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THE EFFECTS ON CARDIAC MUSCLE OF β-RECEPTOR ANTAGONISTS IN RELATION TO THEIR ACTIVITY AS LOCAL ANAESTHETICS

BY

A. MORALES-AGUILERA AND E. M. VAUGHAN WILLIAMS

From the Department of Pharmacology, University of Oxford

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Pronethalol, introduced by Black & Stephenson (1962) as a drug whose main action was the blockade of β-receptors, was found to reduce the toxicity of ouabain (Vaughan Williams & Sekiya, 1963) and to be more active than quinidine in conventional tests for antifibrillatory action (Sekiya & Vaughan Williams, 1963a, b). Pronethalol is also a powerful local anaesthetic, nearly twice as active as procaine (Gill & Vaughan Williams, 1964). Since many antifibrillatory agents are also local anaesthetics, it was of interest to know whether the antifibrillatory potency of pronethalol and other similar compounds ran parallel to their action as local anaesthetics or as β-receptor antagonists. The introduction of propranolol, as a β-receptor antagonist several times more active than pronethalol, has provided an opportunity to study this problem, and the present paper is concerned with estimating the relative potencies in several different tests of propranolol, pronethalol and lignocaine (the local anaesthetic activity of which is approximately equal to that of pronethalol).

METHODS

Tests for local anaesthetic action were made in guinea-pigs by the wheal method of Bülbring & Wajda (1945). The results presented give the mean values of experiments on eighteen guinea-pigs. The other tests were carried out on rabbit isolated atria with apparatus and methods already described (Vaughan Williams & Szekeres, 1961; Szekeres & Vaughan Williams, 1962). In the experiments summarized in Figs. 2 and 3, control readings were taken after the atria had been beating for 1 hr, and measurements were again made after 1 hr exposure to a given drug concentration (total, fifty-one exposures). The drugs used were procaine hydrochloride (B.D.H.), lignocaine hydrochloride (Xylocaine, Astra-Hewlett); pronethalol (Alderlin) and propranolol (Inderal, I.C.I., 45520) were kindly supplied by I.C.I. Pharmaceutical Division.

RESULTS

Local anaesthetic activity. Pronethalol had been assayed against procaine as standard (Gill & Vaughan Williams, 1964) and had been found to have 1.8-times the potency of procaine (log $R=0.27\pm0.05$). Propranolol was, therefore, also assayed against procaine, and was found to be 2.3-times as active (5% fiducial limits 2.47 and 2.17; log $R=0.365\pm0.014$). The difference in local anaesthetic potency (28%) between pronethalol and propranolol was thus on the borderline of statistical significance ($P<0.1>0.05$).

Tests for antifibrillatory action. In a previous investigation the performance of five drugs which had been reported to possess antifibrillatory activity was compared in a number of tests (Vaughan Williams & Szekeres, 1961). These same tests have been employed in the present study. In control experiments, in which records were obtained from atria without exposure to any drugs, the results of the tests were very stable (Fig. 1). The effects of lignocaine, pronethalol and propranolol have been compared on the spontaneous heart rate and height of contractions in Fig. 2, and on the electrical threshold and maximum driving frequency in Fig. 3. Measurements of the action of pronethalol in similar tests

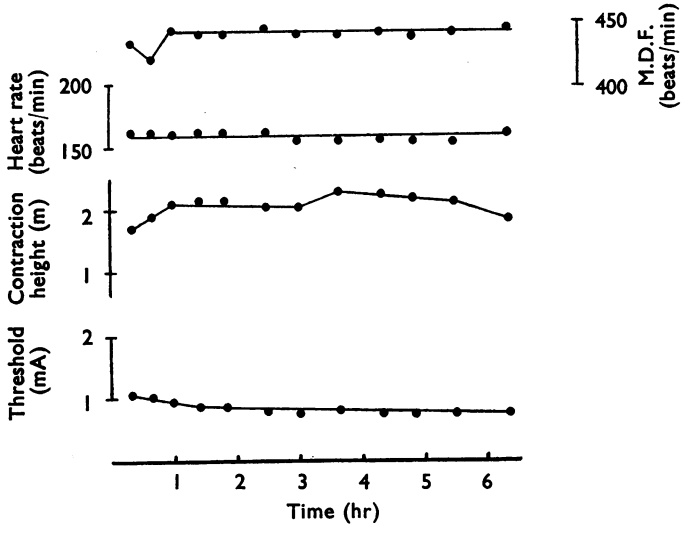

Fig. 1. Repeated measurements to illustrate stability of tests. Successive measurements for more than 6 hr were made of spontaneous heart rate, maximum driving frequency (M.D.F.), electrical threshold and the height of contractions.

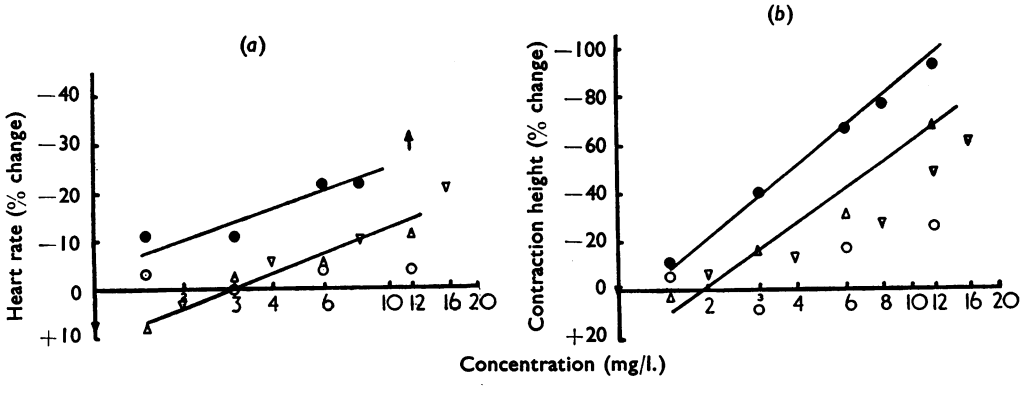

Fig. 2. Effects of lignocaine (○), pronethalol (△) and propranolol (●) on: (*a*) the spontaneous rate; (*b*) the height of contractions of rabbit isolated atria. Ordinate: percentage decrease. Abscissa: drug concentration on log scale. The results with pronethalol (indicated by ▽) are from Sekiya & Vaughan Williams (1963b). The lines in Figs. 2 and 3 have been drawn to fit regressions calculated from the data of Morales-Aguilerá & Vaughan Williams only.

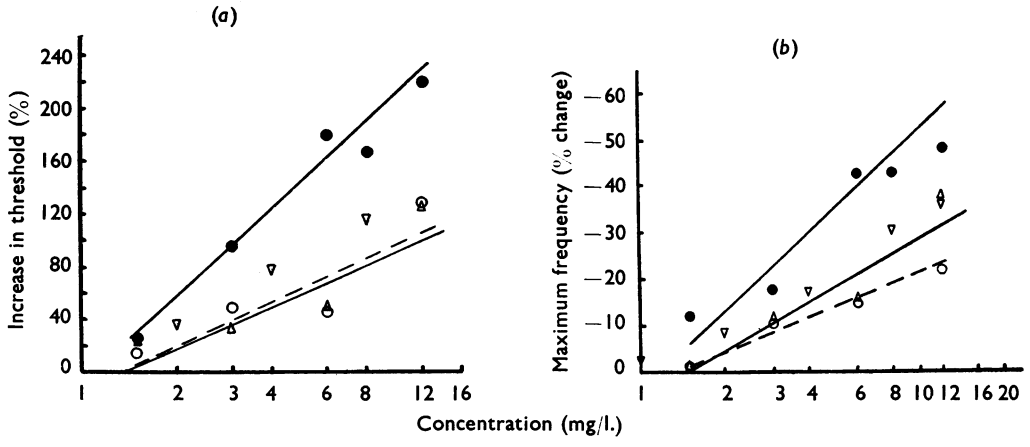

Fig. 3. Effects of lignocaine, pronethalol and propranolol on: (*a*) electrical threshold; (*b*) maximum frequency at which atria would follow electric stimuli. Ordinate: percentage change from controls. Abscissa: drug concentration on log scale. Symbols as in Fig. 2.

were made previously by Sekiya & Vaughan Williams (1963b), and these results also have been replotted in the Figures with different symbols. It is evident that lignocaine had no significant effect on the spontaneous frequency, and had less effect than pronethalol on contractions. In raising threshold current and reducing maximum driving frequency, however, lignocaine and pronethalol were of comparable potency. It is also clear that propranolol was much more active than was pronethalol in all the tests. The ratio (R) of activities was greatest on the spontaneous frequency (R=4.7, fiducial limits 9.4 to 2.2), and least on contractions (R=1.9). There was thus a very marked contrast between the results of the tests for " antifibrillatory activity " (electrical threshold, R=3.7, and maximum driving frequency, R=2.2), in which propranolol was clearly much more active than pronethalol, and the tests for local anaesthetic potency, in which the difference between the drugs was barely significant.

Effects on intracellular potentials. The difference in activity between propranolol and pronethalol was even more marked when their effects on intracellular cardiac potentials were measured. Pronethalol had already been shown (Sekiya & Vaughan Williams, 1963b) to be about twice as active as quinidine in reducing the rate of entry of depolarizing current. Antifibrillatory drugs interfere with depolarization, without significantly affecting the resting potential, the rate of repolarization (Vaughan Williams, 1958a; Szekeres & Vaughan Williams, 1962) or the active extrusion of sodium (Goodford & Vaughan Williams, 1962). The effects of propranolol on various parameters of intracellularly recorded action potentials have been presented in Table 1 and Fig. 4 which may be compared with Fig. 2 in Vaughan Williams (1958a) illustrating the action of quinidine. Some experiments were also done in this investigation on the effects of pronethalol on intracellular potentials, and the results were in close agreement with those shown in Table 1 of Sekiya & Vaughan Williams (1963b). Comparison of the two Tables indicates that propranolol (3×10^{-6}) was more active than pronethalol (8×10^{-6}), and that its mode of action was similar, that is there was little change in the resting potential and the phase of repolarization was actually shorter than normal, but the rate of rise of the action potential and the " overshoot " were greatly

TABLE 1

PARAMETERS OF INTRACELLULARLY RECORDED ACTION POTENTIALS

The means and standard errors are given. n=number of fibres, but the value for each fibre was itself the mean of measurements from several photographs taken at intervals of a few seconds. All fibres in which such records showed substantial differences were rejected, since stability was the criterion for a " good entry " (Vaughan Williams, 1959)

Condition	Resting potential (mV)	Action potential (mV)	Maximum rate of rise (V/sec)	Mean rate of rise (V/sec)	Time for repolarization to 50% (msec)	Time for repolarization to 95% (msec)
Control	62·9±1·2 (n=34)	80·0±1·3 (n=48)	76·5±4·5 (n=43)	44·8±2·3 (n=44)	28·4±0·5 (n=42)	67·4±1·2 (n=42)
Propranolol, 3×10^{-6}	60·6±1·0 (n=15)	58·6±1·9 (n=24)	28·7±4·9 (n=16)	17·5±3·2 (n=18)	22·6±0·5 (n=19)	57·3±2·1 (n=19)
Difference from controls	−2·3	−21·4	−47·8	−27·3	−5·8	−10·1
Probability	0·2>*P*>0·1	*P*<0·001	*P*<0·001	*P*<0·001	*P*<0·001	*P*<0·001

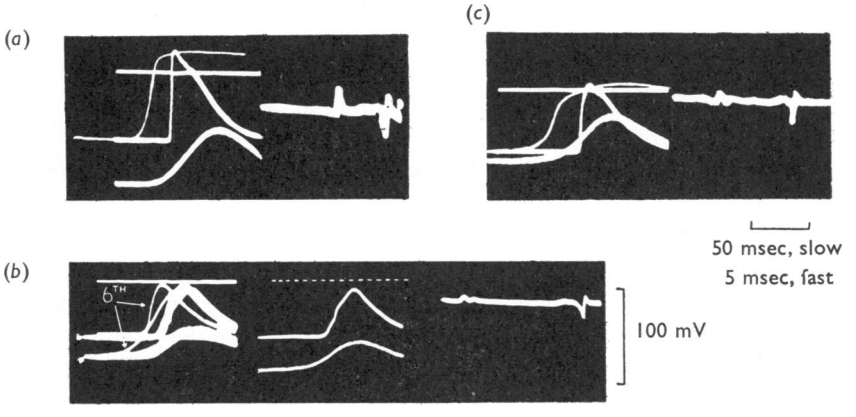

Fig. 4. Intracellularly recorded action potentials. (*a*) Controls. The lower trace records the contraction, the upper trace the intracellular potential. Three records have been superimposed ; at a fast sweep to show the rate of rise of the action potential, and at a slow speed to measure its total duration. The third trace was taken immediately after the electrode was withdrawn from the fibre. The trace on the right shows extracellularly recorded action potentials from right and left atria, from which conduction velocity could be calculated. (*b*) After 1 hr in propranolol (3×10^{-6}). Left: several records superimposed. The atrium failed to respond to the fifth stimulus, so that a longer interval occurred between beats. The response to the sixth showed a faster rate of rise, and the contraction was larger. The overshoot was absent. (*c*) After 70-min washing, recovery was still incomplete.

decreased. A second series of experiments was also done on atria exposed to propranolol (1.5×10^{-6}). The results were similar, but the differences from the controls were smaller and more variable, so that they have not been presented in detail.

Conduction velocity. Conduction velocity was reduced by all three drugs, but the results were too variable to make quantitative comparisons worth while.

Duration of action. There were important differences between the drugs in the duration of their effects. Lignocaine acted rapidly, reaching its maximum effect within 30 min, and the measurements returned to control values equally rapidly on washing. After pronethalol, the effects did not reach a maximum until about 1 hr, and the effects of propranolol reached about 80% of maximum after 1 hr and continued to increase slowly during the next hour.

Though pronethalol was more slowly eliminated than lignocaine, the measurements did eventually return to normal, but after propranolol, if the concentration had been greater than 3×10^{-6}, the measurements never returned to their original control values (Fig. 4).

DISCUSSION

Pronethalol has been shown to have potent antifibrillatory actions. It completely prevented the development of ventricular fibrillation during intoxication by ouabain, abolished fibrillation already established, and doubled the lethal dose of ouabain in guinea-pigs (Vaughan Williams & Sekiya, 1963). Pronethalol has also been shown to be an effective antiarrhythmic agent in man during anaesthesia with chloroform and halothane (Payne & Senfield, 1964), and during digitalis intoxication (Stock & Dale, 1963). Pronethalol is also, however, a powerful local anaesthetic, about twice as active as procaine, and the object of the present work was to compare the activities of pronethalol and of the more recent and more potent β-receptor antagonist, propranolol, as local anaesthetics and as antiarrhythmic drugs.

In the guinea-pig wheal test pronethalol was 1.8-times as active as procaine, and propranolol was 2.3-times as active. The two β-receptor antagonists were not compared directly for local anaesthetic activity on the same animals but, on the basis of the assays with procaine as standard, the difference of 28% between their activities was on the borderline of statistical significance, and they were thus both comparable in local anaesthetic potency with lignocaine (Löfgren, 1948).

In spite of being of similar potency as local anaesthetics, lignocaine, pronethalol and propranolol had actions on cardiac muscle which were far from equal. Lignocaine had no significant effect on frequency or contractions, and was equipotent with pronethalol in tests of electrical threshold and maximum driving frequency. Propranolol was much more active in all the tests. Thus it appeared that another factor was involved in the cardiac effects of pronethalol and propranolol additional to or independent of their local anaesthetic activity in peripheral nerve. From previous work it was concluded that antiarrhythmic drugs acted by reducing the availability of the channel for depolarizing current (presumably sodium) opened when the membrane is active, without interfering with the potassium channel through which current flows during repolarization and at rest. The present experiments indicate that propranolol acts in a similar way, and does not lend support to the view of West & Amory (1960) that antiarrhythmic agents act by prolonging the duration of the action potential, because in the presence of propranolol the duration was actually shorter than in the controls ($P<0.001$).

Catechol amines increased the rate of rise of the action potential and the overshoot in the frog sinus venosus (Hutter & Trautwein, 1956) but those experiments were not done at constant frequency, and the concomitant increase in heart rate may have been a factor. In rabbit atria driven at constant frequency, adrenaline produced a 50% increase in force of contraction without altering the action potential at all. Also, the rate of rise and overshoot in atria from rabbits previously treated with reserpine (5 mg/kg) were not less than normal (Vaughan Williams, 1958b). These observations would not support the view that part of the action of pronethalol and propranolol on cardiac excitability can be attributed to block of endogenous catechol amines.

Propranolol has been reported to be ten- to fifteen-times more active than pronethalol as

a β-receptor antagonist (Duncan & Shanks, communication to British Pharmacological Society, July, 1964), whereas in our own experiments propranolol was only about three-times as active as pronethalol in direct actions on the cardiac muscle membrane. Are these direct actions, then, relevant to the antiarrhythmic properties of the drugs, or is the block of β-receptors the important factor? Possibly both are involved, reduction in excitability and competition for β-receptor sites. It is not known, in any case, how catechol amines produce arrhythmias, and it is possible that a very small change in the availability of the sodium channel, produced by concentrations whose effects might be too small to be measurable in terms of a change of rate of rise of the normal action potential, might nevertheless be adequate to block the development of an *abnormal* impulse.

SUMMARY

1. The recently introduced β-receptor antagonist, propranolol, was 2.3-times more active than procaine as a local anaesthetic, and approximately equal in potency to lignocaine and pronethalol. The actions of propranolol, pronethalol and lignocaine on isolated rabbit atria have been compared.

2. Lignocaine had no significant effect on the spontaneous frequency, and was less active than pronethalol in depressing contractions. Lignocaine and pronethalol had equal effects in raising the electrical threshold and in reducing the maximum driving frequency.

3. Propranolol was more active than pronethalol in all tests: ratio=4.7 for spontaneous frequency; 3.7 for maximum driving frequency; 2.2 for electrical threshold; and 1.9 for contractions.

4. Propranolol had effects on intracellularly recorded cardiac action potentials qualitatively similar to those of pronethalol, but was about three-times more active. There was no significant change in resting potential, but the rate of rise of the action potential and the " overshoot " were greatly reduced. The phase of repolarization was significantly shortened.

5. The results were consistent with the view that propranolol, like other antiarrhythmic agents previously studied, interfered with the entry of depolarizing current.

REFERENCES

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

BÜLBRING, E. & WAJDA, I. (1945). Biological comparison of local anaesthetics. *J. Pharmacol. exp. Ther.*, **85**, 78–84.

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

GOODFORD, P. J. & VAUGHAN WILLIAMS, E. M. (1962). Intracellular Na and K concentrations of rabbit atria, in relation to the action of quinidine. *J. Physiol. (Lond.)*, **160**, 483–493.

HUTTER, O. F. & TRAUTWEIN, W. (1956). Vagal and sympathetic effects on the pacemaker fibres in the sinus venosus of the heart. *J. gen. Physiol.*, **39**, 715–733.

LÖFGREN, N. (1948). *Studies on Local Anaesthetics. Xylocaine, a new synthetic drug.* Stockholm: Ivar Haeggströms.

PAYNE, J. P. & SENFIELD, R. M. (1964). Pronethalol in the treatment of ventricular arrhythmias during anaesthesia. *Brit. med. J.*, **i**, 603–604.

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 antifibrillatory actions and effects on intracellular cardiac potentials of pronethalol, disopyramide and quinidine. *Brit. J. Pharmacol.*, **21**, 473–481.

STOCK, J. P. P. & DALE, N. (1963). Beta-adrenergic receptor blockade in cardiac arrhythmias. *Brit. med. J.*, **ii**, 1230–1233.

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

VAUGHAN WILLIAMS, E. M. (1958a). The mode of action of quinidine on isolated rabbit atria interpreted from intracellular records. *Brit. J. Pharmacol.*, **13**, 276–287.

VAUGHAN WILLIAMS, E. M. (1958b). Some observations concerning the mode of action of acetylcholine on isolated rabbit atria. *J. Physiol.* (*Lond.*), **140**, 327–346.

VAUGHAN WILLIAMS, E. M. (1959). The effect of changes in extracellular potassium concentration on the intracellular potentials of isolated rabbit atria. *J. Physiol.* (*Lond.*), **146**, 411–427.

VAUGHAN WILLIAMS, E. M. & SEKIYA, A. (1963). Prevention of arrhythmias due to cardiac glycosides by block of β-sympathetic receptors. *Lancet*, i, 420–421.

VAUGHAN WILLIAMS, E. M. & SZEKERES, L. (1961). A comparison of tests for antifibrillatory action. *Brit. J. Pharmacol.*, **17**, 424–432.

WEST, T. C. & AMORY, D. W. (1960). Single fiber recording of the effects of quinidine at atrial and pacemaker sites in the isolated right atrium of the rabbit. *J. Pharmacol. exp. Ther.*, **130**, 183–193.