Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5122–5125, August 1983
Neurobiology

Antischizophrenic drugs of the diphenylbutylpiperidine type act as calcium channel antagonists

(pimozide/fluspirilene/[^{3}H]nitrendipine/neuroleptic drug/dopamine receptor)

ROBERT J. GOULD, KENNETH M. M. MURPHY, IAN J. REYNOLDS, AND SOLOMON H. SNYDER*

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

Contributed by Solomon H. Snyder, May 5, 1983

ABSTRACT **Antischizophrenic neuroleptic drugs of the diphenylbutylpiperidine class, which includes pimozide, fluspirilene, penfluridol, and clopimozide, inhibit [^{3}H]nitrendipine binding with IC_{50} values of 13–30 nM. This inhibition involves receptors for the verapamil/prenylamine class of calcium channel antagonists. These diphenylbutylpiperidines also inhibit potassium-induced calcium-dependent contractions of rat vas deferens at concentrations of 40–350 nM. Other phenothiazine and butyrophenone neuroleptics lack such potent calcium-antagonist actions. Diphenylbutylpiperidines also differ from other neuroleptics in their ability to relieve negative symptoms of schizophrenia, such as emotional withdrawal, as well as the positive symptoms which respond to all neuroleptics. We suggest that these unique antischizophrenic actions are related to a blockade by diphenylbutylpiperidines of voltage-operated calcium channels.**

The neuroleptic drugs are chemically diverse compounds that share the ability to relieve schizophrenic symptoms. Their antischizophrenic actions are thought to involve blockade of dopamine receptors, since their relative potencies at D_2 dopamine receptors correlate closely with clinical efficacy (1, 2). Although the influences of various neuroleptics on the positive symptoms of schizophrenia, delusions and hallucinations, are similar, other actions vary among the drugs. Neuroleptics have various tendencies to elicit extrapyramidal parkinsonian side effects related to their various muscarinic anticholinergic actions (3, 4). Sedating and hypotensive effects of certain neuroleptics are associated with blockade of α-1 adrenergic receptors (1, 5).

Most neuroleptic drugs relieve the positive symptoms of schizophrenia but are less effective for the negative symptoms, such as emotional withdrawal and poverty of speech and affect (6). One neuroleptic class, the diphenylbutylpiperidines (DPBPs), including pimozide, clopimozide, fluspirilene, and penfluridol, do ameliorate negative as well as positive schizophrenic symptoms (7–14).

Voltage-dependent calcium channels that mediate functions of smooth muscle and cardiac muscle and neural tissue are blocked by calcium channel-antagonist drugs (15, 16). Receptor sites for the dihydropyridine class of calcium channel-antagonist drugs can be labeled with ligands such as [^{3}H]nitrendipine (17–19). Calcium channel-antagonist drugs of the verapamil and diltiazem class do not interact directly with sites labeled by [^{3}H]nitrendipine but act at a distinct site that is allosterically linked to [^{3}H]nitrendipine sites (18, 19). The DPBPs display structural similarities to verapamil-like drugs (Fig. 1). Whereas most neuroleptics augment prolactin release from the pituitary by blocking dopamine receptors, the DPBPs can inhibit prolactin release and smooth muscle contraction, possibly by impairing calcium influx (20–23). In this paper, we report that DPBP neuroleptics are calcium channel antagonists with potencies comparable with their potencies in blocking dopamine receptors. We suggest that the unique psychoactive effects of these drugs are related to calcium channel antagonism in the brain.

MATERIALS AND METHODS

Binding assays in membranes prepared from male Sprague–Dawley rat (Charles River Breeding Laboratories) cerebral cortices, filtration, and liquid scintillation counting were carried out as described (17, 18). Drug solutions (1 mM) were prepared fresh daily in 2.5 mM HCl/50% ethanol. Appropriate dilutions were made from these stock solutions. Pimozide, clopimozide, and fluspirilene were obtained from Janssen Pharmaceutica (Beerse, Belgium). Penfluridol was from McNeil Pharmaceutical (Spring House, PA). Other suppliers were [^{3}H]nitrendipine, [^{3}H]spiperone, and [^{3}H]WB-4101, New England Nuclear; nifedipine, Miles, New Haven, CT.

Vasa deferentia from male Sprague–Dawley rats were removed following cervical dislocation and exsanguination. After being dissected free of mesentery and blood vessels, each vas was mounted in an organ bath containing high-K saline buffer [composition (mM), NaCl, 47; KCl, 80; $NaHCO_3$, 25; glucose, 11; KH_2PO_4, 0.9] under an initial tension of 0.5 g at 37°C and gassed with 95% O_2/5% CO_2. The tissue was allowed to equilibrate for 60 min during which time, it was washed several times.

Contractions, measured isometrically and displayed on a Grass polygraph, were elicited by the cumulative addition of $CaCl_2$ at four or five concentrations. The tissue was then washed and after 20 min a second dose–response relationship for $CaCl_2$ was obtained. Twenty minutes after addition of the diphenylbutylpiperidines, a dose–response for $CaCl_2$ relationship was determined.

The interaction between $CaCl_2$ and the drugs was analyzed using a Schild plot (24). The dose ratio (DR) is the ratio of the agonist concentrations required to produce equal responses in the presence and absence of inhibitor. A plot of logarithm (DR-1) vs. logarithm (inhibitor concentration) that yields a straight line with a slope of 1.0 indicates a competitive interaction between inhibitor and receptor. When logarithm (DR-1) is equal to zero, the intersection of the slope gives the pA_2 value of the inhibitor, where the pA_2 is the concentration of inhibitor that gives a dose ratio of 2 and approximates the affinity (K_{app}) of the drug for its receptor.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DPBP, diphenylbutylpiperidine; DR, dose ratio.

* To whom reprint requests should be addressed.

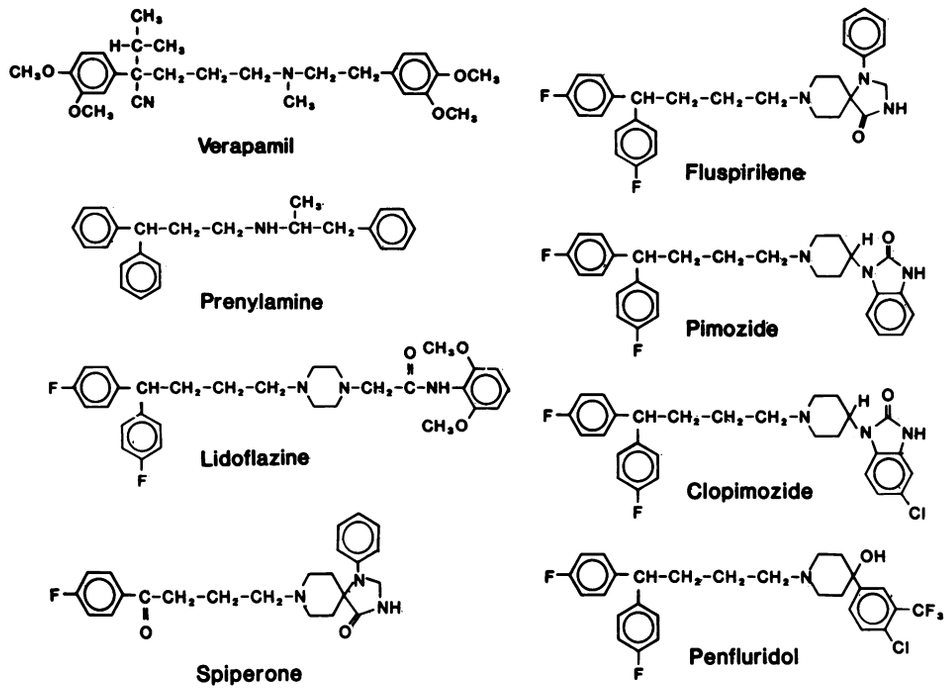

FIG. 1. Calcium channel antagonists and neuroleptic drugs.

RESULTS

The DPBP neuroleptic drugs inhibit binding of [^{3}H]nitrendipine (Table 1 and Fig. 2) with 50% inhibition by pimozide, clopimozide, fluspirilene, and penfluridol at 13–30 nM. These DPBP effects appear unique as the neuroleptics spiperone and haloperidol, which are structurally similar to the DPBPs (Fig. 1), are only 1–2% as potent at [^{3}H]nitrendipine sites. The phenothiazine neuroleptics trifluoperazine, prochlorperazine, and *cis*-clopenthixol (data not shown) are also ≈1% as potent as the DPBP neuroleptics while the benzamide neuroleptic sulpiride is ineffective at 100 μM.

The DPBP neuroleptics have similar potencies at [^{3}H]nitrendipine sites and at D_2 dopamine receptors labeled with [^{3}H]-spiperone (Table 1). By contrast, with the exception of thioridazine, other neuroleptics are at least 100 times as potent at dopamine receptors than at [^{3}H]nitrendipine sites. At α-1 adrenergic receptors labeled with [^{3}H]WB-4101, the DPBPs bind relatively weakly (Table 1) (1).

Earlier, we showed that calcium antagonists of the verapamil class influence [^{3}H]nitrendipine binding in an allosteric fashion even though some of these, such as lidoflazine, seem competitive because they displace [^{3}H]nitrendipine binding completely with a pseudo Hill coefficient of 1.0. The DPBP neuroleptics also inhibit [^{3}H]nitrendipine binding completely with competition curves that are parallel to those of the dihydropyridines and have pseudo Hill coefficients of 0.8–1.0 (Fig. 2).

Lidoflazine allosterically modulates [^{3}H]nitrendipine binding by competing with verapamil and related drugs at a single site (18). Thus, the inhibition of [^{3}H]nitrendipine binding by lidoflazine and flunarizine can be reversed by methoxyverapamil (D-600) (18). To ascertain whether the DPBP neuroleptics act at the verapamil sites or at the dihydropyridine binding site, we explored the influence of methoxyverapamil on the inhibition of [^{3}H]nitrendipine binding by DPBPs (Fig. 3). Inhibition of [^{3}H]nitrendipine binding by fluspirilene and pimozide (Fig. 3) as well as by clopimozide and penfluridol (data not shown) is reversed by methoxyverapamil. Methoxyverapamil alone maximally inhibits [^{3}H]nitrendipine binding about

Table 1. Effects of neuroleptic drugs on dopamine, calcium-antagonist, and α-1 adrenergic receptor binding sites

		Inhibition of ^{3}H-labeled ligand binding (IC_{50}), nM		
	Schizophrenic symptom targets	[^{3}H]Spiperone (striatum; dopamine)	[^{3}H]Nitrendipine (cortex; calcium channels)	[^{3}H]WB4101 (cortex; α-1 adrenergic receptors)
Diphenylbutylpiperidines	Negative, positive			
Pimozide		4	13	200
Clopimozide		10	17	250
Fluspirilene		2.2	21	350
Penfluridol		16	30	1,250
Diphenylbutylpiperazine				
Lidoflazine	ND	940	650	ND
Phenothiazines	Positive			
Thioridazine		125	1,250	7.1*
Trifluoperazine		10	2,500	67*
Butyrophenones	Positive			
Haloperidol		8	2,100	14*
Spiperone		2	2,500	14*
Benzamide	Positive			
Sulpiride		600	NE	ND

Concentrations necessary to inhibit 50% of the binding obtained with 0.2 nM [^{3}H]nitrendipine, [^{3}H]spiperone, or [^{3}H]WB-4101 were determined. Nonspecific binding was defined as that which occurred in the presence of 100 nM nifedipine, 5 μM chlorpromazine, or 0.1 mM norepinephrine, respectively. Values are means of two or three experiments, done in triplicate, using five to seven drug concentrations. The positive symptoms of schizophrenia are typically seen in acute (type I) schizophrenia or schizophreniform disorder and include hallucinations, delusion, and thought disorder. The negative symptoms, characteristic of chronic or type II schizophrenia, are affective flattening, poverty of speech, and loss of drive (6). NE, no effect up to 0.25 mM; ND, not determined.

*Taken from ref. 1.

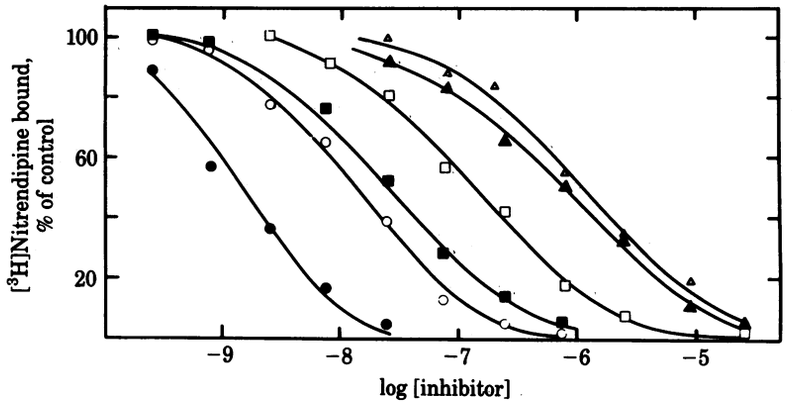

FIG. 2. Inhibition of [^{3}H]nitrendipine binding by nifedipine (●), pimozide (○), fluspirilene (■), prenylamine (□), lidoflazine (▲), and spiperone (△). Dose–response curves for the inhibition of 0.2 nM [^{3}H]nitrendipine binding were constructed. Values are means of two or three experiments, each performed in triplicate.

40%. However, in the presence of concentrations of DPBPs that themselves greatly reduce or abolish [^{3}H]nitrendipine binding, methoxyverapamil restores binding to a level comparable with the inhibition observed with methoxyverapamil alone. These findings indicate that methoxyverapamil and the DPBPs act at the same site and that the DPBPs are more efficacious in allosterically reducing the affinity for the [^{3}H]nitrendipine binding site. Numerous calcium channel-antagonist

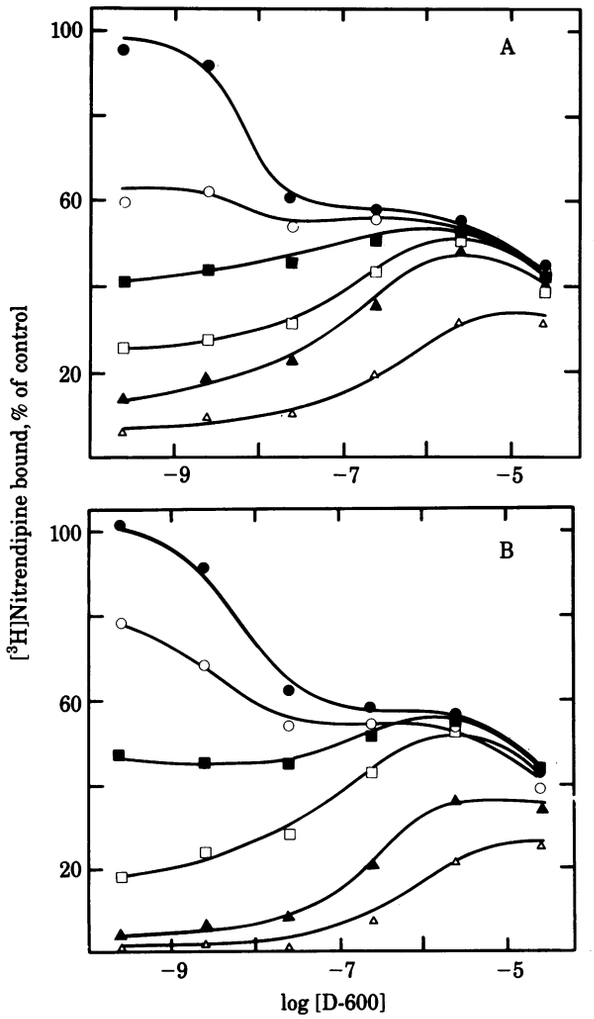

FIG. 3. Methoxyverapamil (D-600) reverses the inhibition of [^{3}H]-nitrendipine binding produced by fluspirilene (*A*) or pimozide (*B*). Dose–response curves for the influence of D-600 on [^{3}H]nitrendipine binding to membranes were obtained with six concentrations of D-600 and 0.2 nM [^{3}H]nitrendipine. [^{3}H]Nitrendipine binding was determined at each concentration of D-600 in the absence (●) or presence of 7.5 nM (○), 25 nM (■), 75 nM (□), 250 nM (▲), or 750 nM (△) fluspirilene or pimozide.

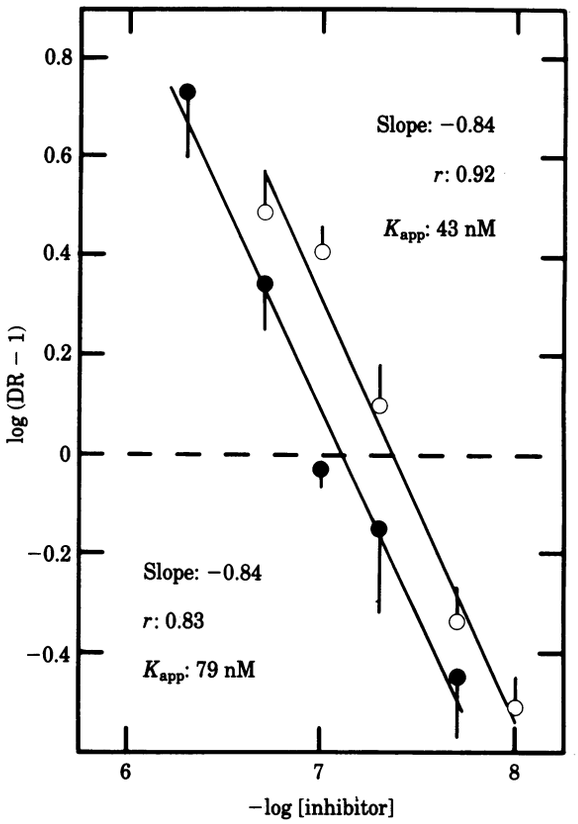

FIG. 4. Effect of pimozide and fluspirilene on potassium-induced calcium-dependent contractions of rat vas deferens. The inhibition of $CaCl_2$-dependent contractions of vasa deferentia depolarized by 80 mM KCl was plotted by the method of Schild (24). For each estimation, $CaCl_2$ was added at 2 min intervals. Three estimations were made to obtain a mean for the control curve while single determinations were used for the response in the presence of fluspirilene (●) or pimozide (○). The dose ratio (DR) is the ratio of the concentration of $CaCl_2$ required to elicit responses equal to 50% of the maximum control response observed. Each point represents mean ± SEM ($n = 6$). Fluspirilene gave a slope of −0.84, a correlation coefficient (r) of 0.83, and K_{app} = 79 nM; pimozide gave a slope of −0.84, r = 0.92, and K_{app} = 0.43 nM. These slopes are consistent with competitive interaction.

drugs act at the verapamil site but have various efficacies in allosterically reducing [^{3}H]nitrendipine binding (18). The calcium channel antagonist diltiazem also acts at this site but allosterically enhances [^{3}H]nitrendipine binding (18).

To determine whether the influences of DPBPs on [^{3}H]nitrendipine binding reflect pharmacologically relevant calcium channel blockade, we monitored drug effects on potassium-induced contractions of the rat vas deferens. Potassium depolarization elicits contractions of the rat vas deferens that are absolutely dependent on calcium. The DPBP neuroleptics block these contractions in a fashion that is reversed by higher concentrations of calcium. Schild plots indicate that these drugs compete with calcium (slopes of 0.84 for pimozide and fluspirilene) (Fig. 4). The pA_2 values, 7.1 and 7.4 for fluspirilene and pimozide, respectively, reflect K_{app} values of 79 nM and 43 nM. In similar experiments, clopimozide and penfluridol display apparent K_{app} values of 40 ± 10.6 and 371 ± 92 nM, respectively. Thus, the DPBP neuroleptics are comparable with, and in some instances substantially more potent than, clinically used calcium channel-antagonist drugs (15, 16, 25). Since the rat vas deferens does not possess dopamine receptors (26), blockade of such sites is not related to the DPBP actions. Moreover, in the range of concentrations eliciting complete blockade of dopamine receptors (10–100 nM), other neuroleptic drugs, including phenothiazines such as chlorpromazine and butyrophenones such as haloperidol and spiperone, do not influence rat vas deferens contractions (data not shown).

DISCUSSION

The major finding of the present study is that DPBP neuroleptic drugs are potent calcium channel antagonists. These influences on voltage-sensitive calcium channels are apparent both in receptor binding studies and in blocking calcium-dependent smooth muscle contractions. The reversal by methoxyverapamil of the inhibition of [^{3}H]nitrendipine binding indicates that the DPBPs act at the same site as verapamil-like drugs. Our findings on the effects on rat vas deferens contractions substantiate earlier observations that DPBP neuroleptics antagonize calcium-dependent contractions of rat caudal artery and guinea pig taenia caeci (20, 23). Although neuroleptics including DPBPs bind to calmodulin, such effects cannot explain the effects on smooth muscle as the potencies are 1–2% of those of DPBPs at calcium channels (27).

The similar potencies of DPBPs at calcium channel-antagonist binding sites and dopamine receptors (Table 1) indicate that at therapeutic doses these drugs will influence calcium channels. Moreover, in animals, doses that are therapeutic in man provide brain concentrations sufficient to occupy 50% of calcium channel-antagonist binding sites (28). Accordingly, behavioral alterations related to calcium channel modulation should occur following treatment with therapeutic doses of DPBP neuroleptics but not neuroleptics lacking calcium channel-antagonist actions. While all neuroleptics relieve the positive symptoms of schizophrenia, some clinical actions appear unique to DPBPs. DPBPs are substantially more effective than other neuroleptics in reversing emotional withdrawal and poverty of affect and speech, the negative schizophrenic symptoms (6–14). Such effects have been reported consistently in independent studies of DPBPs such as pimozide (7–10), penfluridol (11, 12), and fluspirilene (13, 14). In several studies, these actions were reported as unique for the DPBPs when compared in the same study with neuroleptics of other chemical classes (8, 9, 11, 13). We suggest that this selective effect of DPBPs on schizophrenic negative symptoms derives from calcium channel influences. How the affected calcium channels modulate schizophrenic behavior is unclear. Conceivably, the relief of negative symptoms by DPBPs may be related to the stimulant side effects, such as insomnia and nervousness, observed with these drugs (7, 11, 13).

Cardiovascular side effects related to calcium antagonism have not been described with the DPBP neuroleptics, perhaps because the high partitioning of the drug into the brain keeps peripheral levels relatively low. Alternatively, these drugs may show relative specificity for neuronal calcium channels. One would expect that combined administration of DPBPs with calcium channel-antagonist drugs used for cardiovascular therapy may elicit synergistic effects.

The unique clinical spectrum of DPBP antischizophrenic actions suggests that voltage-dependent calcium channel activity in the brain may influence behavior along an activation continuum. Conceivably, centrally acting calcium channel-antagonist drugs that influence the verapamil site will have therapeutic utility independent of dopamine receptor blockade.

We thank Greg Mack for technical assistance and Nancy Bruce for manuscript preparation. This work was supported by Public Health Service Grants DA-00266, MH-18501, and NS-16375; Research Scientist Award DA-00074 to S.H.S.; Training Grant MH-15330 to R.J.G.; and Training Grant GM-07309 to K.M.M.M.

1. Peroutka, S. J. & Snyder, S. H. (1980) *Am. J. Psychiatry* **137,** 1518–1522.
2. Seeman, P. (1981) *Pharmacol. Rev.* **32,** 229–313.
3. Snyder, S. H., Greenberg, D. A. & Yamamura, H. I. (1974) *Arch. Gen. Psychiatry* **31,** 58–61.
4. Miller, R. J. & Hiley, C. R. (1974) *Nature (London)* **248,** 596–597.
5. Peroutka, S. J., U'Prichard, D. C., Greenberg, D. A. & Snyder, S. H. (1977) *Neuropharmacology* **16,** 549–556.
6. Crow, T. J. (1980) *Br. Med. J.* **280,** 66–68.
7. Pinder, R. M., Brogden, R. N., Sawyer, P. R., Speight, T. M., Spencer, R. & Avery, G. S. (1976) *Drugs* **12,** 1–40.
8. Haas, S. & Beckmann, H. (1982) *Pharmacopsychiatry* **15,** 70–74.
9. Kudo, Y. (1972) *Acta Psychiatr. Belg.* **72,** 685–697.
10. Lapierre, Y. D. & Lavallee, J. (1975) *Curr. Ther. Res. Clin. Exp.* **18,** 181–188.
11. Lapierre, Y. D. (1978) *Am. J. Psychiatry* **135,** 956–959.
12. Shopsin, B., Klein, H., Gerbino, L. & Selzer, G. (1977) *Psychopharmacology* **55,** 157–164.
13. Frangos, H., Zissis, N. P., Leontopoulos, I., Diamantas, N., Tsitouridis, S., Gavriil, I. & Tsolis, K. (1978) *Acta Psychiat. Scand.* **57,** 436–446.
14. Singh, A. N. (1973) *Can. Psychiatr. Assoc. J.* **18,** 415–419.
15. Triggle, D. J. (1982) in *Calcium Blockers, Mechanisms of Action and Clinical Applications,* eds. Flaim, S. F. & Zelis, R. (Urban and Schwarzenberg, Baltimore), pp. 121–134.
16. Fleckenstein, A. (1971) *Annu. Rev. Pharmacol. Toxicol.* **17,** 149–166.
17. Gould, R. J., Murphy, K. M. M. & Snyder, S. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3656–3660.
18. Murphy, K. M. M., Gould, R. J., Largent, B. L. & Snyder, S. H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 860–864.
19. Ehlert, F. J., Roeske, W. R., Itoga, E. & Yamamura, H. I. (1982) *Life Sci.* **30,** 2191–2204.
20. Denef, C., Van Neuten, J. M., Leysen, J. E. & Janssen, P. A. J. (1979) *Life Sci.* **25,** 217–226.
21. MacLeod, R. M. & Lamberts, S. W. J. (1978) *Endocrinology* **103,** 200–203.
22. West, B. & Dennies, P. S. (1979) *Endocrinology* **104,** 877–880.
23. Spedding, M. (1982) *Naunyn-Schmiedeberg Arch. Pharmacol.* **318,** 234–240.
24. Kenakin, T. P. (1982) *Can. J. Physiol. Pharmacol.* **60,** 249–265.
25. Nordstrom, L. A. & Gobel, F. L. (1978) *Chest* **74,** 50–54.
26. Leedham, J. A. & Pennefather, J. N. (1982) *Br. J. Pharmacol.* **77,** 293–299.
27. Prozialeck, W. C. & Weiss, B. (1982) *J. Pharmacol. Exp. Ther.* **22,** 509–516.
28. Janssen, P. A. J., Soudijn, W., van Wijngaarden, I. & Dreen, A. (1968) *Arzneim-Forsch/Drug Res.* **18,** 282–287.