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The relevance of β-receptor blockade to ouabain-induced cardiac arrhythmias

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1. (+)-propranolol and (±)-propranolol are comparable in their potency as a local anaesthetic on the intact and desheathed frog sciatic nerve.

2. (±)-propranolol is much more potent than (+)-propranolol as a β-receptor blocking agent and also more effective than the latter in protecting guinea-pigs against ouabain-induced ventricular fibrillation.

3. In isolated rabbit atria (−)-, (+)- and (±)-propranolol, and I.C.I. 50172, which has hardly any local anaesthetic activity, greatly reduce the rate of rise of the intracellularly recorded action potential at concentrations which have no significant effect on electrical threshold, contractions, spontaneous frequency, maximum driving frequency, repolarization time or conduction velocity.

In 1961 Mendéz, Aceves & Mendéz reported that in sympathectomized animals toxic doses of cardiac glycosides did not produce ventricular fibrillation. A pharmacological cardiac sympathetic block was made possible by the introduction of pronethalol (Black & Stephenson, 1962), which had little β-receptor excitatory activity. Vaughan Williams & Sekiya (1963) found that pronethalol protected anaesthetized guinea-pigs from digitalis-induced arrhythmias, and abolished those already established. The anti-arrhythmic action of pronethalol was confirmed in animals (Erlij & Mendéz, 1963) and in man (Stock & Dale, 1963; Payne & Senfield, 1964). The implication was that β-receptor excitation was to some extent involved in the production of arrhythmias not only in the presence of anaesthetics and other sensitizing agents (Moore & Swain, 1960), but even after digitalis.

Subsequently, however, it was found that pronethalol (Gill & Vaughan Williams, 1964) and its successor propranolol (Morales-Aguilerá & Vaughan Williams, 1965) were powerful local anaesthetics, with activities about double that of procaine, estimated by the guinea-pig wheal method of Bülbring & Wajda (1945). Furthermore, it was found that pronethalol and propranolol had effects on the intracellularly recorded cardiac action potential (Sekiya & Vaughan Williams, 1963; Vaughan Williams, 1966) which were very similar to those produced by quinidine (Vaughan Williams, 1958a) and other anti-arrhythmic compounds (Szekeres & Vaughan Williams, 1962).

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It was thus possible that pronethalol and propranolol were merely good local anaesthetics with some specificity for cardiac membranes, and that their β-receptor blocking activity was incidental and irrelevant. Compounds became available such as INPEA (N-isopropyl-*p*-nitrophenyl-ethanolamine) and I.C.I. 50172, which were claimed to be without local anaesthetic activity, but to possess β-receptor blocking actions. Somani & Lum (1965) stated: "failure of INPEA to inhibit the ventricular tachycardia induced by ouabain clearly demonstrates that this arrhythmia does not depend on an adrenergic mechanism". Lucchesi (1965) concluded from a comparison of the effects of (+)- and (±)-pronethalol that "with respect to digitalis-induced arrhythmias the anti-arrhythmic action of pronethalol is not related to its ability to produce beta adrenergic receptor blockade." Benfey & Varma (1966), however, in contrast to the results of Lucchesi & Hardman (1961), concluded that "DCI did not significantly increase the lethal dose of the glycoside." In view of these and other conflicts of evidence (*vide* discussion) it seemed worthwhile to re-investigate the relevance of β-blockade to ouabain toxicity, especially when the dextro and laevo isomers of propranolol were kindly made available to us by Imperial Chemical Industries.

Methods

Local anaesthetic activity

A frog (*Rana pipiens*) sciatic nerve was mounted in a Perspex bath with three chambers: in the end chambers the nerve rested in moist air on pairs of platinum wires connected to stimulating and recording assemblies; in the central chamber, which was grounded, the nerve was immersed in Ringer, with or without drug. The stimulus was 1 msec, frequency 1/sec, strength at least twice supra-maximal. The Ringer contained (per l.): NaCl, 7·0 g; KCl, 0·14 g; $NaHCO_3$, 0·2 g; $CaCl_2$, 0·6 ml. of 20% w/v solution. 10 ml. of Tris (Sigma) buffer pH 7·0 was added.

Protection against ouabain

The method was similar to that described by Vaughan Williams & Sekiya (1963) but was automated by a 0·5 rev./min motor, closing contacts for 5 and 30 sec per revolution. Guinea-pigs of either sex were given 1·6 g/kg of urethane intraperitoneally; artificial respiration (suck and thrust) was applied. Temperature was maintained at 37° C by a heated copper plate thermostatically controlled from an intrarectal thermistor (Mullard, VA 3410; 47K at 20° C). The electrocardiogram was recorded for 5 sec every 2 min. Ouabain 3·3 μg was infused during 30 sec every 2 min (the motor driven syringe was on for 30 sec, off for 90 sec). Five minutes before the start of the infusion, the drug to be tested was given intravenously over a 2 min period.

Intracellular potentials

The method was similar to that described by Szekeres & Vaughan Williams (1962). Atria from young rabbits (about 1·2 kg) of either sex were suspended horizontally with the inner surface uppermost in a bath through which solution flowed

continuously. The fluid in the bath was maintained at 32° C and recirculated by the gas mixture. The atria were viewed by transmitted light, and the microelectrodes inserted under observation at 25 or 40 magnification. Contractions were recorded with a RCA 5734 transducer. Conduction velocity was calculated from records obtained with external bipolar leads on the left and right atrium. The left atrium was stimulated at a frequency 10% above the spontaneous rate by 1 msec square-wave shocks, strength at least twice threshold. All solutions were equilibrated with 95% oxygen and 5% carbon dioxide, and contained (g/l.) ; NaCl, 7·32 ; KCl, 0·42 ; $NaHCO_3$, 2·1 ; $CaCl_2$, 0·24 ; glucose, 10·0 ; pH, 7·4.

Drugs

(+)-Propranolol (I.C.I. 47319), (−)-propranolol (I.C.I. 47320), (±)-propranolol (I.C.I. 45520), and I.C.I. 50172 were all supplied by I.C.I. Pharmaceuticals. Strophanthin G (B.D.H.) and procaine HCl (B.D.H.) were obtained commercially.

Results

Local anaesthetic activity

Two kinds of preparation were used ; whole frog sciatic nerves, and nerves from which the sheath had been stripped for 10 or more mm in the central portion. The stimuli were strong enough to excite all fibres, so that several action potential waves were seen, representing the activity of nerves of different conduction velocities. For the purposes of the assay, the height of the first peak was measured, so that the effect estimated was the depression of conduction in the largest and fastest nerves only. Two experimental procedures were followed. In the first, the nerve was exposed to the drug for 30 min, or longer if the action potential height was still falling ; it was then washed for 30–60 min with Ringer, and the process was repeated with another drug concentration. In the second type of experiment, the nerve was exposed to a low concentration of procaine for 30 min ; the drug concentration was then increased and recordings made for a further 30 min without intermediate

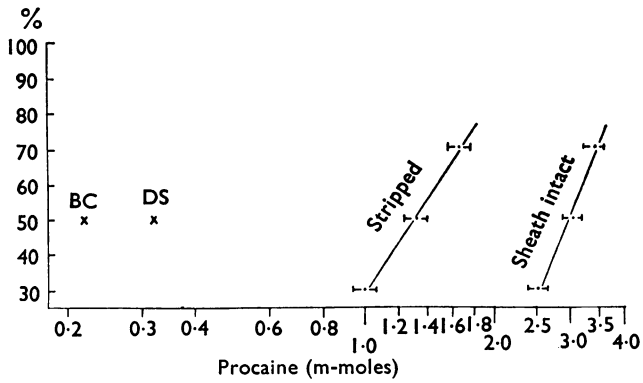

FIG. 1. Effect of procaine on sheathed and stripped nerves. Ordinate, height of action potential expressed as percentage of control. Abscissa, concentration of procaine in m-moles l. BC, Results of Barrett & Cullum (1968) ; DS, results of Dunlop & Shanks (1968).

washing, and the process repeated until at least a 70% block was achieved. The nerve was then washed for a prolonged period and the same sequence followed with another drug. The order in which the drugs were given did not effect the results. Both (+)- and (±)-propranolol took about three times as long as procaine to achieve their full effects, and a correspondingly longer time was required to wash them out.

In Fig. 1, two dose-response curves for procaine are shown depicting results obtained from sheathed and desheathed nerves respectively. The change in sensitivity caused by removal of the nerve sheath is, of course, well known (Ritchie, Ritchie & Greengard, 1965) and the only reason for presenting these results in detail is that they are quantitatively not in agreement with some recently published findings that the average concentration of procaine required to reduce the height of the action potential of frog sciatic nerves (with sheaths intact) by 50% was 85 μg/ml. (0·312 mM) (Dunlop & Shanks, 1968), and 61 μg/ml. (0·223 mM) (Barrett & Cullum, 1968). The corresponding figure in our results was 834 μg/ml. (3·05 mM ±0·11).

In view of this large discrepancy (Fig. 1) the experiments were repeated (*a*) with a new sample of procaine, (*b*) with procaine that had been 10 yr in the department and (*c*) with a sample of procaine supplied by Dr. Barrett. The results were the same as with the procaine used originally. Experiments were also carried out at pH 7·4 instead of pH 7·0 when it was learned that the I.C.I. workers had used a solution at pH 7·4 (personal communication). Although the activity of the procaine was increased, there was still a fivefold difference between their results and ours, which is perhaps attributable to regional differences in the frogs used. Attention has been drawn to these quantitative differences because they illustrate the danger of drawing conclusions about the mode of action of a drug in man on the basis of

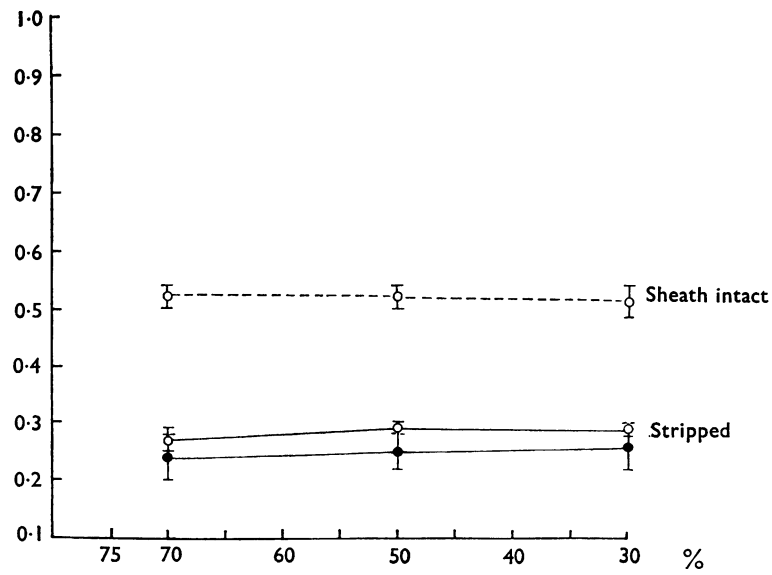

FIG. 2. Effect of (+)- and (±)-propranolol on sheathed and stripped nerve. Ordinate, ratio of molar concentration of test drug to that of procaine producing the same reduction of action potential height in the same nerve. Abscissa, height of action potential expressed as percentage of control. ○, (±)-propranolol; ●, (+)-propranolol.

results obtained with a certain dose in animals (namely a "beta-blocking dose," a "cardiac depressant dose" etc.).

The results of all the experiments have been pooled and are presented in Fig. 2. The ordinate is the molar concentration of (+)- or (±)-propranolol expressed as a fraction of that of procaine (=1·0) producing the same reduction in the height of the action potential. Three features are evident: (1) the drug ratios remain reasonably constant for all levels of local anaesthesia; (2) the removal of the sheath makes a greater difference to the sensitivity of the nerve to propranolol than to procaine; consequently the effectiveness of propranolol relative to procaine is smaller in the intact than in the stripped nerves; (3) the activity of (+)-propranolol is not very different from that of the racemic compound. Thus if either the (−)- or (±)-compound proved more active than the (+)-compound in protecting against digitalis intoxication, this property could fairly be attributed to their greater potency as β-receptor blocking agents.

Ouabain-induced arrhythmias

The lethal dose of cardiac glycosides is notoriously variable, and an intermittent infusion method was introduced by Vaughan Williams & Sekiya (1963), in an attempt to improve the accuracy of the assay. The lethal dose in control animals was then found to be 336±15·3 μg/kg, and in the present series it was 326±10·3 μg/kg. The amounts of ouabain required to produce (1) the first signs of abnormality of rhythm (unequal intervals between atrial beats), (2) extrasystoles and (3) ventricular fibrillation were also very similar in the previous and present series (Table 1). Table 1 also gives the effects of the intravenous administration of (±)-propranolol 0·75 and 3·0 mg/kg, and of (+)-propranolol 3·0 and 12.0 mg/kg, 5 min before the start of the ouabain perfusion.

In the control series all animals developed ventricular fibrillation, but after pretreatment with (±)-propranolol 0·75 mg/kg (i.e., 0·375 mg/kg of the (−) form)

TABLE 1. *Comparison of effects of* (+)- *and* (±)-*propranolol on ouabain-induced cardiac arrhythmias*

Control	Unequal intervals	Extra-systoles	Ventricular fibrillation	Cardiac arrest
Vaughan Williams & Sekiya (1963) $n=13$	128±13·8	187±10·9	272±14·6	336±15·3
Control. This paper $n=16$	118±14·7	192±16·5	272±13·4	326±10·3
0·75 mg/kg (±)-Propranolol $n=18$	102±15·2	201±18·5	7/18 only 242±23·8 for survivors	291±11·7 $0·05>P>0·02$
3·0 mg/kg (+)-Propranolol $n=13$	84±8·3	166±10·9	12/13 only 256±13·5 for survivors	293±10·4 $0·05>P>0·02$
3·0 mg/kg (±)-Propranolol $n=13$	84±13·8	193±23·1	None	381±20·6 $0·02>P>0·01$
12·0 mg/kg (+)-Propranolol $n=16$	114±11·8	235±16·9	15/16 only 315±14·3	353±10·9

The amounts of ouabain (μg/kg) required to produce the stated effects are given together with the standard errors. When these amounts are significantly different from the controls the P values also are given (that is, when $P<0·05$).

only seven out of eighteen fibrillated; after (±)-propranolol 3·0 mg/kg none of the twelve animals fibrillated, and the dose of ouabain required to produce cardiac arrest was significantly increased ($P<0.02$).

In contrast, after (+)-propranolol 3·0 mg/kg, which is much less active as a β-receptor blocking agent but is at least as potent a local anaesthetic, twelve out of thirteen animals fibrillated, and after 12·0 mg/kg fifteen out of sixteen fibrillated. With neither dose was there a statistically significant increase in the lethal dose of ouabain. On the contrary, a surprising finding was that both after (±)-propranolol 0·75 mg/kg and after (+)-propranolol 3·0 mg/kg, the lethal dose of ouabain was significantly reduced.

The results show that the compound with β-receptor blocking activity is much more effective than the one without in preventing digitalis-induced ventricular fibrillation.

TABLE 2A AND B. *Effects on intracellularly recorded potential and other properties of isolated rabbit atria*

TABLE 2A

Drug	Concentration		Resting potential	% Difference	Action potential	% difference	% difference: Repolarization	% difference: Threshold	% difference: Conduction velocity
Laevo-propranolol	0·15 mg/l. ($5·075\times10^{-7}$ M)	C	61·8 (4)		86·6 (9)				
		E	62 (4)	+0·3	83·2 (8)	−3·5	+4	0	−9
		R	61·8 (4)		82·0 (6)				
		C	65 (5)		84·6 (13)				
		E	62 (4)	−4·6	79·5 (6)	−6·0	+13	+5	0
Dextro-propranolol	0·15 mg/l. ($5·075\times10^{-7}$ M)	C	69·2 (5)		87 (7)				
		E	68·2 (6)	−1·4	86 (11)	−1·2	+1·6	0	−4
		R	64·0 (4)		86·5 (4)				
		C	61·5 (6)		82 (6)				
		E	61·5 (6)	0	80 (8)	−2·3	−2	+3·5	0
		R	60·5 (2)		80 (2)				
Laevo-propranolol	0·3 mg/l. ($1·015\times10^{-6}$ M)	C	70·2 (6)		99·6 (7)	***			
		E	66·8 (6)	−4·5	85·1 (7)	−14·5	−1	0	−4
		R	66·9 (7)		94 (12)				
		C	64·3 (4)		86·7 (7)	**			
		E	63·8 (10)	−0·7	80·3 (12)	−7·2	−9	+38	0
		R	69 (1)		86·2 (5)				
Dextro-propranolol	0·3 mg/l. ($1·015\times10^{-6}$ M)	C	57·1 (7)		78·3 (13)	**			
		E	57·6 (6)	+0·8	70·3 (6)	−10·3	+4	+17	−11
		R	60·8 (7)		79·0 (7)				
		C	70·1 (10)		89·0 (11)				
		E	72·6 (6)	+3·4	88·3 (6)	−0·8	+0·6	0	−14
		R	64·3 (7)		82·8 (10)				
		C	68 (5)		89·9 (7)				
		E	66·8 (5)	−1·6	87·4 (9)	−2·7	+7	0	
Dextro-propranolol	3·2 mg/l. ($1·08\times10^{-5}$ M)	C	59·7 (6)		80·9 (12)				
		E	63·4 (5)	+6·2	81·6 (7)	+0·5	+5	0	−27
		R	62·3 (3)		81·3 (6)				
Racemic propranolol	3·0 mg/l. ($1·015\times10^{-5}$ M)	C	62·9 (34)		80 (48)	***			
		E	60·6 (15)	−3·5	58·6 (24)	−26·6	−15		

In the columns indicating parameters of intracellular records, the means are given with the number of individual fibres in brackets. C, Results obtained during control period. E, Effects obtained after administration of drug. R, Results obtained after 1 hr recovery from drug. The resting and action potentials are given in mV, the maximum and mean rates of rise in V/sec. Statistical significance (*t* test) of difference of effect from control; *=$0·05>P>0·01$; **=$0·01>P>0·001$; ***=$P<0·001$. ±-propranolol figures quoted from Morales-Aguilerá & Vaughan Williams (1965).

Intracellularly recorded potentials

Records were obtained from isolated rabbit atria of contractions, conduction velocity and intracellular potentials. Measurements were made from the intracellular records of resting potential, total action potential height, repolarization time from peak to 50% and to 90% of diastolic potential, and of maximum and mean rates of rise of the action potential (Vaughan Williams, 1958b). Measurements were also made of the electrical threshold (current required to drive atria with 1 msec shocks at frequency 10% above spontaneous rate), and the maximum driving frequency (1 msec shocks, strength more than twice threshold).

The results of experiments with isomers of propranolol are given in Table 2A and B. There was no consistent effect on the contractions or on the 50% repolarization time and so these measurements have been omitted. (+)- (−)- and (±)-propranolol, and I.C.I. 50172 had no significant effect on the resting potential at any of the concentrations studied. This is in line with previous observations of the activity of drugs used as anti-arrhythmics (Szekeres & Vaughan Williams, 1962). All four compounds greatly reduced both the maximum and mean rates of rise of the action potential.

TABLE 2B

Drug	Concentration		Maximum rate of rise of action potential	% Difference	Mean rate of rise of action potential	% Difference	% difference: Spontaneous frequency	% difference: Maximum driven frequency
Laevo-propranolol	0·15 mg/l. ($5{\cdot}075\times10^{-7}$ M)	C	134 (7)	*	94 (7)	*		
		E	95 (8)	−29	68 (5)	−27	−10	+5
		R	105 (9)		68 (6)			
		C	120 (13)	*	53 (13)	**		
		E	92 (6)	−23	32 (6)	−38	0	0
Dextro-propranolol	0·15 mg/l. ($5{\cdot}075\times10^{-7}$ M)	C	118 (7)	***	74 (6)	**		
		E	83 (9)	−30	39 (6)	−47	−5	−8
		R	138 (4)		78 (4)			
		C	104 (9)	**	68 (6)	*		
		E	68 (8)	−37	54 (6)	−21	−6	0
		R	93 (2)		73 (2)			
Laevo-propranolol	0·3 mg/l. ($1{\cdot}015\times10^{-6}$ M)	C	170 (7)	***	50·1 (7)	***		
		E	85 (7)	−50	31·6 (7)	−39	−3·1	
		R	161 (12)		50 (12)			
		C	105 (7)	***	27·4 (7)	**		
		E	73·4 (12)	−31	22·6 (10)	−18	0	−28·2
		R	95 (5)		27.6 (5)			
Dextro-propranolol	0·3 mg/l. ($1{\cdot}015\times10^{-6}$ M)	C	90 (14)	***	56 (14)	***		
		E	36 (6)	−60	34 (6)	−40	−1·5	0
		R	110 (6)		58 (6)			
		C	137 (11)	**	61 (11)	**		
		E	95·5 (6)	−30	49 (6)	−20	0	0
		R	135 (10)		58·5 (11)			
		C	78·7 (6)	**	45·5 (6)	*		
		E	60·4 (8)	−23	34·5 (8)	−24	0	0
Dextro-propranolol	3·2 mg/l. ($1{\cdot}08\times10^{-5}$ M)	C	84·6 (19)	***	42·5 (19)	***		
		E	47·3 (18)	−44	21·8 (18)	−48	0	0
		R	54·5 (11)		34 (11)			
(±)-propranolol	3·0 mg/l. ($1{\cdot}015\times10^{-5}$ M)	C	76·5 (43)	***	44·8 (44)	***		
		E	28·7 (16)	−62·5	17·5 (18)	−61		

Perhaps the most interesting feature of Table 2 is that it shows that the rate of rise of the action potential was reduced by concentrations of the drugs which had either no effect, or only a very small effect, on the electrical threshold, the conduction velocity, the spontaneous frequency and the maximum driving frequency. It was surprising how large an effect on the rate of rise of the action potential was exerted by concentrations of propranolol as low as 0·15 mg/l. ($5{\cdot}075 \times 10^{-7}$M). Furthermore I.C.I. 50172 also reduced the rate of rise of the action potential although its local anaesthetic activity on nerve is extremely weak. It is true that within the time schedule of the experiments (in which recordings were made approximately 1 hr after changes in conditions) the rate of rise had not always returned to the control values, but the rates of rise did invariably increase again after being reduced by drug action. It was evident that the measurement of the rate of rise of the intracellularly recorded action potential was by far the most sensitive test of the activity of the drugs on cardiac function.

Discussion

Both (−)- and (+)-propranolol significantly reduced the rate of rise of the action potential at a concentration of 0·15 mg/l. ($5{\cdot}075 \times 10^{-7}$M). The concentration of (±)-propranolol required to cause a 30% reduction in the height of the frog stripped nerve action potential at pH 7·4 was 39 mg/l. ($1{\cdot}32 \times 10^{-4}$M), two hundred and sixty times as much. I.C.I. 50172, which is reported to be devoid of local anaesthetic activity (Dunlop & Shanks, 1968), also reduced the rate of rise of the cardiac action potential, although the concentration required was more than twenty times that of propranolol. These concentrations produced negligible effects on spontaneous frequency, maximum driving frequency, contractions, electrical threshold and conduction velocity. Measurement of the rate of rise of the action potential thus provided the most sensitive test of the activity of these compounds on cardiac muscle, but did not distinguish between (+)- and (−)-propranolol. In this context it must be noted that although (−)-propranolol is a much more potent β-receptor blocking agent than the (+)-compound, the latter is far from being devoid of activity and has been estimated to be similar in potency to pronethalol in antagonizing the chronotropic action of adrenaline on isolated rabbit atria. The αA_2 values were $1{\cdot}2 \times 10^{-7}$M for pronethalol, and $1{\cdot}9 \times 10^{-7}$M for (+)-propranolol ($3{\cdot}2 \times 10^{-8}$ for (±)-propranolol) (Raper & Jowett, 1967).

It is not possible to say whether the ratio of the concentrations producing β-receptor blockade and reducing the rate of rise of the cardiac potential is the same for I.C.I. 50172 as for propranolol, because the relative status of I.C.I. 50172 as a β-receptor blocking agent is in some doubt. Dunlop & Shanks (1968) stated that I.C.I. 50172 had one-third to one-quarter the activity of propranolol in human volunteers, however, Brick, Hutchison, McDevitt, Roddie & Shanks (1968) found I.C.I. 50172 had much less than 1/20th the activity of propranolol in antagonizing the chronotropic action of infused isoprenaline, and Jackson (1968) in experiments on electrically driven frog ventricles estimated I.C.I. 50172 to have 1/70th the activity of propranolol.

Local anaesthetic and anti-arrhythmic activity

As mentioned in the introduction, although pronethalol and propranolol were

found to be potent anti-arrhythmic compounds, even against ouabain-induced arrhythmias, the relevance of the β-receptor blocking action to the anti-arrhythmic properties was placed in doubt by the finding that they were also good local anaesthetics. Lucchesi (1965) stated that the anti-arrhythmic action of pronethalol was "not related to its ability to produce beta-adrenergic receptor blockade". This conclusion was based on the finding that "the effective anti-arrhythmic dose of (+)-pronethalol, in both the isolated rabbit heart and anaesthetized dog receiving toxic doses of digitalis, was found to be very similar to the effective dose of (±)-pronethalol, reported in a previous publication (Lucchesi, 1964)". Both isomers are potent local anaesthetics, however, so it would be expected that the protective action of large doses would be similar, and in order to decide whether the β-receptor blocking action of one isomer affords any additional protection, it would be necessary to make a strict quantitative comparison, particularly in the lower dose ranges. Similarly, Benfey & Varma (1966) supported the view that β-receptor blocking activity was irrelevant to protection against ouabain-induced arrhythmias on the basis of a comparison of the effects of pronethalol and propranolol (which latter they found 16·5 times more active than pronethalol as an antagonist of the augmentation by noradrenaline of contractions of isolated guinea-pig atria). In the lower dose range, however, (below 5·0 mg/kg) their results showed without exception that propranolol provided greater protection than pronethalol, and even after 5·0 mg/kg it was striking that ventricular fibrillation occurred in six out of eight animals after pronethalol and in none of five after propranolol.

Our own experiments indicated that (+)-propranolol, which had marginally greater potency as a local anaesthetic than the (±)-compound, was significantly less active than (±)-propranolol in protecting against ouabain-induced arrhythmias. The implication was that the much greater β-receptor blocking activity of the (±)-compound contributed to this protective effect. This conclusion would be consistent with the findings of Mendéz, Aceves & Mendéz (1961) that acetyl digitoxin no longer caused ventricular fibrillation in dogs after sympathectomy and adrenalectomy, and with the evidence of Cairoli, Reilly & Roberts (1961) that cat papillary muscles normally beat spontaneously in the presence of ouabain, but not when taken from reserpinized animals. In a subsequent paper Cairoli, Reilly, Ito & Roberts (1962) reported that reserpine pretreatment in dogs significantly increased the amount of acetyl-strophanthidin required to produce ventricular arrhythmias. Ciofalo, Levitt & Roberts (1967) found that the onset of arrhythmias induced by ouabain in cats was delayed by pretreatment with reserpine. Finally, Barrett & Cullum (1968) showed that (−)-propranolol was more effective than (+)-propranolol in reversing ouabain-induced ventricular tachycardia in dogs.

Significance of intracellular records

From the functional point of view, decrease in the rate of rise of the action potential could account for anti-arrhythmic action. Depolarization increases sodium permeability and becomes self-recruiting since net inward current causes further depolarization. The depolarization, however, also sets in motion other opposing processes—for example, inactivation of sodium permeability. Reactivation follows repolarization, and if this were delayed or incomplete as a consequence of drug action, the threshold for re-excitation would be raised.

The ionic composition of the depolarizing current in cardiac muscle is still not

completely elucidated. It was observed 10 years ago that concentrations of quinidine above 10 mg/l. ($1{\cdot}28 \times 10^{-5}$M) progressively reduced the rate of rise until a " step " appeared in the upstroke of the action potential, a fast phase being followed by a slower phase, which nevertheless continued to reach an " overshoot " potential (interior of cell positive to exterior). At that time some doubt had been thrown upon the view that the inward-going depolarizing current was carried by sodium ions, by the observation of Coraboeuf & Otsuka (1956) that replacement of external sodium ions by choline chloride had little effect on the overshoot of the guinea-pig ventricle. As an explanation of the " step " revealed by quinidine it was suggested that " the early fast phase of the step represents the operation of the sodium-carrier, and that the second slower phase is produced by current carried by other ions " (Vaughan Williams, 1958a). In 1962 Noble showed that the shape of the Purkinje fibre action potential could be simulated by a computer fed with modifications of the Hodgkin-Huxley equations for nerve, and it was supposed that sodium ions carried the depolarizing current of the cardiac action potential, assisted by an instantaneous cut-off of potassium current. More recently, however, Reuter (1967) provided evidence that " calcium ions carry an appreciable membrane current in the inward direction when the membrane of the Purkinje fibre is depolarized ". Since this current began to be switched on when the membrane was depolarized to between -60 to -40 mV, was driven by a concentration gradient with an equilibrium potential of $+150$ mV, and was of a duration which extended into the plateau region, it would account very well for the slow phase of the " step " revealed by large concentrations of quinidine.

The action of anti-arrhythmic drugs, and perhaps also the action of adrenaline (Vaughan Williams, 1967) may be attributed to specific interference with the mechanism by which the channels for individual ions are switched on (and off) by the voltage across the membrane. Whereas anti-arrhythmics decrease the rate of rise of the action potential, sympathetic stimulation or catecholamines increase it, especially, or perhaps only, when it has become subnormal as a result of drug action or for other reasons, (Hutter & Trautwein, 1956 ; Hoffman & Singer, 1967). Thus drugs such as quinidine or procaine are anti-arrhythmic in high dosage, but their effects on the rate of rise could be nullified or even reversed in situations in which there is a high concomitant sympathetic drive. Compounds which also possess β-receptor blocking activity, however, eliminate the sympathetic " restorative " effect, and thus their anti-arrhythmic properties are unopposed even at low dosage. It is also possible that the occupation of β-receptors by blocking drugs could itself affect the availability of channels for depolarizing current independently of the block of access by the natural transmitter to these sites.

REFERENCES

BARRETT, A. M. & CULLUM, V. A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BENFEY, B. G. & VARMA, D. R. (1966). Antisympathomimetic and antifibrillatory effects of pronethalol and propranolol. *Br. J. Pharmac. Chemother.*, **26**, 3–8.

BLACK, J. W. & STEPHENSON, J. S. (1962). Pharmacology of a new adrenergic beta-receptor-blocking compound, Nethalide. *Lancet*, **2**, 311–314.

BRICK, I., HUTCHISON, K. J., MCDEVITT, D. G., RODDIE, I. C. & SHANKS, R. G. (1968). Comparison of the effects of I.C.I. 50172 and propranolol on the cardiovascular responses to adrenaline, isoprenaline and exercise. *Br. J. Pharmac.*, **34**, 127–140.

BÜLBRING, E. & WAJDA, I. (1945). Biological comparison of local anaesthetics. *J. Pharmac.*, **85**, 87–84.

CAIROLI, V., REILLY, J., ITO, R. & ROBERTS, J. (1962). The relation of the digitalis induced arrhythmia to catecholamine release. *Fedn Proc.*, **21**, 127.

CAIROLI, V., REILLY, J. & ROBERTS, J. (1961). The effect of reserpine pretreatment on the positive inotropic action of ephedrine and ouabain. *Fedn Proc.*, **20**, 122.

CIOFALO, F., LEVITT, B. & ROBERTS, J. (1967). Some factors affecting ouabain-induced ventricular arrhythmia in the reserpine-treated cat. *Br. J. Pharmac. Chemother.*, **30**, 143–154.

CORABOEUF, E. & OTSUKA, M. (1956). L'action des solutions hyposodiques sur les potentials cellulaires de tissue cardiaque de Mammifères. *C.r. Acad. Sci., Paris*, **243**, 441–444.

DUNLOP, D. & SHANKS, R. G. (1968). Selective blockade of adrenoceptive beta receptors in the heart. *Br. J. Pharmac. Chemother.*, **32**, 201–218.

ERLIJ, D. & MENDÉZ, R. (1963). Modification of digitoxin intoxication by exclusion of the sympathetic system. *Fedn Proc.*, **22**, 184.

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

HOFFMAN, B. F. & SINGER, D. H. (1967). Appraisal of the effects of catecholamines on cardiac electrical activity. *Ann. N.Y. Acad. Sci.*, **139**, 914–931.

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

JACKSON, I. M. (1968). Beta adrenergic receptor blockade by ICI 50172. *J. Physiol., Lond.*, **198**, 28*P*.

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

LUCCHESI, B. R. (1965). The effects of pronethalol and its *dextro* isomer upon experimental cardiac arrhythmias. *J. Pharmac. exp. Ther.*, **148**, 94–99.

LUCCHESI, B. R. & HARDMAN, H. F. (1961). The influence of Dichloro-isoproterenol (DCI) and related compounds upon ouabain and acetylstrophanthidin induced cardiac arrhythmias. *J. Pharmac.*, **132**, 372–381.

MENDÉZ, C., ACEVES, J. & MENDÉZ, R. (1961). Inhibition of adrenergic cardiac acceleration by cardiac glycosides. *J. Pharmac.*, **131**, 191–198.

MOORE, J. I. & SWAIN, H. H. (1960). Sensitization to ventricular fibrillation. *J. Pharmac.*, **128**, 243–251.

MORALES-AGUILERÁ & VAUGHAN WILLIAMS, E. M. (1965). The effects on cardiac muscle of β-receptor antagonists in relation to their activity as local anaesthetics. *Br. J. Pharmac. Chemother.*, **24**, 332–338.

NOBLE, D. (1962). A modification of the Hodgkin–Huxley equations applicable to Purkinje fibre action and pace-maker potentials. *J. Physiol., Lond.*, **160**, 317–352.

PAYNE, J. P. & SENFIELD, R. M. (1964). Pronethalol in the treatment of ventricular arrhythmias during anaesthesia. *Br. med. J.*, **1**, 603–604.

RAPER, C. & JOWETT, A. (1967). Anti-fibrillary and anti-adrenaline activity of β-receptor blocking drugs. *Eur. J. Pharmac.*, **1**, 353–362.

REUTER, H. (1967). The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. *J. Physiol., Lond.*, **192**, 479–492.

RITCHIE, J. M., RITCHIE, B. & GREENGARD, P. (1965). The active structure of local anaesthetics. The effect of the nerve sheath on the action of local anaesthetics. *J. Pharmac. exp. Ther.*, **150**, 152–164.

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

SOMANI, P. & LUM, B. K. B. (1965). The antiarrhythmic actions of beta-adrenergic blocking agents. *J. Pharmac. exp. Ther.*, **147**, 194–204.

STOCK, J. P. P. & DALE, N. (1963). Beta-adrenergic receptor blockade in cardiac arrhythmias. *Br. med. J.*, **2**, 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. *Br. J. Pharmac. Chemother.*, **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. (1966). Mode of action of beta-receptor antagonists on cardiac muscle. *Am. J. Cardiol.*, **18**, 399–405.

VAUGHAN WILLIAMS, E. M. (1967). The action of beta-receptor blocking agents on cardiac muscle. In *Int. Symp. on the Coronary Circulation and Energetics of the Myocardium, Milan*, pp. 118–126. Basel: Karger.

VAUGHAN WILLIAMS, E. M. & SEKIYA, A. (1963). Prevention of arrhythmias due to cardiac glycosides by block of β-sympathetic receptors. *Lancet*, **1**, 420–421.

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