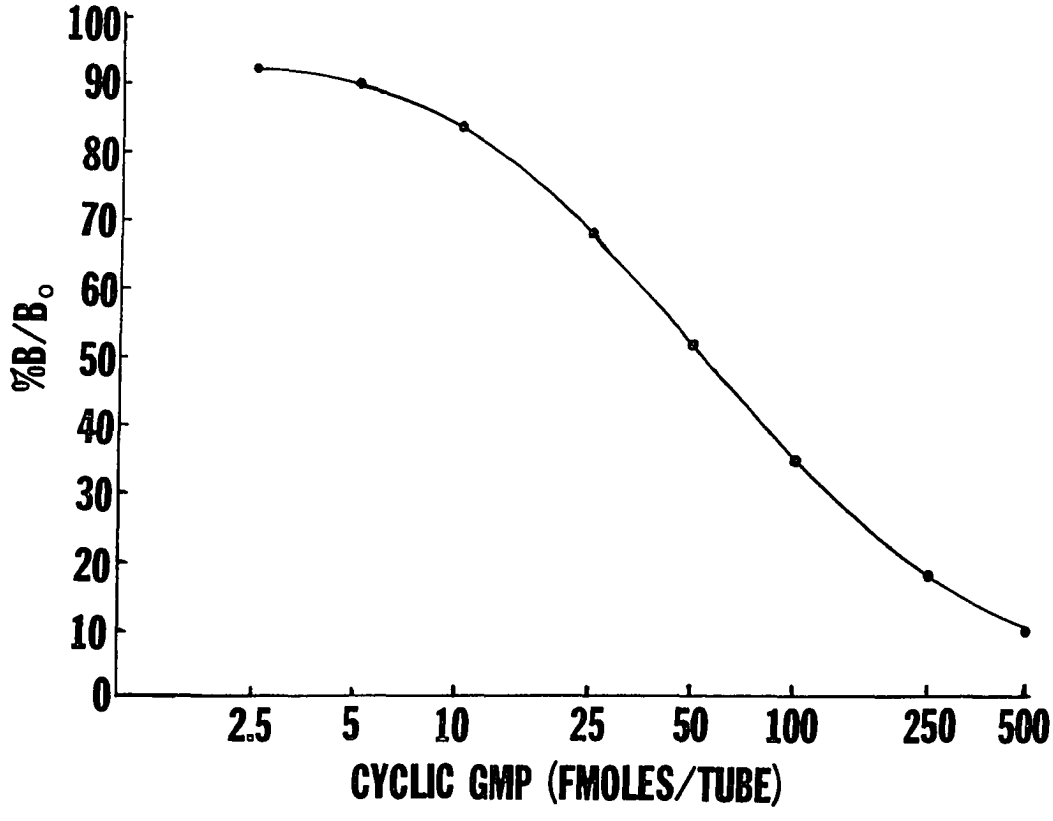

Figure 1. Typical standard curve of "normalized percent bound" versus cGMP content of RIA standard tubes.

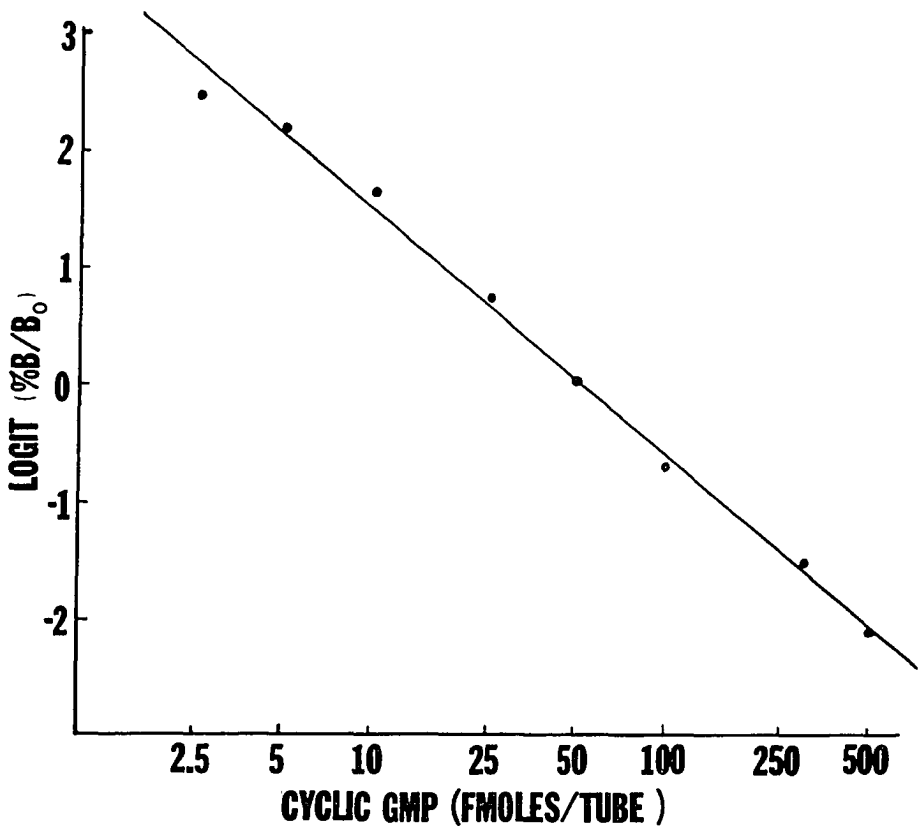

Figure 2. Typical standard curve of the logit transformed "normalized percent bound" versus cGMP content of RIA standard tubes.

sion of the logit values (prior to averaging) versus the natural log of the cyclic nucleotide contents of the standard tubes. The program weights an individual point's contribution to the regression as an inverse function of its deviation from the regression line. Calculations were made on a PDP-8/E laboratory computer using BASIC language.

To determine the accuracy of the assay and the possibility of interference from other cellular constituents, the following experiment was conducted. The supernatants of the homogenates from 2 control strips of HUA were split into 4 parts. One part was assayed unchanged; a second part was assayed after the addition of 50 fmole of cAMP (100 fmole of cGMP), a third part was assayed after the addition of 250 fmole of cAMP (500 fmole of cGMP); and the fourth part was assayed after treatment with cyclic nucleotide phosphodiesterase (to hydrolyze the cAMP and cGMP in the sample). The cyclic nucleotide phosphodiesterase treatment was carried out according to the procedure of Steiner et al. (1972b); i.e., samples were incubated for 1 hour at 37°C in acetate buffer (50 mM Na acetate, pH 6.2) containing 1.6 mM $MgCl_2$ and cyclic nucleotide phosphodiesterase with excess activity. The reaction was stopped by boiling for 2 min.

The results from supernatants that had been taken through Procedure A (i.e., with column chromatography, see above) are listed below:

RIA for cAMP:

Part 1 = 78 fmole/assay tube

Part 2 - Part 1 = 45 fmole (50 fmole was added)

Part 3 - Part 1 = 320 fmole (250 fmole was added)

Part 4 = 24 fmole/assay tube = 31% of Part 1

RIA for cGMP:

Part 1 = 39 fmole/assay tube

Part 2 - Part 1 = 110 fmole (100 fmole was added)

Part 3 - Part 1 = 710 fmole (500 fmole was added)

Part 4 = 0 fmole = 0% of Part 1

Supernatants taken through Procedure B (i.e., without column chromatography, see above) showed considerably less interference with the cAMP-RIA. For example, Part 4 from Procedure B was assayed to have 12% of control cAMP content as compared to 31% with Procedure A. In addition, Part 4 from Procedure B was assayed to have 11% of control cGMP content.

Tissue levels of cAMP or cGMP were calculated from the RIA data as follows:

Cyclic nucleotide level (fmole/mg of protein) = (cAMP in RIA [fmole/tube] - ^{3}H-cAMP[fmole/tube]) X 10 ÷ % recovery X sampling factor ÷ protein (mg)

The sampling factors were 4/0.5 for cAMP and 4/3.5 for cGMP, based upon the 0.5 ml aliquot for cAMP determination and the 3.5 ml aliquot for cGMP determination from the total 4.0 ml of supernatant (see steps 4 and 5 of Procedure A of Preparation of strips for radioimmunoassay). The factor of 10 in the above equation corrects for the 0.1 ml aliquot of the total 1.0 ml sample (in acetate buffer) taken for RIA.

Statistics

Data from the experiments on cyclic nucleotide levels and indomethacin effects were analyzed by paired t-tests using 4 to 9 pairs of strips (HUA or SUA) from individual umbilical cords. The one exception was the

comparison of cyclic nucleotide levels in HUA strips from male versus female in which 6 HUA strips from male neonates were compared to 10 HUA strips from female neonates using an unpaired t-test. All statistical calculations were done on a Wang 600 computerized calculator.

List of Chemicals, Drugs, and Materials and their Manufacturers

Chemicals

Acetic acid (A.C.S.)	Fisher Scientific Co. Chemical Manufacturing Div. Fair Lawn, N.J.
Charcoal (Norit A, No. C-176)	
Folin Reagent (Phenol Reagent, 2N)	
Glucose (Dextrose, anhydrous, A.C.S.)	
KCl (A.C.S.)	
$MgSO_4$ (A.C.S.)	
NaCl (A.C.S.)	
Na_2CO_3 (A.C.S.)	
$NaHCO_3$ (A.C.S.)	
Perchloric acid (70%, A.C.S.)	
PPO (2,5-diphenyloxazole, scintanalyzed)	
Toluene (A.C.S.)	
Trichloroacetic acid (A.C.S.)	
Bovine serum albumin (No. A-4378)	Sigma Chemical Co. P.O. Box 14508 St. Louis, Mo.
Bovine serum albumin (Fraction V, No. A-4503)	
Cyclic nucleotide phosphodiesterase (No. P-0134)	

Pyruvic acid (sodium salt, Type II, No. P-2256)	
Tris (tris(hydroxymethyl)aminomethane, No. T-1503)	
Triton X-100	
Cyclic AMP [^{125}I] RIA kit	New England Nuclear 549 Albany St. Boston, Mass.
Cyclic GMP [^{125}I] RIA kit	
Cyclic AMP [^{125}I] tracer (adenosine 3',5'-cyclic phosphoric acid, (^{125}I)-2'-O-succinyl(iodotryosine methyl ester))	
Sodium acetate buffer (50 mM, pH 6.2)	
Formic acid (88%, A.C.S.)	J. T. Baker Chemical Co. Phillipsburg, N.J.
KH_2PO_4 (A.C.S.)	
$CuSO_4 \cdot 5H_2O$ (analytical)	Mallinckrodt Chemical Works St. Louis, Mo.
Na_2EDTA (analytical)	
Alumina (aluminum oxide, Woelm neutral, activity grade I)	M. Woelm Eschwege, Germany
AG 1-X8 (200-400 mesh, formate form, analytical)	Bio-Rad Laboratories 2200 Wright Ave. Richmond, Ca.
$CaCl_2 \cdot H_2O$ (A.C.S.)	Allied Chemical Corp. Specialty Chemical Division P.O. Box 1087R Morristown, N.J.
Ethanol (absolute)	Chemistry Stores Iowa State University Ames, Iowa

POPOP (1,4-bis-(2-(5-phenyloxazolyl))-benzene)	Packard Instrument Co. 2200 Warrenville Rd. Downers Grove, Ill.
Potassium sodium tartrate	Matheson Colemen and Bell Norwood, Ohio
Triethylamine	Eastman Organic Chemicals Eastman Kodak Company Rochester, N.Y.

Drugs

Adenosine (No. A-9251)	Sigma Chemical Co. P.O. Box 14508 St. Louis, Mo.
ADP (disodium salt, No. A-0127)	
AMP (Type V, No. A-2127)	
Arachidonic acid (Grade I, No. A-6382)	
ATP (disodium salt, No. A-3127)	
8-Bromo-cAMP (sodium salt, No. B-7880)	
8-Bromo-cGMP (sodium salt, No. B-1381)	
Cyclic AMP (crystalline, No. A-9501)	
Cyclic GMP (sodium salt, No. G-6129)	
1-methyl-3-isobutyl xanthine (No. I-5879)	
$N^6,O^{2'}$-dibutyryl cAMP (Grade II, No. D-0627)	
$N^2,O^{2'}$-dibutyryl cGMP (sodium salt, No. D-3510)	
Papaverine (HCL salt, No. P-3510)	
PGE_1	The Upjohn Company Kalamazoo, Mi.
PGE_2	
$PGF_{2\alpha}$	

Histamine (HCl, B grade)	Calbiochem-Behring Corp. P.O. Box 12087 San Diego, Ca.
5-Hydroxytryptamine (creatinine sulfate complex, B grade)	
Aminophylline (theophylline ethylene-diamine injection, U.S.P.)	The Vitarine Co., Inc. New York, N.Y.
Caffeine (citrate)	ICN Pharmaceutical Inc. Life Science Group 26201 Miles Road Cleveland, Ohio
Indomethacin	Merck, Sharp and Dohme Research Laboratories West Point, Pa.
Nitroglycerin (1/100)	Eli Lilly Indianapolis, Ind.

Materials

Biogamma II (solid scintillation) counter	Beckman Instrument, Inc. Scientific Instrument Div. Irvine, Ca.
Centrifuge (J-21B)	
Centrifuge rotor (JA-21)	
Liquid scintillation vials (20 ml, glass)	
RIA vials (Bio-vials)	
Spectrophotometer (DB-GT)	
Liquid scintillation counter (Tri-carb, Model 2425)	Packard Instrument Co., Inc. 220 Warrenville Road Downers Grove, Ill.
Tissumizer (tissue homogenizer, Model SDT-100EN)	Tekmar Company P.O. Box 37202 Cincinnati, Ohio

RESULTS

Cyclic Nucleotide Levels

Endogenous levels of cAMP and cGMP in strips of human umbilical artery (HUA) were found to be essentially the same whether the strips were taken from male or female neonates (Table 1). Therefore, no distinction between male and female was made in subsequent experiments.

Figure 3 illustrates the data from experiments that were conducted to determine correlations between changes in cyclic nucleotide levels and changes in contractility induced by 5-HT. Strips of HUA were frozen at the peak of contraction (B and D in Figure 3) following cumulative doses (10^{-9} M - 10^{-6} M, added in half-log increments) of 5-HT or a single dose (10^{-5} M) of 5-HT. Strips under similar conditions, but not exposed to 5-HT, were frozen at times A and C (Figure 3) and served as controls.

Table 1. Comparison of cyclic nucleotide levels in isolated human umbilical arteries from male versus female neonates

	Cyclic nucleotide levels[a] (pmoles/mg protein)	
	cAMP	cGMP
Female	0.71 ± 0.07 ($\overline{10}$)	0.20 ± 0.02 ($\overline{10}$)
Male	0.95 ± 0.20 ($\overline{6}$)	0.22 ± 0.03 ($\overline{6}$)

[a]Cyclic nucleotide levels are reported as mean ± SEM. The numbers in parentheses indicate the numbers of arteries from individual umbilical cords that were analyzed. The data in this table were analyzed using unpaired t-tests. No significant differences were found between cyclic nucleotide levels in arteries from male versus female neonates.

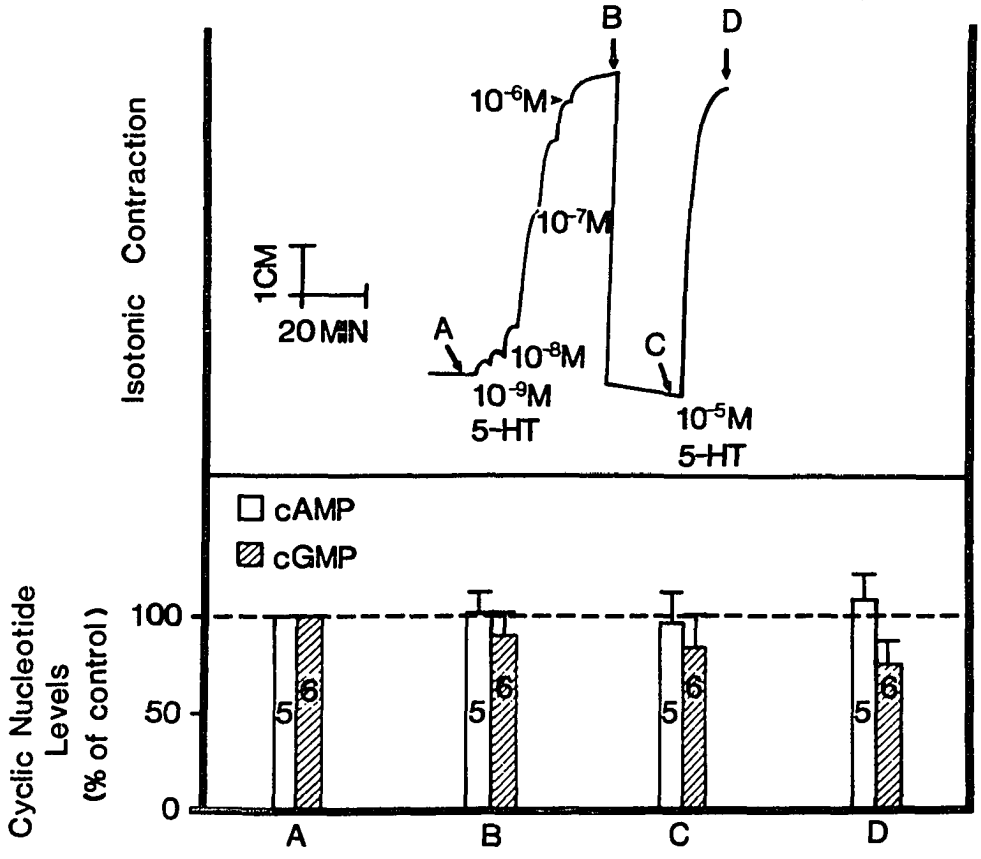

Figure 3. Cyclic nucleotide levels in isolated strips of human umbilical artery during contractions induced by cumulative doses (10^{-9} M -10^{-6} M, added at half-log increments) or single doses (10^{-5} M) of 5-hydroxytryptamine (5-HT). The upper graph is a tracing of a typical recording of isotonic contractions induced by 5-HT. An upward deflection on the tracing represents a shortening of the arterial strip with a 10-fold amplification. Points A-D on both upper and lower graphs represent the times at which the strips of arteries were frozen. The cyclic nucleotide levels ± SEM of these strips are shown in the lower graph. The number of arteries from individual umbilical cords that were analyzed in each group is indicated. No significant changes in either cAMP or cGMP levels were found.

Cyclic nucleotide levels of all strips were reported as the percent of control (at time A). Comparisons (paired t-tests) were made between cyclic nucleotide levels in the strips at: B versus A, C versus A, D versus A, and D versus C. No significant differences in cyclic nucleotide levels were found. However, cGMP levels appeared to decrease continually throughout the duration of the experiments (Figure 3), although this decrease was not significant at the 5% probability level.

Table 2 shows the levels of cyclic nucleotides in HUA strips under 1 g resting tension at the beginning (15 sec) and at the peak (420 sec) of contractions elicited by a single dose of 5-HT (10^{-5} M). No significant changes in cyclic nucleotide levels were noted. Table 3, in contrast, shows that cGMP levels were significantly ($p < .05$) elevated in HUA strips under no resting tension following exposure to 5-HT (10^{-5} M) for 7 min. Apparently the resting tension under which the strips are placed has an influence on the ability of 5-HT to elevate cGMP levels. Table 3 also shows that strips placed under 1 g resting tension appear to have higher cGMP levels than strips under no tension, although this difference was found to be not significant ($p > .05$), perhaps due to the large SEM value (see Table 3).

In contrast to HUA strips, strips from sheep umbilical arteries (SUA) showed an elevation in cAMP levels during 5-HT-induced contractions (Figure 4). The levels of cAMP became significantly ($p < .05$) different from control levels at 20 sec and reached their highest levels at 660 sec (peak of contraction) after the addition of 5-HT (10^{-5} M). However, contractions of the strips began at 5-10 sec after 5-HT addition and, there-

Table 2. Cyclic nucleotide levels in human umbilical arteries during isotonic contractions induced by 5-hydroxytryptamine (5-HT)

Treatment[b]	Contact time (sec)	Cyclic nucleotide levels[a] (% of control)	
		cAMP	cGMP
Control	---	100 (6)	100 (7)
5-HT (10^{-5}M)	15	102 + 13 (6)	98 + 17 (6)
5-HT (10^{-5}M)	420	108 + 9 (6)	101 + 21 (7)

[a]See Table 1 for partial explanation of data representation. Note that in Table 2 the levels of cyclic nucleotides are expressed as percent of control levels rather than as pmoles/mg protein. This is to emphasize the relative changes in cyclic nucleotide levels. Large variations in actual tissue levels (pmoles/mg protein) of cyclic nucleotides were commonly observed between strips of arteries from different umbilical cords. In the statistical analysis of the data, paired t-tests were used to compare cyclic nucleotide levels in pmoles/mg protein in paired strips (one control and one treated) from each umbilical cord. No significant changes in cyclic nucleotide levels were observed in strips isotonically contracted with 5-HT for 15 seconds or 420 seconds (peak of contraction). Contractions began between 5 and 10 seconds after 5-HT additions.

[b]Strips were placed under 1g resting tension and were allowed to relax for 3-4 hours before freezing of control strips or before the addition of 5-HT to treated strips. Control strips were handled in an identical manner as the 5-HT-treated strips, except that no 5-HT was added.

Table 3. Cyclic nucleotide levels in human umbilical arteries under no resting tension before and after exposure to 5-hydroxytryptamine and comparison to arteries under 1 g resting tension

Additions	Resting tension (gram)	Cyclic nucleotide levels[a] (% of control) cAMP	cGMP
None	0	100 (6)	100 (7)
5-HT (10^{-5}M) for 7 minutes	0	102 + 11 (6)	150 + 15* (7)
None	1	98 + 11 (6)	190 + 44 (6)

[a]See Table 2 for explanation of data representation. Control in Table 3 refers to those strips of arteries with no additions and no resting tension. Paired t-tests were used to compare cyclic nucleotide levels in pmoles/mg protein between control and 5-HT-treated strips from each umbilical cord. Using a similar statistical analysis for effects of resting tension, no significant changes were observed between cyclic nucleotide levels in strips with no resting tension versus strips with 1g resting tension.

*$p < .05$ versus control.

fore, the cAMP elevations lagged behind the beginning of contractions. No significant changes in cGMP levels were noted in SUA. Figure 5 shows that the elevation of cAMP in SUA strips was dependent upon the dose of 5-HT; i.e., 3×10^{-8} M 5-HT produced a smaller elevation of cAMP than that produced by 10^{-5} M 5-HT.

Figure 6 shows that cGMP levels in HUA strips were increased at 60 sec (3.5-fold) and 240 sec (7-fold) after addition of histamine (4 μM). Although large rises in cGMP levels were noted in some strips at 60 sec (see Table 4), these rises were found to be not significant ($p > .05$)

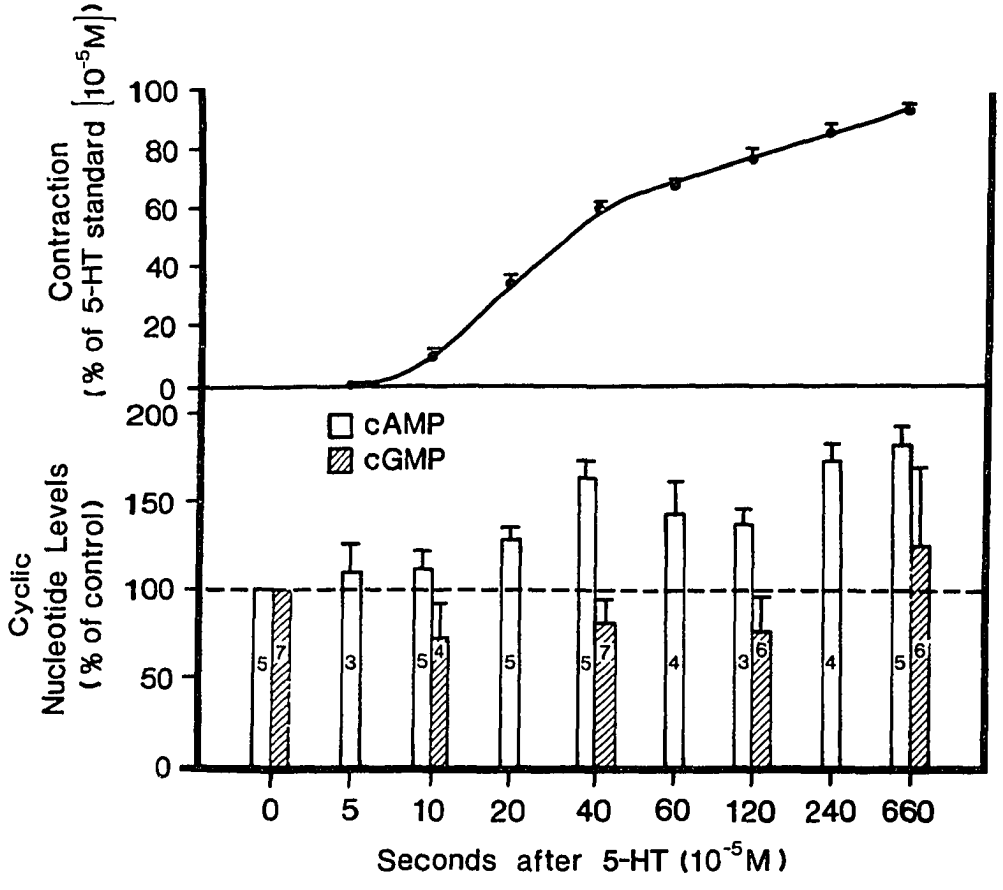

Figure 4. Cyclic nucleotide levels in isolated strips of sheep umbilical artery at selected times during contractions induced by 5-HT (10^{-5} M). The upper graph shows the amounts of isotonic contraction of strips at selected times after additions of 5-HT. The lower graph shows the cyclic nucleotide levels ± SEM in these strips at the selected times. The number of arteries from individual umbilical cords that were analyzed is indicated within each bar. Cyclic AMP levels were significantly elevated at 20, 40, 60, and 120 seconds ($p < .05$) and at 240 and 660 seconds ($p < .01$) after 5-HT additions. No significant changes in cGMP levels were noted.

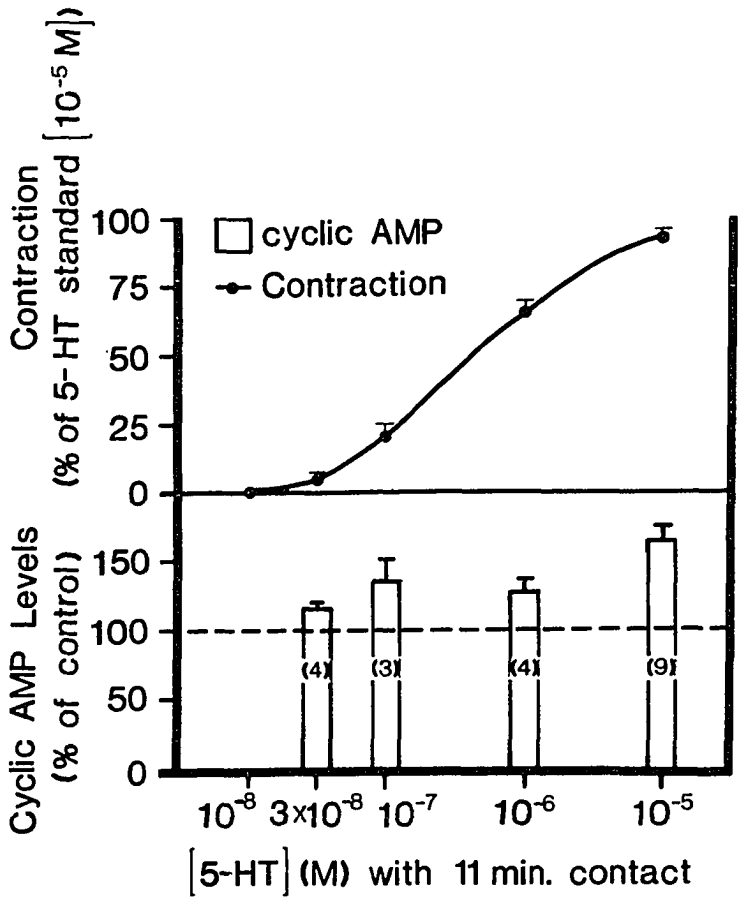

Figure 5. Cyclic AMP levels in isolated strips of sheep umbilical artery during contractions induced by selected doses of 5-HT. The upper graph shows the amounts of isotonic contractions of strips at 11 minutes (peak of contractions) after additions of 5-HT (10^{-8} M - 10^{-5} M). The lower graph shows the cAMP levels ± SEM in these strips at the selected doses of 5-HT. The number of arteries from individual umbilical cords that were analyzed is indicated. Levels of cAMP were significantly elevated by 5-HT at doses of 3 x 10^{-8} M ($p < .05$), 10^{-6} M ($p < .05$), and 10^{-5} M ($p < .01$).

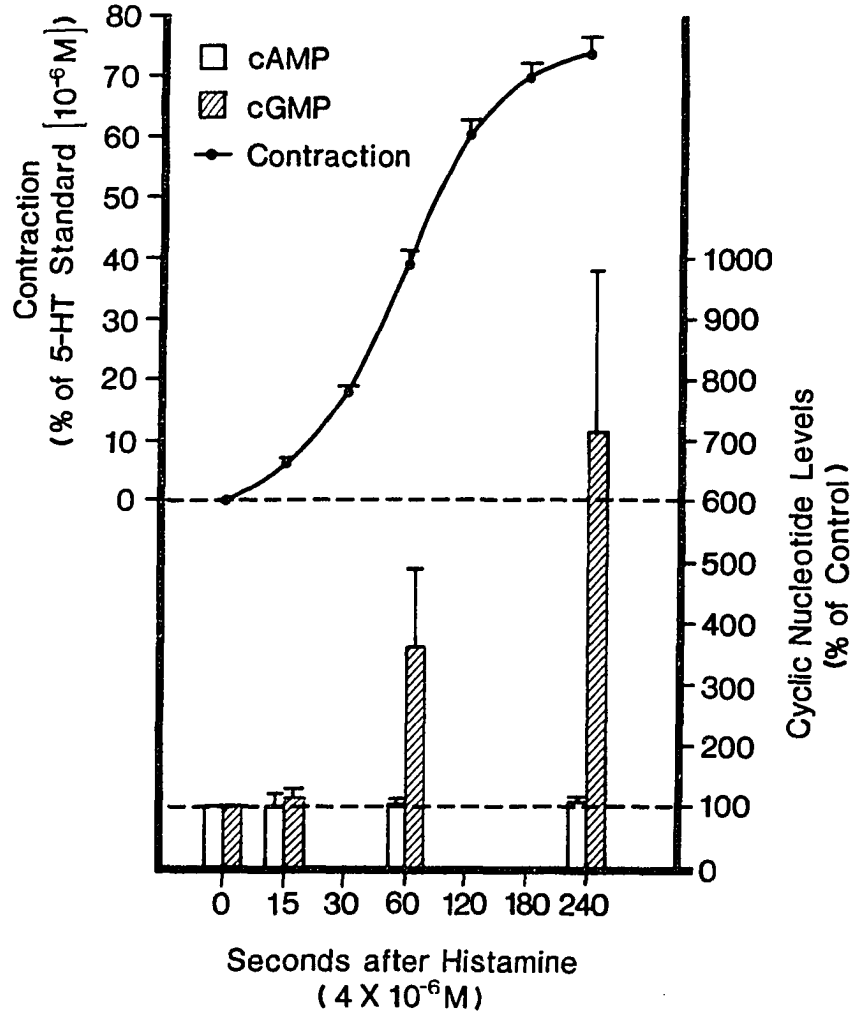

Figure 6. Cyclic nucleotide levels in isolated strips of human umbilical artery during contractions induced by histamine (4 μM). The upper graph shows the amounts of isotonic contractions of strips at selected times after additions of histamine. Measured contractions at 30 sec were from the strips subsequently frozen at 60 sec. Measured contractions at 120 and 180 sec were from the strips subsequently frozen at 240 sec. The lower graph shows the cyclic nucleotide levels ± SEM in the strips at 0, 15, 60, and 240 sec after histamine additions. Six strips of arteries from individual umbilical cords were analyzed in each time group. No significant changes in cAMP levels were noted. For statistical analysis of the cGMP levels, see Table 4.

Table 4. Cyclic GMP levels in human umbilical arteries during isotonic contractions induced by histamine

Arteries (date of delivery)	Cyclic GMP levels (fmoles/mg protein)			
		Histamine		
	Control	15 sec	60 sec	240 sec
5-10-78	236	422	276	662
5-11-78	148	112	105	485
6-01-78	124	156	294	2080
6-05-78	287	354	444	4240
6-07-78	214	148	1620	778
6-08-78	230	225	1911	316
Mean ± SEM	206 ± 25	236 ± 51	775 ± 320	1420 ± 620
N	6	6	6	6
Paired t-test		$p > .05$	$p > .05$	$p > .05$
Logarithmic transformation of data[a]:				
$\overline{\log Y}$	2.30	2.32	2.68	2.97
Antilog ($\overline{\log Y}$)	198	210	476	942
95% confidence limits	142-277	121-364	147-1540	341-2601
Paired t-test		$p > .05$	$p > .05$	$p < .05$

[a] Y = cGMP level in fmole/mg protein. Antilog ($\overline{\log Y}$) = geometric mean of data. Note that the elevations of cGMP levels became significant ($p < .05$) at 240 seconds after histamine addition when a logarithmic transformation is performed on the data.

after statistical analysis of the data and of the logarithmic transformed data. However, the rises of cGMP levels at 240 sec were found to be significant ($p < .05$) using the logarithmic transformed data, but not the untransformed data (see Table 4). Logarithmic transformation of the data can be justified (Snedecor and Cochran, 1967), based upon the fact that the SEM values were dependent upon the mean values in the untransformed data (see Table 4). Since all strips had begun contracting at 5-10 sec after histamine addition, the elevation of cGMP levels appeared to lag behind the beginning of contractions.

Table 5 shows the effects of PGs, indomethacin, and ethanol on the contractility and cyclic nucleotide levels of HUA strips. Ethanol at a concentration of 0.1% (V/V) was found to significantly ($p < .01$) lower the levels of cGMP after exposure for 4 min (40% of control levels) and 60 min (47% of control levels). The same concentration of ethanol caused a small but persistent contraction of HUA strips (see Table 5 and Figure 15). Both responses elicited by ethanol were of interest, because ethanol (0.1% V/V, final bath concentration) was used as the vehicle for the additions of all PGs and indomethacin. In the statistical analysis of the effects of PGs and indomethacin, cyclic nucleotide levels of strips treated with ethanol (at appropriate contact times) were used as control.

PGE_1 (1 μg/ml) after 0.5 and 4 minutes contact and PGE_2 (1 μg/ml) after 4 minutes contact caused significant ($p < .05$) elevations of cAMP levels (Table 5). No other changes in cyclic nucleotide levels were found after additions of PGE_1, PGE_2, or $PFG_{2\alpha}$ (each at 1 μg/ml) when compared to solvent controls. However, when strips exposed to PGE_1, PGE_2, or $PGF_{2\alpha}$

Table 5. Effects of PGE_1, PGE_2, $PGF_{2\alpha}$, indomethacin and solvent (ethanol) on cyclic nucleotide levels and contractility of isolated human umbilical arteries

Additions[b]	Contact time (min)	Contraction (+) or relaxation (-) (% of standard 5-HT[10^{-6}M])	Cyclic nucleotide levels[a] (% of control)	
			cAMP	cGMP
None	---	---	100 (7)	100 (8)
Ethanol	0.5	+0.8 ± 0.5	90 + 9 (4)	77 + 7 (4)
(0.1%[v/v])	4.0	+4.8 ± 1.4	87 + 11 (5)	40 + 11** (5)
	60	+10 ± 2	79 + 10 (6)	47 + 7** (4)
PGE_1	0.5	+1.2 ± 0.9	146 + 15* (5)	102 + 21 (5)
(1µg/ml)	4.0	+29 ± 12[c]	159 + 27* (6)	36 + 9 (6)
PGE_2	0.5	+1.7 ± 0.8	107 + 11 (5)	79 + 11 (5)
(1µg/ml)	4.0	+44 ± 10[d]	134 + 15* (5)	29 + 4 (5)

$PGF_{2\alpha}$	0.5	+2.0 ± 0.6	121 ± 14 (6)	94 ± 27 (5)
(1μg/ml)	4.0	+62 ± 7[e]	95 ± 13 (6)	26 ± 3 (7)
Indomethacin	60	-14 ± 7	88 ± 11 (7)	36 ± 3 (8)
(10μg/ml)				

[a]Control levels of cAMP = 0.82 ± 0.10 pmoles/mg protein and cGMP = 0.21 ± 0.02 pmoles/mg protein. Numbers in parentheses indicate the numbers of arteries from individual umbilical cords that were analyzed. Paired t-tests were used to compare cyclic nucleotide levels in pmoles/mg protein between control (no additions) and ethanol-treated strips. Similar analyses were used to compare cyclic nucleotide levels in pmoles/mg protein in prostaglandin-treated and indomethacin-treated strips versus their solvent controls (ethanol additions with appropriate contact times).

[b]Ethanol was used as the solvent for additions of the prostaglandins and of indomethacin and was added to a final bath concentration of 0.1% (v/v).

[c]Four strips contracted, 1 strip relaxed, and 1 strip showed no response.

[d]Four strips contracted, 1 strip showed only slight contraction (2% of standard 5-HT [10^{-6}M]).

[e]All strips contracted.

*$p < .05$ versus ethanol (solvent control) at appropriate contact times.

**$p < .01$ versus none.

were compared to strips with no additions, the cGMP levels were found to be significantly lowered ($p < .01$) after 4 minutes with PG contact. This response is similar to that of the solvent (ethanol) (see Table 5). With the exception of two strips exposed to PGE_1, all strips contracted after additions of PGE_1 (1 μg/ml), PGE_2 (1 μg/ml), and $PGF_{2\alpha}$ (1 μg/ml). However, the magnitudes of these contractions were observed to be inversely related to the elevations in cAMP levels elicited by each PG; i.e., PGE_1 caused greatest elevations of cAMP and smallest contractions, $PGF_{2\alpha}$ caused no elevation of cAMP and largest contractions, and the actions of PGE_2 were intermediate (see Table 5).

Indomethacin (10 μg/ml) was found to relax strips of HUA but was found to have no significant effects on cyclic nucleotide levels when compared to solvent control (Table 5). The cGMP levels in strips exposed to indomethacin for 60 min was significantly lower ($p < .01$) when compared to strips with no additions, but this response was also observed with addition of the ethanol alone.

The effects of nitroglycerin on cyclic nucleotide levels and contractility of HUA strips are shown in Table 6. These strips were first exposed to 30 mM KCl, which caused persistent and near maximal contractions. Levels of cyclic nucleotides were not altered by KCl (30 mM). Nitroglycerin (3 μM) caused highly significant increases in cGMP levels after exposure for 0.5 minutes ($p < .001$) and 4 minutes ($p < .01$). A small, but significant, increase ($p < .05$) in measured cAMP levels was found in strips exposed to nitroglycerin for 4 minutes. Table 7 shows the

Table 6. Effect of nitroglycerin on cyclic nucleotide levels and contractility of human umbilical arteries previously contracted with KCl

Additions	Contact time (min)	Contraction (+) or relaxation (-) (% of standard 5-HT[10^{-6}M])	Cyclic nucleotide levels[a] (pmoles/mg protein)	
			cAMP	cGMP
None	---	---	0.64 + 0.08 ($\bar{5}$)	0.24 + 0.03 ($\bar{5}$)
KCl (30mM)	30-40	+94 + 3 ($\bar{5}$)	0.69 + 0.13 ($\bar{5}$)	0.29 + 0.08 ($\bar{4}$)
KCl (30mM) + Nitroglycerin (3μM)	30-40 0.5	0[b] (5)	0.89 + 0.14 ($\bar{5}$)	8.4 + 2.2*** ($\bar{5}$)
KCl (30mM) + Nitroglycerin (3μM)	30-40 4.0	-13 + 3 ($\bar{5}$)	1.14 + 0.24* ($\bar{5}$)	38 + 4** ($\bar{5}$)

[a]See Table 1 for explanation of data representation. See Table 7 for statistical analysis of cGMP data.

[b]Detectable relaxations began between 30 and 45 seconds after addition of nitroglycerin (3μM).

*$p < .05$ vs KCl (30mM).

**$p < .01$ vs KCl (30mM).

***$p < .001$ vs KCl (30mM).

Table 7. Cyclic GMP levels in KCl-contracted human umbilical arteries during nitroglycerin-induced relaxation

Arteries (date of delivery)	Cyclic GMP levels (fmoles/mg protein)			
			KCl (30mM) + Nitroglycerin (3μM)	
	Control	KCl (30mM) (30-40 min)	30 sec	4 min
7-25-78	289	436	11,060	35,240
8-01-78	288	181	4,620	50,270
8-11-78	260	429	11,960	35,560
8-16-78	186	118	1,723	43,460
8-17-78	155	---	12,590	26,430
Mean ± SEM	236 ± 28	291 ± 82	8,390 ± 2,200	38,200 ± 4,000
N	5	4	5	5
Paired t-test		p>.05 vs Control	p>.05 vs KCl	p<.005 vs KCl
Logarithmic transformation of data[a]:				
$\overline{\log Y}$	2.40	2.40	3.76	4.61
antilog $(\overline{\log Y})$	251	251	5,750	40,700
95% confidence limits	180-350	89.3-707	1,360-24,300	31,100-53,400
Paired t-test		p>.05 vs Control	p<.001 vs KCl	p<.005 vs KCl

[a] Y = cGMP level in fmole/mg protein. Antilog $(\overline{\log Y})$ = geometric mean of data. Note that the elevations of cGMP levels became significant ($p < .001$) at 30 seconds after nitroglycerin additions when logarithmic transformations are performed on the data. For justification of logarithmic transformations, see Snedecor and Cochran (1967).

raw data and statistical analysis of nitroglycerin's effect on cGMP levels in HUA strips. Both untransformed and logarithmic transformed data were analyzed.

Cyclic Nucleotide Derivatives

Figure 7 shows the relaxation effects of adenosine and cyclic nucleotide derivatives on KCl-contracted strips of HUA. KCl (21 mM) produced a sustained contraction (~50% of standard 5-HT (10^{-6} M)) upon which was superimposed smaller spontaneous transient contractions. Adenosine (0.1 mM) produced a rapid reduction in the sustained contraction induced by KCl. Relaxations of the KCl-contracted strips were also caused by the cyclic nucleotide derivatives, dibutyryl cAMP (dBcAMP), dibutyryl cGMP (dBcGMP), 8-bromo-cAMP (8BcAMP), and 8-bromo-cGMP (8BcGMP), each at 0.1 mM. With the exception of dBcGMP, all cyclic nucleotide derivatives caused relaxations of KCl-contracted strips approximately equal to that elicited by adenosine. The relaxation produced by dBcGMP was considerably smaller in magnitude.

In addition to their relaxant effects in KCl-contracted strips of HUA, adenine nucleotides and nucleoside also caused contraction in relaxed (non-KCl-contracted) HUA strips (Table 8). When each adenine compound was added at a concentration of 0.1 mM, ATP caused the largest contractions (on the average), whereas AMP and cAMP caused the smallest contractions (Table 8). The contractions elicited by these agents were very transient, lasting only 1-2 min (see the contractile responses to ATP in Figure 15 as an example). The data, therefore, illustrate that adenosine

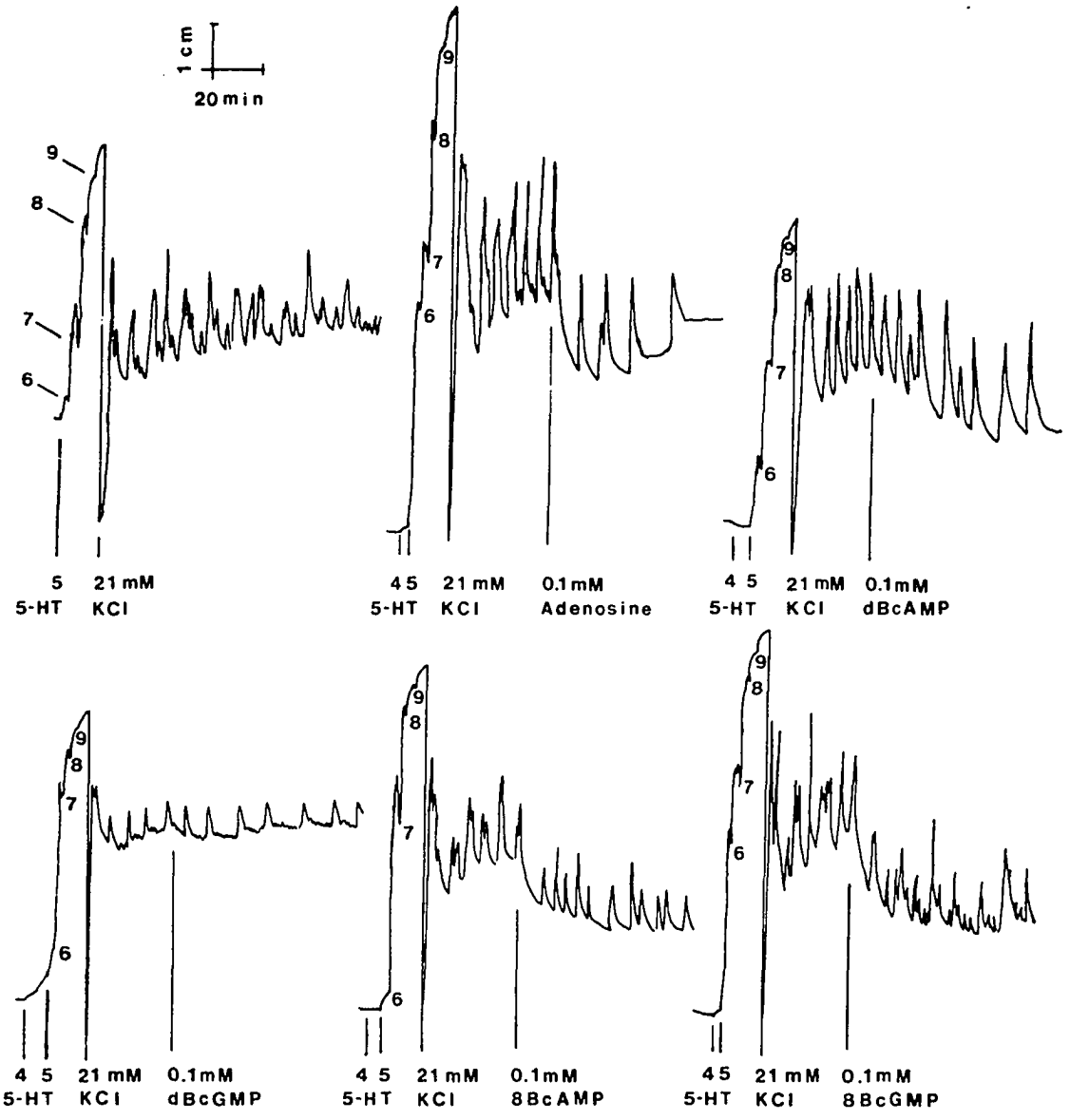

Figure 7. Relaxation of isolated human umbilical arteries (HUA) induced by cyclic nucleotide derivatives and by adenosine. The strips were first contracted with cumulative doses of 5-HT (4 = 3 x 10^{-9} M, 5 = 10^{-8} M, 6 = 3 x 10^{-8} M, 7 = 10^{-7} M, 8 = 3 x 10^{-7} M, 9 = 10^{-6} M) to establish a standard response. The strips were then washed and allowed to relax before addition of KCl (21 mM). Cyclic nucleotide derivatives (0.1 mM) and adenosine (0.1 mM) were added approximately 30 min following the addition of KCl. Strips from four other umbilical arteries responded similarly.

Table 8. Isotonic contractions of isolated human umbilical arteries induced by adenine nucleotides and a nucleoside

Adenine nucleotide or nucleoside (0.1 mM)	Contraction (% of standard 5-HT [10^{-6} M])
ATP	47±6.8 (8)
ADP	41±5.7 (6)
AMP	21±11 (6)
cAMP	27±10 (4)
adenosine	45±9.6 (6)

and the adenine nucleotides act as vasodilators in contracted HUA but as vasoconstrictors in relaxed HUA.

Inhibitors of Cyclic Nucleotide Phosphodiesterase

Figures 8 and 9 illustrate the effects of the cyclic nucleotide phosphodiesterase inhibitors, aminophylline, caffeine, papaverine, and 1-methyl-3-isobutyl xanthine (MIX), on KCl-contracted strips of HUA. Aminophylline, at the highest concentrations (1.8 and 3.6 mM), produced a small increase in the sustained contraction and an attenuation of the superimposed spontaneous contractions (Figure 8). In contrast, caffeine, at the highest concentration (3 mM) caused relaxation of the sustained contraction (Figure 8). At the lower concentrations (0.1, 0.3, and 1 mM), caffeine appeared to slightly facilitate contractions of HUA. Papaverine and MIX, at concentrations ranging from 10-100 μM and 30-300 μM, respectively, produced dose-dependent relaxations of the KCl-contracted strips.

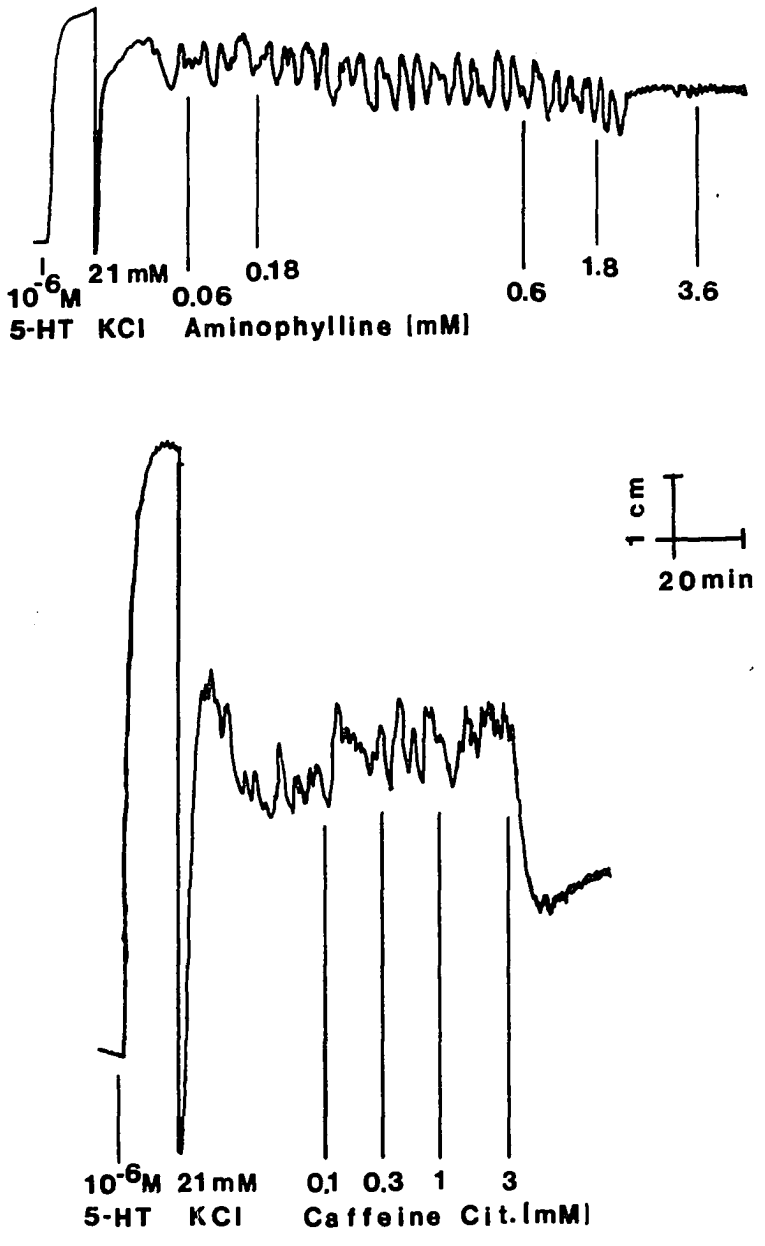

Figure 8. Effects of aminophylline and caffeine on KCl-contracted strips of human umbilical artery. The strips were first contracted with 5-HT (10^{-6} M) to establish a standard response. The strips were then washed and allowed to relax before addition of KCl (21 mM). Aminophylline and caffeine citrate (cit.) were added at approximately 30 min following the addition of KCl. Strips from four other umbilical arteries responded similarly.

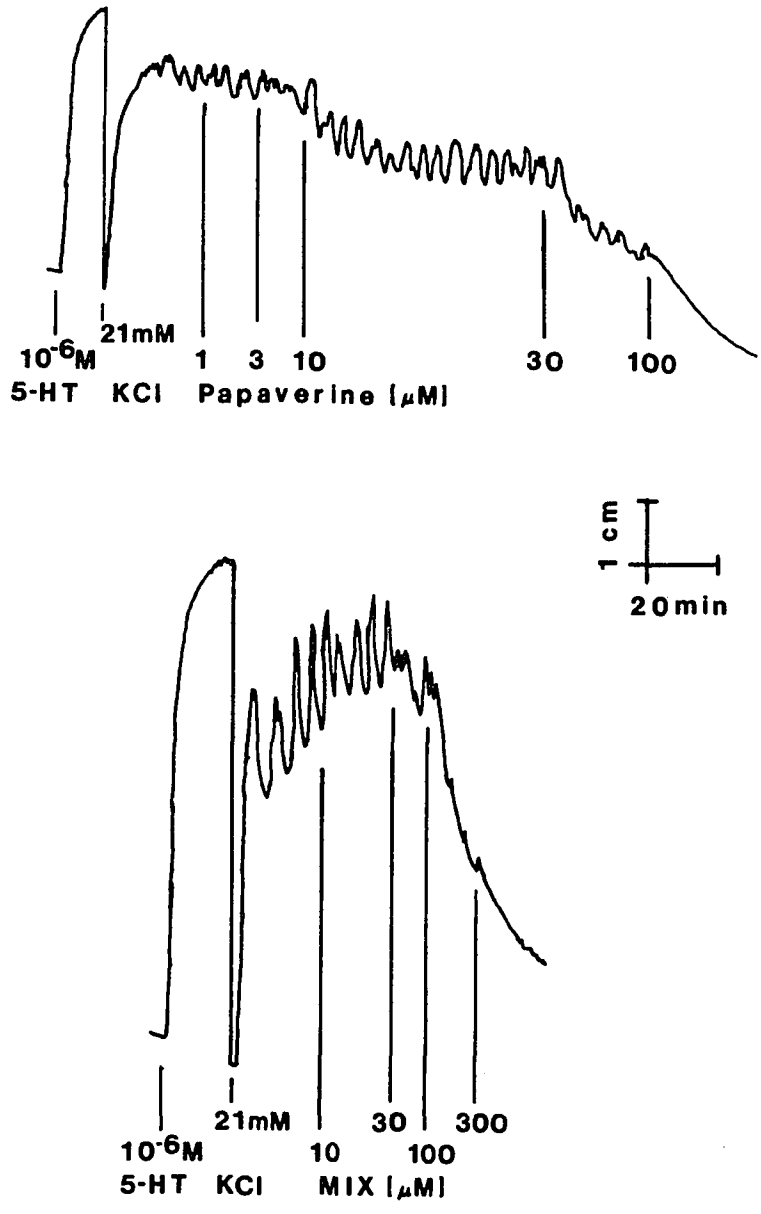

Figure 9. Effects of papaverine and 1-methyl-3-isobutyl xanthine (MIX) on KCl-contracted strips of human umbilical artery. See Figure 8 for explanation of the procedure. Strips from four other umbilical arteries responded similarly.

Effects of Indomethacin on Contractility

Indomethacin, a potent inhibitor of PG synthesis (Flower, 1974), was used to determine the influence of released PGs in the contractile responses in HUA elicited by a variety of agonists (5-HT, KCl, $PGF_{2\alpha}$, and ATP). Preliminary experiments were conducted to determine the concentrations of indomethacin that caused the specific effect (PG synthesis inhibition) and the concentrations which caused nonspecific effects (smooth muscle relaxation unrelated to PG synthesis inhibition). The specific effect of indomethacin was tested on HUA strips contracted by arachidonic acid (precursor for the synthesis of vasoconstrictor PGs). The nonspecific effects of indomethacin were tested on KCl-contracted strips of HUA.

Figure 10 illustrates the effects of indomethacin on contractions of HUA strips induced by cumulative doses of arachidonic acid (1, 10, 100 µg/ml). The magnitude of the arachidonic acid-induced contractions (calculated as percent of standard 5-HT [10^{-6} M]) were observed to vary considerably from one umbilical cord to the next. For this reason, the data were reported as the percent of maximal contraction produced by arachidonic acid (1, 10 or 100 µg/ml) in strips from the same umbilical cord. Indomethacin (1, 2.5, and 10 µg/ml) was observed to produce a dose-dependent reduction in the contractions induced by arachidonic acid. Statistical analysis of the data shows that indomethacin (2.5 µg/ml) produced a significant ($p < .05$) reduction in the contractions to 1 µg/ml and 10 µg/ml of arachidonic acid. Statistical analysis was not performed on the data with indomethacin at 1 µg/ml or 10 µg/ml due to the small number of observations. Thus, the data above illustrate that indomethacin, at

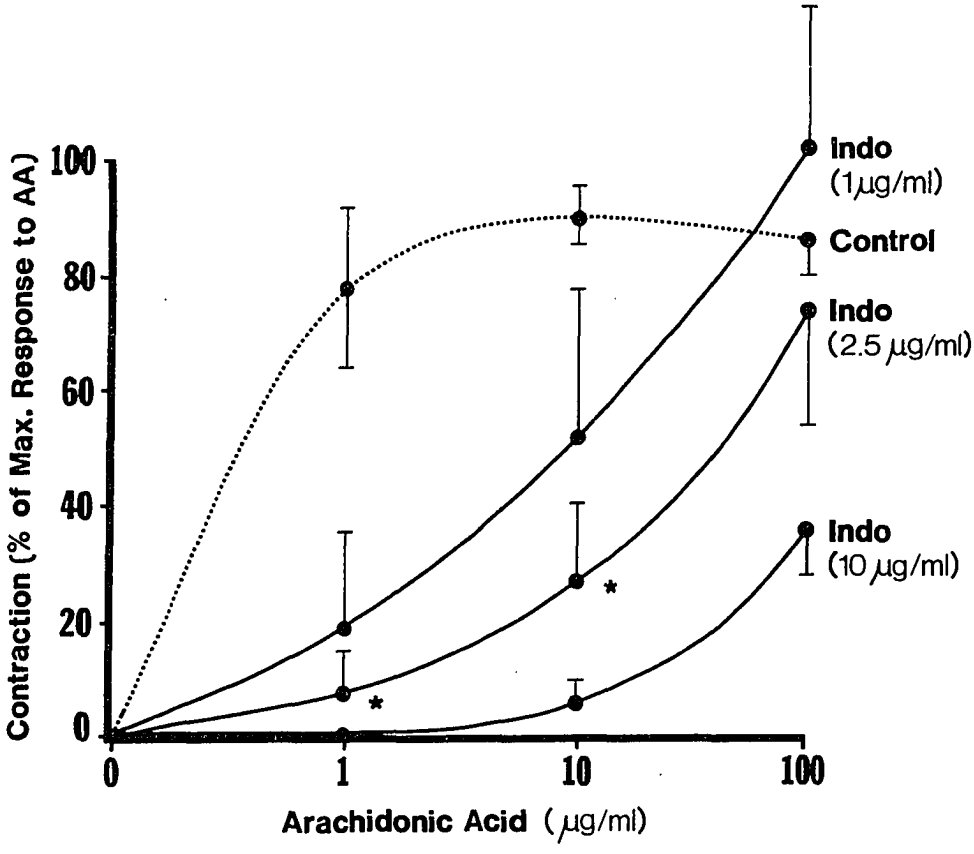

Figure 10. Effects of indomethacin (Indo) on the cumulative dose-response curves to arachidonic acid (AA) on isolated human umbilical arteries. Five strips of artery were preincubated for 30 min in indomethacin (2.5 μg/ml) and three strips were preincubated for 30 min in indomethacin at 1 μg/ml and 10 μg/ml. The mean ± SEM of contractions was reported as the percent of maximal response elicited by arachidonic acid (1, 10 or 100 μg/ml). Indomethacin (2.5 μg/ml) significantly reduced the contractions to 1 and 10 μg/ml of arachidonic acid. Statistics were not performed on data with indomethacin at 1 and 10 μg/ml, because of the small number of strips. *$p < .05$ versus control.

concentrations as low as 2.5 µg/ml, severely depressed HUA contractions induced by an agent acting through the effects of released PGs.

In Figure 11, it can be seen that indomethacin at 25 µg/ml and 65 µg/ml, but not at 10 µg/ml (or lower), caused relaxation of KCl-contracted strips of HUA. Since relaxation of HUA occurred only at concentrations (25-65 µg/ml) of indomethacin considerably higher than needed to inhibit PG synthesis, this response may be attributed to a nonspecific effect of indomethacin.

Contractions of HUA strips induced by cumulative doses of 5-HT (Figure 12), KCL (Figure 13), and $PGF_{2\alpha}$ (Figure 14) were antagonized by the higher concentrations (25-65 µg/ml) of indomethacin. In all cases, the dose-response curves were shifted to the right and the maximum contractile responses were reduced. Indomethacin (10 µg/ml) antagonized the contractions induced by vasoconstrictor agonists at some, but not all, of the concentrations (see Figures 12, 13, and 14). Indomethacin (2.5 µg/ml), which had significantly antagonized the contractions induced by arachidonic acid (Figure 10), had no effect on contractions induced by 5-HT (Figure 12), KCl (Figure 13), and $PGF_{2\alpha}$ (Figure 14).

Contractions of HUA strips induced by ATP (0.1 mM) were completely inhibited by indomethacin at concentrations as low as 1 µg/ml (Figure 15). In fact, preincubation of HUA strips in indomethacin (1 µg/ml) for 30 min changed the contractile response to ATP into a relaxation (Figure 15). Interestingly, preincubation of HUA strips in ethanol (0.2% V/V) alone appeared to potentiate the contractile response to ATP.

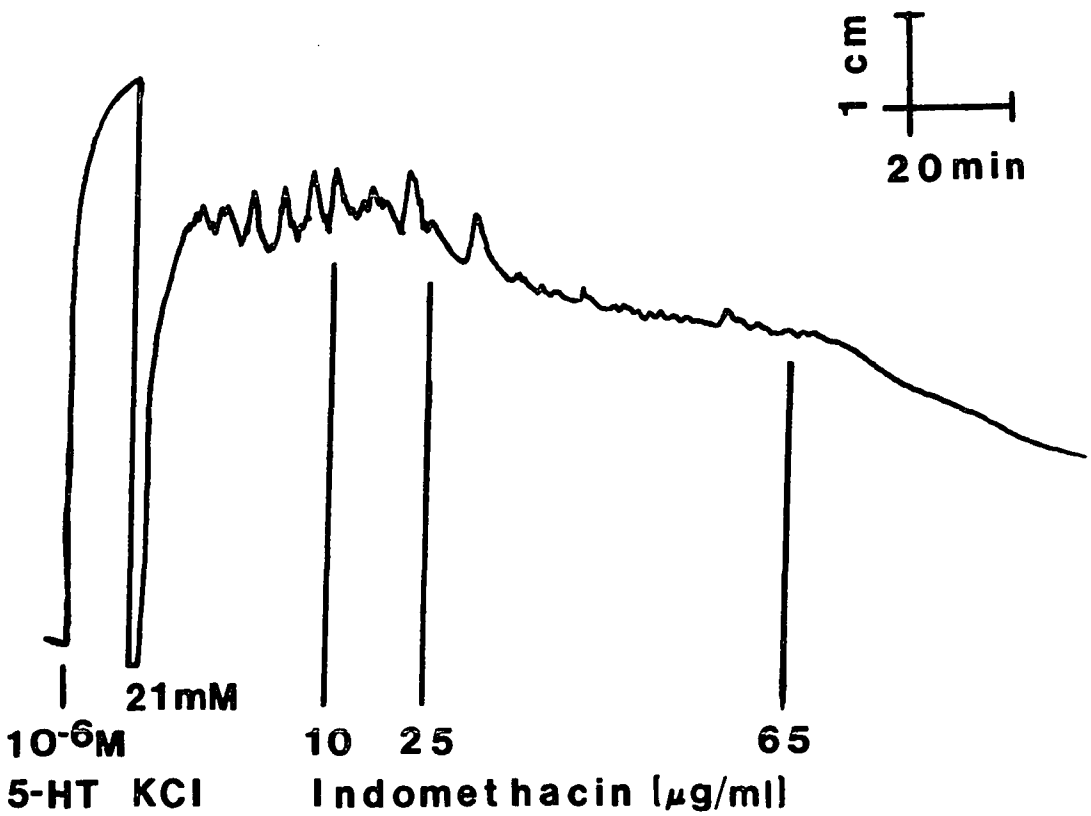

Figure 11. Effects of indomethacin on KCl-contracted strips of human umbilical artery. See Figures 8 and 9 for explanation of procedure. Strips from four other umbilical arteries responded similarly.

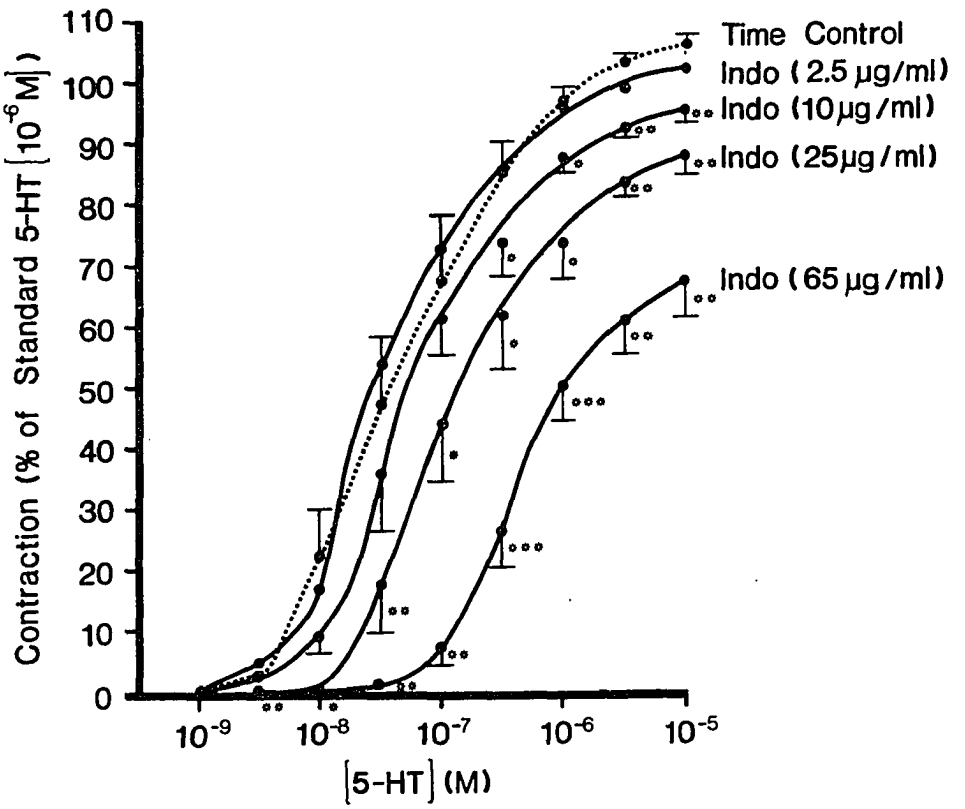

Figure 12. Effects of indomethacin (Indo) on cumulative dose-response curves to 5-HT on isolated human umbilical arteries. Preincubations for 30 minutes in 25 µg/ml or 65 µg/ml of indomethacin caused a significant reduction in the contractions induced by all cumulative doses of 5-HT. Indomethacin (10 µg/ml) reduced the maximal contractions induced by the highest doses of 5-HT, but did not alter responses to lower doses of 5-HT. Indomethacin (2.5 µg/ml) had no effect on the cumulative dose-responses to 5-HT. The data represent the mean ± SEM of contractions of six arteries from individual umbilical cords. For clarity, the standard error values for the contractions of strips preincubated in indomethacin (2.5 µg/ml) are not shown. *$p < .05$ versus time control. **$p < .01$ versus time control. ***$p < .001$ versus time control.

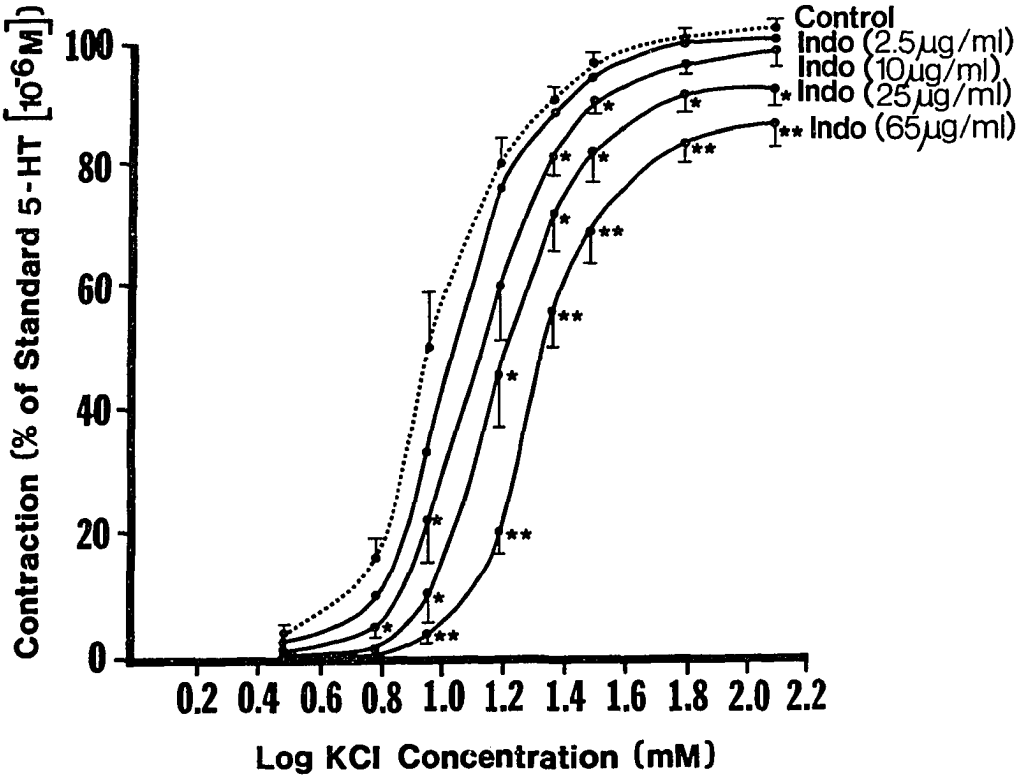

Figure 13. Effects of indomethacin (Indo) on cumulative dose-response curves to KCl on isolated human umbilical arteries. Preincubation for 30 min in indomethacin at 25 or 65 µg/ml significantly reduced the contractions induced by all cumulative doses of KCl. Indomethacin (10 µg/ml) reduced contractions to some of the concentrations of KCl. Indomethacin (2.5 µg/ml) had no significant effect on the cumulative dose-responses to KCl. The data represent the mean ± SEM of contractions in arteries from six individual umbilical cords. For clarity, the SEM values are not shown for the contractions of strips preincubated in 2.5 µg/ml indomethacin. *$p < .05$ versus control. **$p < .01$ versus control.

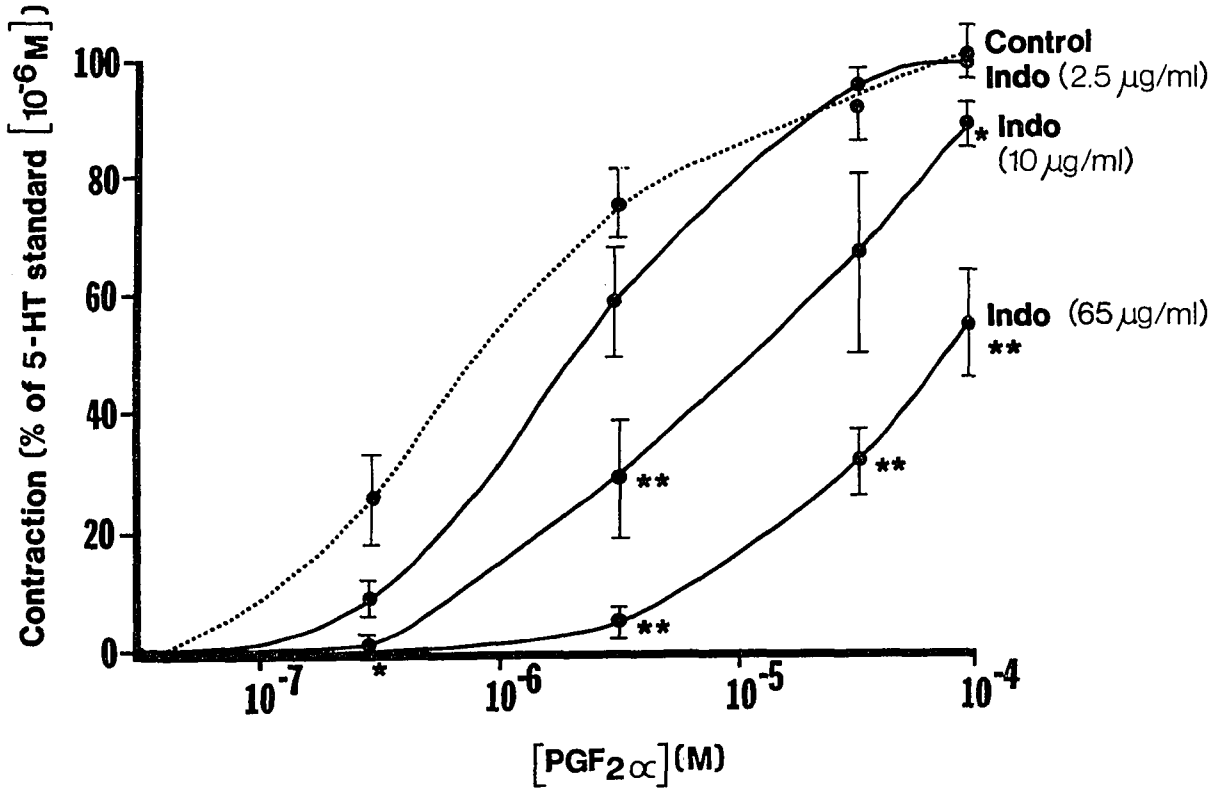

Figure 14. Effects of indomethacin (Indo) on cumulative dose-response curves to $PGF_{2\alpha}$ on isolated human umbilical arteries. Preincubation for 30 min in indomethacin at 65 μg/ml significantly reduced the contractions induced by all concentrations of $PGF_{2\alpha}$. Indomethacin (10 μg/ml) significantly reduced contractions to some of the concentrations of $PGF_{2\alpha}$. Indomethacin (2.5 μg/ml) had no significant effect on the cumulative dose-response curves to $PGF_{2\alpha}$. The data represent the mean ± SEM of contractions in arteries from five individual umbilical cords. *$p < .05$ versus control. **$p < .01$ versus control.

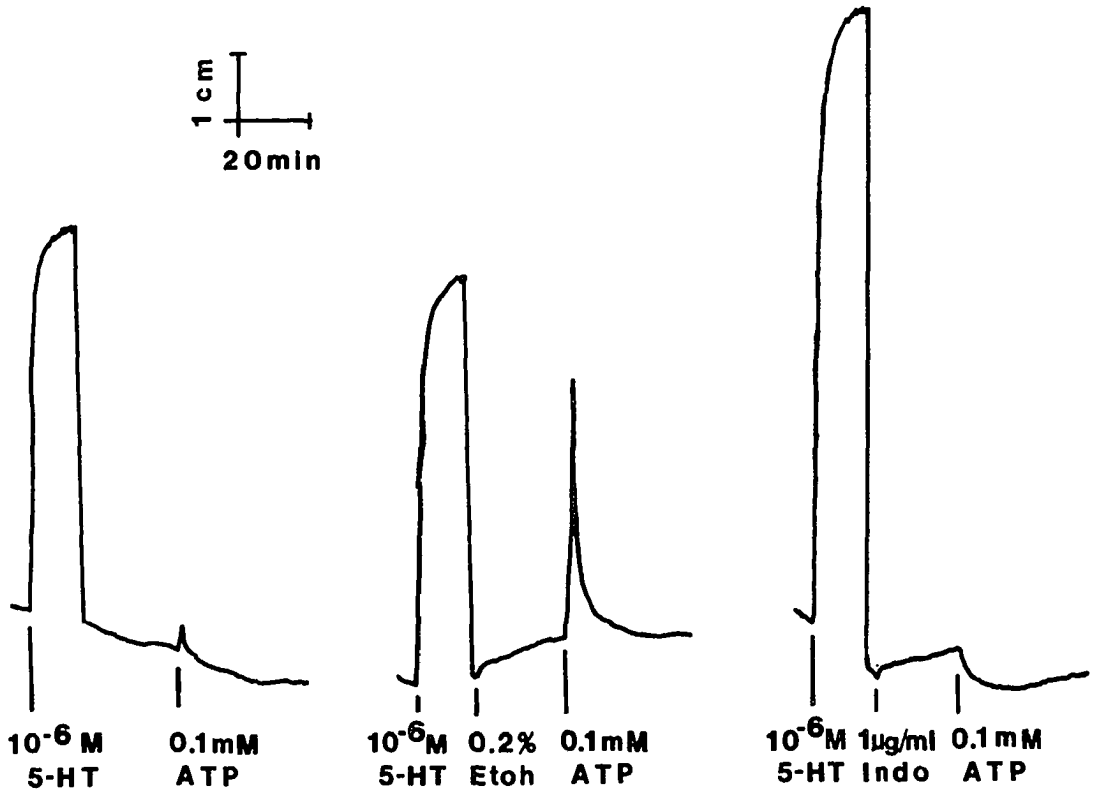

Figure 15. Effect of indomethacin (Indo) on ATP-induced contractions of isolated strips of human umbilical artery. See Figures 8 and 9 for explanation of procedure. Indomethacin (1 µg/ml), dissolved in absolute ethanol, was added to the strip on the right; final bath concentration of ethanol equaled 0.2% (V/V). Absolute ethanol alone was added to the middle strips to give a final bath concentration of 0.2% (V/V). Note that a slight contraction occurred in strips exposed to ethanol. Indomethacin (1 µg/ml) changed the contraction induced by ATP (0.1 mM) into a relaxation. Ethanol (0.2% V/V) alone appeared to potentiate ATP-induced contractions. Strips from two other umbilical arteries responded similarly. In addition, indomethacin at 2.5 µg/ml and 10 µg/ml also completely blocked the contractile response to ATP in strips from three other umbilical arteries.

DISCUSSION

Cyclic Nucleotide Levels

In a review of the biological actions of cAMP, Sutherland et al. (1968) proposed that relaxation of smooth muscle may depend on the elevation of cellular levels of cAMP. Support for this concept was presented by the findings of Volicer and Hynie (1971), Lee et al. (1972), Triner et al. (1972a), Schultz et al. (1973a), Andersson (1973a,b), and Sheperd et al. (1973). However, other reports have argued against a universal role for cAMP in smooth muscle relaxation (Collins and Sutter, 1975; Daniel and Crankshaw, 1974; Ljung et al., 1975; Lau and Lum, 1977, 1978; Diamond, 1977, 1978).

The hypothesis that cGMP may mediate vascular smooth muscle contraction whereas cAMP may mediate vascular smooth muscle relaxation was proposed by Dunham et al. (1974). Findings from the laboratories of Kadowitz et al. (1975) and Clyman et al. (1975a,b,c) have supported this hypothesis. Furthermore, the studies by Amer and coworkers have provided strong evidence which supports the idea that elevated levels of cGMP (with or without reductions in cAMP levels) are involved in controlling the tone and sensitivity of vascular smooth muscle and are an important factor in the etiology of many forms of experimental hypertension (Amer, 1977).

Recent studies, however, have provided evidence that illustrates a dissociation between increases in cGMP levels and smooth muscle contraction (Diamond and Hartle, 1974; Diamond and Holmes, 1975; Diamond and Blisard, 1976; Diamond, 1977, 1978; Katzuki and Murad, 1977; Schultz et al., 1977a). In all but one of these studies, however, smooth muscle from

other than vascular sources was analyzed. There is obviously a need for a thorough study of the involvements of cAMP and cGMP in vascular smooth muscle. Indeed, in a recent review by Namm and Leader (1976), it was emphasized that there is a lack of information about the regulation and the function of cGMP in blood vessels. Although voluminous, the literature of cAMP functions in vascular tissue is full of confusing and often conflicting results. Namm and Leader (1976) emphasized that a systematic approach is needed to elucidate the involvement of cyclic nucleotides in vascular smooth muscle. Such an approach was attempted in the present study.

In addition to a systematic approach, it was also necessary to select a suitable experimental model that would be appropriate for both biochemical and pharmacological studies. The human umbilical artery was selected because of the following unique characteristics: (1) The placental half (the part used in the present study) has been reported to be noninnervated (Ellison, 1971). Although the majority of investigators in this field believe this to be the case (Roach, 1973), not all are in agreement (Fox and Jacobson, 1969). If HUA is indeed free of innvervation, release of endogenous neurotransmitters would not be a complicating problem. (2) HUA has a high proportion of smooth muscle compared to other vascular components. Indeed, Roach (1973) stated that umbilical arteries are probably the most muscular arteries that occur in mammals. She further stated that HUA contains very little elastin and essentially no collagen, although ground substance is found in larger amounts in HUA than in other arteries.

(3) HUA is a human blood vessel that is readily obtainable for research in a relatively healthy state.

Although the extensive studies by Clyman et al. (1975a,b,c) utilized the unique characteristics of HUA and were conducted in a relatively systematic manner (i.e., cyclic nucleotide levels were measured in arterial strips exposed to a variety of vasoconstrictor and vasodilator agents in the presence of varying Ca^{2+} and O_2 levels), they failed to measure contractile changes during the experiments and were, therefore, unable to make direct comparisons between changes in cyclic nucleotide levels and changes in contractility. Nevertheless, their results have been of great value in selecting appropriate vasoactive agents with characteristics that would be useful in elucidating cyclic nucleotide function in vascular contractility. The agents that were selected are: 5-HT, histamine, PGE_1, PGE_2, $PGF_{2\alpha}$, indomethacin, and nitroglycerin.

The working hypotheses for the first part of the present study were:

(A) Cyclic GMP mediates and/or modulates contractions in vascular smooth muscle.

(B) Cyclic AMP mediates and/or modulates relaxation in vascular smooth muscle.

The two vasoactive amines, 5-HT and histamine, were selected because of the distinct difference in their ability to elevate cGMP levels in HUA. For example, the elevation of cGMP levels induced by histamine (like ACh, bradykinin, and K^+) was found to be totally dependent on extracellular Ca^{2+} (Clyman et al., 1975a). In contrast, 5-HT-induced elevation of cGMP was independent or even slightly inhibited by extracellular Ca^{2+} (Clyman et al., 1975a). Second, the magnitude of the cGMP increase caused by each

agent is noticeably different. For example, histamine, at a concentration (3 μM) that is known to produce approximately half-maximal contractions of isolated HUA strips (Dyer et al., 1972; Nair and Dyer, 1974), caused a large (~17-fold) increase in cGMP levels in incubated HUA. 5-HT, on the other hand, at a concentration (10 μM) which produces maximal contractions of HUA (Park et al., 1972), caused a much smaller increase (~3-fold) in the cGMP levels in HUA incubated without resting tension (Clyman et al., 1975c)

Under the experimental conditions of the present study, no significant changes in cAMP or cGMP levels were observed during isotonic contractions induced by cumulative doses (1 nM - 1 μM) or by single doses (10 μM) of 5-HT (Figure 3). Furthermore, in strips that were clamp-frozen at 15 sec (beginning of contraction) and at 7 min (peak of contraction) after addition of 5-HT, no changes in cAMP or cGMP levels were seen (Table 2). In all cases, 5-HT-treated strips began to contract within 5-10 sec after addition of 5-HT. These data clearly illustrate a dissociation between contraction of HUA and increases in cGMP levels and are therefore inconsistent with working hypothesis (A) above. These findings are obviously in conflict with those reported by Clyman et al. (1975c). One reason for this difference may be that the HUA strips were handled differently in the two studies. For example, strips of HUA in the present study were suspended under 1 g resting tension for 3-4 hours in an oxygenated Krebs-bicarbonate solution prior to the addition of the test agonist (see Methods). This procedure was found to be necessary if full contractile responses were to be measured, since full relaxation of HUA strips did not

occur until the strips had been incubated for at least 3-4 hours under 1 g tension. Since the experiments by Clyman et al. (1975c) were conducted with no tension on the HUA strips, it was hypothesized that tension may have an influence on the 5-HT-induced elevations of cGMP levels. To test this hypothesis, strips of HUA were incubated under conditions similar to those of the previous experiment, but with no tension put on the strips. At 7 min after the addition of 5-HT (10 μM), the strips were clamp-frozen (as above) and analyzed for cyclic nucleotide levels. In this experiment the cGMP levels were significantly elevated (by 50%) with no changes in cAMP levels (Table 3). However, it should be noted that this increase in cGMP is considerably smaller than the 300% increase reported by Clyman et al. (1975c). There are several possible explanations for this discrepancy. First, Clyman et al. (1975c) incubated the HUA strips in a Krebs-tris buffer equilibrated with room air, whereas the strips in the present study were incubated in a modified Krebs-bicarbonate solution aerated with 95% O_2-5% CO_2. Although the effect of the different perfusing solutions on tissue cGMP levels cannot be predicted at this time, it is likely that the differences in the gases used in each laboratory may have influenced the outcome of the data. To illustrate this point, Clyman et al. (1975b) found that high O_2 tension has an inhibitory effect on the 5-HT-induced elevations of cGMP levels in HUA.

A second possible explanation for the different results obtained is that different homogenization procedures were followed. In contrast to the procedure used in the present study (see Methods), Clyman et al. (1975c) homogenized HUA strips along with its medium. Therefore, Clyman

et al. (1975c) were measuring total (tissue plus medium) content of cGMP rather than tissue cGMP levels. If significant amounts of cGMP had been released by the HUA strips into the medium during the incubation period or following drug additions, total cGMP content, as measured by Clyman's method, would be obviously greater than tissue content.

A third and perhaps most intriguing possibility that can explain the differences between my results and those of Clyman et al. (1975c) is now presented. This possibility is based upon the concept that the cGMP elevations in HUA induced by 5-HT are mediated through the actions of an endogenous substance which is released by 5-HT and which in turn activates cGMP accumulation. The existence of such a mediator of the 5-HT response was predicted, based upon the above data and upon the differences in the experimental methods employed by Clyman et al. (1975c) and in the present study. For example, the strips of HUA (300-400 mg) used by Clyman et al. (1975c) were considerably larger than those (~100 mg) used in the present study. In addition, the strips in the study by Clyman et al. (1975c) were incubated in 4 ml of medium as compared to 50 ml of medium used in the present study. If an endogenous substance were released from these strips in an amount proportional to the weight of the strips, then the experimental conditions of the present study present a dilution (of the released substance) that is 40-50-fold greater than in the experiments by Clyman et al. (1975c). Therefore it would be expected that the response (increase in cGMP levels) induced by this released substance would be diminished considerably by the conditions of the present study.

Although direct evidence is lacking, findings from other laboratories have provided indirect evidence for the existence and identity of such a substance; i.e., one which is released by 5-HT and which stimulates cGMP accumulation. For example, 5-HT has been shown to increase the release of prostaglandins (PGs) from various tissues, including rat stomach (Coceani et al., 1968), isolated guinea pig lung (Alabaster and Bakhle, 1970), and isolated guinea pig ileum (Famaey et al., 1977a,b). In addition, agents such as angiotensin II and bradykinin, which have vasoconstrictive properties similar to that of 5-HT, have been found to increase the release of PGs from isolated HUA (Terragno et al., 1977) and from cultured endothelial cells from human umbilical veins (Gimbrone and Alexander, 1975). Alexander and Gimbrone (1976) further demonstrated that bradykinin, angiotensin II, histamine, and 5-HT could stimulate the release of PGs from cultured smooth muscle cells isolated from human umbilical veins. Further, Tuvemo et al. (1976a) demonstrated that isolated strips of HUA are capable of spontaneously synthesizing PG-endoperoxides (intermediates in the synthesis of all PGs) and thromboxane A_2 (TxA_2).

It was recently shown that the PG-endoperoxides can stimulate guanylate cyclase activity and the accumulation of cGMP in splenic cells (Goldberg et al., 1978) and platelets (Glass et al., 1977a,b). Glass et al. (1977a,b) further demonstrated that arachidonic acid or collagen could induce platelet aggregation and elevate cGMP in platelets--both responses were inhibited by prior incubation of the platelets with prostaglandin synthetase inhibitors, aspirin or indomethacin. Likewise, Schoepflin et al. (1977) found that 5-HT (0.5-16 μM) caused a dose-dependent increase in

cGMP levels in human platelets: This response was observed to be independent of extracellular Ca^{2+}, analogous to the 5-HT-induced elevation of cGMP in HUA (Clyman et al., 1975a). They further observed that the cGMP elevation in platelets by 5-HT was inhibited by PG-synthetase inhibitors, aspirin and indomethacin. These findings suggest that the 5-HT-induced elevation of cGMP levels in platelets is mediated through the release and subsequent action of a PG-like substance (probably PG-endoperoxide or TxA_2). An analogous mechanism may also be involved in HUA; i.e., PGG_2, PGH_2 and/or TxA_2 may be the endogenous substances that are released by 5-HT and in turn cause cGMP accumulation in HUA. It will be interesting to see if PG-endoperoxides or TxA_2 are capable of inducing cGMP accumulation in isolated HUA.

There is still the question, however, of why the 5-HT-induced elevation of cGMP in HUA is dependent on the amount of resting tension under which the strip is placed. A possible explanation is that the release of the endogenous mediator (PG-endoperoxide or TxA_2) is already stimulated prior to the addition of 5-HT in the strips under tension. Indeed, this hypothesis is supported by the present finding that cGMP levels appear to be elevated in HUA strips incubated under 1 g tension (even before 5-HT additions) when compared to strips under no tension (see Table 3). Although the elevated cGMP levels are not statistically significant at the 5% probability levels (due to the large standard error), they are considerably higher than the elevated levels of cGMP produced by 5-HT in strips under no tension (Table 3). In other words, the 1 g tension appears to stimulate cGMP accumulation in HUA. Like the 5-HT-induced

elevation in cGMP levels, the response to tension (stretch) may be mediated through the release of PG-like substances. Indeed, stretching of rat stomach (Bennett et al., 1967) and uterine tissue (Horton et al., 1971; Southern, 1972) has been shown to trigger the release of PGs. Furthermore, Piper and Vane (1971) reported that other forms of mechanical stimulation, such as gentle stroking of guinea pig lung, stimulated the release of histamine, rabbit aorta contracting substance (a mixture of PG-endoperoxides and TxA_2) and PGs. It is possible, therefore, that 5-HT is unable to further increase the release of PG-like substances or, alternatively, the guanlyate cyclase in HUA may be already maximally stimulated by released PG-like substances associated with stretch.

This proposed mechanism for cGMP accumulation may also afford an explanation for the apparent time-dependent decline in cGMP levels in HUA strips under 1 g tension (see Figure 1). Again, this effect is not statistically significant at the 5% probability level, yet there appears to be a continuous depletion of the cGMP levels throughout the length of the experiment. This decrease in cGMP levels may be due to the depletion of the endogenous PG-like substance (or more likely its precursor) following continual stimulation via stretch and/or intermittent stimulation with 5-HT.

Also of interest in this study was the effect of 5-HT on cyclic nucleotide levels in isolated strips of sheep umbilical arteries (SUA). The pharmacology of this vascular preparation has been extensively studied (Lewis, 1968; Dyer, 1970a,b) and has become of increasing interest because of the frequent use of fetal lambs as models in physiological and pharmacological studies of fetal circulation (see review by Rudolph and Heymann,

1974; Coceani and Olley, 1978). In addition, SUA has been of particular interest because of its extreme sensitivity to the vasoconstrictive effects of many hallucinogens (LSD, mescaline, bufotenine, psilocin and psilocybin) and its usefulness in studying the interactions of these agents with 5-HT receptors (Dyer and Gant, 1973; Dyer, 1974).

In the present study, 5-HT caused a time-dependent (Figure 4) and dose-dependent (Figure 5) increase in cAMP levels (without changing cGMP levels) in isolated strips of SUA. A significant rise in cAMP occurred at the lowest concentration (30 nM) of 5-HT that had produced a significant contractile response (Figure 5). However, contractions began between 5-10 sec following the addition of 5-HT (10 μM), but significant elevations in cAMP levels were not observed until 20 sec after 5-HT. Thus, the elevation in cAMP clearly lagged behind the contraction.

It can be hypothesized that 5-HT-induced increase in cAMP levels in SUA may be mediated through the release of a PG-like substance as was suggested for the cGMP rise in HUA. Indeed, many of the arguments used above could apply in this case as well. However, since cAMP levels rose in SUA, it can be assumed that the mediator is a PG such as PGE_1, PGE_2, or PGI_2--agents that are known to activate adenylate cyclase and cause accumulation of cAMP in a variety of tissues (for review, see Samuelsson et al.,1978a). The data of the present study have further demonstrated that PGE_1 and PGE_2 can cause accumulation of cAMP in HUA (see below). The recently discovered PGI_2 has been shown to stimulate the activity of adenylate cyclase and to increase cAMP levels in tissues such as platelets (Best et al., 1977; Gorman et al., 1977; Tateson et al., 1977) and in 3T3 fibroblasts

(Claesson et al., 1977). In addition, Terragno et al. (1978) has recently found that PGI_2 is the major PG synthesized by all vascular preparations that they studied from fetal, maternal and nonpregnant cows. They found that exceptionally high levels of 6 keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, was formed in fetal vessels (aorta, pulmonary artery and ductus arteriosus). Similar findings were made by Pace-Asciak and Rangaraj (1978) using the same blood vessels from fetal lambs and they concluded that PGI_2 (and perhaps PGE_2 as well) may be important factors in regulating vascular tone during fetal life.

It is possible that 5-HT preferentially stimulates the release of the PGEs or PGI_2 in SUA, whereas in HUA, 5-HT primarily stimulates the release of PG-endoperoxides or TxA_2. The reason for the different responses in the two umbilical arteries may be related to a species difference and/or to the difference in gestational age. To illustrate this difference, it should be emphasized that HUA was obtained at full term after normal vaginal delivery, whereas SUA was obtained from fetal lambs after Cesarean section on day 120-125 of gestation (full gestation in sheep $\simeq$ 147 days) (see Methods). Change in the pattern of PG synthesis has been found to occur throughout the duration of pregnancy, especially during the last few days or hours immediately before and after parturition (Challis et al., 1978; Clyman et al., 1978).

Other investigators have reported findings which indicate that PG-like substances may be mediating some of the cyclic nucleotide changes induced by vasoactive agents. For example, Andersson et al. (1975) reported that preincubation of bovine tracheal smooth muscle with indomethacin

blocked the increases in both cAMP and cGMP caused by carbachol. He further found that indomethacin could block the increase in cAMP levels but not the increase in cGMP levels in rabbit colon contracted with carbachol. In addition, Stoner et al. (1973) found that indomethacin blocked the increase in cAMP levels in guinea pig lung caused by ACh. However, results contrary to these have also been reported. For example, Schultz and Hardman (1976) were unable to detect any effect of preincubation of indomethacin on ACh-induced increases in cGMP levels in rat vas deferens.

The second vasoactive amine studied, histamine (4 μM), caused a large increase in cGMP levels but no change in cAMP levels (Figure 6); this is in agreement with the findings of Clyman et al. (1975c). However, the data of the present study clearly illustrate that the histamine-induced increase in cGMP levels lags behind the contractile effect; i.e., significant contractions but no change in cGMP levels were observed at 15 sec following addition of histamine (Figure 6). Therefore, a dissociation between contraction and increases in cGMP levels has again been demonstrated, this time based upon the time-course of the two events. The increases in cGMP levels that were observed may reflect the histamine-induced increases in intracellular Ca^{2+} concentrations, as suggested by Schultz and Hardman (1976), or may reflect the stimulatory effects of released PG-like substances, as was suggested for the actions of 5-HT above.

Another class of vasoactive agents that appeared to be potentially useful in demonstrating dissociations between changes in cyclic nucleotide levels and changes in contractility were the PGs: PGE_1, PGE_2 and $PGF_{2\alpha}$. Clyman et al. (1975c) found that PGE_1 elevated cAMP levels in HUA without

changing the levels of cGMP. Since PGE_1 had been previously shown to relax isolated strips of HUA (Hillier and Karim, 1968), Clyman et al. (1975c) proposed that relaxation of HUA caused by PGE_1 may be mediated by the increase in cAMP. However, PGE_1 does not always cause relaxation in HUA. Indeed, Park et al. (1972) showed that PGE_1 (0.1-10 μM) produced a dose-dependent contraction of isolated strips of HUA. Tuvemo et al. (1976a) has suggested that this discrepancy can be attributed to a dose-related phenomena. They found that the PGE_1 effects on HUA were biphasic; i.e., PGE_1 caused relaxation at 0.28 to 8.5 μM and contractions at 28 to 106 μM. However, no such simple relationship between PGE_1 concentrations and contractile effects were found in the present study (see below).

Clyman et al. (1975c) also found that $PGF_{2\alpha}$ (14 μM) had no effect on cGMP levels in incubated segments of HUA. Yet this concentration of $PGF_{2\alpha}$ has been associated with near maximal contractions of isolated strips of HUA (Park et al., 1972). It was therefore of interest to see if these PGs (PGE_1 and $PGF_{2\alpha}$) as well as PGE_2 could cause contractions of HUA without raising cGMP levels under the experimental conditions of the present study. In addition, it was also of interest to see if indomethacin could influence the cyclic nucleotide levels in HUA. As stated above, Tuvemo et al. (1976a) has suggested that PGs and PG-like substances are spontaneously released from isolated HUA strips and are presumed to be involved in the maintenance of vascular tone observed in this preparation. If the released PGs are in sufficient concentrations to alter cyclic nucleotide levels in HUA, then indomethacin would be expected to have an effect on cyclic nucleotide levels.

Ethanol (50 µl) was used as the vehicle for the additions of PGs and indomethacin into the 50 ml baths. Ethanol alone at a concentration (0.1% V/V) that was attained when used as the vehicle caused a dramatic reduction in cGMP levels at 4 min (40% of control levels) and at 60 min (47% of control levels) (Table 5). The decrease in cGMP and the contraction caused by ethanol occurred with a similar time-course; i.e., both events were not significantly different from control at 30 sec, but were significantly different from control at 4 and 60 min following the addition of ethanol. Reduced levels of cGMP following exposure to ethanol have also been observed in rat brain (Redos et al., 1976; Hunt et al., 1977; Volicer and Hurter, 1977; Breese et al., 1978) and in rat heart (Vesely et al., 1978). In addition, ethanol has been shown to inhibit guanylate cyclase activity in rat heart (Vesely et al., 1978) and in homogenized mouse mammary gland (Rillema, 1978). Furthermore, Stierlé et al. (1978) reported that ethanol (8-13%) stimulated the cGMP-PDE activity from the media layer of bovine aorta. Thus, it appears that ethanol may be lowering cGMP levels by inhibiting guanylate cyclase and/or stimulating cGMP-PDE activity. However, the latter response is of questionable significance, since such high concentrations (8-13%) of ethanol were used in that study (Stierlé et al., 1978).

The cGMP-depleting effect of ethanol may not be universal for all tissues, since Schultz et al. (1977b) were unable to detect any effect of ethanol on the cGMP or cAMP levels in strips of rat vas deferens. However, in their study these strips were incubated in a calcium-free medium, which by itself is known to lower cGMP levels (Schultz et al., 1973b; Schultz and Hardman, 1975, 1976). It seems likely that the ethanol, in

the study by Schultz et al. (1977b), was unable to further lower cGMP levels in the rat vas deferens because of the already low guanylate cyclase activity in the absence of Ca^{2+}. Stoner et al. (1973) also reported that ethanol had no effects on either cGMP or cAMP levels in slices of guinea pig lung. However, in this case, the lack of ethanol effects may have been due to the short incubation time (2 min).

PGE_1 at 1 μg/ml (2.8 μM) caused contractions in 4 out of 6 HUA strips (one relaxed and one showed no contractile response) and elevated cAMP levels in all strips (Table 5). The increase in cAMP levels occurred at 30 sec (beginning of contraction) as well as at 4 min (peak of contraction). PGE_2 at 1 μg/ml (2.8 μM), on the other hand, caused contractions in all 5 strips and elevated cAMP levels at 4 min but not a 30 sec following PGE_2 addition. The elevations in cAMP levels in HUA by PGE_2 appeared to develop more slowly and with less magnitude than the elevations caused by an equimolar concentration of PGE_1. $PGF_{2\alpha}$ at 1 μg/ml (2.8 μM) caused contractions in all strips and had no effect on cAMP levels. Since ethanol (the vehicle) had no influence on cAMP levels, the elevations in cAMP levels induced by PGE_1 and PGE_2 can be assumed to be due to the effects of the PGs themselves. It should be noted that the magnitude of contractions caused by the PGs were inversely related to the elevations of cAMP levels; i.e., PGE_1 caused greatest elevation of cAMP but smallest contraction, $PGF_{2\alpha}$ caused no increase in cAMP levels and largest contractions, and the actions of PGE_2 were intermediate (Table 5).

The results of the present study are in partial agreement with the findings of Dunham et al. (1974) and Kadowitz et al. (1975) who reported increases in cAMP levels in canine lateral saphenous vein by PGE_2 and in

canine lobar artery and vein by PGE_1 (10 μM), respectively. The concentration of PGs used in the study by Dunham et al. (1974) could not be easily determined because they used a superfusion technique. In contrast to the preparations used in the above studies, HUA did not relax (in most cases) following exposure to PGE_1 or PGE_2 under the experimental conditions of the present study. Therefore, it appears that there is a dissociation between increases in cAMP levels and smooth muscle relaxation. This situation is analogous to the one described by Daniel and Crankshaw (1974), who reported that norepinephrine elevated cAMP levels in rabbit pulmonary artery yet caused contractions of that vessel. They used this evidence as support for their contention that increases in cAMP are neither necessary nor sufficient for relaxation to occur in vascular smooth muscle. However, their argument is considerably diminished by the fact that norepinephrine is probably acting on two distinct receptors: 1) an α-adrenergic receptor which is associated with increased intracellular concentrations of Ca^{2+} but either no change or a decrease in cAMP levels and 2) β-adrenergic receptors which are associated with activation of adenylate cyclase and increases in cAMP levels (Bär, 1974; Namm and Leader, 1976). Indeed, Triner et al. (1972a) found that epinephrine, an agonist that acts on both α- and β-adrenergic receptors, caused both contraction and an increase in cAMP levels in rat aorta and rabbit ear artery. However, in the presence of propranolol (a β-receptor blocker) epinephrine caused a greater contraction than before and caused no increases in cAMP levels. They suggested that the contractile effect to epinephrine is modified by its action on cAMP levels.

Based upon the similarities between the effects of catecholamines and those of the E-type PGs, it is proposed that the PGs may also be acting on two distinct receptors--one involved in initiating contractions and the other involved in activating adenylate cyclase. $PGF_{2\alpha}$ can be viewed as an agonist that acts selectively on the first receptor resulting in a contraction without an elevation in cAMP levels. Data supporting the existence of multiple, distinct receptors for PGs have been presented in several recent reviews (Jones, 1977; Samuelsson et al., 1978a). Manku et al. (1978) have recently suggested that PGE_1, PGE_2, and PGI_2 are acting at distinct binding sites based upon their effects on the contractile activity of isolated rat mesenteric artery.

Levels of cGMP in HUA were not altered by any of the PGs (PGE_1, PGE_2 or $PGF_{2\alpha}$, each at 1 μg/ml [2.8 μM]) as compared to ethanol controls (Table 5). However, when compared to strips not exposed to ethanol, the cGMP levels in PG-treated strips were significantly lower at 4 min but not at 30 sec after PG addition. Since the ethanol controls also showed lowered cGMP levels at 4 min but not at 30 sec following the addition of ethanol, the lowered cGMP levels in PG-treated strips can be attributed to the ethanol effect. It appears that PGs have no effect on cGMP levels at 30 sec. This interpretation must be viewed with caution, however, since ethanol may have an influence on the ability of guanylate cyclase to be stimulated without showing an effect on cGMP levels at 30 sec. Nevertheless, the results of the present study are in direct opposition to those reported by Dunham et al. (1974) and Kadowitz et al. (1975). My results are in agreement, however, with other reports that show that $PGF_{2\alpha}$ does not alter cGMP levels in: HUA incubated without resting tension (Clyman

et al., 1975c); bovine tracheal smooth muscle (Katsuki and Murad, 1977); rat myometrium (Angles d'Auriac and Worcel, 1975; Harbon et al., 1978); and rabbit uterus (Kovách and Rubányi, 1978).

Levels of cAMP or cGMP were not significantly changed by 60 min exposure of the strips to indomethacin at 10 μg/ml (28 μM) as compared to ethanol controls. However, similar to the results obtained with the PGs, cGMP levels were significantly lower in strips preincubated in indomethacin than in those not exposed to ethanol. Therefore, the effect of indomethacin (alone) on cGMP levels could not be determined. The data indicate that indomethacin is without effect on cAMP levels in HUA under the present conditions.

The above data emphasize the need for several types of controls when utilizing PGs, indomethacin, or other lipid soluble agents. Unfortunately, this procedure is not commonly practiced. In the study by Dunham et al. (1974), no mention was made of the solvent used for PG additions. In the study by Kadowitz et al. (1975), PGs were dissolved in ethanol (100%) and added to the baths to give a final concentration of up to 0.1%. They stated that this concentration of ethanol had "no significant effect" (presumably contractile effect) on lobar vessels when administered alone. However, no mention was made of the effects of ethanol (alone) on cyclic nucleotide levels of lobar vessels or whether ethanol had been added to control strips. For these reasons, it is difficult to make direct comparisons of the effects of PGs on vascular smooth muscle reported from different laboratories.

Of particular interest in this study was the effects of nitroglycerin on cyclic nucleotide levels and contractility of isolated HUA strips.

Kimura et al. (1975) and DeRubertis and Craven (1976a,b) reported that agents such as NaN_3, NH_2OH, $NaNO_2$ and the nitroamines could stimulate guanylate cyclase activity and cause cGMP accumulations in a variety of tissues. Diamond and Blisard (1976) reported that nitroglycerin, a vasodilator with pharmacological properties similar to $NaNO_2$ and NH_2OH, caused a large (1540%) increase in cGMP levels in canine femoral arteries.

In the present study, isolated strips of HUA, previously contracted with KCl (30 mM), were exposed to nitroglycerin (3 μM) for either 30 sec or 4 min (Table 6). The KCl pretreatment had no effect on cyclic nucleotide levels. Thus, another example of a dissociation between contraction and increase in cGMP levels has been shown. Nitroglycerin caused a 29-fold increase in cGMP levels after 30 sec and a 130-fold increase in cGMP levels after 4 min (peak of relaxation) of exposure. Levels of cAMP were unchanged at 30 sec and slightly elevated at 4 min following addition of nitroglycerin. Since relaxation of each strip began between 30 and 45 sec after nitroglycerin addition, the elevation in cGMP levels clearly preceded the relaxation. Therefore, not only do the results further illustrate the dissociation between increase in cGMP and contraction; it raises the intriguing possibility that increases in cGMP levels may, in fact, mediate certain types of smooth muscle relaxation. Such a role for cGMP has been proposed by Schultz et al. (1977b).

The small but significant increase in cAMP levels at 4 min following the addition of nitroglycerin may be more apparent than real. For example, it is very likely that the extremely high levels of cGMP in the nitroglycerin-treated strips cross-reacted with the cAMP antibody during the RIA measurement of cAMP. This is a strong possibility, since the

cyclic nucleotides were not separated in this part of the study (see Methods). However, the synthesis of cAMP from ATP was recently shown to be catalyzed by the NaN_3-activated guanylate cyclase (Mittal and Murad, 1977). A related mechanism may be the cause of the increase in cAMP levels in HUA during nitroglycerin treatment.

In a recent abstract by Holzmann et al. (1978), it was reported that sodium nitroprusside (another NO_2-like vasodilator) produced a dose-dependent rise in cGMP levels that closely correlated ($r = 0.98$) with relaxation of bovine circular coronary strips. They also noted that the rise in cGMP levels caused by the highest concentration of sodium nitroprusside preceded the relaxation. Based upon these results and the results of the present study, it appeared that increases in cGMP levels may be involved in relaxation of vascular smooth muscle induced by the NO_2-like vasodilators.

Cyclic Nucleotide Derivatives

In addition to the measurement of cyclic nucleotide levels, the contractile actions of some cyclic nucleotide derivaties were also investigated on isolated strips of HUA. The derivatives of cyclic nucleotides were originally synthesized to overcome some of the problems associated with studying effects of exogenously applied cAMP (or cGMP). For example, in many isolated tissue preparations, it was found that exogenous cAMP at concentrations as much as 100 times the intracellular concentrations were without effect or were only weakly effective in eliciting responses normally associated with increases in intracellular levels of cAMP (Simon et al., 1973). The weak actions of extracellular cAMP was attributed to:

(1) poor transport of cAMP into whole cells and (2) destruction of cAMP by either extracellular or intracellular cyclic nucleotide phosphodiesterases (Simon et al., 1973). Because of its structural similarity to cAMP, exogenously applied cGMP can be assumed to share these problems.

Dibutyryl cAMP has been observed to mimic the actions (vasodilation) of agents that elevate intracellular levels of cAMP in vascular smooth muscle (Triner et al., 1971). In addition, the 8-bromo derivatives of both cAMP and cGMP have also been found to be inducers of smooth muscle relaxation (Szaduykis-Szadurski and Berti, 1972; Szaduykis-Szadurski et al., 1972). However, there are problems with interpretations of the actions of these agents. For example, adenosine has been shown to be equipotent to the cyclic nucleotide derivatives in inducing relaxation in some vascular preparations; thus, it has been suggested that the cyclic nucleotide derivatives may be acting in a nonspecific (adenosine-like) manner. Indeed, the vasodilatory actions of adenosine and other adenine compounds (e.g., ATP) on coronary (Feigl, 1974) and skeletal muscle (Rowell, 1974) blood vessels have been well-documented. Burnstock (1972, 1975) has reviewed the numerous findings that indicate the existence of "purinergic receptors" in vascular tissue as well as in many other types of tissue.

Mutschler and Tulenko (1976) reported that dibutyryl cAMP (4 mM) causes relaxation of HUA strips previously contracted with KCl or 5-HT. They further reported that this response was potentiated by theophylline (0.1 mM) and they suggested that formation of cAMP mediated relaxation in HUA. They did not, however, report the effects of adenosine or other

adenine compound on the contracted HUA strips, and therefore a nonspecific action of dibutyryl cAMP cannot be ruled out.

The results of the present study illustrate that adenosine can indeed relax KCl-contracted strips of HUA (Figure 7). The relaxations induced by dibutyryl cAMP, 8-bromo-cAMP and 8-bromo-cGMP (each at 0.1 mM) were observed to be similar in magnitude to the relaxation induced by adenosine (0.1 mM). Therefore, it is likely that the vasodilation caused by the cyclic nucleotide derivatives is due, at least in part, to the adenosine-like action of these agents. The above data are of little value in elucidating the involvement of cAMP and cGMP in the regulation of HUA contractility. However, these findings do point out the importance of using appropriate controls in experiments of this type and of taking into consideration the multiple mechanisms of action of the cyclic nucleotide derivatives.

Inhibitors of Cyclic Nucleotide Phosphodiesterase

The contractile effects of four CN-PDE inhibitors (aminophylline, caffeine, papaverine, and MIX) were studied on isolated strips of HUA. In some vascular preparations, the CN-PDE inhibitors have been found to cause relaxation and to cause inhibition of CN-PDE in similar concentration ranges (see Literature Review). However, there has been controversy concerning the interpretation of the results of experiments that utilize CN-PDE inhibitors (Namm and Leader, 1976). In addition to their inhibitory effects on CN-PDE activity, the methyl xanthines are known to alter Ca^{2+} binding and fluxes at muscle membrane (Ritchie, 1975) and papaverine has been reported to have local anesthetic properties (Andersson, 1973b).

In the present study, caffeine (3 mM) (Figure 8), papaverine (10-100 μM) (Figure 9) and MIX (30-300 μM) (Figure 9) were observed to cause relaxation of KCl-contracted strips of HUA. These concentrations are similar to the concentrations known to cause 50% inhibition of CN-PDE activity in other tissues (see Literature Review). Therefore, these data may be used as evidence favoring the hypothesis that cyclic nucleotides (either cAMP or cGMP or both) mediate relaxation in vascular smooth muscle. However, aminophylline (theophylline ethylenediamine), at concentrations up to 3.6 mM, was found to be ineffective as a relaxant on KCl-contracted strips of HUA (Figure 8). Since theophylline is known to inhibit CN-PDE activity with a potency equal to or greater than that of caffeine (Butcher and Sutherland, 1962), it appears that aminophylline may be acting by a mechanism unrelated to inhibition of CN-PDE activity. The possibility that the other agents (caffeine, MIX and papaverine) may be acting by mechanisms other than inhibition of CN-PDE must be kept in mind.

Speculations on the Involvement of Cyclic Nucleotides in the Regulation of Contractility

Besides the cyclic nucleotide phosphodiesterases, the only other cellular protein that has been found to possess a high affinity binding site for cAMP is the regulatory subunit of cAMP-dependent protein kinase (Nimmo and Cohen, 1977). This observation coupled with the observed widespread occurrence of cAMP-dependent protein kinase led to the proposal that most if not all of the actions of cAMP in mammalian systems are mediated by a cAMP-dependent protein kinase-catalyzed phosphorylation of cellular proteins (Kuo and Greengard, 1969). There are numerous

endogenous substrates for cAMP-dependent protein kinase and this fact may be related to the multiple actions of cAMP within cells (Nimmo and Cohen, 1977). The substrates of protein kinase which are of particular interest with regards to smooth muscle contractility will be discussed below.

A protein kinase, which was specifically dependent upon cGMP, was isolated and partially purified by Kuo and Greengard (1970). The enzyme was found to have a substrate specificity that overlapped that of cAMP-dependent protein kinase (Lincoln and Corbin, 1978). However, Casnellie and Greengard (1974) found that the cGMP-dependent protein kinase in three smooth muscle preparations (guinea pig ductus deferens, guinea pig uterus, and rabbit small intestine) specifically catalyzed the phosphorylation of two endogenous proteins: protein G-I (130,000 dalton) and protein G-II (100,000 dalton). A half-maximal increase in the phosphorylation of these proteins occurred with 20-30 nM cGMP and approximately 10-fold higher concentrations of cAMP were required to produce a similar increase. Despite these observations, the functional role of cGMP and cGMP-dependent protein kinase in smooth muscle as well as in other tissues still remains obscure (Lincoln and Corbin, 1978).

It has been postulated that cAMP regulates intracellular concentrations of Ca^{2+} in vascular smooth muscle; this in turn regulates the contractile state of the tissue (Andersson, 1973a). Calcium ion appears to be an essential component in the regulation of contractility in smooth muscle (Somlyo and Somlyo, 1968, 1970; Hurwitz and Suria, 1971; Fleckenstein, 1977), as well as in cardiac (Fozzard, 1977) and skeletal (Ebashi, 1976) muscle. In general, changes in the concentration of Ca^{2+} that is in con-

tact with the contractile proteins leads to changes in the contractile state of the muscle (Somlyo and Somlyo, 1968). However, in smooth muscle, changes in the concentration of Ca^{2+} in this pool can result from the translocation of Ca^{2+} from many sources: extracellular Ca^{2+} and/or intracellular Ca^{2+} associated with the plasma membrane, sarcoplasmic reticulum, or mitochondria (Hurwitz, 1977). The source of Ca^{2+} involved in contractile changes appears to differ depending upon the type of smooth muscle and upon the form of stimulation (e.g., electrical, K^{+}, hormone, etc.) (Hurwitz, 1977).

In cardiac muscle, it was found that cAMP stimulated the uptake of Ca^{2+} into a membranous fraction (microsomes) (Kirchberger et al., 1972; Katz et al., 1975). The stimulation of this Ca^{2+} uptake has been associated with a cAMP-dependent phosphorylation of a 22,000-dalton protein, named phospholamban (Katz et al., 1975). It was suggested that this cAMP-activated mechanism may mediate the increased rate of relaxation of cardiac muscle following exposure to agents (e.g., catecholamines) that increase cAMP levels (Katz et al., 1975). A similar mechanism may be involved in the smooth muscle relaxation associated with increases in cAMP (or cGMP) levels. For example, Bhalla et al. (1978) recently found that cAMP-dependent protein kinase catalyzes the phosphorylation of a 44,000-dalton protein and increases the Ca^{2+} uptake in rat aortic microsomes. They also showed that the phosphorylation of this protein was reversed by an intrinsic phosphoprotein phosphatase. Thus the reversible nature of this mechanism was demonstrated and therefore adds support to the hypothesis that this mechanism is of physiological significance. A recent report by Thorens and Haeusler (1978) indicates that both cAMP and cGMP can

influence the phosphorylation and Ca^{2+} uptake into rat aortic microsomes. However, Clyman et al. (1976) was unable to detect any effects of cAMP or cGMP (in the presence or absence of added cyclic nucleotide-dependent protein kinase and kinase modulator) on Ca^{2+} uptake into microsomal and mitochondrial fractions prepared from human umbilical arteries. Therefore, there does not appear to be agreement on a role for cAMP or cGMP in regulating the uptake of Ca^{2+} in membranous preparations from vascular smooth muscle.

A second possible site for the involvement of cAMP (or cGMP) in regulating smooth muscle contractility is at the contractile proteins themselves. Numerous articles have recently appeared that report the isolation and characterization of the contractile protein from vertebrate smooth muscle (Adelstein et al., 1976; Perry, 1976; Bremel et al., 1977; Hartshorne and Aksoy, 1977; Hartshorne et al., 1977; Sobieszek, 1977; Adelstein, 1978; Sherry et al., 1978). These authors agreed that the Ca^{2+}-regulation of actomyosin in smooth muscle involves the myosin component, as was found earlier in invertebrate muscle (Kendrick-Jones et al., 1970). This regulation system proposed for smooth muscle is in direct contrast to the complex interactions of the troponin-tropomyosin-actin complex that regulates the contractile state in skeletal and cardiac muscle (Ebashi, 1976).

In smooth muscle from chicken gizzard (Aksoy et al., 1976; Górecka et al., 1976; Sobieszek and Small, 1977), pig stomach (Small and Sobieszek, 1977) and guinea pig vas deferens (Chacko et al., 1977), it was found that the 20,000-dalton light chain of myosin was phosphorylated by a

specific Ca-dependent myosin light chain kinase and that this phosphorylation was essential for the actin-activated ATPase activity. However, this proposed mechanism has been challenged by Takashi et al. (1977).

Myosin light chain phosphatase activity (i.e., dephosphorylation of myosin light chain) has been observed in smooth muscle preparations from bovine carotid artery and stomach (Frearson et al., 1976), chicken gizzard (Dabrowska et al., 1977) and pig stomach (Small and Sobieszek, 1977). In general, myosin phosphorylation appears to be regulated by a balance between the activities of myosin light chain kinase and myosin light chain phosphatase.

An exciting finding was recently reported by Adelstein et al. (1978) which provides evidence for a direct link between changes in cAMP levels and changes in contractile protein interactions in vertebrate smooth muscle. They found that the catalytic subunit of cAMP-dependent protein kinase phosphorylates the myosin light chain kinase from turkey gizzard smooth muscle. This phosphorylation of the myosin light chain kinase resulted in a 2-fold decrease in the rate at which the enzyme phosphorylates the 20,000-dalton light chain of myosin. Since phosphorylation of the light chain has been shown to be essential for actin-activated ATPase activity and probably for smooth muscle contraction, it was proposed that an increase in cAMP levels, which in turn activates the cAMP-dependent protein kinase, could decrease the activity of myosin light chain kinase and therefore reduce the activation of the contractile machinery. In addition, the reduced activity of myosin light chain kinase would allow the myosin light chain phosphatase to dephosphorylate the 20,000-dalton light chain and lead to relaxation. It will be interesting to see if

myosin light chain kinase can serve as a substrate for cGMP-dependent protein kinase as well.

Another substrate of cAMP-dependent protein kinase that may influence the interactions of contractile proteins in smooth muscle is filamin. Filamin is a high molecular weight (~240,000 dalton) protein that is abundant in smooth muscle, binds to actin in vitro, and is associated with the microfilament bundles in intact smooth muscle cells (Wang et al., 1975; Wang, 1977; Wang and Singer, 1977). Filamin has been shown to markedly reduce the actin activation of heavy meromyosin ATPase activity (Davies et al., 1977). In addition, Wallach et al. (1978) recently reported that the phosphorylation of filamin is stimulated by cAMP, but not by cGMP or by Ca^{2+}. However, at the present time, it has not been determined whether the phosphorylation of filamin alters its ability to bind to actin or its ability to inhibit actin activation of heavy meromyosin ATPase activity (personal communication with Dr. Peter J. Bechtel, Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa).

It therefore appears that, potentially, cAMP and cGMP may be involved at several sites in the regulation of smooth muscle contractility. However, further evidence is needed to establish a mediator or modulator role for cAMP and cGMP in contractile changes in smooth muscle.

Effects of Indomethacin on Contractility

Tuvemo (1978) has reported that released PG-like substances (probably PG-endoperoxides and TxA_2) are responsible for the maintenance of muscle tone in isolated strips of HUA. He showed that indomethacin (8-40 µg/ml) caused relaxation of HUA strips and inhibited the accumulation

of PGs and TxB_2 in the surrounding medium. Alexander and Gimbrone (1976) have reported that vasoconstrictors, such as bradykinin, angiotensin II, histamine, and 5-HT, stimulated the release of PGs from cultured vascular smooth muscle cells isolated from human umbilical veins.

In the present study, experiments were conducted to determine the influence of released PG-like substances in the vasoconstrictive actions of several agonists (5-HT, KCl, $PGF_{2\alpha}$, and ATP) on isolated strips of HUA. Strips were incubated for 30 min in indomethacin prior to the addition of the vasoconstrictors. To determine the concentrations of indomethacin which inhibit PG synthesis in isolated strips of HUA under the experimental conditions of the present study, strips were incubated in indomethacin at 1, 2.5, and 10 μg/ml and the strips were then exposed to cumulative doses of arachidonic acid, the precursor for the synthesis of the vasoconstrictor PG-like substances. Contractions induced by arachidonic acid at 1 μg/ml and at 10 μg/ml were significantly reduced by indomethacin (2.5 μg/ml) to 10% and 30% of control contractions, respectively (Figure 10). The data indicate that the capacity of HUA strips to synthesis PG-like vasoconstrictor substances is severely depressed following 30 min of incubation in indomethacin at a concentration of 2.5 μg/ml.

It has been reported, however, that indomethacin has many other actions in biological preparations in addition to its ability to inhibit PG synthesis (Ferreira and Vane, 1974). One of the actions of indomethacin is to interfere with Ca^{2+} uptake, binding, and translocation at the smooth muscle membrane (Northover, 1971, 1973, 1977). Northover (1977) has attributed these actions of indomethacin to the general depressant effects of indomethacin on vascular preparations. In fact, there have been

numerous reports that indomethacin antagonizes the vasoconstrictor effects of various agonist (epinephrine, norepinephrine, histamine, bradykinin, 5-HT, angiotensin II, arginine vasopressin, K^+, Ca^{2+}, and Ba^{2+}) in vascular preparations (Northover, 1967a,b, 1968; Manku and Horrobin, 1976). In addition, Görög and Kovàcs (1970) reported that indomethacin inhibits the superprecipitation and Mg^{2+}- and Ca^{2+}-ATPase activity of vascular actomyosin; thus, a direct effect of indomethacin on contractile proteins has been demonstrated. However, these nonspecific effects (i.e., effects other than PG synthesis inhibition) of indomethacin were observed at concentrations (20-500 µg/ml) considerably higher than the concentration (2.5 µg/ml) needed to inhibit PG synthesis (Whitehouse, 1964; Northover, 1971; Ferreira and Vane, 1974).

To determine the concentrations of indomethacin that are associated with nonspecific vasodilatory effects in HUA, indomethacin (10, 25 and 65 µg/ml) was added to KCl-contracted strips of HUA. Indomethacin at 10 µg/ml was found to be ineffective as a vasodilator, but indomethacin at 25 and 65 µg/ml caused noticeable relaxation of the KCl-contracted strips of HUA (Figure 11). It appears, therefore, that relaxation of KCl-contracted strips of HUA induced by indomethacin is a nonspecific effect, since it occurred at concentrations (25 and 65 µg/ml) of indomethacin much higher than are needed to inhibit PG synthesis.

Contractions of HUA strips induced by cumulative doses of 5-HT (Figure 12), KCl (Figure 13), and $PGF_{2\alpha}$ (Figure 14) were significantly reduced by indomethacin at high concentrations (25 and 65 µg/ml). In general, the dose-response curves of these vasoconstrictors appear to be

shifted to the right and the maximal responses were reduced in the presence of indomethacin (25 and 65 μg/ml). Indomethacin at 10 μg/ml significantly reduced the contractions elicited by the vasoconstrictors at some, but not all, of the agonist concentrations tested. However, indomethacin at 2.5 μg/ml had no effect on the 5-HT-, KCl-, or $PGF_{2\alpha}$-induced contractions of HUA strips (Figures 12, 13 and 14). The data indicate that contractions of HUA induced by 5-HT, KCl, and $PGF_{2\alpha}$ are not significantly influenced by the inhibition of PG synthesis and, therefore, probably are not mediated by the actions of released PG-like substances. Since indomethacin at the highest concentrations (25 and 65 μg/ml) did significantly reduce the contractions to these three agents, a general depressant effect to indomethacin, similar to that observed by Northover (1967a,b, 1968) and by Manku and Horrobin (1976), was probably involved in the present study.

In should be noted that indomethacin (10 and 65 μg/ml) appeared to be more effective in antagonizing contractions induced by $PGF_{2\alpha}$ than those induced by 5-HT or KCl. This observation is similar to the report by Sorrentino et al. (1972), who found that indomethacin at 10-40 μg/ml inhibited contractions of isolated rat uterus and guinea pig ileum induced by PGE_2, but that concentrations of 40-160 μg/ml of indomethacin were needed to produce equivalent antagonism of contractions induced by histamine, 5-HT, and bradykinin. The greater susceptibility of $PGF_{2\alpha}$-induced contractions to indomethacin antagonism may reflect differences in the mechanisms of excitation-contraction coupling stimulated by these vasoconstrictors. For example, $PGF_{2\alpha}$, when compared to KCl, has been shown to be more dependent

on the release of intracellularly stored Ca^{2+} than on increased influx of extracellular Ca^{2+} (Ishizawa and Miyazaki, 1978). Indomethacin may be more effective in inhibiting the release of intracellularly stored Ca^{2+} than in blocking Ca^{2+} influx; although partial antagonism of the latter response also appears to occur based upon the present data with KCl-induced contractions (Figure 13). An alternative explanation may be that indomethacin antagonizes the interaction of $PGF_{2\alpha}$ at the PG receptor.

The contractions induced by ATP (0.1 mM) in HUA strips were completely inhibited by indomethacin at 1, 2.5, and 10 μg/ml (Figure 15). In fact, the contractile response to ATP in the presence of indomethacin (1-10 μg/ml) was changed to a relaxation (Figure 15). The data indicate that the vasoconstrictive action of ATP may be mediated through the actions of released PGs or TxA_2. The ATP-induced contractions of HUA are very transient, returning to resting levels within 2-3 min (Figure 15). Since the contractions produced by PG-endoperoxides or TxA_2 are also transient, in contrast to the more sustained contractions induced by the PGs (Tuvemo, 1978), it is proposed that PG-endoperoxide and/or TxA_2 are mediators of the contractile response to ATP.

The data of the present study are consistent with the findings of Needleman et al. (1974), who found that ATP (as well as ADP, but not AMP or adenosine) stimulated the release of PGs from a variety of isolated organs (kidney, spleen, spleen fat pads, heart, liver, and lung). In fact, of all of the stimulating agents (angiotensin, epinephrine, and ischemia) tested, ATP was the only one that consistently stimulated PG release from all of the tissues. Isakson et al. (1976, 1978) have found that indomethacin (infused at 1 μg/min) completely inhibited the PG release

stimulated by ATP, angiotensin, bradykinin and ischemia from heart and kidney. In addition, Burnstock et al. (1975) found that indomethacin at 20-50 μM (7.1-18 μg/ml) completely abolished the "rebound contractions" of the guinea pig taenia coli following stimulation of "purinergic" nerves or following the addition of exogenous ATP (1.7 μM). They stated that indomethacin at 10 μM (3.8 μg/ml) and at 5 μM (1.9 μg/ml) produced 50-70% and 25% inhibition of "rebound contractions" respectively. Thus, it appears that the actions of ATP on smooth muscle may be mediated by the actions of released PG-like substances.

It is interesting that the vehicle (ethanol) for indomethacin delivery, when given alone, appeared to potentiate the ATP-induced contractions of isolated strips of HUA (Figure 15). Based upon the limited evidence, it is suggested that ethanol may facilitate the ATP-induced release of the vasoconstrictor PG-like substances.

SUMMARY

1) 5-Hydroxytryptamine, in cumulative doses or single doses sufficient to produce near maximal contractions, had no effect on either cAMP or cGMP levels in isolated strips of human umbilical arteries (HUA) under 1 g tension. Therefore, a dissociation between contractions and increases in cGMP levels was demonstrated.
2) In strips of HUA with no resting tension, 5-HT induced a small (50%) increase in cGMP levels.
3) In isolated strips of sheep umbilical arteries, 5-HT initiated a dose-dependent and time-dependent increase in cAMP levels with no change in cGMP levels. Again, a dissociation between contractions and increases in cGMP levels was demonstrated.
4) The histamine-induced contractions of HUA strips were associated with a large (7-fold) increase in cGMP levels with no change in cAMP levels. However, since the increase in cGMP levels lagged behind the contractile response, a mediator role for cGMP in histamine-induced contractions is not indicated by the data.
5) Ethanol induced a small but persistent contraction of HUA strips and lowered the cGMP levels to 40% of control levels with no change in cAMP levels.
6) PGE_1 and PGE_2 induced an increase in cAMP levels but did not change cGMP levels when compared to ethanol controls.
7) $PGF_{2\alpha}$ did not change cAMP or cGMP levels when compared to ethanol controls.

8) KCl (30 mM) which produced near maximal contractions of isolated strips of HUA had no effect on cAMP and cGMP levels.

9) Nitroglycerin caused a large (29-fold) increase in cGMP levels that preceded relaxation and a still larger (130-fold) increase in cGMP levels at the peak of relaxation. The data are consistent with a mediator role for cGMP in nitroglycerin-induced relaxation.

10) The dibutyryl and 8-bromo derivatives of cAMP and cGMP relaxed KCl-contracted strips of HUA. However, since adenosine (at the same concentration) similarly relaxed these strips, the actions of these derivatives may be due to interactions at nonspecific sites.

11) Adenosine and adenine nucleotides (including cAMP) initiated contractions of previously relaxed strips of HUA.

12) The cyclic nucleotide phosphodiesterase inhibitors, papaverine, 1-methyl-3-isobutyl xanthine, and caffeine (listed in order of decreasing potency), relaxed KCl-contracted strips of HUA. Another cyclic nucleotide phosphodiesterase inhibitor, aminophylline (3.6 mM), appeared to potentiate the sustained contractions induced by KCl.

13) Contractions of HUA strips induced by arachidonic acid (the precursor for the synthesis of vasoconstrictor PG-like substances) were severely antagonized by preincubation in indomethacin at 2.5 μg/ml.

14) Much higher concentrations (25 and 65 μg/ml) of indomethacin were needed to relax KCl-contracted strips of HUA. This observation was used as an example of the nonspecific action of indomethacin on vascular smooth muscle.

15) Contractions induced by 5-HT, KCl, and $PGF_{2\alpha}$ were antagonized by indomethacin at 25 and 65 μg/ml, but not at 2.5 μg/ml. These data

indicate that the contractile responses to 5-HT, KCl, and $PGF_{2\alpha}$ are probably not mediated by released PG-like substances. However, the contractions induced by these agents may be antagonized by the non-specific actions of indomethacin at high concentrations (25 and 65 μg/ml).

16) Contractions of HUA strips induced by ATP were completely inhibited by indomethacin (1 μg/ml) and therefore the contractile responses to ATP may be attributed to the actions of released PG-like substances.

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