Brit. J. Pharmacol. (1963), **21**, 473–481.

A COMPARISON OF THE ANTIFIBRILLATORY ACTIONS AND EFFECTS ON INTRACELLULAR CARDIAC POTENTIALS OF PRONETHALOL, DISOPYRAMIDE AND QUINIDINE

BY

A. SEKIYA AND E. M. VAUGHAN WILLIAMS

From the Department of Pharmacology, University of Oxford

(*Received August* 3, 1963)

A quantitative comparison of the effects of quinidine, pronethalol and γ-di-isopropylamino-α-phenyl-α-pyrid-2-ylbutyramide (disopyramide) has been made on rabbit isolated atria. All three drugs raised the electrical threshold and reduced the contractions, the conduction velocity and the maximal frequency at which the atria would follow a stimulus. The descending order of potency was pronethalol, quinidine and disopyramide, but the range was small, pronethalol having about twice the activity of disopyramide. Both the new compounds affected intracellular potentials in the same way as quinidine, causing little change in the resting potential or duration of the action potential, but reducing the overshoot potential and slowing the rate of rise of the action potential. These results support the view that interference with depolarization is an essential feature of antifibrillatory activity.

In a previous paper it was shown that the β-receptor blocking agent pronethalol (Nethalide), completely prevented the development of fibrillation due to intoxication with ouabain in anaesthetized and artificially ventilated guinea-pigs (Vaughan Williams & Sekiya, 1963). Dichloroisoprenaline and the synthetic quinidine substitute γ-di-isopropylamino-α-phenyl-2-pyrid-2-ylbutyramide (disopyramide, SC7031) (Sekiya & Vaughan Williams, 1963b) also had some protective action, and quinidine has been employed by clinicians to alleviate digitalis intoxication. It was, therefore, of interest to know how the newer compounds compared with quinidine in conventional tests for antifibrillatory activity. There was clearly some difference in their mode of action since the β-receptor blocking agents had a much greater effect than disopyramide in lowering the spontaneous heart-rate in anaesthetized guinea-pigs, and isoprenaline reversed the protective action of disopyramide against ouabain, but not that of dichloroisoprenaline.

Several compounds of widely different chemical structure have been reported to possess antifibrillatory activity. All the compounds previously studied (Szekeres & Vaughan Williams, 1962) had a number of common actions, causing (1) reduction in the maximal frequency at which atria would follow a stimulus; (2) increased electrical threshold; (3) increased "fibrillation threshold"; and (4) decreased conduction velocity. Most of them also depressed the contractions of isolated atria.

In addition they all affected intracellular cardiac potentials in a similar way, producing large reductions in the rate of rise of the action potential and in the "overshoot" potential, but causing no change in resting potential or in the time taken for the membrane to repolarize to within 50% of its resting value. The time taken to repolarize to 95% of the resting value was either unchanged or prolonged by only a few milliseconds. These results implied that antifibrillatory drugs had very little effect on permeability to potassium, but owed their antifibrillatory action to an interference with the entry of depolarizing (sodium) current (Vaughan Williams, 1958).

There were thus two main reasons for carrying out several different measurements of the activity of the new compounds. First, it was probable that any two drugs which had the same order of potency in all the tests had the same mode of action. Secondly, if the new and highly active antifibrillatory compound pronethalol, introduced as a β-receptor blocking agent, nevertheless turned out to have the same effects as the other drugs on the various parameters of the intracellular action potential, this would support the hypothesis that interference with depolarization was the essential feature of antifibrillatory activity.

METHODS

The apparatus and methods were similar to those previously described (Vaughan Williams & Szekeres, 1961; Szekeres & Vaughan Williams, 1962). Atria from small rabbits were preferred (700 to 1,500 g), and the preparations were maintained at 34° C. The spontaneous frequency was measured by observing the contractions on an oscilloscope, and then adjusting the sweep frequency, triggered by the stimulator, until they were synchronous. The spontaneous frequency could then be read off from the stimulator frequency calibration. The animals were injected with 5 mg of heparin intravenously before the dissection.

RESULTS

Spontaneous frequency

It is well known that quinidine reduces the spontaneous frequency of the heart, and it was found that disopyramide and pronethalol had similar effects, the former being a little less potent than quinidine (Fig. 1). Quinidine did not cause an increase in rate at any concentration. The solid line drawn in Fig. 1, to give the best fit by eye to the points for pronethalol, has a steeper slope than that drawn for disopyramide (broken line), and cuts the baseline at 2.5×10^{-6} g/ml. The explanation is probably that pronethalol does retain some sympathomimetic activity, although it is very small in comparison with that of dichloroisoprenaline, and that at low concentrations this effect becomes apparent, whereas at higher concentrations the blocking action is paramount.

There was one other point of interest with regard to the spontaneous frequency. In the isolated atrium, even 16 mg/l. of pronethalol reduced the spontaneous frequency by no more than 20%, and pronethalol was only about twice as active as disopyramide. From Figs. 1 and 2 of the previous paper (Sekiya & Vaughan Williams, 1963b) it can be seen that in the whole animal 5 mg/kg of pronethalol reduced the spontaneous frequency by 40%, whereas 15 mg/kg of disopyramide caused a fall of only 7%. The explanation of this difference in the results from the isolated organ and the whole animal is, perhaps, that the pacemaker is depressed by both

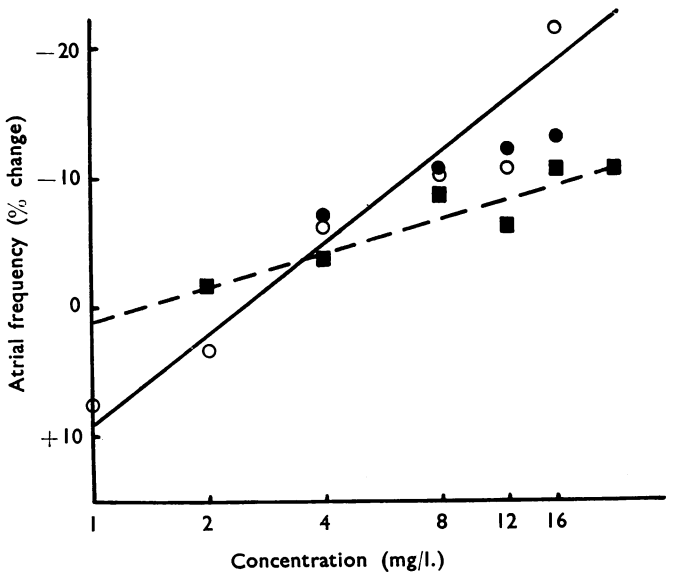

Fig. 1. Relationship between spontaneous frequency of isolated atria and drug concentration. Ordinate: percentage change in frequency; abscissa: concentration, in mg/l. on log scale. Filled circles, quinidine; empty circles, solid line, pronethalol; and squares, broken line disopyramide. At low concentrations pronethalol had some excitatory action on the pacemaker.

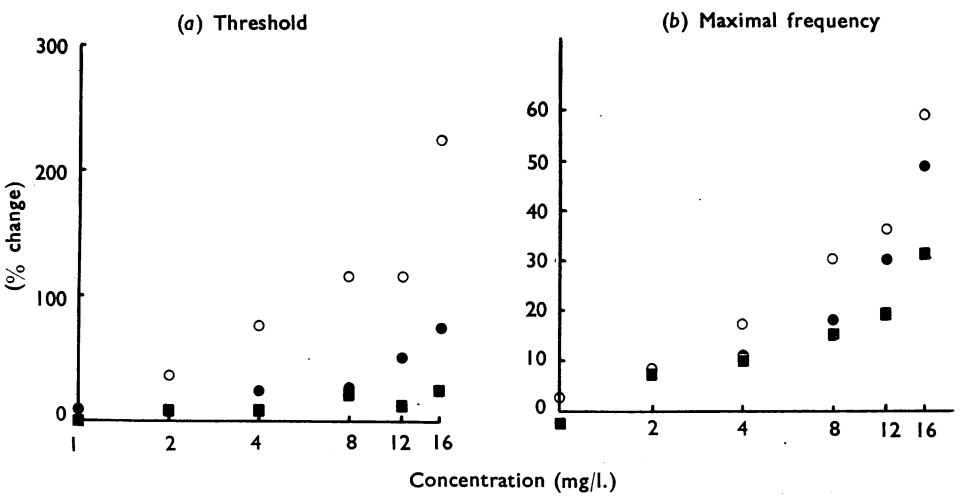

Fig. 2. Relationship between percentage change in electrical threshold (*a*), and percentage change in maximal frequency at which atria would follow a stimulus (*b*) and drug concentration (abscissa). Filled circles, quinidine; empty circles, pronethalol; and squares, disopyramide.

drugs, with a resultant reflex increase in sympathetic tone. In the presence of disopyramide this increase in tone is effective, but with pronethalol both reflex and basal sympathetic tone is blocked.

Maximal driving frequency and electrical threshold

In order to measure the threshold current required to drive the atria, the stimulus frequency was set a few beats/min faster than the spontaneous frequency, and the current was then increased until the atria followed the stimulus. All three compounds increased the threshold. The differences in activity were small but definite, the descending order of potency being pronethalol, quinidine and disopyramide at all concentrations studied (Fig. 2*a*).

To measure the maximal driving frequency, the stimulus strength was set to three-times the threshold, and the frequency increased until the atria no longer followed the stimulus. This test also gave an unequivocal result, and placed the drugs in the same order of potency (Fig. 2*b*).

Contraction and conduction velocity

Since compounds acting on sympathetic β-receptors increase cardiac contractions, it might have been expected, by analogy with its effects on the spontaneous rate, that pronethalol would have increased contractions at low concentrations and

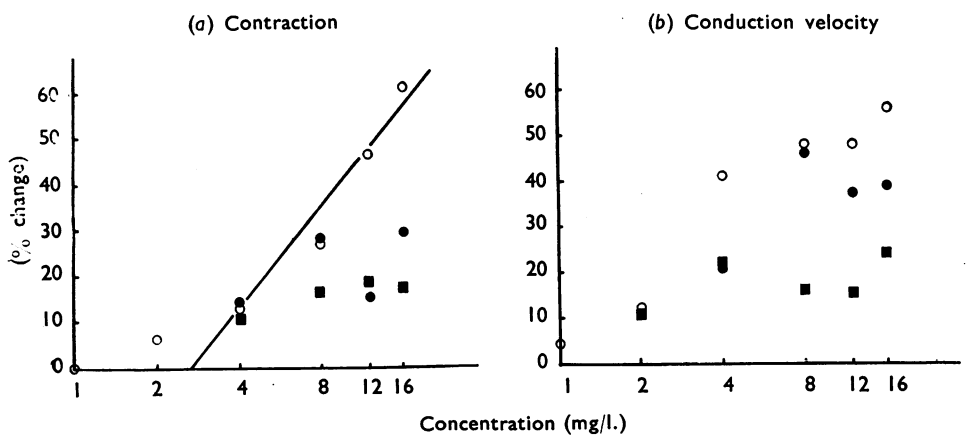

Fig. 3. Relationship between percentage change in contractions (*a*), and percentage change in conduction velocity (*b*) and drug concentration (abscissa). Filled circles, quinidine; empty circles, solid line, pronethalol; and squares, disopyramide. At concentrations above 3 mg/l. the dose/response relation for pronethalol was similar to that shown in Fig. 1, yet there was negligible excitatory action on contractions at low concentrations.

depressed them at higher concentrations. It is true that a line drawn to give the best fit to points above 3 mg/l. (Fig. 3*a*) has a steep slope and when extrapolated to zero cuts the baseline at approximately the same place as in Fig. 1 (2.6 mg/l.). It could still be maintained, therefore, that a part of the action of pronethalol is to block basal and reflex sympathetic tone. However, the contractions were unaltered

by 1 mg/l. of pronethalol in all the experiments. At 2 mg/l., the contractions were smaller in two out of four, and larger in one experiment only. Thus, although pronethalol seems to possess some β-receptor excitatory action on the pacemaker at low concentrations, the excitatory action on contractions is negligible.

The effects on contractions and conduction velocity were more variable, and did not give an unequivocal order of potency. At concentrations above 4 mg/l., however, the conduction velocity test put the drugs in the same order of potency (Fig. 3*b*).

Intracellular potentials

Quinidine greatly reduces the " overshoot " and rate of rise of the action potential, but hardly affects the duration of the phase of repolarization (Vaughan Williams,

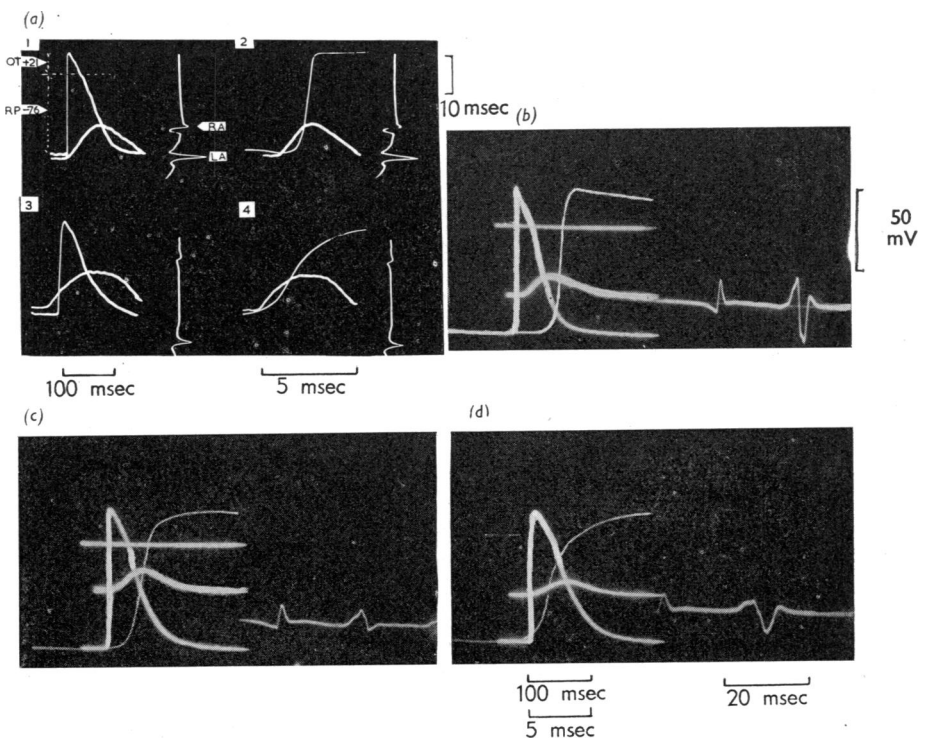

Fig. 4. Intracellular potentials, contractions and conduction velocity. (*a*) 1, Control intracellularly recorded potential at slow sweep speed, showing resting potential (RP) and overshoot potential (OT), and contraction at the same sweep-speed. The vertical trace shows extracellularly recorded potentials from the left atrium (LA) and right atrium (RA). 2, As for 1, but the sweep for the intracellular potential has been quickened, to show the rate of rise. 3 and 4, as 1 and 2, but in presence of quinidine (9×10^{-6}). (*b*), Control. Superimposed traces of intracellular potentials at slow and fast sweep speeds, and of the zero potentials, with three superimposed traces of contractions, and externally recorded action potentials from right and left atria (on right). (*c*) Effect of disopyramide (5×10^{-6}). (*d*) Effect of disopyramide (8×10^{-6}). The scales under (*d*) also apply to (*b*) and (*c*).

1958, from which Fig. 4*a* has been reproduced). Since the effects of disopyramide in the tests described above were close to those of quinidine, it was not surprising to find that intracellular potentials were altered in a similar fashion, also. Control potentials, and records made in the presence of 5 and 8 mg/l. of disopyramide, are shown (Fig. 4*b*, *c* and *d*).

From a study of several antifibrillatory compounds (Vaughan Williams & Szekeres, 1961 ; Szekeres & Vaughan Williams, 1962) it was suggested that the essential feature of antifibrillatory action was an interference with the entry of depolarizing current. Pronethalol has powerful β-receptor blocking activity, and it was quite

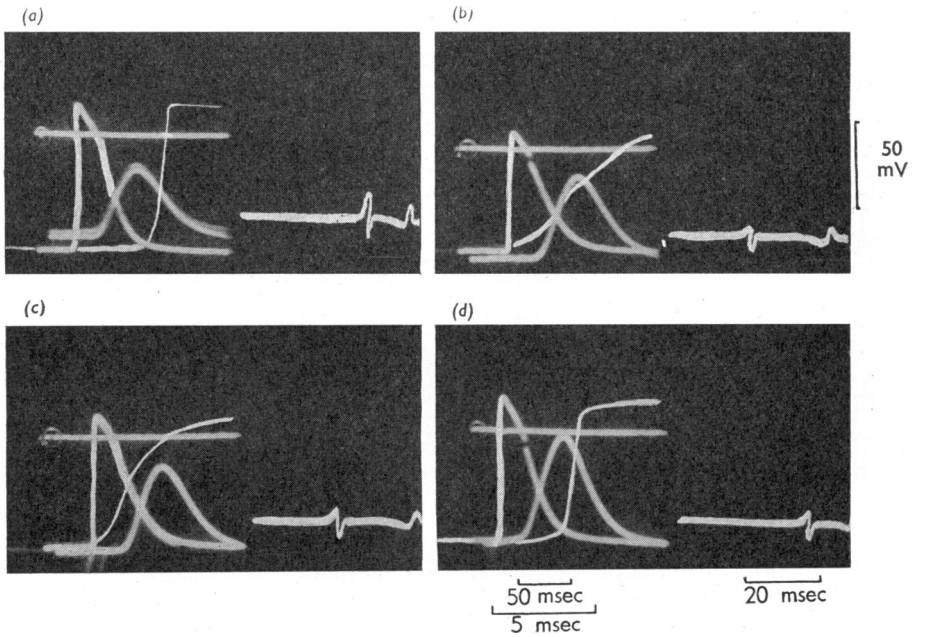

Fig. 5. Effect of pronethalol on intracellularly recorded potentials. (*a*) Control; (*b*) after 20 min and (*c*) after 47 min in the presence of pronethalol (8×10^{-6}); (*d*) records after 30 min washing with control solution. Traces as in Fig. 4, *b*, *c* and *d*.

possible that its mode of action as an antifibrillatory agent was different from that of quinidine. It might, for example, have prolonged the duration of the action potential, without affecting the rate of rise, a change which should in theory also result in antifibrillatory action (Burn, 1960). Thus pronethalol, an entirely new compound with little chemical resemblance to quinidine, offered the opportunity of subjecting to a critical test the hypothesis that interference with depolarization was the essential feature of antifibrillatory action.

The effect of pronethalol (8 mg/l.) is shown in Fig. 5, and the mean results of experiments with pronethalol and disopyramide are presented in Table 1 (compare Table 1 in Szekeres & Vaughan Williams, 1962). It is evident that pronethalol, so far from acting differently, is actually more specific as an impediment to depolarization than is disopyramide. The latter, in addition to the effect on

TABLE 1

PARAMETERS OF INTRACELLULARLY RECORDED POTENTIALS

Each group of values consists of: uppermost, mean with standard error; middle, difference from control; and lowest, probability of statistical significance of difference

Treatment	Resting potential (mV)	Action potential (mV)	Time for 50% repolarization (msec)	Time for 95% repolarization (msec)	Maximal rate of rise (V/sec)	Mean rate of rise (V/sec)
Control	67·5±4·3	86·3±1·9	38·4±1·1	73·8±5·7	86±6	30·3±5·7
Pronethalol 8×10^{-6}	66·4±1·3 −1·1 0·9>*P*>0·8	78·8±2·0 −7·5 0·02>*P*>0·01	37·2±0·4 −1·2 0·4>*P*>0·3	82·7±2·7 +8·9 0·2>*P*>0·1	30·1±3·7 −55·9 *P*<0·001	10·9±1·2 −19·4 *P*<0·001
Control	67·4±0·3	90·05±1·4	46·9±2·8	89·3	121±10	40·3±1·4
Disopyramide $2·5\times10^{-6}$	63·8±0·7 −3·6 0·01>*P*>0·001	86·8±1·3 −3·25 0·2>*P*>0·1	46±0·1 −0·9 0·8>*P*>0·7	98·3±1·9 +9 0·01>*P*>0·001	143±7·1 +22 0·1>*P*>0·05	42·2±2·2 +1·9 *P*=0·5
5×10^{-6}	64·1±1·3 −3·3 0·05>*P*>0·02	84±2·1 −6·05 0·05>*P*>0·02	47·8±0·9 +0·9 0·8>*P*>0·7	111±0·6 +21·7 *P*<0·001	51±2·6 −70 *P*<0·001	15·1±2 −25·2 *P*<0·001
8×10^{-6}	65·4±0·6 −2 *P*=0·02	80·5±0·5 −9·55 *P*<0·001	50±2·8 +3·1 0·5>*P*>0·4	116·2±4·3 +26·9 *P*<0·001	41±2·8 −80 *P*<0·001	9·2±0·4 −31·1 *P*<0·001

depolarization, did cause a small fall in resting potential and a slight lengthening of the tail of the action potential, with the implication of some reduction of permeability to potassium. After quinidine and pronethalol, however, the lengthening of the action potential was small in comparison with the prolongation of the effective refractory period (Table 2).

One difference between quinidine and the two new compounds was of interest. Quinidine is not easily washed out, and it was often found that control values for various measurements were never re-established after the higher concentrations of quinidine (Vaughan Williams, 1958) even after washing for 1 hr. After even 16 mg/l. of pronethalol and disopyramide, however, all measurements rapidly returned to control values on washing out.

DISCUSSION

Two new compounds with antifibrillatory activity, the β-receptor blocking drug, pronethalol, and a substitute for quinidine, disopyramide, have been compared with quinidine in a number of tests. All the drugs reduced the maximal frequency at

TABLE 2

MEAN INCREASES IN EFFECTIVE REFRACTORY PERIOD

Dose (mg/l.)	Increase in refractory period (msec) after		
	Disopyramide	Quinidine	Pronethalol
1	—	—	3
2	—	—	18
4	12·5	21	41·4
8	30	43	71
12	43·5	71·5	124
16	65	166·5	229
32	86	—	—

which rabbit atria would follow a stimulus, and raised the electrical threshold. By both these tests the drugs were placed unequivocally in the descending order of potency pronethalol, quinidine and disopyramide. At a concentration of 16 mg/kg, pronethalol had an effect in lowering the spontaneous frequency about twice as strong as that of disopyramide, but below 2.5 mg/kg pronethalol increased the spontaneous rate. This implied that pronethalol had a small sympathomimetic activity at low concentration, but at higher concentrations block of naturally released transmitter predominated. The small difference in activity on the isolated atria between pronethalol and disopyramide was in contrast with their effects in the whole animal (Sekiya & Vaughan Williams, 1963b). Pronethalol (5 mg/kg) caused a 40% fall in spontaneous heart-rate in anaesthetized guinea-pigs, but disopyramide (15 mg/kg) produced a reduction of only 7%.

Pronethalol and disopyramide were also like quinidine in reducing contractions and conduction velocity. These tests give much more variable results with antifibrillatory drugs (Vaughan Williams & Szekeres, 1961), however, and may reflect actions of the drug on systems other than those directly concerned in their antifibrillatory effects.

In another paper (Vaughan Williams, 1958) it was suggested that the essential feature necessary for antifibrillatory activity was an interference with the mechanism by which depolarizing curent could enter the muscle fibre. The advent of two new compounds with antifibrillatory action provided an opportunity to test the hypothesis. Disopyramide was introduced as a possible substitute for quinidine, and had a comparable performance in the tests employed, so that it was not unexpected that the changes produced in the intracellularly recorded action potential were similar to those produced by quinidine.

Pronethalol, on the other hand, is primarily an antagonist of β-receptor activity. It prevented the development of ventricular fibrillation during intoxication with ouabain (Vaughan Williams & Sekiya, 1963), but it was only tested for this effect as a result of the report by Méndez, Aceves & Méndez (1961) that ventricular fibrillation did not occur during digitalis intoxication after sympathectomy and adrenalectomy. It came as a surprise, therefore, to find that pronethalol was approximately twice as potent as quinidine by conventional tests for antifibrillatory action. Since there is little similarity in chemical structure between pronethalol and quinidine, it was of great interest to know the effect of pronethalol on intracellularly recorded cardiac potentials. If the hypothesis was correct that the essential feature of antifibrillatory action was interference with depolarization, it could be predicted that pronethalol would have no effect on the resting potential or the duration of repolarization, but would decrease the overshoot potential and greatly slow down the rate of rise of the action potential. In the event all these predictions were fulfilled. In fact pronethalol proved to be more specific than disopyramide which, in addition to impeding depolarization, also appeared to restrict permeability to potassium, in that there was a small fall in resting potential and some lengthening of the tail of the repolarization phase of the action potential.

In conclusion, it would appear that pronethalol is the most potent anifibrillatory agent so far studied, and it has already been employed in man (Dornhorst &

Robinson, 1962). From the theoretical point of view, it is surprising that a β-receptor blocking agent should act in the same way as quinidine, and this conclusion raises the question whether other antifibrillatory compounds also block β-receptors. It is possible that some of the actions of pronethalol may not be directly related to its action in blocking β-receptors, and in this connection it is of interest that the drug depresses reflex activity (Sekiya & Vaughan Williams, 1963a), and is a local anaesthetic (Gill & Vaughan Williams, unpublished) twice as potent as procaine.

REFERENCES

BURN, J. H. (1960). The cause of fibrillation. *Brit. med. J.*, **1**, 1379–1384.

DORNHORST, A. C. & ROBINSON, B. F. (1962). Clinical pharmacology of a beta-adrenergic-blocking agent (Nethalide). *Lancet*, **ii**, 314–317.

MÉNDEZ, C., ACEVES, J. & MÉNDEZ, R. (1961). The anti-adrenergic action of digitalis on the refractory period of the A–V transmission system. *J. Pharmacol. exp. Ther.*, **131**, 191–198.

SEKIYA, A. & VAUGHAN WILLIAMS, E. M. (1963a). The effect of Nethalide, an antagonist of β-sympathetic responses, on the flexor reflex in guinea-pigs. *J. Physiol. (Lond.)*, **167**, 56–57*P*.

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

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

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

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.