Steric Aspects of Adrenergic Drugs IV. Antagonism of Norepinephrine Effects by Ephedrine Isomers on the Isolated Rabbit Ileum

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The inhibition of the pendular movements of the isolated rabbit ileum, due to norepinephrine, was not antagonized in the presence of 10^{-5} *M* D(−)-ephedrine, L(+)-ephedrine, L(+)-pseudoephedrine, or D(−)-pseudoephedrine. However, a 10^{-3} *M* concentration of these isomers did antagonize the norepinephrine effects. The stereospecificity in such antagonism is not marked.

In the past a number of investigators have reported that in certain tissues such as rabbit ileum and cat uterus, epinephrine-induced relaxation can be blocked by ephedrine (1–4). This observation was recently confirmed by Van Rossum and Mujic (5). Furthermore, they have suggested that certain "indirectly" acting sympathomimetic amines do not of themselves cause inhibition of the rabbit jejunum, but antagonize the effects of "directly" acting sympathomimetic amines. These reports formed the basis for the present investigation, which was aimed at obtaining information regarding stereospecificity in such antagonistic effects.

EXPERIMENTAL

Twenty-four hour starved rabbits of either sex were sacrificed by a sharp blow on the head. The ileum was isolated and a small piece (about 2 cm.) was suspended in a 25-ml. jacketed tissue chamber which contained Tyrode's solution at 37°. A mixture of oxygen (95%) and carbon dioxide (5%) was continuously bubbled through the Tyrode's solution. The composition of the Tyrode's solution was as follows: NaCl, 8.0 Gm.; KCl, 0.2 Gm.; $CaCl_2$ ($2H_2O$), 0.26 Gm.; $MgCl_2$ ($6H_2O$), 0.188 Gm.; $NaHCO_3$, 1.0 Gm.; NaH_2PO_4, 0.05 Gm.; dextrose anhydrous, 1.0 Gm.; double distilled water to make 1000 ml. Pendular movements of ileum were recorded on a smoked drum *via* a light lever (magnification 1:20, tension approximately 500 mg.). The tissue was washed 3 or 4 times before cumulative dose-response curves were obtained. This method is described in detail by Van Rossum and Mujic (5). For each procedure six to nine pieces of ileum were obtained from three to four different animals. Only one dose response curve was obtained from each piece of ileum. The maximum height of the pendular movements was taken as 100% and inhibition expressed as the per cent of the initial activity. Ephedrine isomers were left in contact with the tissue for 2 min. before cumulative dose-response curves of norepinephrine were determined.

A stock solution of norepinephrine[1] was prepared in a solution containing 0.05% sodium metabisulfite. Ephedrine isomers were obtained by a method described previously (6).

RESULTS AND DISCUSSION

Norepinephrine produces a rapid inhibition of the pendular movements. The inhibition is complete in 10^{-6} *M* norepinephrine. The negative log molar concentration of norepinephrine for producing 50% inhibition is 6.9. This value is lower than that for jejunum which is 7.8 (5). Ephedrine isomers in concentrations of 10^{-5} *M* did not produce any intrinsic effects, nor did they exhibit any antagonism toward norepinephrine effects (Fig. 1 and Table I).

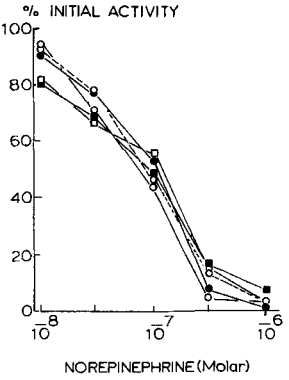

Fig. 1.—Cumulative dose-response curves of norepinephrine on rabbit ileum in the presence of 10^{-5} *M* D(−) - ephedrine (O- - -O); L(+)-ephedrine (O——O); L(+)-pseudoephedrine (□——□); D-(−) - pseudoephedrine (■——■). Norepinephrine control (●——●). Standard errors of the mean were 5 to 7% and showed a marked overlap.

Table I.—Lack of Inhibition of Norepinephrine Effects on Rabbit Ileum by 10^{-5} *M* Concentration of Ephedrine Isomers

	(−)-Norepinephrine −Log ED_{50} with S.E.M.			
Control	D(−)-Ephedrine, 10^{-5} *M*	L(+)-Ephedrine, 10^{-5} *M*	L(+)-Pseudoephedrine, 10^{-5} *M*	D(−)-Pseudoephedrine, 10^{-5} *M*
6.96	6.98	7.16	6.87	7.04
±0.08	±0.17	±0.11	±0.12	±0.14
$n^a = 9^b$	$n = 9^c$	$n = 7^c$	$n = 6^d$	$n = 7^d$
	$P >$ 0.05	$P >$ 0.05	$P >$ 0.05	$P >$ 0.05

[a] *n*, number of observations. [b] Nine animals. [c] Four animals. [d] Three animals.

In higher concentration (10^{-3} *M*), ephedrine showed variable intrinsic effects. D(−)-Ephedrine produced 34% (S.E. ± 4.2) inhibition; L(+)-ephedrine and D(−)-pseudoephedrine produced stimulant effects of 10% (S.E. ± 1) and 29% (S.E. ± 2), respectively, while L(+)-pseudoephedrine did not produce any observable effects.

The inhibitory effects of norepinephrine were antagonized by ephedrine isomers (Fig. 2). The dose-response curves were shifted to the right, *i.e.*, in the presence of ephedrine isomers a higher concentration of norepinephrine was required to

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* National Science Foundation Pharmacy Undergraduate Research Participant, 1965.
[1] Regis Chemical Co.

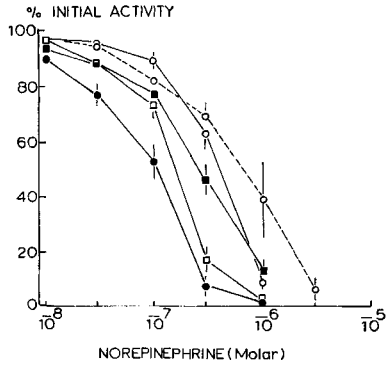

Fig. 2.—Illustrates the cumulative dose-response curves of norepinephrine on rabbit ileum in the presence of 10^{-3} *M* D(−)-ephedrine (○- - -○), L(+)-ephedrine (○——○), L(+)-pseudoephedrine (□——□), D(−)-pseudoephedrine (■——■). Norepinephrine control (●——●). Vertical lines, standard errors of the mean.

produce the same response. It appears that L(+)-pseudoephedrine is < antagonist than D(−)-pseudoephedrine which is ≤ L(+)-ephedrine. The interpretation of the antagonist potency of D(−)-ephedrine is complicated by its intrinsic inhibitory effects. Present results are compatible with the view that sympathomimetic amines may compete for "direct" sites, but the possibility of noncompetitive antagonism must be borne in mind. (Table II.)

TABLE II.—INHIBITION OF NOREPINEPHRINE EFFECTS ON RABBIT ILEUM BY 10^{-3} *M* CONCENTRATION OF EPHEDRINE ISOMERS

	(−)-Norepinephrine −Log ED_{50} with S.E.M.			
Control	D(−)-Ephedrine, 10^{-3} *M*	L(+)-Ephedrine, 10^{-3} *M*	L(+)-Pseudoephedrine, 10^{-3} *M*	D(−)-Pseudoephedrine, 10^{-3} *M*
6.96	6.16	6.41	6.78	6.49
±0.08	±0.13	±0.05	±0.04	±0.08
$n^a = 9^b$	$n = 6^c$	$n = 9^c$	$n = 8^c$	$n = 8^c$
	$P <$ 0.001	$P <$ 0.001	$P <$ 0.1	$P <$ 0.01

[a] *n*, number of observations. [b] Nine animals. [c] Three animals.

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3-Thenyl Nitrogen Mustards

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As potential anticancer agents, three new 3-thenyl nitrogen mustards have been prepared from the corresponding diethanolamine derivatives. The NMR data for these compounds are reported.

IN A SERIES of antihistaminic compounds, Clapp and co-workers (1) have suggested that the inclusion of a halogen atom on the thiophene ring improves the therapeutic ratio. This suggestion has not been exploited in thiophene nitrogen mustards. Indeed, besides dimethyl derivatives (2), no nuclear substituted thiophene nitrogen mustards have been reported.

Campaigne's comparative data (3) suggest that in testing thiophene analogs, there is a high probability that the 3-isomer will be at least as active as the 2-isomer, if not more active. Of the six thiophene nitrogen mustards previously reported (2, 4, 5), four are thenyl derivatives (2, 5) and, of these, only two are 3-thenyl isomers (2).

In this investigation, three 3-thenyl nitrogen mustard derivatives have been prepared as potential anticancer agents. These are denoted by structure I in which R = H, 2-bromo, and 2,5-dichloro.

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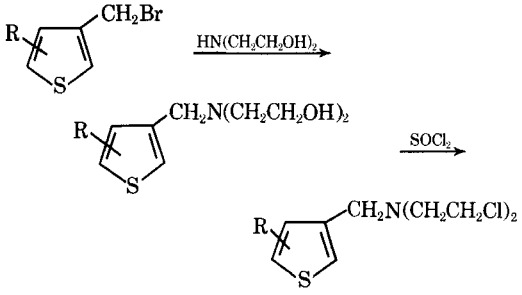
As outlined in Scheme I, the synthetic route employed for the preparation of these compounds is a modification of that of Wilson and Tishler (5).



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Scheme I

The 3-thenyl bromides were obtained by the reaction of *N*-bromosuccinimide with the respective methylthiophenes in the presence of catalytic amounts of benzoyl peroxide (6). The diethanolamine derivatives were prepared by treating the thenyl bromides with diethanolamine.

Preparation of the nitrogen mustard function by halogenation of the appropriate diethanolamine derivative with thionyl chloride (2, 5) or phosphorus oxychloride (4) has been reported in low yields, 12–27%. In each instance, the reaction mixture was cooled during the addition of the halogenating agent, and then it was heated to reflux. In the