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NICOTINE AND THE EFFECT OF ANTISYMPATHOMIMETIC AGENTS ON THE AORTA OF THE RABBIT

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The responses of strips of rabbit aorta to almost maximal doses of nicotine were less readily antagonized by five antisympathomimetic agents than were comparable responses to noradrenaline. The effect was most marked with dibenamine, ergotamine, and tolazoline: approximately twice the dose of noradrenaline was required to match the test dose of nicotine after treatment with the antagonists. Dose/response curves for nicotine before and after phentolamine 10^{-7} indicate that the phenomenon may be reversed with low doses of nicotine and that the release of noradrenaline by nicotine within the tissues is probably a graded response. The pattern of nicotine/phentolamine antagonism in this preparation is consistent with the view that nicotine acts indirectly by releasing a noradrenaline-like substance, and the difficulty found in antagonizing responses to nicotine with antisympathomimetic agents is probably similar to that responsible for failure of atropine to block some parasympathomimetic responses to nicotine.

It is well known that the effects of sympathetic nerve stimulation in various organs are less readily opposed by antisympathomimetic agents than are the effects of circulating adrenaline. This has been demonstrated for piperoxane hydrochloride by Morison and Lissak (1938) using the cat salivary gland as a test object, for tolazoline by Chess and Yonkman (1946) using the cat salivary gland and splanchnic vascular bed, and for dibenamine on the cat nictitating membrane by Nickerson and Nomaguchi (1948).

Bacq and Fredericq (1935) demonstrated differences between benzodioxane blocking agents in this respect; piperoxane was found to be much less effective than compound 883F against effects produced by nerve stimulation. Bovet and Simon (1937) extended these observations, showing that in several series of benzodioxane derivatives increase in the size of the N radical brought about a transition from the 883F to the piperoxane type of compound. Apart from these observations, no direct comparisons of antisympathomimetic agents against effects of nerve stimulation appear to have been made.

The local vasoconstrictor action of nicotine is probably due to the stimulation of sympathetic nerve endings or ganglion cells in the walls of blood vessels (Hilton, 1954). Kottegoda (1953) and Burn and Rand (1958) have shown that this action of nicotine is due to the release of a noradrenaline-like substance and have discussed the question of its release. The responses to nicotine of isolated vascular smooth muscle might therefore be expected to be rather more difficult to antagonize with antisympathomimetic agents, and the relative effectiveness of such agents against these responses might be expected to resemble their effectiveness against that which follows stimulation of nerves.

METHOD

Spiral strips of aorta, 5 cm. long and 2 mm. wide, were prepared as described by Furchgott and Bhadrakom (1953) from rabbits weighing 2 to 2.5 kg. The strips were suspended in oxygenated Ringer-Locke solution in a 50 ml. bath at 37° and movements recorded with a frontal writing lever (magnification $\times 10$, tension 2 g.). The strips were allowed to relax for 1½ hr. before drugs were added.

RESULTS

There were two series of experiments. In the first, the response to a standard dose of nicotine was matched by a response to noradrenaline before and after addition of a blocking agent. In the second, a dose/response curve was plotted for nicotine before and after addition of phentolamine 10^{-7}, to describe more completely the antagonism between a typical antisympathomimetic agent and nicotine.

Comparison of the Effects of Antagonists against Responses to Nicotine and Noradrenaline

Five antisympathomimetic agents were tested. For each, six strips of aorta from three different rabbits were used. Responses to nicotine and noradrenaline were recorded alternately at 30 min. intervals. The noradrenaline doses were added in 4 successive steps at 2.5 min. intervals to give a dose/response curve. Before the addition of the blocking agent, the response to a standard dose of nicotine (3×10^{-5}) was matched twice by a response to noradrenaline. One hr. after the addition of the blocking agent, the amount of noradrenaline required to match the response to the standard dose of nicotine was again determined twice in the presence of the blocking agent. (The aorta was exposed to dibenamine for 20 min., then washed for 40 min. before testing.) The same tests were carried out on a control series of six strips, but with no antagonist present.

For each agent, results from all six strips were combined to estimate the noradrenaline equivalents before and after addition of the drug (Table I).

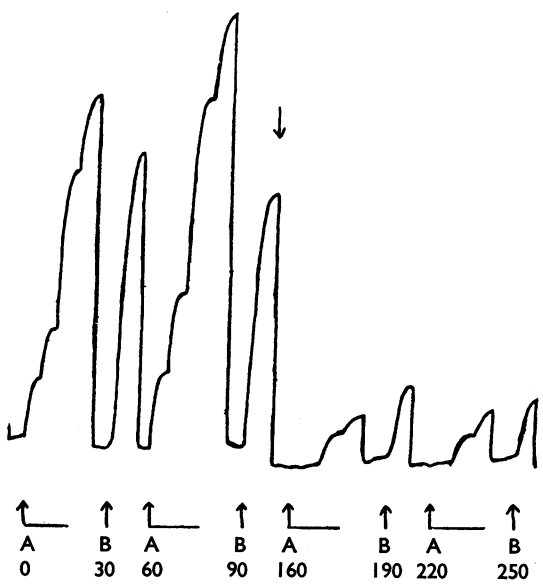

FIG. 1.—Effect of dibenamine on the responses to nicotine and noradrenaline of spiral strips of rabbit aorta. At A, successive doses of (−)-noradrenaline bitartrate (10^{-8}, 3×10^{-8}, 10^{-7}, and 3×10^{-7}) were added at 2.5 min. intervals. At B, nicotine hydrogen tartrate (3×10^{-5}) was added. The numerals give the time in min. The preparation was treated (at the arrow pointing downwards) with dibenamine hydrochloride 3×10^{-7} from 100 to 120 min. The response to nicotine was less readily antagonized by dibenamine than was a comparable response to noradrenaline. Numerals, time in min.

The responses to both noradrenaline and nicotine were reduced by the concentrations of blocking agents used, but the responses to nicotine were less affected than were those to noradrenaline. Thus, after dibenamine, the mean response to nicotine was 1.8 times greater than the response to noradrenaline which initially matched it, and approximately twice the dose of noradrenaline was required to match the test dose of nicotine. The result of a typical experiment on a single strip is shown in Fig. 1.

Nicotine/Phentolamine Antagonism

Dose/response curves were determined for nicotine in eight control strips, and in eight strips treated with phentolamine (10^{-7}) (Fig. 2). Three strips were used each day. In each group of eight, four strips received 0.25, 1, 4, and 16×10^{-5} of nicotine, and four received 0.5, 2, 8, and 32×10^{-5}.

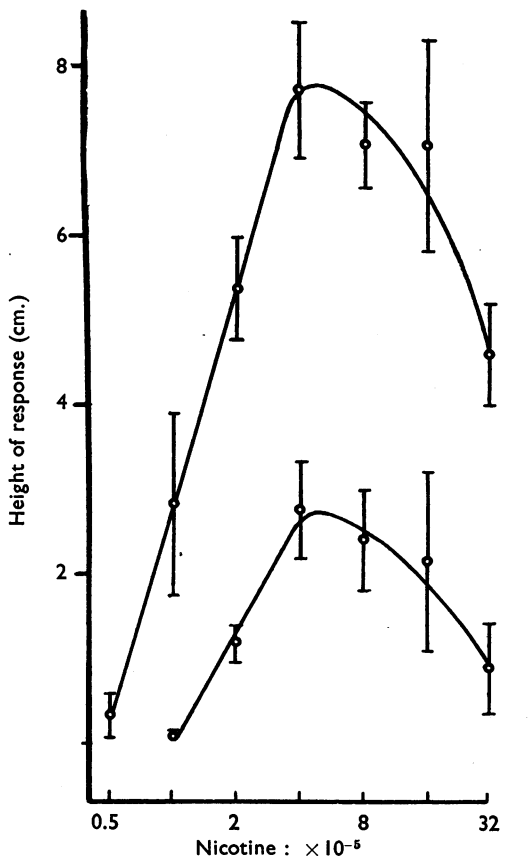

FIG. 2.—Effect of phentolamine on the response to nicotine of spiral strips of rabbit aorta. Each point was based on 4 observations: range marks show S.D. Upper curve: nicotine alone. Lower curve: nicotine after phentolamine (10^{-7}).

TABLE I

EFFECT OF ANTISYMPATHOMIMETIC AGENTS ON THE AMOUNT OF NORADRENALINE REQUIRED TO MATCH THE RESPONSE TO A TEST DOSE OF NICOTINE IN ISOLATED STRIPS FROM RABBIT AORTA

x, the ratio between doses of noradrenaline producing equivalent effects before and after the addition of antagonist, was estimated graphically from the shift of the noradrenaline dose/response curve. Mean results from 6 strips of aorta in parentheses, 95% limits (Finney, 1952, Chapter 4), are given.

Antagonist	Conc.	Noradrenaline Dose Ratio x	Noradrenaline ($\times 10^{-8}$) Required to Match Response to Nicotine 3×10^{-5}, 5% Probability Limits		Ratio B/A
			Before (A)	After (B)	
Dibenamine	3×10^{-7} for 20 min.	8	8·18 (6·29–11·2)	17·8 (13·3–23·8)	2·17
Ergotamine tartrate ..	10^{-7}	13	9·62 (7·25–13·9)	20·8 (13·7–34·1)	2·17
Tolazoline	10^{-6}	6	12·6 (8·49–23·8)	24·8 (17·1–40·0)	1·96
Phentolamine	10^{-7}	6	8·67 (5·99–14·4)	13·6 (10·5–17·3)	1·57
Piperoxane	10^{-6}	10	6·55 (4·81–9·23)	8·62 (5·14–12·1)	1·32
None			17·75 (12·7–25·0)	12·6 (9·01–16·6)	0·71

DISCUSSION

The results described indicate that the response to a near maximal dose of nicotine (3×10^{-5}) was less readily antagonized by antisympathomimetic agents than was a comparable response to noradrenaline. This difference was most marked with dibenamine and ergotamine, where approximately twice the dose of noradrenaline was required to match the test dose of nicotine after treatment with the blocking agents. The other agents examined behaved similarly to some extent, while the control series showed a drift in the opposite direction. Further experiments would be necessary to determine whether these differences between antisympathomimetic agents are real, but it is notable that piperoxane differentiated nicotine and noradrenaline responses least effectively, despite its reputed ineffectiveness against nerve responses.

It was not expected that there would be marked difficulty in blocking the responses to nicotine. Kottegoda (1953) reversed the response to nicotine in the perfused rabbit ear with tolazoline 2×10^{-5}, and Hilton (1954) used phentolamine to demonstrate that nicotine acted sympathomimetically on the blood flow in cat muscle. The concentration of tolazoline used by Kottegoda (1953) was high: the above results show that it would reduce the sensitivity of the preparation to noradrenaline 100-fold.

Haimovici (1948) reported that the responses to nicotine in perfused frog hindquarters were very difficult to block with dibenamine. He claimed that perfusion with dibenamine (4 to 40 mg./ml.) had no effect on the response to nicotine but selectively abolished the response to adrenaline, and used this as evidence that nicotine has a direct vasoconstrictor action in this preparation. The resistance seen in the rabbit aorta was obviously much less marked. The atropine resistance shown by nicotine responses in the isolated rabbit ileum is a similar phenomenon, although more marked. Ambache and Edwards (1951) found that atropine 10^{-4} did not abolish contractions in response to nicotine, although atropine 10^{-6} antagonized large doses of acetylcholine. In the ileum nicotine acts parasympathomimetically (Ambache, 1955), and here, too, the effects of nerve stimulation are antagonized with difficulty. In both cases there are two possible explanations: first that nicotine may liberate high local concentrations of the transmitter within the tissues; second that the transmitter may be released behind a diffusion barrier which is impermeable to both the antagonist and the transmitter substance (see Nickerson and Nomaguchi, 1948). In an isolated organ it is possible that the thickness of the tissue itself might act as a diffusion barrier, nicotine alone of the drugs applied being capable of penetrating throughout the tissue and the others acting only on the surface layers. This seems improbable since the phenomenon was seen for nerve effects *in situ*.

The dose/response curves for nicotine before and after phentolamine 10^{-7} show that the response to nicotine was readily surmountable, as one would expect if it acted by liberating a limited amount of noradrenaline within the tissues. The inhibitory action of large doses of nicotine appeared to be little affected by phentolamine. In the presence of phentolamine 10^{-7} large doses of nicotine (32×10^{-5}) produce a biphasic response in the strips, contraction being followed by relaxation beyond the resting length. Low concentrations of nicotine were apparently not inhibitory, the response to nicotine 10^{-5} (about one-third of the maximal response) being completely abolished by phentolamine 10^{-7}, but the response was not reversed.

Phentolamine 10^{-7} antagonized responses to low doses of nicotine with unexpected ease (Fig. 2). Low doses of nicotine must liberate a uniformly low concentration of noradrenaline within the tissues rather than a high concentration at only a fraction of the muscle fibres, that is to say, the response of the nervous structures stimulated by nicotine must be graded rather than all-or-none. The ease with which low doses of nicotine were antagonized suggests that the amount of difficulty experienced in blocking the response to nicotine may depend on the dose of nicotine. Responses to low doses of nicotine may be more easily blocked than equivalent responses to noradrenaline, although a direct comparison would be required to determine this.

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References

Ambache, N. (1955). *Pharmacol. Rev.*, **7**, 467.
—— and Edwards, J. (1951). *Brit. J. Pharmacol.*, **6**, 311.
Bacq, Z. M., and Fredericq, H. (1935). *C.R. Soc. Biol. (Paris)*, **118**, 183.
Bovet, D., and Simon, A. (1937). *Arch. int. Pharmacodyn.*, **55**, 15.
Burn, J. H., and Rand, M. J. (1958). *Brit. med. J.*, **1**, 903.
Chess, D., and Yonkman, F. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 127.
Finney, D. J. (1952). *Statistical Method in Biological Assay*. London: Griffin & Co.
Furchgott, R. F., and Bhadrakom, S. (1953). *J. Pharmacol. exp. Ther.*, **108**, 129.
Haimovici, H. (1948). *Proc. Soc. exp. Biol., N.Y.*, **68**, 516.
Hilton, S. M. (1954). *J. Physiol. (Lond.)*, **123**, 289.
Kottegoda, S. R. (1953). *Brit. J. Pharmacol.*, **8**, 156.
Morison, R. S., and Lissak, K. (1938). *Amer. J. Physiol.*, **123**, 404.
Nickerson, M., and Nomaguchi, G. M. (1948). *J. Pharmacol. exp. Ther.*, **93**, 40.