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ANTISYMPATHOMIMETIC AND ANTIFIBRILLATORY EFFECTS OF PRONETHALOL AND PROPRANOLOL

BY

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In a previous publication (Benfey & Varma, 1964) it was reported that, although pronethalol inhibits noradrenaline competitively, it inhibits the sympathomimetic effects of butyrylcholine and tyramine on the guinea-pig isolated atrium noncompetitively. With the introduction of the more potent sympathetic β-receptor blocking drug, propranolol (Black, Crowther, Shanks, Smith & Dornhorst, 1964), it became possible to investigate whether the nonspecific (that is noncompetitive and reversible) butyrylcholine and tyramine antagonism is related to the specific (that is competitive) noradrenaline antagonism. Also it appeared of interest to investigate if the nonspecific antisympathomimetic effects of these drugs are related to their antifibrillatory effects.

β-Receptor blocking drugs are known to possess antifibrillatory properties. Thus antifibrillatory effects of dichloroisoprenaline have been described by Gilbert, Lange & Brooks (1959), Dresel (1960), Dresel, MacCannell & Nickerson (1960), Moore & Swain (1960), Lucchesi & Hardman (1961), Moran, Moore, Holcomb & Mushet (1962) and Sekiya & Vaughan Williams (1963a). Antifibrillatory properties of pronethalol have been reported by Sekiya & Vaughan Williams (1963a, b), Erlij & Mendez (1964) and Lucchesi (1964).

Sekiya & Vaughan Williams (1963a) " obtained some evidence that the protective action of pronethalol was related to its β-receptor sympathetic blocking activity," and Erlij & Mendez (1964) " ascribe the modifications of digitalis intoxication to a reduction in adrenergic influences on the heart." However, Lucchesi (1964) " suggested that nethalide possesses an additional pharmacologic effect to account for its anti-arrhythmic action " (Nethalide is pronethalol). A quantitative study of the two β-receptor blocking drugs was expected to show if the antifibrillatory activity was related to the β-receptor blocking property.

METHODS

The guinea-pig atria were set up as described before (Benfey & Varma, 1964), the force of contraction being recorded by a Twin-Viso Sanborn recorder.

Cumulative dose/response curves were determined to establish the potency of the antagonists. Butyrylcholine (0.3, 1, 3, 10 and 30 μg/ml.), tyramine (0.3, 1, 3, 10, 30 and 100 μg/ml.) and noradrenaline (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 μg/ml.) were added alone and 5 min after adding the antagonists. The concentrations remained in the bath until the maximal effect on the force of contraction had been observed. To prevent the parasympathomimetic effect of the highest concentration of butyrylcholine, atropine (0.7 μg/ml.) was added in the butyrylcholine experiments.

Pronethalol and propranolol inhibit butyrylcholine and tyramine noncompetitively (Benfey & Varma, 1964). Therefore, to compare their potency amounts were selected which depressed the maximum of the dose/response curves approximately 50%. In regard to noradrenaline, which is inhibited competitively (Benfey & Varma, 1964), the potency of the antagonists was calculated in terms of the dose-ratios by comparing the effects of noradrenaline in the presence of the antagonist with the initial dose/response curve. The pA_{10} was determined using Fig. 1; pA_{10} is the negative log of the molar concentration of the antagonist which leads to an agonist dose-ratio of 10 (Schild, 1947).

The antifibrillatory potency was determined according to the method of Dawes (1946), using the guinea-pig atrium at 30° C instead of the rabbit atrium at 29° C. Five atria were used whose spontaneous rate was 144±7 beats/min (mean and standard error). Before adding the drugs these atria could be stimulated to a rate of 507±43 beats/min. This may be compared to the spontaneous rate of rabbit isolated atria of 80 to 120 beats/min which could be stimulated to 250 to 350 beats/min (Dawes, 1946). Using rabbit atria, Sekiya & Vaughan Williams (1963b) found little difference in the antifibrillatory activities of pronethalol and quinidine, a result which is similar to that obtained in this study with the guinea-pig atria. Thus guinea-pig atria may be used to determine the effective refractory period. The drugs were added 10 min before the test.

The effect of the drugs on the toxic dose of ouabain was tested according to the method of Sekiya & Vaughan Williams (1963a); guinea-pigs were anaesthetized with urethane and were given infusions of ouabain, 4 μg/30 sec every 2 min. The antagonists were injected slowly over a period of 5 to 10 min and the ouabain infusions were started 5 min later.

The following drugs were used: butyrylcholine iodide, tyramine hydrochloride, (−)-noradrenaline bitartrate monohydrate, atropine sulphate, pronethalol hydrochloride (Alderlin), propranolol hydrochloride (Inderal) and quinidine sulphate. Amounts refer to the salts unless otherwise stated. The statistical calculations were made according to conventional procedures (Mainland, 1952).

RESULTS

Competitive antagonism of noradrenaline

Fig. 1 shows a linear relationship of log (concentration ratio −1) and log molar concentration of antagonist, which indicates a competitive antagonism of noradrenaline by

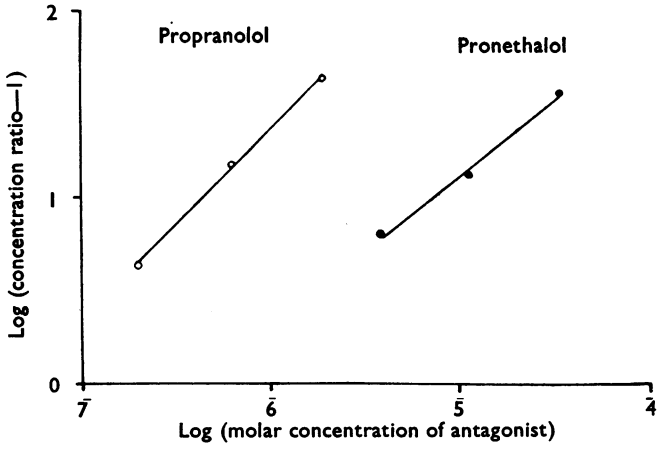

Fig. 1. Guinea-pig atrium. Relation of log (concentration ratio of noradrenaline minus 1) and log molar concentration of propranolol (empty circles) and pronethalol (filled circles). Each value was obtained from three preparations. The plot was obtained from dose/effect curves measuring the effect of noradrenaline on the force of contraction before and after adding the antagonists; it served to obtain the pA_{10} values of pronethalol and propranolol with noradrenaline. The concentration ratio was determined at 50% of the maximal effect.

pronethalol and propranolol. While the pA_{10} of pronethalol with noradrenaline was 5.19, that of propranolol was 6.40, indicating a 16.6-fold greater potency of the latter drug.

Propranolol does not exhibit a particularly high degree of potency, judged from the fact that on the guinea-pig isolated atrium the α-receptor blocking drug, phenoxybenzamine, had a pA_{10} with acetylcholine of 6.8 and atropine had a pA_{10} of 8.4 (Benfey & Grillo, 1963). Thus the α-receptor blocking drug was a more potent antagonist of acetylcholine than was the β-receptor blocking drug of noradrenaline. It may be mentioned that on the guinea-pig isolated atrium α-receptor blocking drugs, for example, phenoxybenzamine and phentolamine, potentiate the action of noradrenaline and inhibit the sympathomimetic actions of butyrylcholine and tyramine (Benfey & Greeff, 1961).

Acetylcholine was more potent in depressing the isolated atrium than was noradrenaline in stimulating it. While a concentration of 0.016 μg/ml. (0.071 μM) of acetylcholine reduced the amplitude of contraction 50% (Benfey & Grillo, 1963), 50% of the maximal increase of the amplitude of contraction was observed in the seventeen atria used in this study with 0.18 ± 0.03 μg/ml. (mean and standard error) of noradrenaline (0.53 μM). However, differences in potency of agonists do not influence *pA* values (Schild, 1947). It may be expected that β-receptor blocking drugs will be found which are more potent than propranolol.

Noncompetitive antagonism of butyrylcholine and tyramine

Pronethalol and propranolol inhibit butyrylcholine and tyramine noncompetitively (Benfey & Varma, 1964). It is seen in Fig. 2 that 2.25 μM-pronethalol and 0.61 μM-propranolol depressed the maximum of the dose/response curve of butyrylcholine approximately 50%, indicating a 3.7-fold difference in potency. The same concentrations had a similar effect on tyramine (Fig. 2).

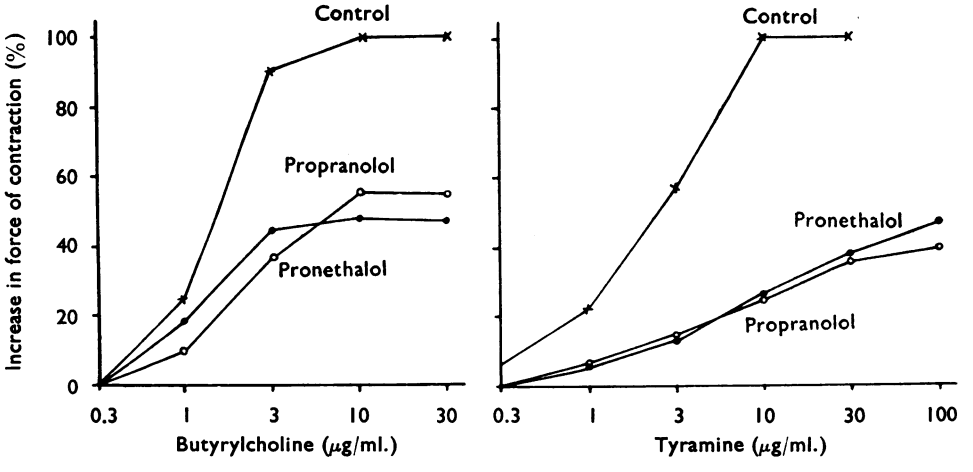

Fig. 2. Effects of butyrylcholine (left-hand graph) and tyramine (right-hand graph) on the force of contraction (percentage increase) of the guinea-pig atrium before (control, crosses) and after the addition of propranolol (0.61 μM, empty circles) and pronethalol (2.25 μM, filled circles). Each value was obtained from three preparations.

It appears that the sympathomimetic effects of butyrylcholine and tyramine are only partly antagonized by a nonspecific (that is noncompetitive and reversible) effect. That propranolol was 3.7-times more potent than pronethalol is probably due to the fact that its β-receptor blocking activity is greater, thus accounting for part of the inhibition.

Antifibrillatory effect

Similar concentrations of quinidine, pronethalol and propranolol had similar effects on the effective refractory period of the guinea-pig atrium. The antifibrillatory potency of the drugs was not significantly different, as seen by the reduction of the maximal rate at which the atrium followed an electrical stimulus (Fig. 3).

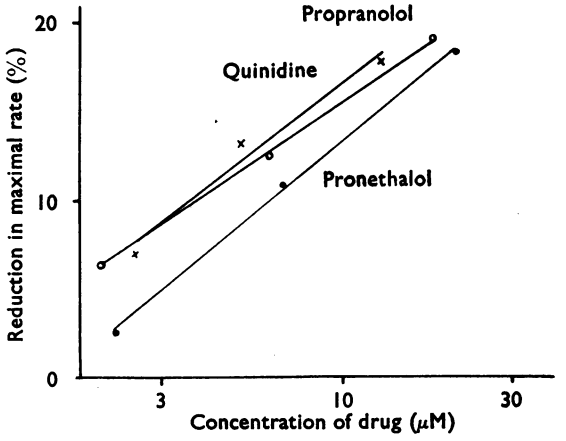

Fig. 3. Guinea-pig atrium. Effects of quinidine (crosses), propranolol (empty circles) and pronethalol (filled circles) on the maximal rate at which the preparation followed an electrical stimulus. Each value was obtained from three preparations.

TABLE 1

PROTECTIVE ACTION OF PRONETHALOL AND PROPRANOLOL AGAINST OUABAIN INTOXICATION

Values are means and standard errors; numbers in parentheses give the number of animals which had fibrillation and the number of animals tested.
* $P<0.05$; † $P<0.01$

Treatment	Dose (mg/kg)	No. of animals	Mean weight (g)	Heart rate: Control (beats/min)	Heart rate: After treatment (% of control)	Amount (μg/kg) of ouabain to produce: Ventricular arrhythmias	Ventricular fibrillation	Cardiac arrest
None	—	11	484	318	—	99±9·5	128±12·9	151±14·9
Pronethalol	1·25	5	500	308	79	140±12·9	227±32·2* (4/5)	260±27·2†
Pronethalol	2·5	6	329	316	63	215±26·5†	242±28·1* (5/6)	266±23·6†
Pronethalol	5·0	8	385	336	58	258±43·2†	280±23·0† (6/8)	332±42·2†
Propranolol	1·25	6	460	292	66	172±35·4	248±30·2† (3/6)	268±25·8†
Propranolol	2·5	6	337	320	64	238±22·6†	245±26·2† (4/6)	305±21·3†
Propranolol	5·0	5	374	342	60	243±23·3†	— (0/5)	294±25·3†

Ouabain antagonism

The protective effect of pronethalol and propranolol against ouabain intoxication is shown in Table 1. The drugs slowed the heart rate. The onset of ventricular arrhythmias, of ventricular fibrillation and of cardiac arrest was slowed and it required significantly greater amounts of ouabain to exert these effects. In contrast to the results of Sekiya & Vaughan Williams (1963a), ouabain produced ventricular fibrillation in some of the animals treated with pronethalol. The protective effect of 1.25 mg/kg of pronethalol was not significantly different from that of 5 mg/kg. Nor was the effect of equal amounts of propranolol different from that of pronethalol. It appears that propranolol is not more potent than pronethalol as an antagonist of ouabain.

Three animals were used to test if propranolol, like pronethalol (Sekiya & Vaughan Williams, 1963a), is capable of reversing ventricular fibrillation. Propranolol, 5 mg/kg, was injected after ventricular fibrillation had developed. Ventricular fibrillation was stopped in all three animals and a normal rhythm returned in two of them.

DISCUSSION

It is evident from the results that there is no direct correlation between the β-receptor blocking potencies of pronethalol and propranolol and their antifibrillatory activities. Although different in potency as receptor antagonists, the drugs were equally potent in lengthening the effective refractory period of the guinea-pig atrium and in increasing the toxic dose of ouabain in guinea-pigs. This supports the contention of Lucchesi & Hardman (1961) and Lucchesi (1964) who could not find a direct relationship between the β-receptor blocking potencies of dichloroisoprenaline and pronethalol and the effects of these drugs against cardiac glycosides. It appears that properties other than β-receptor antagonism are required to antagonize the toxic effects of cardiac glycosides.

β-Receptor blocking drugs may antagonize arrhythmias caused by sympathomimetic agents by a receptor antagonism, as suggested by Moran *et al.* (1962). These authors found that dichloroisoprenaline was effective in antagonizing arrhythmias caused by sympathomimetic drugs in contrast to arrhythmias resulting from coronary artery ligation which are antagonized by quinidine. In addition, dichloroisoprenaline did not prevent the induction of arrhythmias by ouabain and did not significantly increase the lethal dose of the glycoside. It remains to be seen if propranolol as the more potent β-receptor blocking drug is more potent than pronethalol in antagonizing arrhythmias caused by sympathomimetic drugs.

SUMMARY

1. Sympathetic β-receptor blocking drugs are known to possess antifibrillatory properties, and it has been suggested that they owe their antifibrillatory effects to their β-receptor blocking property. When the new β-receptor blocking drug, propranolol, was introduced this hypothesis was tested. We studied if propranolol has antifibrillatory properties and, if so, if these were related to its β-receptor blocking activity, by comparing them to those of the less-potent β-receptor blocking drug, pronethalol.

2. On the guinea-pig isolated atrium pronethalol and propranolol inhibited the effect of noradrenaline competitively, propranolol being seventeen-times more potent than

pronethalol. The sympathomimetic effects of butyrylcholine and tyramine were inhibited noncompetitively; propranolol was four-times more potent than pronethalol.

3. The drugs reduced the maximal rate at which the guinea-pig isolated atrium followed an electrical stimulus; pronethalol, propranolol and quinidine were equally potent.

4. Propranolol was similar in potency to pronethalol in increasing the toxic dose of ouabain in guinea-pigs.

5. It is concluded that pronethalol and propranolol possess an antifibrillatory property which is not directly related to their β-receptor blocking activity.

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REFERENCES

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

BENFEY, B. G. & GRILLO, S. A. (1963). Antagonism of acetylcholine by adrenaline antagonists. *Brit. J. Pharmacol.*, **20**, 528–533.

BENFEY, B. G. & VARMA, D. R. (1964). Inhibition of sympathomimetic effects on the heart. *Brit. J. Pharmacol.*, **23**, 399–404.

BLACK, J. W., CROWTHER, A. F., SHANKS, R. G., SMITH, L. H. & DORNHORST, A. C. (1964). A new adrenergic beta-receptor antagonist. *Lancet*, **i**, 1080–1081.

DAWES, G. D. (1946). Synthetic substitutes for quinidine. *Brit. J. Pharmacol.*, **1**, 90–112.

DRESEL, P. E. (1960). Blockade of some cardiac actions of adrenaline by dichloroisoproterenol. *Canad. J. Biochem.*, **38**, 375–381.

DRESEL, P. E., MACCANNELL, K. L. & NICKERSON, M. (1960). Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane-anesthetized dogs. *Circulation Res.*, **8**, 948–955.

ERLIJ, D. & MENDEZ, R. (1964). The modification of digitalis intoxication by excluding adrenergic influences on the heart. *J. Pharmacol. exp. Ther.*, **144**, 97–103.

GILBERT, J. L., LANGE, G. & BROOKS, C. MCC. (1959). Influence of sympathomimetic pressor drugs on arrhythmias caused by multiple stimuli. *Circulation Res.*, **7**, 417–423.

LUCCHESI, B. R. (1964). The action of Nethalide upon experimentally induced cardiac arrhythmias. *J. Pharmacol. exp. Ther.*, **145**, 286–291.

LUCCHESI, B. R. & HARDMAN, H. F. (1961). The influence of dichloroisoproterenol (DCI) and related compounds upon ouabain and acetylstrophanthidin induced cardiac arrhythmias. *J. Pharmacol. exp. Ther.*, **132**, 372–381.

MAINLAND, D. (1952). *Elementary Medical Statistics*, 1st ed., p. 149. Philadelphia & London: W. B. Saunders.

MOORE, J. I. & SWAIN, H. H. (1960). Sensitization to ventricular fibrillation. I. Sensitization by a substituted propiophenone, U-0882. *J. Pharmacol. exp. Ther.*, **128**, 243–252.

MORAN, N. C., MOORE, J. I., HOLCOMB, A. K. & MUSHET, G. (1962). Antagonism of adrenergically-induced cardiac arrhythmias by dichloroisoproterenol. *J. Pharmacol. exp. Ther.*, **136**, 327–335.

SCHILD, H. O. (1947). *p*A, a new scale for the measurement of drug antagonism. *Brit. J. Pharmacol.*, **2**, 189–206.

SEKIYA, A. & VAUGHAN WILLIAMS, E. M. (1963a). The effects of pronethalol, dichloroisoprenaline and disopyramide on the toxicity to the heart of ouabain and anaesthetics. *Brit. J. Pharmacol.*, **21**, 462–472.

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