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ANTAGONISM OF ACETYLCHOLINE BY ADRENALINE ANTAGONISTS

BY

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Phenoxybenzamine antagonized the inhibitory action of acetylcholine on the guinea-pig isolated atrium. The antagonism was slow in onset, very slowly reversible, and could be overcome by increased concentrations of acetylcholine. In contrast, atropine inhibited the action of acetylcholine quickly, and the effect disappeared soon after withdrawal. The pA_{10} of phenoxybenzamine (2 hr of contact) was 6.8, and that of atropine (30 min of contact) was 8.4. In the presence of atropine phenoxybenzamine did not exert a slowly reversible antagonism, and the dose-ratio of acetylcholine returned to normal soon after withdrawal of both drugs. Phenoxybenzamine also antagonized acetylcholine in the guinea-pig isolated ileum, but with higher concentrations acetylcholine did not overcome the antagonism. The pA_{10} (60 min of contact) was 6.6. The pA_{10} of chlorpromazine in the atrium (2 hr of contact) and ileum (60 min of contact) was 5.9. Phentolamine, 2-diethylaminomethylbenzo-1,4-dioxan hydrochloride (883 F), and yohimbine antagonized acetylcholine in the atrium and ileum but required higher concentrations than chlorpromazine.

As the adrenaline antagonist, phenoxybenzamine, prevents vagal slowing of the heart of the spinal dog (Benfey, 1962) and converts vagal inhibition of the guinea-pig isolated atrium into stimulation (Benfey & Greeff, 1961), it appeared of interest to investigate its antagonism of acetylcholine. Cholinergic blockade by the related drug, dibenamine, in the rat isolated atrium, was briefly mentioned by Furchgott (1954). Other antisympathomimetic drugs were studied for the purpose of comparison.

METHODS

The guinea-pig isolated atrium was prepared by the method of Greeff, Benfey & Bokelmann (1959), but the Tyrode solution contained 10 mg/l. of the ganglion-blocking drug, pempidine, to prevent nicotinic effects of high concentrations of acetylcholine. Throughout the experiment acetylcholine was added to the preparation every 10 min and left in the bath for 1 min. Increasing concentrations of acetylcholine produced progressively greater negative inotropic and chronotropic effects (Fig. 1). The mean dose/response curve for acetylcholine in thirty-two experiments on the atrium was compared with the curve obtained from the same number of experiments on the guinea-pig ileum (Fig. 2). The two curves had similar positions and slopes.

The potencies of the antagonists were calculated in terms of the dose-ratios by comparing the effects of acetylcholine in the presence of an antagonist with the initial dose/response curve. pA_{10} is the negative logarithm of the molar concentration of an antagonist which leads to an acetylcholine dose-ratio of 10 (Schild, 1947).

The guinea-pig ileum was suspended in 50 ml. of Tyrode solution containing 10 mg/l. of pempidine. Throughout the experiment acetylcholine was added every 3 min and left in the

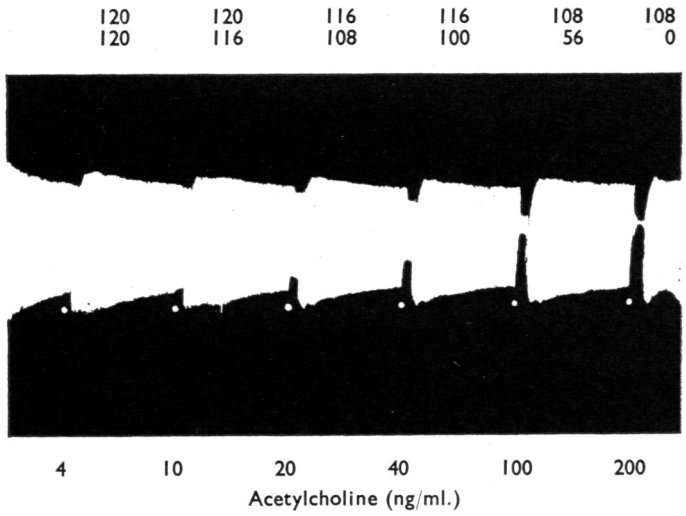

Fig. 1. Effect of acetylcholine on the contractions and rate of the guinea-pig isolated atrium. Acetylcholine was added every 10 min and remained in contact for 1 min, after which the bath liquid was changed. The numerals at the top of the record show the atrial rate of contraction (per min).

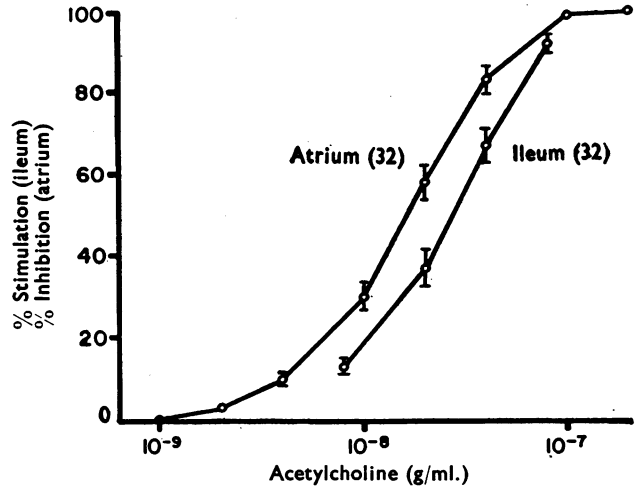

Fig. 2. Dose/response curves for acetylcholine in the guinea-pig isolated atrium and ileum. The numerals in parentheses refer to the numbers of preparations. The vertical bars represent standard errors of means.

bath until the maximum effect was obtained. The dose/response curve of acetylcholine was determined alone and 60 min after adding an antagonist. The concentration of acetylcholine causing 50% of the maximal contraction was then used to calculate the dose-ratio.

Phenoxybenzamine hydrochloride (Dibenzyline) was dissolved in 95% ethyl alcohol containing 0.005 ml. of concentrated HCl/ml. The amount of added solution did not exceed 0.02% of the bath fluid. The hydrochlorides of chlorpromazine, phentolamine, yohimbine, and 2-diethylaminomethylbenzo-1,4-dioxan hydrochloride (Prosympal, 883 F) were dissolved in water. Amounts of acetylcholine refer to the bromide.

RESULTS

In the presence of phenoxybenzamine, the dose-ratio of acetylcholine increased slowly without reaching a maximum and, when phenoxybenzamine was withdrawn after 2 hr, it declined very slowly (Fig. 3). The highest concentration of phenoxybenzamine used (4.7×10^{-7} M, present for 2 hr) did not prevent complete inhibition of the atrium by suitable concentrations of acetylcholine.

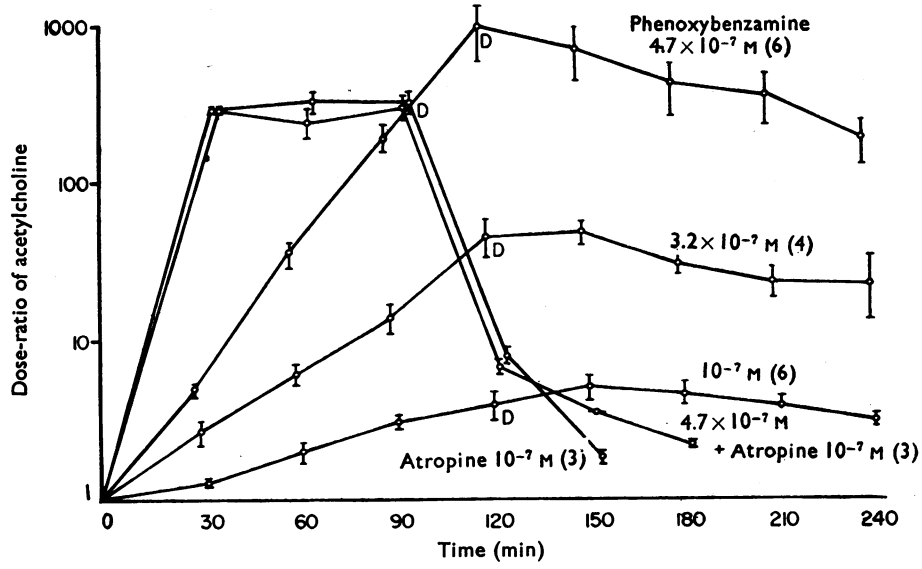

Fig. 3. Relation between dose-ratio of acetylcholine (ordinate, log scale) and time of exposure to antagonist in the guinea-pig isolated atrium. Antagonists were withdrawn at D. The numbers of experiments are in parentheses and the standard errors of the means are represented by the vertical bars.

Atropine (10^{-7} M) led to an immediate rise in the dose-ratio, which remained constant. Following withdrawal of atropine after 90 min, the dose-ratio readily returned to normal (Fig. 3). The pA_{10} of atropine in the guinea-pig atrium (8.4) was similar to that in the frog atrium (8.3), which has been calculated from the results of Clark & Raventos (1937).

When phenoxybenzamine was added together with atropine, there was the same immediate rise in the dose-ratio and, when the two antagonists were withdrawn, the same rapid fall (Fig. 3). At 30 min after withdrawal of the combination of phenoxybenzamine and atropine, the dose-ratio was significantly lower ($P<0.05$) than 30 min after phenoxybenzamine alone.

Phenoxybenzamine also antagonized acetylcholine on the guinea-pig isolated ileum. A concentration of 3.2×10^{-7} M led to a dose-ratio of 14 (s.e. of mean, ± 4) and flattened the slope of the dose/response curve, while 7.6×10^{-7} M (dose-ratio 388 ± 115) depressed its maximum.

Treatment of guinea-pigs with reserpine (1 mg/kg injected intraperitoneally a day before the experiment) did not significantly change the responses of the atrium and ileum to acetylcholine or the effects of phenoxybenzamine thereupon.

The effect of chlorpromazine in the atrium developed faster than that of phenoxybenzamine, and a maximum appeared in approximately 90 min. After withdrawal of chlorpromazine the dose-ratio declined slowly. When chlorpromazine was given together with atropine, the dose-ratio was higher than with atropine alone and, when the two antagonists were discontinued, it declined slowly. The highest concentration of chlorpromazine used (8.4×10^{-6} M, present for 2 hr) did not prevent complete inhibition of the atrium by acetylcholine.

In the ileum 5.6×10^{-7} M-chlorpromazine (dose-ratio 4 ± 1) did not change the slope of the dose-response curve, while 2.3×10^{-6} M (dose-ratio 27 ± 12) flattened it and 4.5×10^{-6} M depressed its maximum below 50%.

Fig. 4 correlates the molar concentrations of the antagonists with the dose-ratios of acetylcholine. The values for atropine in the ileum were taken from Schild (1947) and Arunlakshana & Schild (1959). The plot was used to obtain the pA_{10} values given in Table 1, which shows that phenoxybenzamine was more potent than chlorpromazine.

Other adrenaline antagonists (phentolamine, yohimbine and 883 F) inhibited the action of acetylcholine in the atrium and ileum but required higher concentrations than chlorpromazine. In the atrium on 2 hr contact phentolamine (3.1×10^{-5} M) led to a dose-ratio of 32 (s.e. of mean, ± 16), yohimbine (5.1×10^{-5} M) 3 ± 2, and

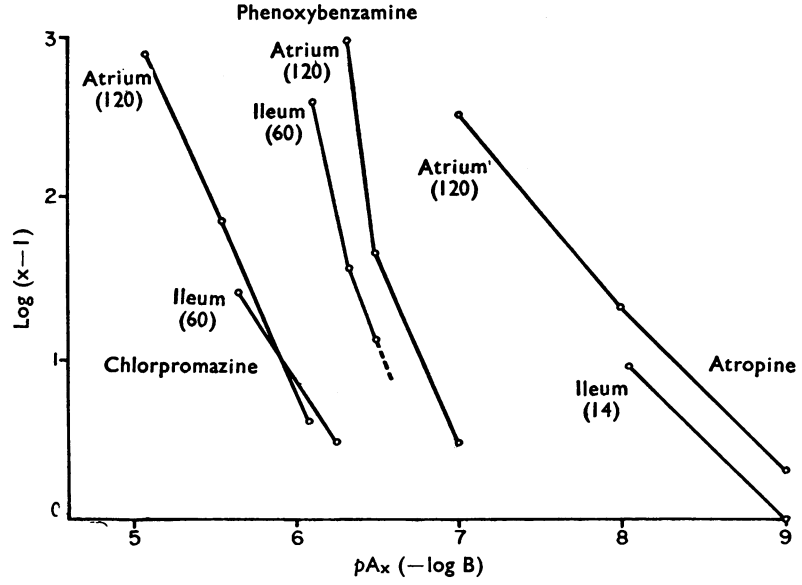

Fig. 4. Relation between the negative logarithm of the molar concentration of antagonist (pA_x) and the logarithm of the dose-ratio of acetylcholine minus 1 (log x − 1). The numbers in parentheses are times (in min) of exposure to antagonist. The pA_x values for atropine in the experiments on the ileum were taken from Schild (1947) and Arunlakshana & Schild (1959).

TABLE 1

pA_{10} VALUES WITH ACETYLCHOLINE IN THE GUINEA-PIG ISOLATED ATRIUM AND ILEUM

*Schild, 1947

Antagonist	Preparation	Time of contact (min)	pA_{10}
Atropine	Atrium	60	8·4
Atropine*	Ileum	14	8·1
Phenoxybenzamine	Atrium	60	6·4
Phenoxybenzamine	Atrium	120	6·8
Phenoxybenzamine	Ileum	60	6·6
Chlorpromazine	Atrium	60	5·8
Chlorpromazine	Atrium	120	5·9
Chlorpromazine	Ileum	60	5·9

883 F (1.6×10^{-4} M) 19 ± 5. The effects were slow in onset and slowly reversible. A higher concentration of yohimbine (1.5×10^{-4} M) inhibited the contractions of the atrium.

In the ileum on 30 min contact phentolamine (3.1×10^{-5} M) led to a dose-ratio of 5 ± 1, yohimbine (1.5×10^{-4} M) 3 ± 1, and 883 F (7.8×10^{-5} M) 3 ± 1. The slopes of the dose/response curves did not change.

The anti-acetylcholine potency of all of these drugs was not related to their anti-adrenaline potency. This was also shown for certain 2-halogenoalkylamines by Graham (1960).

DISCUSSION

It is interesting that the slow onset and long duration of the antagonism of acetylcholine by phenoxybenzamine are also characteristics of its antagonism of adrenaline. Furthermore, the related drug, dibenamine, has a slow onset and long persistence of action against 5-hydroxytryptamine in the rat uterus (Gaddum, Hameed, Hathway & Stephens, 1955), against acetylcholine, histamine and 5-hydroxytryptamine in isolated strips of rabbit aorta, and against acetylcholine in isolated strips of rabbit stomach and rat isolated atria (Furchgott, 1954). Another 2-halogenoalkylamine, *N*-1-naphthylmethyl-*N*-ethyl-β-chloroethylamine, exerts a prolonged antihistamine effect in the guinea-pig ileum (Nickerson, 1956).

It may be worth mentioning that the concentrations of phenoxybenzamine used in this study did not inhibit stimulation of the atrium by histamine or calcium and were approximately 100 times smaller than those needed to potentiate stimulation by noradrenaline (Benfey & Greeff, 1961).

Phenoxybenzamine probably acts at sites which are protected by atropine, as it did not exert a slowly reversible antagonism in the presence of atropine. Furchgott (1954) has shown that atropine also protects the rabbit isolated aorta against the prolonged effects of dibenamine. Thus phenoxybenzamine probably exerts a specific acetylcholine antagonism, a fact which should be considered when employing the drug for its adrenaline antagonism. Using a long time of contact, phenoxybenzamine may have at least 1/40 the potency of atropine.

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