In Vitro Evaluation of a Series of Sympathomimetic Amines and the Beta-Adrenergic Blocking Properties of Cyclopentamine

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Abstract □ A series of sympathomimetic amines of varying structure was studied on the isolated rabbit ileum and uterus in the presence of phentolamine. Norepinephrine was the most potent and cyclopentamine the least potent of the drugs giving responses. The structure required for high activity was discussed. It was demonstrated that cyclopentamine was a very weak alpha-adrenergic receptor stimulant and a weak beta-adrenergic receptor blocker. This drug also potentiated the auto-inhibition produced by high doses of isoproterenol and the effect lasted as long as the auto-inhibition persisted. It was suggested that the blockade by both drugs was the result of a direct action on the beta receptors. Isoproterenol also produced an auto-inhibition of its own responses on rabbit intestine which was gradually reversible with time.

Keyphrases □ Sympathomimetic amines—*in vitro* activity □ Uteri, ileal segments—sympathomimetic amines, effect □ Phentolamine effect—sympathomimetic amines, activity □ Adrenergic activity—sympathomimetic amines

Ahlquist (1) classified adrenergic receptors into alpha and beta types depending upon their responses to structurally related catecholamines. Evidence that the inhibitory effects of sympathomimetic amines on the intestine was the result of stimulation of both types of receptor has been provided by Ahlquist and Levy (2). Furchgott (3) showed that similar effects were produced on the isolated rabbit ileum *in vitro*. Miller (4) has shown that stimulation of both types of receptor in the isolated rabbit uterus caused contraction and the responses to catecholamines were related to the hormonal state of the tissue. Burn and Rand (5) have shown that sympathomimetic agents can act directly by occupying specific receptor sites or indirectly by releasing catecholamines. Van Rossum and Mujic (6) have shown that the rabbit intestine may be used to differentiate between such direct- and indirect-acting alpha sympathomimetics. However, these actions are by no means clear-cut because the beta-receptor agonist, *l*-isoproterenol, can stimulate the alpha receptor (7) and the alpha- receptor agonist, phenylephrine, can block the responses to isoproterenol (8). Moreover, Tothill (9) has shown that *l*-isoproterenol can produce resistance to its own action on the rat uterus (auto-inhibition).

Cyclopentamine, an alpha-receptor agonist, has also been shown to antagonize the intestinal inhibitory action of *l*-norepinephrine (6). However, Schmidt and Fleming (10) believe that cyclopentamine exerts its effect on intestinal peristalsis *via* a papaverine-like effect. Numerous other investigators (11–16) have studied structure-activity relationships in the various sympathomimetic drugs in order to classify their various activities, but the area is still somewhat confused. The present work is an effort to assist in clarifying the issue and to determine if the drugs act only as agonists or whether among the group there are drugs which also act as antagonists on either the alpha or beta-adrenergic receptors.

MATERIALS AND METHODS

Ileal segments and uteri were obtained from rabbits injected with estrogen and progesterone according to the procedure of Leitch and Haley (17). This increased the responsiveness of the uteri by producing the same stage of estrus in all animals. The isolated tissues were suspended in separated 40-ml. baths containing Locke-Ringer solution (NaCl 9 g., KCl 0.42 g., $MgCl_2$ 0.2 g., $CaCl_2$ 0.24 g., $NaHCO_3$ 0.5 g., and dextrose 0.5 g./l.) thermostatically regulated at 37.5° and aerated with 95% O_2 and 5% CO_2. All doses of drugs were administered in a volume of 0.5 to 1 ml. using micromolar concentrations. The doses of the drugs used were: *l*-epinephrine-*d*-bitartrate 4.5×10^{-3} μM,[1] *l*-norepinephrine-*d*-bitartrate 4.7×10^{-3} μM, *l*-phenylephrine hydrochloride 2.4×10^{-2} to 6×10^{-2} μM, *l*-isoproterenol-*d*-bitartrate 8.1×10^{-4} to 1.3×10^{-2} μM, phentolamine methanesulfonate 7×10^{-3} μM, dichloroisoproterenol 5×10^{-3} to 2.5×10^{-2} μM, *p*-methylsulfonamidoisoproterenol 3×10^{-2} to 1.2×10^{-1} μM, methoxamine 1.5×10^{-2} to 10.8×10^{-2} μM, tetrahydrozoline hydrochloride 1.3×10^{-2} to 9.8×10^{-2} μM, xylometazoline 5×10^{-3} to 12.5×10^{-2} μM, methylphenidate hydrochloride 1.2×10^{-1} to 9.5×10^{-1} μM, epinine hydrochloride 2×10^{-2} to 3.5×10^{-1} μM, *dl*-synephrine tartrate 4.5×10^{-1} to 3.4 μM, *dl*-cobefrine hydrochloride 1.1×10^{-2} to 1.2×10^{-1} μM, phenylpropanolamine hydrochloride 7.5×10^{-1} to 11.3 μM, cyclopentamine hydrochloride 1 to 20.4 μM, metaraminol bitartrate 3.5×10^{-2} to 4.2×10^{-1} μM, isopropylmethoxamine hydrochloride 0.25 to 2.5 μM. Doses up to 5 μM of the following drugs were used; tuaminoheptane sulfate, isometheptane mucate, phenylpropylmethylamine hydrochloride, methylhexamine, chlorphentermine, nylidrin hydrochloride, mephentermine sulfate, hydroxyamphetamine hydrobromide, and propylhexedrine. A dose of the antagonist was added to the bath 1 min. prior to administering a dose of the agonist which was allowed to act for 30 sec. Five strips were used for each drug and the results were statistically analyzed by the Litchfield-Wilcoxon (18) method.

RESULTS

The isolated ileal and the uterine preparations of the hormone-treated rabbit showed varying degrees of responsiveness to the sympathomimetic drugs in the presence of phentolamine, although the order of relative potencies was not altered significantly as is evident from Table I. Xylometazoline was ineffective on the uterus whereas it was about as active as phenylephrine on the ileum. On the other hand, tuaminoheptane and cyclopentamine had no activity on the ileal preparation, but they had a weak alpha-receptor stimulating activity on the uterus. Similarly, isopropylmethoxamine had no effect on beta-receptor activity on the intestine but antagonized isoproterenol-induced relaxation of the uterus. Dichloroisoproterenol blocked isoproterenol-induced relaxation in the intestine but consistently relaxed uterine preparations because of its intrinsic sympathomimetic activity. *p*-Methylsulfonamidoisoproterenol was without any sympathomimetic effect and antagonized isoproterenol on both of the preparations.

An interesting observation was the ability of cyclopentamine to reversibly block isoproterenol-induced inhibition of the ileal and uterine smooth muscles. It was about 810 times less potent than DCI on the intestine, and about 150 times less than *p*-methylsulfonamidoisoproterenol on the uterus (Table II). Cyclopentamine (19.8 μM) also blocked epinephrine (4.5×10^{-3} μM) in the presence of phen-

[1] μM designates micromole throughout this article.

Table I—Comparison of Alpha-Receptor Stimulating Properties in the Presence of 7×10^{-3} μM of Phentolamine

Agonist	Ileum: ED_{50} and Range, μM	Ileum: Slope and Range	Ileum: Potency	Uterus: ED_{50} and Range, μM	Uterus: Slope and Range	Uterus: Potency
l-Phenylephrine	0.024 (0.018–0.032)	2.25 (1.61–2.93)	1.0	0.036 (0.019–0.054)	2.41 (1.91–3.42)	1.0
l-Norepinephrine	0.005 (0.002–0.006)	2.70 (1.53–3.54)	5.2	0.004 (0.003–0.005)	2.72 (2.15–3.24)	8.5
dl-Cobefrine	0.040 (0.022–0.065)	1.95 (1.32–3.1)	0.6	0.082 (0.03–0.15)	2.33 (1.62–3.45)	0.45
Tetrahydrozoline	0.042 (0.023–0.061)	2.21 (1.8–3.9)	0.57	0.045 (0.031–0.069)	2.01 (1.30–4.50)	0.8
Xylometrazoline	0.05 (0.025–0.082)	1.83 (1.12–2.96)	0.48	No activity		
Methoxamine	0.059 (0.24–0.097)	1.75 (1.12–3.46)	0.40	0.098 (0.058–0.20)	2.40 (1.73–3.01)	0.37
Metaraminol	0.15 (0.08–0.29)	1.96 (1.31–2.78)	0.16	0.21 (0.12–0.33)	1.87 (1.13–2.76)	0.17
Epinine	0.16 (0.09–0.26)	2.03 (1.45–3.01)	0.15	0.26 (0.15–0.34)	1.93 (1.39–3.4)	0.16
Methylphenidate	0.45 (0.22–0.66)	1.65 (0.98–3.25)	0.053	0.91 (0.69–1.8)	1.82 (1.01–4.60)	0.039
Tuaminoheptane	No activity			3.50 (1.85–5.32)	1.74 (1.21–3.67)	0.015
dl-Synephrine	1.81 (1.22–2.68)	2.18 (1.09–3.20)	0.013	2.51 (1.21–4.08)	2.31 (1.62–3.77)	0.014
Phenylpropanolamine	5.32 (3.2–7.6)	2.27 (1.53–3.98)	0.0045	5.80 (2.93–7.98)	2.08 (1.01–3.79)	0.006
Cyclopentamine	No activity			5.92 (3.69–7.37)	2.31 (1.58–3.24)	0.006

tolamine (7×10^{-3} μM), Fig. 1, but did not affect the tissue responses to alpha-receptor agonists, phenylephrine and norepinephrine. Isoproterenol produced an incomplete autoblockade of its inhibitory effect on the intestinal tissue at a dose of 1.3×10^{-2} μM. This effect could not be overcome by repeated washings but gradually disappeared within 30 min. Successive high doses of isoproterenol produced more nearly complete and longer lasting auto-inhibition of its relaxing response on ileal strips. Cyclopentamine potentiated the isoproterenol-induced auto-inhibition on the isolated ileal preparation. No auto-inhibition was observed on the rabbit uterus. On the spontaneously contracting uterine strips, isoproterenol produced complete relaxation in a dose (1×10^{-3} μM) which was about four times less than (4.1×10^{-3} μM) required for the same effect on ileal strips.

Isometheptane, phenylpropylmethylamine, methylhexamine, chlorphentermine, nylidrin, mephentermine, and propylhexedrine were devoid of adrenergic activity on both *in vitro* preparations.

DISCUSSION

The present work confirms the observations of others (3, 6) that the rabbit intestine is suitable for study of the effects of drugs on the alpha and beta receptors. It has also been shown that *l*-isoproterenol produces resistance to its own action on the rabbit uterus similar to that produced on the rat uterus (9). Under the conditions in this study, it was not possible to confirm Schmidt and Fleming's (10) observation of a papaverine-like action of cyclopentamine.

The drugs examined in this study can be classified into various chemical groups according to their structures. Among the alkylethylamines, activity at the alpha-adrenergic receptor was not high. Straight-chain compound, tuaminoheptane, was more active than the alicyclic cyclopentamine. In straight-chain alkylamines, activtiy at the alpha-adrenergic receptor was found in compounds with seven or eight carbon atoms. Branching of the chain as in methylhexaneamine and the introduction of an ethylenic linkage as in isomethептene destroyed activity.

Among the phenylalkylamine derivatives only one compound, epinine, stimulated the alpha-adrenergic receptor. Epinine has catechol hydroxyl groups and resembles norepinephrine in this regard. The presence of a *para*-hydroxyphenyl moiety in hydroxyamphetamine did not confer activity. This is in agreement with earlier reports that showed that the *meta*-hydroxyl group in sympathomimetic amines was of a greater significance than the *para*-hydroxyl group for activity at adrenergic receptors (19). All substituents at the alpha and beta carbon atoms and the amino nitrogen atom in the side chain consistently produced a decrease in activity.

Compounds derived from the phenylalkanolamine basic structure are closely related to norepinephrine and are more active than phenylalkylamine analogs. The presence of the beta-hydroxyl group in these compounds confers greater activity at adrenergic receptors, indicating that this group facilitates drug-receptor interaction as postulated previously (16). It would appear that the highly specific structural requirements for activity at alpha-adrenergic receptors are met within the norepinephrine structure, and any alteration in this structure simply decreases activity. Starting with norepinephrine, substitution at the terminal amino nitrogen results in a gradually decreasing activity, and bulky groups ultimately destroy all activity at the alpha-adrenergic receptor. Simultaneous methyl substitution at the nitrogen and the carbon atoms next to it produces the same

Table II—Comparison of Beta-Receptor Blocking Properties in the Presence of Isoproterenol

Antagonist	Ileum[a]: ED_{50} and Range, μM	Ileum[a]: Slope and Range	Ileum[a]: Potency	Uterus[b]: ED_{50} and Range, μM	Uterus[b]: Slope and Range	Uterus[b]: Potency
p-Methylsulfonamido-isoproterenol	0.073 (0.034–0.014)	2.62 (1.37–3.69)	1	0.082 (0.035–0.132)	2.34 (1.09–3.62)	1
Dichloroisoproterenol	0.011 (0.006–0.029)	2.46 (1.89–3.65)	6.6	Relaxes		
Isopropylmethoxamine	No activity			0.78 (0.52–1.38)	1.78 (0.92–3.81)	0.105
Cyclopentamine	8.9 (4.93–13.68)	1.73 (0.93–3.81)	0.008	12.1 (6.9–26.2)	2.32 (1.46–3.59)	0.007

[a] 4.1×10^{-3} μM of isoproterenol used on ileum. [b] 1.1×10^{-3} μM of isoproterenol used on uterus.

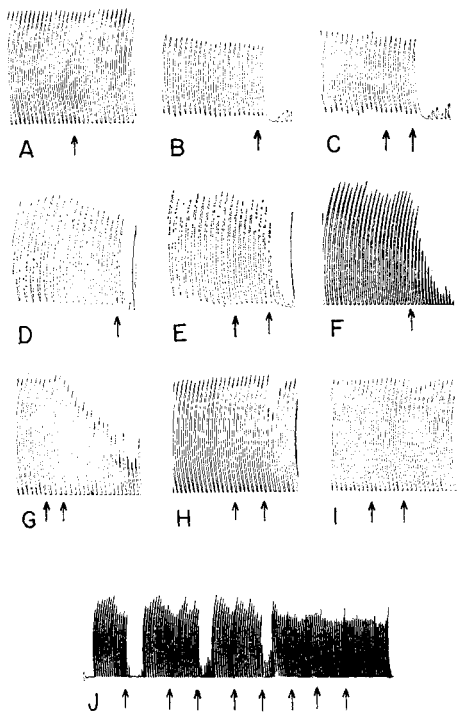

Figure 1.—*Beta-adrenergic blocking activity of cyclopentamine on rabbit ileum. A, cyclopentamine 19.8 μM; B, norepinephrine 4.7 × 10^{-3} μM; C, cyclopentamine 19.8 μM plus norepinephrine 4.7 × 10^{-3} μM; D, phenylephrine 2.4 × 10^{-2} μM; E, cyclopentamine 19.8 μM plus phenylephrine 2.4 × 10^{-2} μM; F, isoproterenol 4.1 × 10^{-3} μM; G, cyclopentamine 4.9 μM plus isoproterenol 4.1 × 10^{-3} μM; H, cyclopentamine 9.8 μM plus isoproterenol 4.1 × 10^{-3} μM; I, cyclopentamine 14.7 μM plus isoproterenol 4.1 × 10^{-3} μM; J, epinephrine 4.5 × 10^{-3} μM, cyclopentamine 14.7 μM, epinephrine 4.5 × 10^{-3} μM, cyclopentamine 19.8 μM, epinephrine 4.5 × 10^{-3} μM, phentolamine 7 × 10^{-3} μM, cyclopentamine 19.8 μM, epinephrine 4.5 × 10^{-3} μM, respectively.*

effect. Here again, the *meta*-hydroxy compound showed greater activity than its *para* isomers.

In tetrahydrozoline and xylometazoline the amino group and the alpha carbon atom are incorporated into a five-membered heterocyclic ring. It seems that the conformaton of these compounds about the amino group is not significantly altered from that in norepinephrine and so the affinity for alpha-adrenergic receptor is maintained. A heterocyclic ring, however, does affect potency according to the size of the ring; methylphenidate with a six-membered heterocyclic ring is less active than its five-membered analogs, tetrahydrozoline and xylometazoline.

Irrespective of the class, in all these compounds —C—C—N< moiety is important for activity at adrenergic receptors, and any alkyl substitution other than hydrogen on any of these atoms decreases activity at alpha receptors.

The dual behavior of cyclopentamine in producing alpha-adrenergic receptor stimulation and beta-receptor blockage is a highly unexpected observation in view of the lack of resemblance of this compound to any of the beta-adrenergic receptor-blocking drugs and to isoproterenol. Thus it is difficult to correlate this finding with the current hypotheses that require the presence of an isopropyl-amino group, a beta-hydroxyl group, and a catechol nucleus in the molecule to have some affinity for beta-adrenergic receptor (12, 15, 16). It may well be that the cyclopentamine structure is unique in this respect since another alicyclic compound propylhexedrine which has a cyclohexyl moiety instead of a cyclopentyl is completely inactive. Although the beta-receptor blocking activity of cyclopentamine is weak, it is to be expected that suitably designed cyclopentamine analogs may prove capable of exhibiting potent beta-receptor blocking property.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 2, 1968, from the *Department of Pharmacology, School of Medicine, University of Hawaii, Honolulu, HI*

Accepted for publication March 14, 1969.

* East-West Center Grantee.

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The authors wish to thank the following companies for the drugs used in this investigation: Ciba Pharmaceuticals, Merck Sharp and Dohme, Eli Lilly and Co., U. S. Vitamin and Pharmaceutical Corp., Burroughs Wellcome and Co., Warner-Lambert, Wyeth Laboratories Inc., McNeil Laboratories, Sterling-Winthrop, and Smith Kline & French Laboratories.