Brit. J. Pharmacol. (1964), **23**, 399–404.

INHIBITION OF SYMPATHOMIMETIC EFFECTS ON THE HEART

BY

B. G. BENFEY AND D. R. VARMA

From the Department of Pharmacology, McGill University, Montreal, Canada

(*Received April* 18, 1964)

The adrenaline β-receptor blocking drug, pronethalol, and the sympathomimetic amines, (−)-ephedrine, (−)-amphetamine, dexamphetamine, (−)-Ψ-ephedrine and tyramine, inhibited the sympathomimetic effects of butyrylcholine and tyramine on the guinea-pig isolated atrium. Being reversible and noncompetitive, this antagonism was unspecific and not due to blockade of adrenaline receptors, although pronethalol inhibited the effect of noradrenaline competitively.

It was shown earlier that adrenaline α-receptor blocking drugs antagonize the sympathomimetic actions of butyrylcholine and tyramine on the guinea-pig isolated atrium in concentrations which potentiate the effect of noradrenaline (Benfey & Greeff, 1961). In continuation of these studies it was observed that sympathomimetic amines have similar properties, and the mechanism of this effect has been studied. It was also found that the β-receptor blocking drug, pronethalol, does not inhibit the sympathomimetic actions of butyrylcholine and tyramine by a specific receptor antagonism.

METHODS

The guinea-pig atrium was suspended at 30° C in a solution containing (%) NaCl 0.8, $NaHCO_3$ 0.1, dextrose 0.1, KCl 0.2, $CaCl_2$ 0.2 and NaH_2PO_4 0.005, which was aerated with 5% carbon dioxide in oxygen. The rate (beats/min) and force of contraction (g) were measured with a Grass force-displacement transducer and recorded either by a Gilson polygraph or by a Twin-Viso Sanborn recorder.

In the first part of the experiments the threshold concentration of the antagonists was determined which significantly inhibited the effect of 3 μg/ml. of butyrylcholine. The concentration of the antagonists was doubled until the degree of inhibition became significant. Butyrylcholine was first added alone and then with the antagonist and left in the bath until the maximal response was obtained. After 7 min from washing out the organ-bath, butyrylcholine was again added alone to determine if the inhibition was reversible. The concentration of the antagonists which significantly inhibited the effect of butyrylcholine was similarly tested against 0.2 μg/ml. of noradrenaline and 5 μg/ml. of tyramine.

In the second part of the experiments cumulative dose/response curves were determined to characterize the type of the inhibition. Butyrylcholine (1, 3, 10, 30 and 100μg/ml.), noradrenaline (0.003, 0.03, 0.3, 3 and 30 μg/ml.) and tyramine (0.3, 1, 3, 10 and 30 μg/ml.) were added alone and in the presence of the antagonists. The concentrations remained in the bath until the maximal effect had been observed. To prevent the parasympathomimetic effects of higher concentrations of butyrylcholine (30 and 100 μg/ml.), atropine was added in these experiments in a concentration of 0.7 μg/ml.

The guinea-pig isolated atrium is stimulated by butyrylcholine, an effect which is inhibited by ganglion-blocking drugs, is similar to that of acetylcholine in the presence of atropine and is mediated by endogenous catechol amines (Holtz & Westermann, 1955; Holtz, 1959).

TABLE 1

THE INFLUENCE OF SOME ANTAGONISTS ON THE EFFECTS OF BUTYRYLCHOLINE, NORADRENALINE AND TYRAMINE ON THE GUINEA-PIG ATRIUM

The effects of butyrylcholine (3 μg/ml), noradrenaline (0·2 μg/ml) and tyramine (5 μg/ml) on the rate (HR, beats/min) and force of contraction (FC, g) are given as increases over the control values. The values are means of three experiments with standard errors .*$P<0.05$. †$P<0.01$.

Antagonist	Concentration (μM)	Control HR	Control FC	Effect of butyrylcholine on HR	Effect of butyrylcholine on FC	Control HR	Control FC	Effect of butyrylcholine and antagonist on HR	Effect of butyrylcholine and antagonist on FC	Control HR	Control FC	Effect of butyrylcholine on HR	Effect of butyrylcholine on FC
Hexamethonium	1·1	149	0·70	40±8	0·53±0·10	145	0·62	11±6*	0·12±0·06*	145	0·60	41	0·48
Pronethalol	3·8	156	0·56	17±4	0·29±0·06	156	0·55	1±1†	0·01±0·01*	151	0·52	7	0·14
Dexamphetamine	54	167	0·54	33±4	0·26±0·02	163	0·58	1±1†	0	175	0·57	35	0·16
				Effect of tyramine				Effect of tyramine and antagonist				Effect of tyramine	
Hexamethonium	1·1	141	0·43	39±11	0·35±0·08	135	0·44	44±14	0·29±0·08	135	0·47	44	0·23
Pronethalol	3·8	140	0·48	23±5	0·17±0·02	142	0·50	5±4*	0·08±0·02*	139	0·53	7	0·07
Dexamphetamine	54	177	0·55	24±5	0·11±0·02	177	0·56	3±3*	0	186	0·60	16	0·04
				Effect of noradrenaline				Effect of noradrenaline and antagonist				Effect of noradrenaline	
Hexamethonium	1·1	143	0·49	44±11	0·31±0·05	139	0·46	48±10	0·33±0·06	143	0·44	44	0·37
Pronethalol	3·8	151	0·49	17±1	0·21±0·04	149	0·49	15±4	0·18±0·01	150	0·49	15	0·21
Dexamphetamine	54	176	0·55	43±8	0·18±0·02	179	0·53	37±8	0·19±0·02	192	0·57	25	0·13

The highest effective concentration of tyramine was 30 μg/ml., 100 μg/ml. causing depression of the amplitude of contraction.

The following drugs were used: butyrylcholine iodide, tyramine hydrochloride, (−)-noradrenaline bitartrate monohydrate, hexamethonium bromide, atropine sulphate (U.S.P.), pronethalol hydrochloride (Alderlin), (−)-ephedrine sulphate (U.S.P.), (−)-amphetamine sulphate, dexamphetamine sulphate (Dexedrine) and (−)-Ψ-ephedrine hydrochloride (L. Light). Amounts refer to the salts.

The statistical calculations were made according to conventional procedures (Mainland, 1952). In all of the experiments the antagonists were added immediately before the agonists.

RESULTS

The concentrations of hexamethonium, pronethalol and dexamphetamine which significantly inhibited the sympathomimetic effect of butyrylcholine on the rate and force of contraction of the atrium are listed in Table 1. The concentrations (μM) of the other drugs which exerted a significant inhibition under the same conditions are as follows: atropine 7.2, cocaine 18, (−)-ephedrine 47, (−)-amphetamine 54, (−)-ψ-ephedrine 200 and tyramine 1200. Thus, on a molar basis pronethalol is approximately three-times less potent than hexamethonium and the potency of the sympathomimetic amines ranges between 0.025- and 0.001-times that of hexamethonium.

The antagonism was reversible. The concentrations effective against butyrylcholine did not inhibit the action of noradrenaline. It appears that, while the onset of the blockade of butyrylcholine and tyramine is immediate, pronethalol requires a few minutes to establish its antagonism against 0.2 μg/ml. of noradrenaline. The effect of tyramine was significantly and reversibly inhibited by pronethalol and the sympathomimetic amines.

Cumulative dose/response curves reveal the type of the antagonism. As the drugs were employed under different conditions (a series of increasing concentrations of the agonist instead of a single concentration applied immediately after the antagonist, see Methods), the quantitative results shown in Table 1 and Figs. 1 and 2 may slightly vary.

Hexamethonium inhibited the effect of butyrylcholine noncompetitively, as the slope of the dose/response curve becomes progressively flatter (Fig. 1). Almost identical curves were obtained with atropine (7.2, 14 and 29 μM). In contrast amphetamine, the other sympathomimetic amines and cocaine flattened the dose/response curves greatly. But being noncompetitive the type of antagonism of all the drugs was similar, although hexamethonium and atropine were effective in concentrations which did not depress the maximum of the dose/response curves.

Fig. 2 shows the behaviour of pronethalol. The drug exerted a noncompetitive antagonism of butyrylcholine in a concentration which inhibited the action of noradrenaline competitively. When the effective concentration of pronethalol (3.8 μM) was doubled, the contractions of the atrium were depressed, butyrylcholine had no effect, but larger concentrations of noradrenaline overcame the block.

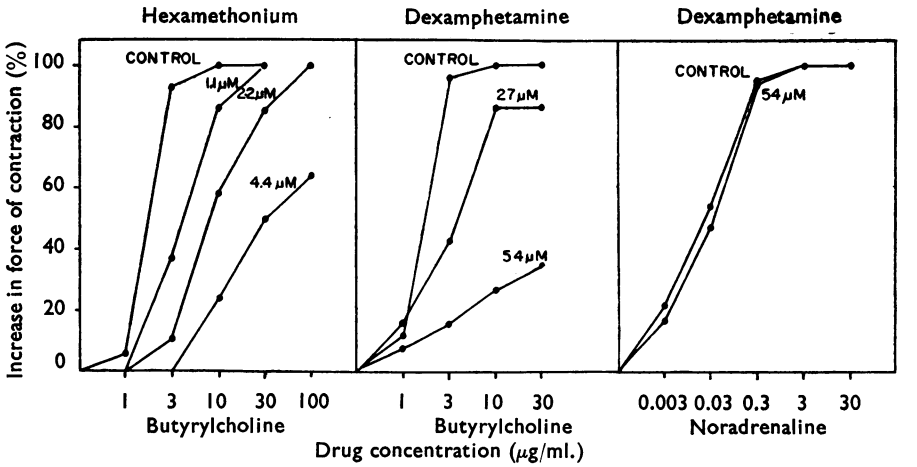

Fig. 1. Guinea-pig atrium. Dose/response curves of butyrylcholine and noradrenaline with hexamethonium (1·1, 2·2 and 4·4 μM) and dexamphetamine (27 and 54 μM). The control curves were in the absence of the antagonists. Each value was obtained from three to five preparations

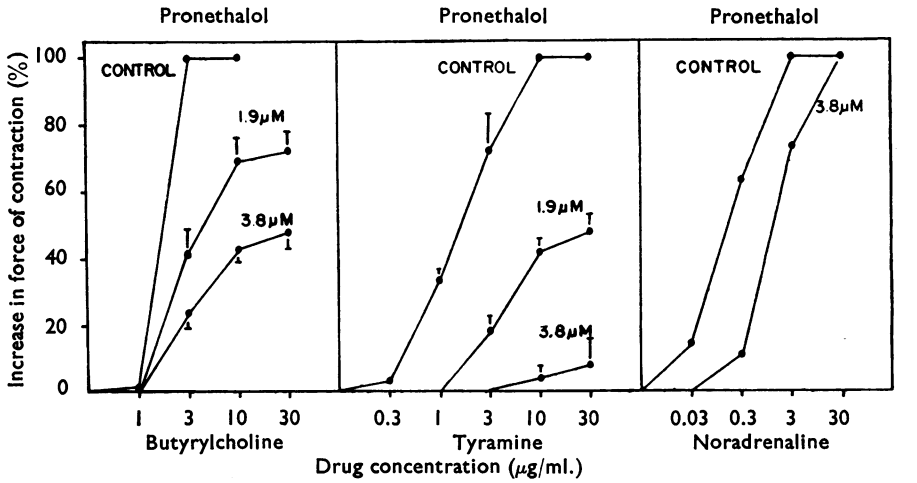

Fig. 2. Guinea-pig atrium. Dose/response curves of butyrylcholine, tyramine and noradrenaline with pronethalol (1·9 and 3·8 μM). The control curves were in the absence of pronethalol. The vertical bars represent standard errors of means. Each value was obtained from three or four preparations.

With regard to tyramine, dexamphetamine (54 μM) inhibited all concentrations entirely although it did not significantly antagonize noradrenaline (Fig. 1). Pronethalol antagonized tyramine noncompetitively (Fig. 2).

DISCUSSION

The sympathomimetic drugs, cocaine, pronethalol and hexamethonium antagonized the sympathomimetic effects of butyrylcholine on the heart by a similar mechanism, a reversible, noncompetitive antagonism.

Reversible, noncompetitive antagonists are known to produce unspecific effects which do not follow the mass law (Arunlakshana & Schild, 1959). They may interfere with ionic fluxes through cell membranes (Shanes, 1958). The sympathomimetic effect of butyrylcholine may involve stimulation of postganglionic sympathetic fibres. Thus, Ferry (1963) found that acetylcholine stimulates the sympathetic postganglionic nerves of the spleen near their endings, an effect which is inhibited by hexamethonium, and Ritchie (1963) reported that acetylcholine depolarizes C-fibres. This occurs through an increase in permeability of the fibre membrane to ions such as sodium and calcium and is inhibited by many quarternary and tertiary nitrogen-containing compounds including hexamethonium, atropine and local anaesthetics.

Pronethalol was reported by Gill & Vaughan Williams (1964) to possess local anaesthetic properties 1.8-times greater than does procaine. As an anti-arrhythmic drug pronethalol is aproximately twice as potent as quinidine (Sekiya & Vaughan Williams, 1963). According to Szekeres & Vaughan Williams (1962) the essential feature of antifibrillatory action is an interference with the mechanism of depolarization.

That sympathomimetic drugs may inhibit effects of acetylcholine on autonomic ganglia has been known for some time. Thus, Marrazzi (1939) found that adrenaline and ephedrine inhibit transmission in the cat superior cervical ganglion, an effect which could be overcome by strengthening the preganglionic stimuli. Bülbring (1944) reported that large amounts of adrenaline inhibit the contractions of the nictitating membrane of cats in response to stimulation of the preganglionic fibres of the superior cervical ganglion and to acetylcholine injected into the perfusion circuit. Paton & Thompson (1953) showed that, similarly to procainamide, adrenaline interferes with ganglionic transmission both by diminishing the release of acetylcholine in response to preganglionic stimulation and by depressing the action of acetylcholine on the ganglion cell. Reinert (1960) reported that dexamphetamine produces depolarization and block in the cat superior cervical ganglion, identical to that seen after nicotine, while ephedrine and tyramine reduce transmission but do not alter the shape of the action potential.

The mechanism of action of tyramine is still in debate. Rather large concentrations of the drug are required to increase the rate of the guinea-pig atrium (at least 1 μg/ml. of the free base), but it depresses the rate in a concentration of 100 μg/ml., while noradrenaline stimulates in a concentration of 0·01 μg/ml. and depresses in a concentration of 2 mg/ml. (Trendelenburg, Gomez Alonso de la Sierra & Muskus, 1963). It may be assumed that this, at least partly, accounts for the phenomenon of tachyphylaxis. Tachyphylaxis may appear when the relatively large amount of tyramine has to stimulate a heart which is already on the verge of depression due to the previous exposure to the amine which causes tachyphylaxis. Dexamphetamine and (—)-ephedrine, which depress the rate of the atrium in a concentration of 10 μg/ml. (Trendelenburg *et al.*, 1963), may inhibit the action of tyramine by cross-tachyphylaxis. As pronethalol is chemically related to isoprenaline and has been reported by Black & Stephenson (1962) to depress the contractions of the cat papillary muscle in concentrations only ten-times greater than the blocking concentration, the drug may be assumed to possess the depressant property of the

sympathomimetic amines in addition to its specific noradrenaline antagonism. Therefore, the antagonism of tyramine by pronethalol, which has been shown not to be due to blockade of adrenaline receptors, may be called cross-tachyphylaxis.

NOTE ADDED IN PROOF

The new adrenaline β-receptor blocking drug propranolol (Inderal, I.C.I.) behaved in a similar way to pronethalol but was more potent both as a competitive inhibitor of the inotropic action of noradrenaline on the guinea-pig isolated atrium and as a noncompetitive inhibitor of the sympathomimetic actions of butyrylcholine and tyramine.

This work was supported by a grant from the Medical Research Council of Canada and was presented in part at the June, 1963, meeting of the Canadian Federation of Biological Societies at London, Ontario. Drugs were kindly provided by Ayerst, McKenna & Harrison and Poulenc.

REFERENCES

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). Some quantitative uses of drug antagonists. *Brit. J. Pharmacol.*, **14**, 48–58.

BENFEY, B. G. & GREEFF, K. (1961). Interactions of sympathomimetic drugs and their antagonists on the isolated atrium. *Brit. J. Pharmacol.*, **17**, 232–235.

BLACK, J. W. & STEPHENSON, J. S. (1962). Pharmacology of a new adrenergic beta-receptor-blocking compound (Nethalide). *Lancet*, **ii**, 311–314.

BÜLBRING, E. (1944). The action of adrenaline on transmission in the superior cervical ganglion. *J. Physiol. (Lond.)*, **103**, 55–67.

FERRY, C. B. (1963). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol. (Lond.)*, **167**, 487–504.

GILL, E. W. & VAUGHAN WILLIAMS, E. M. (1964). Local anaesthetic activity of the β-receptor antagonist, pronethalol. *Nature (Lond.)*, **201**, 199.

HOLTZ, P. (1959). Allgemeine Physiologie der nervalen und humoralen Regulation des Kreislaufs. *Verh. dtsch. Ges. Kreisl.-Forsch.*, **25**, 36–53.

HOLTZ, P. & WESTERMANN, E. (1955). Versuche mit Acetyl-, Propionyl- und Butyrylcholin am isolierten Herzvorhofpräparat. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **225**, 421–427.

MAINLAND, D. (1952). *Elementary Medical Statistics*, 1st edit, pp. 149 and 154. Philadelphia & London : W. B. Saunders.

MARRAZZI, A. S. (1939). Adrenergic inhibition at sympathetic synapses. *Amer. J. Physiol.*, **127**, 738-744.

PATON, W. D. M. & THOMPSON, J. W. (1953). The mechansm of action of adrenaline on the superior cervical ganglion of the cat. *Abstr. 19th Int. Physiol. Congr.*, p. 664.

REINERT, H. (1960). The depolarizing and blocking action of amphetamine in the cat's superior cervical ganglion. *In Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 373–379. London : Churchill.

RITCHIE, J. M. (1963). The action of acetylcholine and related drugs on mammalian non-myelinated nerve fibres. *Biochem. Pharmacol.*, **12**, suppl. 3.

SEKIYA, A. & VAUGHAN WILLIAMS, E. M. (1963). A comparison of the antifibrillatory actions and effects on intracellular cardiac potentials of pronethalol, disopyramide and quinidine. *Brit. J. Pharmacol.*, **21**, 473–481.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.*, **10**, 59–273.

SZEKERES, L. & VAUGHAN WILLIAMS, E. M. (1962). Antifibrillatory action. *J. Physiol. (Lond.)*, **160**, 470–482.

TRENDELENBURG, U., GOMEZ ALONSO DE LA SIERRA, B. & MUSKUS, A. (1963). Modification by reserpine of the response of the atrial pacemaker to sympathomimetic amines. *J. Pharmacol. exp. Ther.*, **141**, 301–309.