Brit. J. Pharmacol. (1963), **21**, 462–472.

THE EFFECTS OF PRONETHALOL, DICHLOROISOPRENALINE AND DISOPYRAMIDE ON THE TOXICITY TO THE HEART OF OUABAIN AND ANAESTHETICS

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(*Received August* 3, 1963)

An intermittent infusion of ouabain, 4 μg during 30 sec every 1.5 min, regularly caused ventricular fibrillation in guinea-pigs. The β-receptor blocking drug, pronethalol (5 mg/kg), increased the dose of ouabain required to produce extrasystoles, completely prevented fibrillation, and significantly raised the lethal dose of ouabain. Dichloroisoprenaline had similar effects, but a dose of 15 mg/kg was required. When fibrillation had already been produced by ouabain, pronethalol (3 to 4 mg) administered slowly restored a regular rhythm, but rapid injection sometimes produced cardiac arrest. As much as 20 to 25 mg/kg of pronethalol could be given to animals deeply anaesthetized with urethane or pentobarbitone, but with light chloroform or ether anaesthesia, 5 mg/kg of pronethalol caused a large fall in blood pressure and complete heart-block.

Atrial fibrillation can usually be terminated by quinidine or procainamide, if this is considered desirable. Ventricular fibrillation is rapidly fatal and, though it may be obedient to the techniques of the cardiac surgeon, remains a hazard during the induction of anaesthesia, as a result of treatment with cardiac glycosides and other drugs, or as a terminal event in pathological conditions. The sympathetic system has long been implicated in the production of ventricular fibrillation. Intravenous injection of adrenaline or isoprenaline can alone induce arrhythmias, which may progress to fibrillation, especially if the cardiac muscle has been sensitized by chloroform, cyclopropane or a number of other substances (Dawes, 1952; Moore & Swain, 1960). More recently it was shown (Méndez, Aceves & Méndez, 1961b) that after sympathectomy and adrenalectomy overdoses of cardiac glycosides no longer caused fibrillation. It seemed worth while, therefore, to investigate whether the recently introduced β-receptor blocking drugs (Powell & Slater, 1958; Black & Stephenson, 1962) had some protective action against agents liable to induce ventricular fibrillation.

METHODS

Guinea-pigs weighing 400 to 600 g were used, and were artificially ventilated after the induction of anaesthesia. For the experiments with ouabain the anaesthetic was 1.6 g/kg of urethane given intraperitoneally. Ouabain (80 μg/ml. in saline) was injected into a vein from a motor-driven syringe, 4 μg being infused during 30 sec every 1.5 min. For experiments with ether and chloroform, the anaesthetic was delivered from EMO (Epstein-Mackintosh-Oxford)

vaporizers especially calibrated for small animals by Dr Epstein. The soluble anaesthetics were administered intraperitoneally in amounts sufficient to permit the preparation to be carried out, and further amounts were given intravenously as required. The electrocardiogram was recorded with a Cossor 1314 electrocardiograph. Pronethalol (Nethalide), dichloroisoprenaline and γ-di-isopropylamino-α-phenyl-α-pyrid-2-ylbutyramide (disopyramide, SC7031, Mokler & Van Arman, 1962) were injected intravenously.

RESULTS

The block of β-receptor sympathomimetic effects by pronethalol has been fully established both in animals and in man by Black & Stephenson (1962) and by Dornhorst & Robinson (1962). In guinea-pigs, the most immediately striking effect of pronethalol was a large fall in the spontaneous heart-rate, which could not be raised to anything approaching control levels by even large doses of isoprenaline. Nevertheless, isoprenaline still had an effect on the pacemaker, and if the increase in frequency was calculated as a percentage of the abnormally low initial frequency, the antagonism of isoprenaline by pronethalol was not striking. These points are

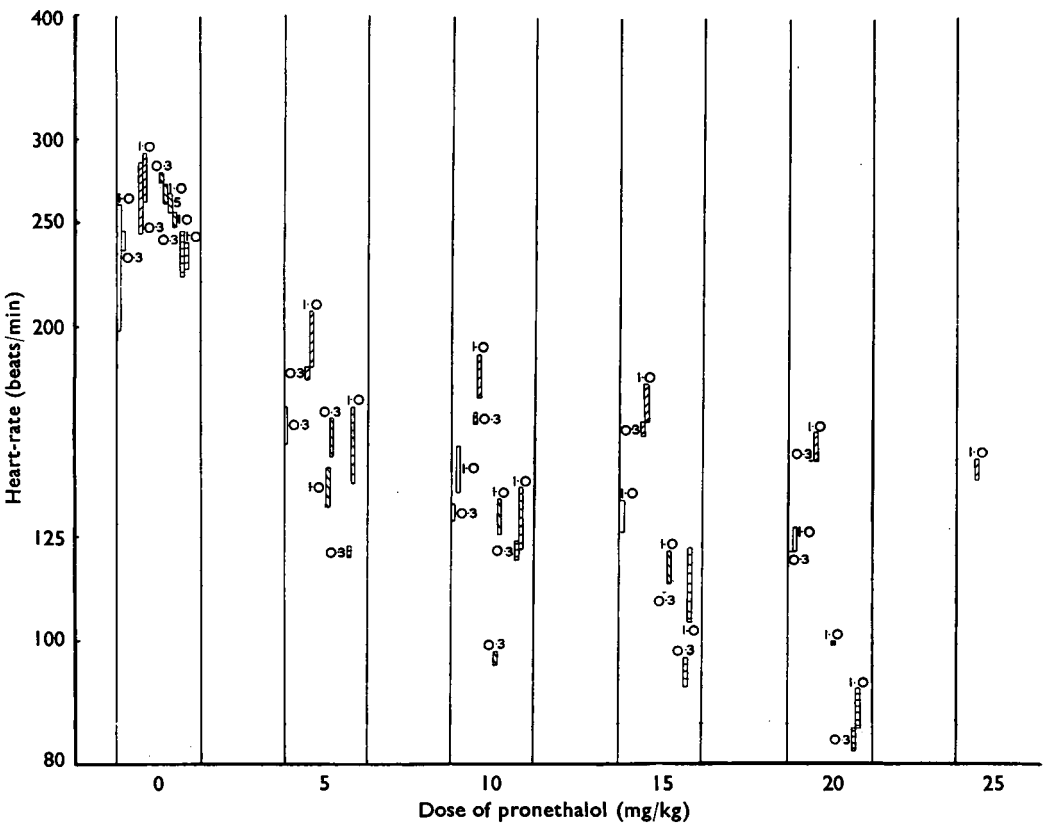


Fig. 1. Effect of pronethalol on the spontaneous heart-rate and the response to isoprenaline. Ordinate: heart-rate in beats/min on a log scale. The bottom and top of each column represent the heart-rates before and after an injection of isoprenaline, the dose in μg being indicated by the numeral beside the column. Different animals are indicated by different patterns of hatching in the columns, and the dose of pronethalol previously administered is shown along the abscissa.

illustrated in Fig. 1, in which the ordinate gives the frequency of the heart on a log scale, in order that equal percentage increases caused by various doses of isoprenaline would be represented by vertical columns of equal height, whatever the initial frequency. The numbers beside each column show the doses of isoprenaline administered intravenously. It is obvious that pronethalol produced very little change in the percentage increase in rate caused by isoprenaline unless about 20 mg/kg of pronethalol was injected, a dose sufficient to halve the spontaneous rate.

When ouabain (80 μg/ml. in saline) was infused into a vein from a motor-driven syringe, the first indication of its activity was a slowing of the spontaneous heart-rate, followed by a prolongation of the P-R interval and an irregularity in the duration of diastole. These effects have often been attributed in part to " sensitization " of vagal reflexes, but it is well known that cardiac glycosides still reduce the heart-rate in the presence of atropine. As the infusion proceeded extrasystoles were observed, and eventually the A-V node lost control and a purely ventricular rhythm was established. Ectopic extrasystoles then occurred, soon to be followed by ventricular fibrillation and cardiac arrest. At each of these successive stages in the intoxication a note was made of the amount of ouabain administered, and in order to sharpen the endpoint at each step, the infusion was made intermittent; 4 μg of oubain was infused into the vein during 30 sec, and the motor was then stopped for 1 min.

In several subsequent series of experiments, the same procedure of ouabain infusion was repeated after intravenous injection of various doses of pronethalol, dichloroisoprenaline and a new antifibrillatory agent, disopyramide (Fig. 4).

Effects of pronethalol

In one series of experiments, 5 mg/kg of pronethalol was injected, and in another 15 mg/kg. At the start of the experiments the mean heart-rates in the control series and the two pronethalol series were 278, 273 and 253 beats/min. Pronethalol, 5 mg/kg, caused a fall in rate from 273 to 168 beats/min, and 15 mg/kg from 253 to 135 beats/min. The P-R interval, on the other hand, was either unchanged by pronethalol or only prolonged by a few milliseconds. When the ouabain was infused after pronethalol there was no further fall in rate, and only a gradually developing increase was seen (Fig. 2). This absence of a fall in rate after ouabain in the presence of pronethalol is probably attributable to the rate having already been greatly reduced by the removal of the normal sympathetic background tone to the heart. Méndez, Aceves & Méndez (1961a) provided evidence of an antagonism between cardiac glycosides and sympathetic activity. The experiments with pronethalol would strongly support the view that the non-vagal component of cardiac slowing caused by ouabain is due to an antagonism to the sympathetic nervous system. If the sympathetic pathways are already blocked, no further slowing can occur from this cause.

Pronethalol greatly reduced the toxicity of ouabain. The amounts of ouabain required to produce successive stages in intoxication in the presence of pronethalol are presented in Table 1. Although there was some increase in the dose of ouabain required to produce " vagal " effects (first and second columns in Table 1) it was

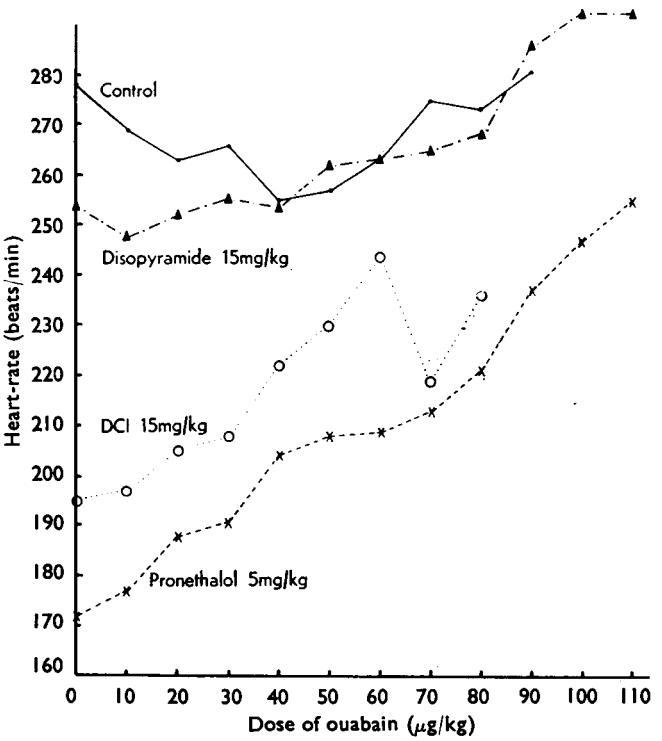


Fig. 2. Effect of pronethalol, dichloroisoprenaline (DCI) and disopyramide on the response to intravenous ouabain. Ordinate: heart-rate in beats/min. Abscissa: amount of ouabain administered in μg/kg. Pronethalol and dichloroisoprenaline, but not disopyramide, caused a large reduction in spontaneous heart-rate. After pronethalol and dichloroisoprenaline, ouabain caused an increase in heart-rate only, but the actual rate was always well below that of the controls.

not statistically significant. The large fall in the spontaneous heart-rate compared with the small change in the P-R interval (which should be lengthened by sympathetic blockade if sympathetic tone were high) may perhaps imply that background sympathetic activity is normally higher in the S-A than in the A-V node. The most striking effects of pronethalol were a highly significant increase in the dose of ouabain required to produce extrasystoles and a ventricular rhythm, the complete absence of fibrillation, and a significant increase in the lethal dose.

The question naturally arose whether pronethalol would reverse fibrillation that was already established. In the sixth column of Table 1 there were only ten instead of thirteen control results. In these ten the additional amount of ouabain (mean and standard error) required to produce cardiac arrest after fibrillation had started was 47 ± 11.4 μg/kg, or $17.7 \pm 4.5\%$ of the dose necessary to produce fibrillation. In the remaining three experiments, after ouabain had already induced fibrillation, pronethalol was cautiously infused into the vein. 3 to 4 mg/kg of pronethalol converted the fibrillation into a regular rhythm (Fig. 3). A total of 5 mg/kg of pronethalol was administered, and the infusion of ouabain was then

TABLE 1

EVIDENCE FOR THE PROTECTIVE ACTION OF PRONETHALOL, DICHLOROISOPRENALINE (DCI) AND DISOPYRAMIDE AGAINST OUABAIN INTOXICATION

The top line in each group gives the mean dose (with standard error) of ouabain in μg/kg required to produce the effect described at the top of the column; the middle line (figures in parentheses) gives the number of animals in which the effect was seen; the bottom line gives the difference from the controls and its significance

Treatment	Total No. of animals	Prolonged P–R interval	Unequal intervals	Ventricular rhythm	Ectopic extrasystoles	Ventricular fibrillation	Cardiac arrest
Control	13	124±13·8 (12)	128±11·9 (13)	168±11·4 (12)	187±10·9 (13)	272±14·6 (13)	336±15·3 (10)
Pronethalol (5 mg/kg)	6	137±18·8 (6) +13 (P=0·6)	140±16·7 (6) +12 (P>0·9)	255±17·2 (6) +87 (P<0·001)	214±11·2 (6) +27 (P=0·1)	None	415±22·6 (6) +79 (P=0·01)
Pronethalol (15 mg/kg)	5	172±50·5 (3) +48 (0·4>P>0·3)	177±33·1 (5) +49 (0·2>P>0·1)	366±31·4 (3) +198 (P<0·001)	399±42·9 (5) +212 (P<0·001)	None	465±40·1 (5) +129 (0·01>P>0·001)
DCI (15 mg/kg)	6	102±28·2 (6) −22 (0·5>P>0·4)	108±28·1 (6) −20 (0·6>P>0·5)	237±19·3 (6) +69 (0·01>P>0·001)	239±14·8 (6) +52 (P=0·01)	369 (1) +97	415±25·9 (6) +79 (0·02>P>0·01)
Disopyramide (5 mg/kg)	5	110±36·5 (5) −14 (0·8>P>0·7)	72·4±22·2 (5) −56 (0·05>P>0·02)	195±24·5 (5) +27 (0·4>P>0·3)	203±18·1 (5) +15 (0·5>P>0·4)	285±14·4 (4) +13 (0·6>P>0·5)	329±15·1 (5) −7 (0·8>P>0·7)
Disopyramide (15 mg/kg)	4	108±16·5 (4) −16 (0·5>P>0·4)	119±17·5 (4) −7 (0·8>P>0·7)	207±41 (4) +39 (0·4>P>0·3)	251±31·8 (4) +64 (0·1>P>0·05)	360 (Periodic in 2) +88	410±21 (4) +74 (0·02>P>0·01)

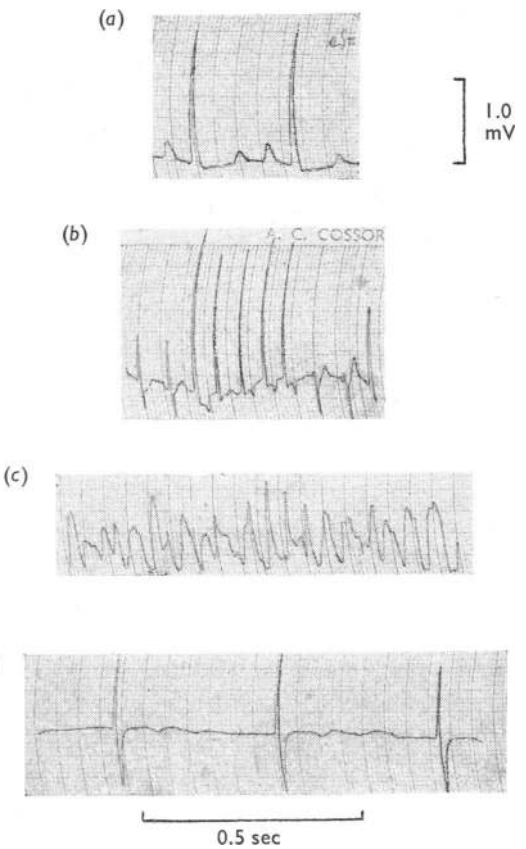
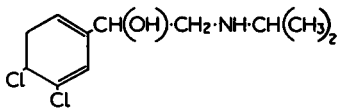


Fig. 3. Restoration of regular rhythm to a fibrillating heart by pronethalol, 4 mg/kg. (*a*) control electrocardiogram. (*b*) A–V nodal beats and ectopic extrasystoles induced by ouabain, 195 μg/kg. (*c*) fibrillation induced by ouabain, 280 μg/kg. (*d*) effect of pronethalol, 4 mg/kg.

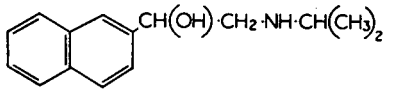
continued. There was no further fibrillation, and the mean additional dose of ouabain required to arrest the heart was 199 μg/kg or 93% of the dose producing the original fibrillation. In two other experiments, when a strong solution of pronethalol (10 mg/ml.) was injected rapidly after fibrillation had been established, not only did the fibrillation cease but the heart stopped altogether.

Dichloroisoprenaline and disopyramide

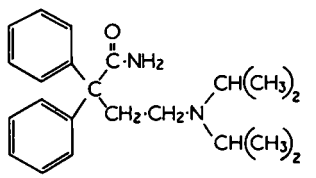
If dichloroisoprenaline is given slowly by intravenous infusion, its initial sympathomimetic activity is transient and negligible, but its β-receptor blocking action persists, with the result that the spontaneous heart-rate falls. In a series of nine experiments the mean heart-rate was reduced from 276 to 195 beats/min by a dose of 15 mg/kg. In six of these experiments ouabain was infused, and it can be seen from Table 1 that dichloroisoprenaline significantly reduced the toxicity of ouabain. In another series, it was found that 7.5 mg/kg of dichloroisoprenaline was without a significant effect.



Dichloroisoprenaline



Pronethalol



Disopyramide

Fig. 4. Structural formulae of compounds referred to in the text.

A new antifibrillatory agent, disopyramide, has been described (Mokler & Van Arman, 1962) which appears to have some advantages over quinidine. The structure of the compound is shown in Fig. 4. This compound caused little slowing of the spontaneous heart-rate (Fig. 2), even a dose of 15 mg/kg only reducing the mean heart-rate from 276 to 256 beats/min, although this dose produced some reduction in the toxicity of ouabain. As can be seen from Table 1, however, 5 mg/kg was without significant effect on ouabain toxicity.

In three experiments, when dichloroisoprenaline (15 mg/kg) had been injected, isoprenaline was also given (3, 21 and 26 μg) before the ouabain infusion was started. The isoprenaline did not, however, reduce the protective action of dichloroisoprenaline against the toxicity of the ouabain (Table 2). In another three experiments, after 15 mg/kg of disopyramide, isoprenaline was injected (14, 2 and 2 μg), and this not only abolished the protective action of disopyramide but increased the toxicity of ouabain above that of the controls.

Effect of pronethalol on anaesthetic toxicity

An antifibrillatory agent is likely to be of value during the induction of anaesthesia, and it was decided to investigate whether pronethalol could safely be used in combination with various anaesthetics. To obtain an objective record of the depth of anaesthesia the withdrawal of the right leg was measured in response to an electric shock applied to the skin over the right ankle. With 1.6 g/kg of urethane, an amount more than sufficient to block the flexor reflex (which failed at 0.9 to 1.3 g/kg of urethane), as much as 20 to 25 mg/kg of pronethalol could be given intravenously without evidence of circulatory failure. It came as a surprise, therefore, to find that with extremely light chloroform anaesthesia (0.6 to 0.8%), which

TABLE 2

EFFECT OF ISOPRENALINE ON THE PROTECTIVE ACTION OF DICHLOROISOPRENALINE (DCI) AND DISOPYRAMIDE

Arrangement as in Table 1 except that the third line of each group of figures gives the difference and its significance between the groups with and without isoprenaline. The line marked C gives the difference between the group given disopyramide and isoprenaline from the controls

Treatment	No. of animals	Prolonged P–R interval	Irregular intervals	Ventricular rhythm	Ectopic extrasystoles	Ventricular fibrillation	Cardiac arrest
DCI (15 mg/kg) without isoprenaline	6	102±28·2 (6)	108±28·1 (6)	237±19·3 (6)	239±14·8 (6)	369 (1)	415±25·9 (6)
DCI (15 mg/kg) with isoprenaline	3	129±27 (3)	141±54·5 (3)	350±93·4 (3)	320±81 (3)	None	436±83 (3)
		+27 (P=0·5)	+33 (P=0·6)	+113 (P=0·1)	+81 (0·4>P>0·3)		+21 (0·9>P>0·8)
Disopyramide (15 mg/kg) without isoprenaline	4	108±16·5 (4)	119±17·5 (4)	207±41 (4)	251±31·8 (4)	360 (2)	410±21 (4)
Disopyramide (15 mg/kg) with isoprenaline	3	66±6·8 (3)	74±2 (3)	128±10·4 (3)	138±8·5 (3)	206±4·5 (2)	243±15·2 (3)
		−42 (P=0·05)	−46 (0·05>P>0·02)	−79 (P=0·1)	−113 (P=0·01)	−154	−168 (P<0·001)
	C	−52 (0·01>P>0·001)	−54 (P<0·001)	−49 (0·01>P>0·001)	−40 (0·05>P>0·01)	−66	−93 (P<0·001)

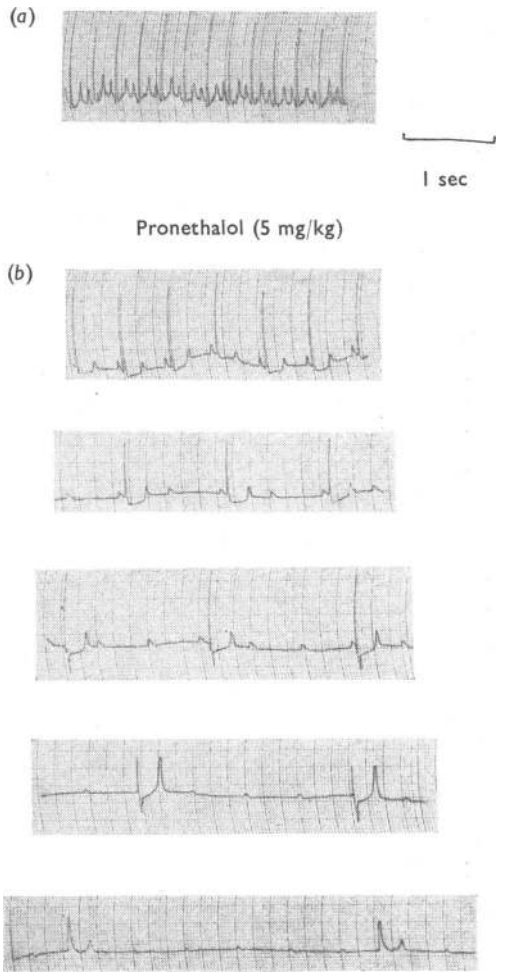


Fig. 5. Effect of pronethalol during light chloroform anaesthesia. A vigorous flexor reflex was present. (*a*) control electrocardiogram during 0.7% chloroform anaesthesia and after atropine 2 mg/kg. (*b*) development of heart-block after pronethalol, 5 mg/kg.

permitted the retention of vigorous flexor reflexes, a small dose of pronethalol precipitated a profound fall of blood pressure, complete heart-block and eventual cardiac arrest (Fig. 5). Ether anaesthesia (5%) produced the same result, but no heart block occurred with pentobarbitone sufficient to block the flexor reflex. It appeared, therefore, that with the volatile anaesthetics the circulation became less competent to withstand block of β-receptors or perhaps some other action of pronethalol. The cardiac effects were doubtless influenced by the low diastolic pressure, since the animal survived for an extended period, albeit with a very slow heart-rate, if the blood pressure was restored by an infusion of noradrenaline or of posterior pituitary extract.

DISCUSSION

Individual responses to cardiac glycosides are notoriously variable, and accurate assays were impossible until adequate statistical methods were developed for dealing with biological variation. Méndez *et al.* (1961b) made the interesting observation that after sympathectomy and adrenalectomy, overdoses of cardiac glycosides no longer caused ventricular fibrillation. It seemed possible, therefore, that dichloroisoprenaline might afford some protection against digitalis intoxication. A series of experiments was undertaken in 1961 on cats (Vaughan Williams, unpublished) given infusions of ouabain and digitoxin. Administration of dichloroisoprenaline during the infusions sometimes precipitated fibrillation. Schull, Berry & Villarreal (1960) did not remark on any arrhythmia-producing effect of dichloroisoprenaline, but found that it had a protective action against the arrhythmias produced by noradrenaline in dogs anaesthetized with cyclopropane. In the experiments with cats, large doses of dichloroisoprenaline given before the infusion caused a small increase in the lethal dose of ouabain, but the difference was not statistically significant and it was felt that the matter was not worth pursuing.

With the introduction of pronethalol, which was reported to block sympathetic β-receptors without exciting them (Black & Stephenson, 1962), an opportunity was offered of taking up the investigation once more. On this occasion guinea-pigs were used, and an infusion of ouabain was administered intermittently in the hope of sharpening the endpoint of the assay. Ouabain regularly produced fibrillation after a mean dose of 272 μg/kg, with a standard error of ± 14.6 μg/kg or little more than 5%. Pronethalol greatly reduced the toxicity of ouabain. The most striking effects were to produce a highly significant increase in the dose of ouabain required to induce extrasystoles, to prevent fibrillation altogether, to restore a regular rhythm in hearts already fibrillating, and to increase considerably the lethal dose of ouabain.

Some evidence was obtained that the protective action of pronethalol was related to its β-receptor sympathetic blocking activity. Both pronethalol and dichloroisoprenaline lowered the spontaneous heart-rate, and dichloroisoprenaline also significantly increased the lethal dose of ouabain. A dose of 15 mg/kg was necessary, however, and even at this dose fibrillation still occurred in one out of six animals. Isoprenaline did not abolish the protective action of dichloroisoprenaline against ouabain, whereas it reversed the protective action of disopyramide, a compound with similar actions and potency to quinidine (Sekiya & Vaughan Williams, 1963).

Large doses of pronethalol (20 to 25 mg/kg) could be given to animals deeply anaesthetized with urethane or pentobarbitone, but even 5 mg/kg of pronethalol caused a large fall of blood pressure and complete heart-block during light chloroform or moderate ether anaesthesia. Also, in two experiments in which ventricular fibrillation had already been produced by ouabain, the rapid injection of a strong solution of pronethalol (10 mg/ml.) caused cardiac arrest, whereas a slow administration of 3 to 4 mg/kg restored a regular rhythm. In conclusion, it was apparent that pronethalol was a potent antifibrillatory agent, which might well be of value in digitalis intoxication. Its protective action against cardiac glycosides has recently been shown in man by Stock (unpublished).

One further point of interest was that ouabain did not cause any initial slowing of the heart-rate after pronethalol. Méndez *et al.* (1961a) presented evidence of an antagonism between digitalis and sympathetic activity. The present experiments lend support to the view that the non-vagal component of the slowing of heart-rate produced by cardiac glycosides can be attributed to an antagonism to the sympathetic nervous system. When the β-receptor sympathetic effects were blocked by pronethalol, the heart-rate was already very low, and no further slowing could, therefore, be produced by antagonism to sympathetic action.

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