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A third class of anti-arrhythmic action. Effects on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ 1999 and AH 3474

B. N. SINGH* AND E. M. VAUGHAN WILLIAMS

Department of Pharmacology, University of Oxford

Summary

1. Both MJ 1999 and AH 3474 protected guinea-pigs anaesthetized with urethane against ouabain-induced ventricular fibrillation.

2. MJ 1999 had 1/90, and AH 3474 1/30, of the activity of procaine in reducing the height of the action potential of frog sciatic nerve.

3. MJ 1999 and AH 3474 reduced the rate of rise of intracellularly recorded action potentials at concentrations in excess of 160×10^{-6}M (50 mg/l.). It was concluded that direct depression of depolarization could have contributed little to the protection against ouabain-induced fibrillation.

4. MJ 1999, but not AH 3474, greatly prolonged the duration of the action potential in acute experiments on isolated atrial and ventricular muscle, and prolonged the Q-Tc interval of the electrocardiogram in anaesthetized guinea-pigs. It is suggested that this effect contributes to anti-arrhythmic activity.

5. At concentrations up to 80×10^{-6}M AH 3474 had positive chronotropic and inotropic effects on isolated rabbit atrial muscle, but at higher concentrations these were superseded by negative effects. MJ 1999 was depressant at all concentrations studied, the threshold concentrations being 19×10^{-6}M for chronotropic, and 162×10^{-6}M for inotropic effects.

Introduction

Quantitative studies in recent years of the effect of anti-arrhythmic agents on various parameters of cardiac function *in vitro* and *in vivo* have made it possible to classify the drugs into groups which do or do not have certain clearly definable pharmacological actions (Vaughan Williams, 1970b), though these actions do not necessarily determine anti-arrhythmic effectiveness. The first class of action associated with anti-arrhythmic properties, and possessed by drugs long in use such as quinidine, procainamide and lignocaine, is a reduction in the maximum rate of rise of cardiac action potentials in the absence of significant effects on resting potential or action potential duration. Such drugs interfere directly with cardiac depolarization, and have local anaesthetic actions on nerve also. They may depress contractions, but only do so at concentrations higher than the threshold concentra-

* Nuffield Dominions (New Zealand) Demonstrator, University of Oxford.

tion at which the maximum rate of depolarization (MRD) is reduced (Singh & Vaughan Williams, 1970a).

The second class of action reduces sympathetic activation. Sympatholytic drugs, whether acting presynaptically or by competition, would naturally be expected to reduce the incidence of arrhythmias associated with sympathetic activity, for example, those occurring during hypercapnia or induction of anaesthesia, or in the presence of other sensitizing agents, but whether they also reduce the incidence of other arrhythmias is more controversial (Dohadwalla, Freedberg & Vaughan Williams, 1969).

A third type of action associated with protection against arrhythmia was recently described (Singh & Vaughan Williams, 1970b). In hypothyroidism, cardiac arrhythmias, apart from occasional ventricular extrasystoles associated with a slow heart rate, are extremely rare and it was found that in thyroidectomized animals the duration of the cardiac action potential was greatly prolonged (Freedberg, Papp & Vaughan Williams, 1970). A similar prolongation is produced by the drug amiodarone (Singh & Vaughan Williams, 1970b), which also has class II sympatholytic effects (Charlier, 1970). The present experiments have indicated that MJ 1999, but not AH 3474, possesses this third type of action to an even greater degree than amiodarone.

Methods

The methods used did not differ from those described in previous papers (Singh & Vaughan Williams, 1970a and b). Very briefly, local anaesthetic action was assayed by measuring the depression of the height of the action potentials of frog sciatic nerves. Protection against ouabain-induced arrhythmias was estimated by measuring the doses of ouabain, infused from a motor-driven syringe, required to produce irregularity of sinus rhythm, ventricular extrasystoles, persistent ventricular tachycardia, ventricular fibrillation and cardiac arrest in guinea-pigs anaesthetized with urethane (1·6 g/kg, intraperitoneally), with and without pretreatment with various doses of the drugs studied. Electrical threshold, conduction velocity, spontaneous and maximum driven frequency (MDF), contraction heights and intracellular potentials were measured on the isolated atria of rabbits, and on the papillary muscles of cats (except MDF), suspended horizontally at 32° C in nutrient fluid gassed with 95% oxygen and 5% carbon dioxide, pH 7·4.

Drugs used

MJ 1999 (Sotalol), DL-4-(2-isopropylamino-1-hydroxyethyl) methane sulphonanilide, HCl (Allen and Hanbury) (Fig. 1, I); AH 3474, DL-5-(2-*t*-butylamino-1-hydroxyethyl) salicylamide, HCl (Allen and Hanbury) (Fig. 1, II); Strophanthin G

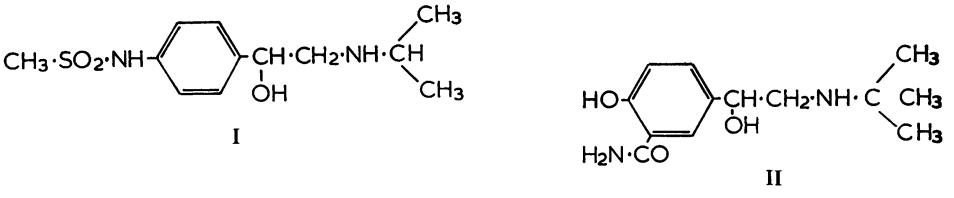

FIG. 1. Structures of MJ 1999 (I) and AH 3474 (II).

(ouabain) (B.D.H.); urethane (Hopkin and Williams); procaine HCl (B.D.H.); pentobarbitone Na (Savory and Moore). Weights are expressed in terms of the salts. Statistical evaluation was by Student's t test, and by χ^2 test (for incidence of ventricular fibrillation).

Results

Local anaesthesia

Both MJ 1999 and AH 3474 had some local anaesthetic action on frog nerve. Though the effects were weak, it cannot be said that the drugs were devoid of local anaesthetic action. The detailed results of the assay, with procaine as standard, are given in Table 1. MJ 1999 had about 1/90 and AH 3474 1/30 of the activity of procaine as a local anaesthetic on frog nerve.

Ouabain-induced arrhythmias

MJ 1999 and AH 3474 reduced the incidence of ouabain-induced ventricular fibrillation in anaesthetized guinea-pigs at doses which were low in relation to their local anaesthetic activities on nerve (Table 2).

TABLE 1. *Comparison of the activities of MJ 1999 and AH 3474 with that of procaine on frog sciatic nerves, stripped of their sheaths*

% reduction of action potential height	Concentrations (mM)			Activity ratios, procaine = 100	
	Procaine $n=15$	MJ 1999 $n=8$	AH 3474 $n=7$	MJ 1999	AH 3474
25	0·45±0·025	47·44±1·92	15·90±1·43	0·95	2·83
50	0·56±0·023	51·52±1·95	17·32±1·38	1·09	3·23
75	0·76±0·023	59·58±1·85	19·91±1·35	1·27	3·82

TABLE 2. *Effect of AH 3474 and MJ 1999 on ouabain-induced cardiac arrhythmias in anaesthetized guinea-pigs*

Compound and dose 10^{-6} mol/kg (mg/kg)	n	Amounts of ouabain (μg/kg i.v.) required to produce				
		Unequal R-R intervals	Ventricular ectopic beats	Persistent ventricular tachycardia	Ventricular flutter-fibrillation	Cardiac arrest
Control	30	88·5±48	20·47±10·6	226·0±11·8	240·7±11·7 (29/30)	311·2±12·7
AH 3474						
2·6 (0·75)	10	80·3±4·7	212·3±12·7	230·2±14·3	247·9±15·5 (9/10)	287·1±12·6
5·19 (1·5)	10	83·6±7·6	206·6±11·6	222·0±10·8	264·0±9·9 (5/10)**	299·3±9·0
10·39 (3·0)	10	86·5±9·3	227·3±29·5	275·7±16·3*	275·8±13·3 (4/10)**	307·0±26·2
20·77 (6·0)	10	73·2±10·2	238·4±5·6	244·6±31·3	276·3±29·6 (2/10)**	313·9±22·9
41·54 (12·0)	10	92·0±8·2	270·1±11·3**	299·7±10·4**	None	346·9±9·5*
MJ 1999						
2·43 (0·75)	10	84·4±3·6	204·7±10·6	228·2±4·3	248·6±3·6 (9/10)	309·6±8·4
4·86 (1·5)	10	89·2±3·0	202·3±2·7	214·5±5·8	241·4±17·2 (5/10)**	307·5±7·5
9·72 (3·0)	10	90·0±5·3	217·8±5·4	263·5±12·9*	278·6±6·2 (3/10)**	334·1±16·5
19·44 (6·0)	10	79·0±4·6	244·7±11·5	287·2±8·5**	None	330·9±9·6

Statistical significance of the difference from control: *$P<0.05$; **$P<0.001$.

Effects on heart rate and the electrocardiogram in anaesthetized guinea-pigs

Both the compounds studied slowed the spontaneous heart rate in guinea-pigs anaesthetized with urethane (Table 3). The initial transient increase in spontaneous rate caused by AH 3474 in isolated rabbit atria (Table 4) was not observed in five anaesthetized guinea-pigs given $20{\cdot}77 \times 10^{-6}$ mol/kg (6 mg/kg) intravenously or in another five given $41{\cdot}54 \times 10^{-6}$ mol/kg intravenously. Low doses ($2{\cdot}43$–$4{\cdot}86 \times 10^{-6}$ mol/kg) of MJ 1999 intravenously did not alter the Q-Tc interval of the electrocardiogram, but $9{\cdot}72 \times 10^{-6}$ mol/kg (3·0 mg/kg) prolonged the Q-Tc interval by 13·3% ($n=10$, $P<0{\cdot}05$), and $19{\cdot}44 \times 10^{-6}$ mol/kg prolonged Q-Tc by 19·3% ($n=10$, $P<0{\cdot}01$), but neither dose altered the P-R interval. The effect of MJ 1999 on the e.c.g. in a typical experiment is shown in Fig. 2, which also illustrates the anti-

TABLE 3. *Effects of MJ 1999 and AH 3474 on the heart rate of guinea-pigs anaesthetized with urethane*

MJ 1999			AH 3474		
Dose 10^{-6} mol/kg (mg/kg)	n	% change from control	Dose 10^{-6} mol/kg (mg/kg)	n	% change from control
2·43 (0·75)	10	−15·6±3·6	2·6 (0·75)	10	−14·6±2·7
4·86 (1·5)	10	−19·7±4·2	5·19 (1·5)	10	−16·4±3·3
9·72 (3·0)	10	−24·3±2·9	10·39 (3·0)	10	−16·9±4·1
19·44 (6·0)	10	−28·1±4·4	20·77 (6·0)	10	−18·9±2·3
			41·54 (12·0)	10	−21·2±4·4

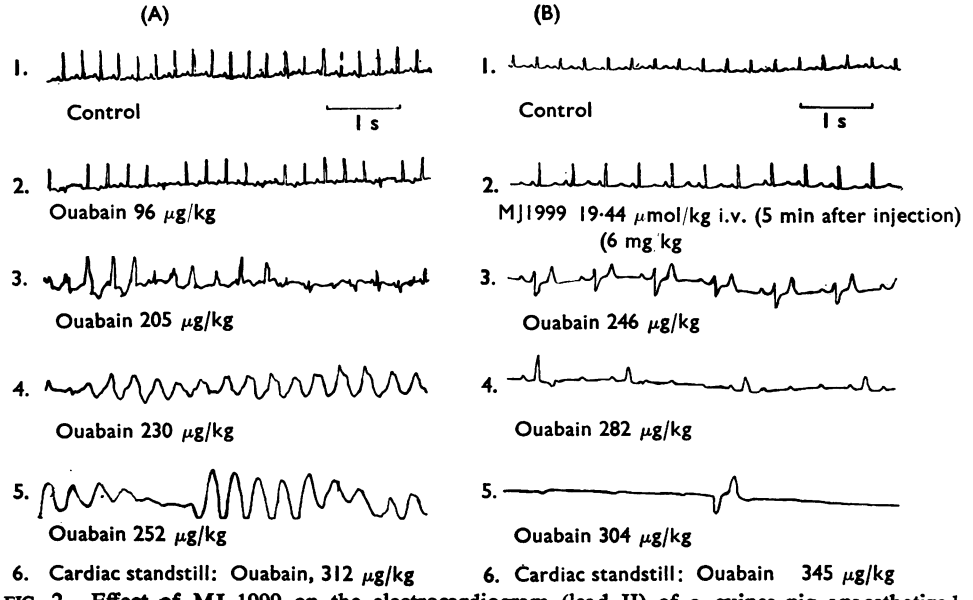

FIG. 2. Effect of MJ 1999 on the electrocardiogram (lead II) of a guinea-pig anaesthetized with urethane, and its protective action against ouabain-induced arrhythmia. Below each e.c.g. record the amount of ouabain is shown which had been infused at the time the record was taken. A: Effect of ouabain alone. B: 2, effect of MJ 1999 alone; 3–5, Effects of ouabain infusion after MJ 1999. The dose given could have had virtually no class I action on the heart, so that this result confirms the view that β-adrenoceptor blockade contributes to protection against ouabain-induced arrhythmia.

fibrillatory action of the compound given in a dose which prolonged the Q-Tc interval. No significant changes on the P-R or Q-Tc intervals were produced by AH 3474 in doses up to 346×10^{-6} mol/kg (100 mg/kg).

It was thought that our failure to observe in anaesthetized guinea-pigs the positive chronotropic action of AH 3474 recorded in isolated rabbit atria might have been connected with the choice of anaesthetic. $41{\cdot}5 \times 10^{-6}$ mol/kg of AH 3474 was accordingly injected intravenously into three guinea-pigs anaesthetized with pentobarbitone $53{\cdot}4 \times 10^{-3}$ mol/kg intraperitoneally, but there was still no initial acceleration of heart rate, only a bradycardia in all of them.

TABLE 4. *Effects on spontaneous frequency, maximum driven frequency, electrical threshold, conduction velocity and contractions in isolated rabbit atria of various concentrations of MJ 1999 and of AH 3474.*

Effect on	Concentration $\times 10^{-6}$M (mg/l.)	Time of exposure to drug (min) 10	30	60	120
(A) MJ 1999					
1. Spontaneous frequency	19·44 (6)	None	−4·3	−7·7	−10·8
	38·88 (12)	−4·8	−13·6	−17·9	−20·6
	77·76 (24)	−3·2	−8·4	−18·2	−22·5
	162 (50)	−10·0	−18·9	−28·4	−33·7
	324 (100)	−7·8	−20·9	−27·9	−34·1
2. Maximum driven frequency	77·76	−1·1	−4·5	−6·1	−9·3
	162	−2·0	−11·4	−18·7	−23·8
	324	−3·6	−13·8	−22·3	−25·6
3. Electrical threshold	324	+2·9	+4·1	+4·3	+4·3
4. Conduction velocity	162	−2·0	−7·0	−8·4	−8·7
	324	−8·6	−17·8	−23·4	−24·8
5. Contractions	162	−4·7	−8·8	−11·5	−11·6
	324	−5·0	−7·9	−9·5	−10·0
(B) AH 3474					
1. Spontaneous frequency	41·54 (12)	+2·3	+2·6	+3·4	+3·8
	83·08 (24)	+7·2	+11·0	+12·5	+11·2
	173 (50)	+5·0	+4·4	−1·8	−7·0
	346 (100)	+3·0	−2·5	−6·7	−11·6
2. Maximum driven frequency	173	−3·8	−5·3	−6·3	−6·8
	346	−5·5	−7·4	−9·8	−19·2
3. Electrical threshold	173	None	None	+2·2	+3·5
	346	+3·7	+5·4	+8·2	+12·9
4. Conduction velocity	173	None	None	−3·3	−3·5
	346	−3·1	−5·0	−11·6	−23·2
5. Contractions	41·54	+2·6	+3·0	+3·3	+4·2
	83·08	+3·7	+4·8	+4·8	+5·3
	173	+4·2	+1·9	−6·2	−8·5
	346	+4·5	−8·9	−17·7	−21·1

Figures show mean per cent differences from measurements made before exposures to the drug, but only changes in excess of 1% have been recorded. (n=3, for each concentration.)

Spontaneous frequency, MDF, electrical threshold, conduction velocity and contractions

The effects of MJ 1999 and AH 3474 on the above features of the function of isolated rabbit atria are presented in Table 4. The sections recording spontaneous frequency and contractions indicate that at the lower concentrations studied AH 3474 had positive actions, and that these were the only actions. At higher concentrations and after more prolonged exposures to the drug, the positive effects were superseded by depressant effects, not only on spontaneous frequency and contractions, but also on conduction velocity, maximum driven frequency, and electrical threshold. In contrast MJ 1999 had no stimulant effects at all, and the slowing of spontaneous frequency, although it took some time to develop, appeared at a concentration of $19{\cdot}44 \times 10^{-6}$M (6 mg/l.).

Rabbit atrial intracellular potentials

MJ 1999 and AH 3474 had significant direct class I actions on cardiac intracellular potentials at concentrations of 162 and 173×10^{-6}M respectively (50 mg/l.) and above (Table 5). The maximum rate of rise of the action potential and the overshoot potential were reduced, but the resting potential was unaffected. In this respect AH 3474 had approximately 1/300 the activity of propranolol, and MJ 1999 was a little weaker still (Figs. 3 and 4). The most striking property of MJ 1999, not shared by AH 3474, propranolol or any other β-adrenoceptor blocking drug so far studied, was to prolong the action potential. Furthermore repolarization was delayed by a concentration only one fourth of that required to reduce the rate of depolarization.

TABLE 5. *Rabbit atrial intracellular potentials*

Concentration 10^{-6}M (mg/l.)	No. of fibres		Resting potential mV	Action potential mV	Maximum rate of rise V/s	% difference from control	Time for repolarization to 50% of resting potential	Time for repolarization to 90% of resting potential	% difference from control
(A) MJ 1999									
38·88	31	C	66·1	90·2	107·3		53·8	109·9	
(12)	33	E	66·6	91·8	108·6		53·0	114·2	+4
	13	R	65·6	90·4	105·3		56·5	111·7	
77·76	27	C	62·9	86·8	95·4		54·4	102·1	
(24)	37	E	62·0	87·5	92·9		59·1*	122·5**	+20
	14	R	62·2	88·7	90·4		56·1	112·2	
162	29	C	65·3	91·2	88·8		54·1	102·6	
(50)	37	E	65·3	88·8	80·0*	−9	64·3**	130·6**	+30
	20	R	66·1	93·9	94·2		52·6	103·6	
324	40	C	66·9	91·2	92·6		54·0	102·6	
(100)	45	E	66·6	84·6*	58·5**	−36	73·9**	159·7**	+55·5
	19	R	66·7	90·6	84·9		59·9	121·9	
(B) AH 3474									
173	32	C	64·6	90·2	86·2		58·9	112·1	
(50)	36	E	62·9	91·4	65·9**	−23·6	59·2	110·7	
	18	R	65·1	89·3	90·3		56·1	115·6	
346	30	C	65·9	89·7	105·2		47·3	98·5	
(100)	32	E	64·4	80·1*	50·9**	−42·2	46·8	97·2	
	15	R	64·7	91·2	95·4		49·2	97·6	

The effects of exposures to 20·77 and $41{\cdot}54 \times 10^{-6}$M AH 3474, and of $19{\cdot}44 \times 10^{-6}$M MJ 1999 were also studied, but have been omitted because no statistically significant changes were produced. C, Control period; E, effect of 60–90 min exposure to drug; R, records after 60–120 min recovery period in absence of drug. Significance for difference from control: $*P<0{\cdot}01$; $**P<0{\cdot}001$.

Intracellular potentials from cat papillary muscle

The above result, and the lengthening by MJ 1999 of the Q-Tc interval in the electrocardiogram of anaesthetized guinea-pigs, suggested that the extent to which ventricular potentials also were lengthened by MJ 1999 ought to be investigated. Although successful impalements have been made in strips of rabbit ventricle (Singh & Vaughan Williams, 1970b), rabbit ventricular muscle is easily damaged, and it was decided to use cat papillary muscles instead.

The results are shown in Table 6. The delay in repolarization by comparable concentrations of MJ 1999 was relatively greater in the cat papillary muscle even than in the rabbit atrium, while the effect on depolarization was about the same (Fig. 5). The degree of prolongation of the ventricular potential may be appreciated by noting that whereas the contraction outlasted the action potential by about

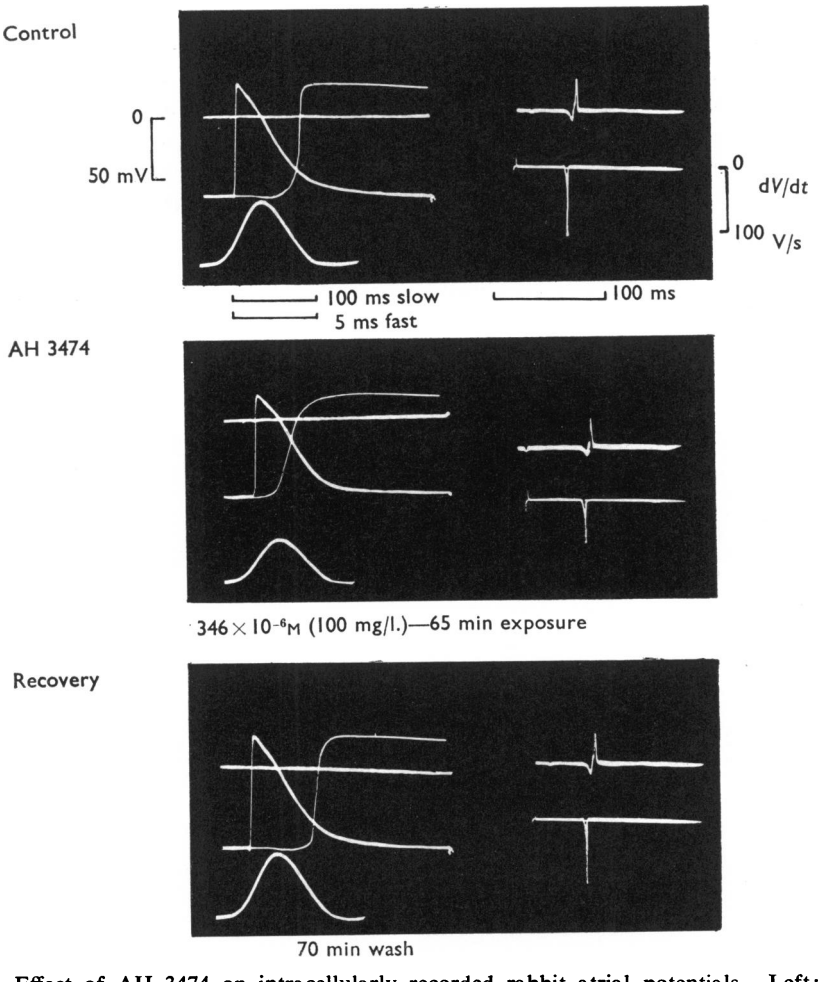

FIG. 3. Effect of AH 3474 on intracellularly recorded rabbit atrial potentials. Left: Horizontal trace, zero potential with electrode outside fibre; middle traces, intracellular records at slow and fast sweep speeds; bottom trace, contraction. Right: Upper trace, interval between stimulus and surface action potential for measurement of conduction velocity; lower trace, differentiation of intracellular record. Spike depth is proportional to rate of rise of action potential.

160 ms in the control period, in the presence of 324×10^{-6}M MJ 1999 the action potential outlasted the contraction by more than 220 ms, even though the duration of the contraction itself had increased by 170 ms.

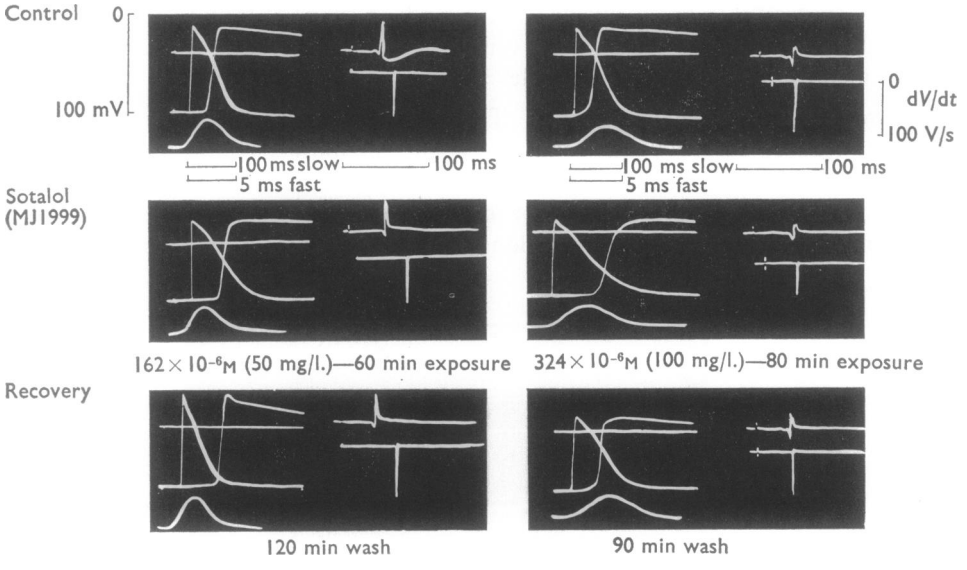

FIG. 4. Effect of MJ 1999 on intracellularly recorded rabbit atrial potentials. Traces as described for Fig. 3.

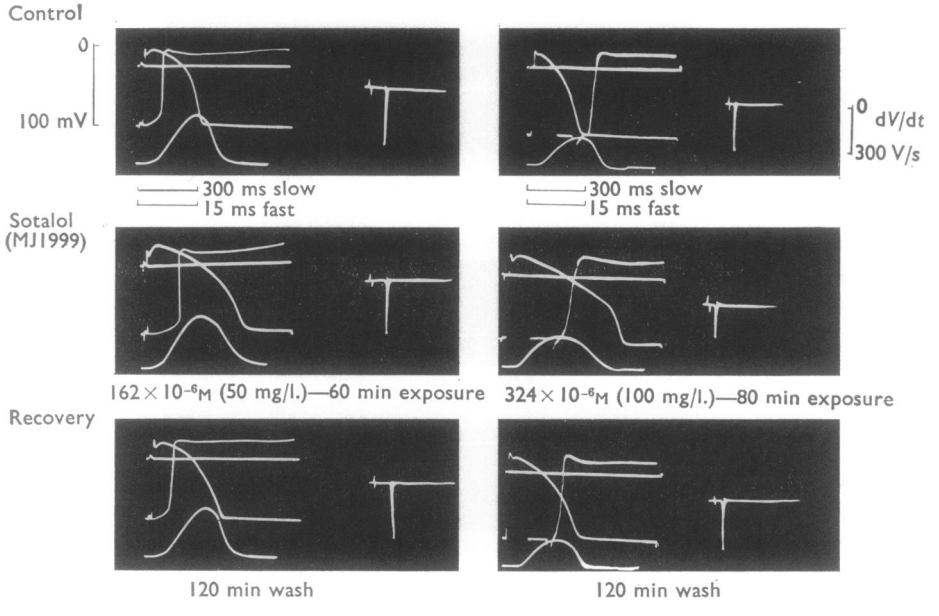

FIG. 5. Effect of MJ 1999 on intracellularly recorded potentials of cat papillary muscle. Traces as described for Fig. 3, except that the surface record has been omitted from the frames on the right.

TABLE 6. *Effect of MJ 1999 on intracellular potentials and contractions of cat papillary muscles (n=3 for each concentration)*

Concentration $\times 10^{-6}$M	No of fibres		Duration of contraction ms	Resting potential mV	Action potential mV	Maximum rate of rise V/s	% difference from control	Time for repolarization to 50% of resting potential (ms)	Time for repolarization to 90% of resting potential (ms)	% difference from control
162	34	C	535·5	82·4	102·2	380·4		292·4	362·2	
	28	E	603·9*	86·1	100·9	390·7		479·9*	584·5*	+61·5
	16	R	525·1	84·9	104·3	384·7		320·6	403·8	
324	30	C	516·7	80·5	104·9	360·8		286·4	346·6	
	36	E	686·3*	78·7	101·1	256·4*	−29	610·2	802·4*	+132
	15	R	625·2	81·3	97·9	344·9		430·7	576·5	

Symbols as in Table 5. Statistical significance: *$P<0.001$.

Discussion

Anti-arrhythmic action

On the basis of a quantitative assessment of various pharmacological properties of anti-arrhythmic drugs it is possible to classify them in three, or possibly four, categories (Vaughan Williams, 1970b). Many well known drugs (quinidine, procaine, lignocaine, etc.) have a direct action on the cardiac membrane, and interfere with depolarization in nerve as well as in cardiac muscle. Such actions (class I) have often been called non-specific, with the implication that they are due to some physical property of the compounds, such as oil-water partition coefficient. However, Levy (1968a) demonstrated that lipid solubility was correlated neither with local anaesthetic activity on nerve nor with negative inotropic action on cardiac muscle. Furthermore, both tetrodotoxin and propranolol block depolarizing current in nerve and cardiac muscle, but the ratio of the concentrations required to do so in nerve and heart respectively is one to several hundred for tetrodotoxin (Dudel, Peper, Rubel & Trautwein, 1967), but two hundred and sixty to one for propranolol (Dohadwalla *et al.*, 1969). Thus the class I action of anti-arrhythmic drugs in reducing the rate of rise of the action potential is fairly specific for cardiac muscle.

The second class of action is sympatholytic, in the sense of reduced activation of sympathetic receptors by whatever means. (Some authors have suggested that the term adrenolytic be used for competitive receptor blockade, and sympatholytic for neurone block (Folle & Aviado, 1965), but there is nothing in the etymology of the latter word to express such a limited meaning.) Although not all authors have been able to show the effect, there is much evidence that reduction of sympathetic activation decreases the probability not only of catecholamine-induced cardiac arrhythmias, but also of ouabain-induced ventricular fibrillation. Surgical removal of sources of sympathetic transmitters (Méndez, Aceves & Méndez, 1961) and sympathetic neurone blocking drugs devoid of class I actions (Papp & Vaughan Williams, 1969a) protect against ouabain-induced ventricular fibrillation, and *laevo*-propranolol is more effective in this respect than the *dextro*-form, which has only 1/100 the β-adrenoceptor blocking activity of the *laevo*-isomer (Barrett & Cullum, 1968), although both isomers are equipotent in their class I actions. The recent evidence of Raper & Wale (1968) and of Blackburn, Byrne, Cullum, Farmer & Levy (1970) support the view that β-adrenoceptor blockade contributes to protection against ouabain-induced arrhythmias.

Many β-adrenoceptor blocking drugs (DCI, pronethalol, propranolol, alprenolol, oxprenolol, LB 46 (Sandoz)) in addition to exerting class II (sympatholytic) actions, have powerful direct class I actions on the cardiac membrane, and are more potent local anaesthetics than procaine on nerve. Four β-adrenoceptor blocking drugs (MJ 1999, practolol, AH 3474 and INPEA) have electron-withdrawing groups in the ring, and have only weak local anaesthetic activity on nerve. Indeed MJ 1999 has been said to be devoid of local anaesthetic activity (Schmid & Hanna, 1967). Certainly when tested on cornea, MJ 1999 is not a local anaesthetic but neither is procaine. We have found MJ 1999 to have about 1/90 the activity of procaine on frog nerve, but Raper & Wale (1968) reported that MJ 1999 had 1/3 of the activity of procainamide on the mouse tail. There is evidence, however (reviewed by Vaughan Williams, 1970b), that the *in vitro* β-adrenoceptor blocking activity of the β-adrenoceptor blocking agents with weak local anaesthetic action has been reduced in approximately the same proportion, so that no significant net gain in the ratio

of class II to class I actions has been achieved. Blinks (1967) recorded a pA_2 of 8·7 for propranolol, from combined measurements of the block of the chronotropic action of isoprenaline on atria and of its inotropic effect on papillary muscle, but the figure for MJ 1999 was only 6·4, a potency ratio of 200. A similar pA_2 value for MJ 1999 was recorded by Aramendia & Kaumann (1967). We have found that MJ 1999 had about 1/300 the activity of propranolol both as a local anaesthetic and in its class I action on the rate of rise of the action potential.

In vivo, however, when compared with propranolol as standard, the β-adrenoceptor blocking drugs with electrophilic groups on the ring appear to be relatively more active than *in vitro*. Practolol had 40% of the potency of propranolol in protecting guinea-pigs against ouabain-induced ventricular fibrillation (Papp & Vaughan Williams, 1969b). The ratios of the activities of MJ 1999 and propranolol as β-adrenoceptor blocking agents in animals have been variously reported as 1/11 (Levy & Richards, 1965), 1/17 (Raper & Wale, 1968), 1/3 to 1/4 (Farmer & Levy, 1968a, b), 1/5 (Hoffmann & Grupp, 1969), and 1/3 (Åberg, Dzedin, Lundholm, Olsson & Svedmyr, 1969). In man the activities of propranolol and of MJ 1999 are nearly equal, especially after oral dosage (Lish, Shelanski, Labudde & Williams, 1967; Frankl & Soloff, 1968; Kofi Ekue, Lowe & Shanks, 1970; Svedmyr, Jakobsson & Malmberg, 1969; Svedmyr, Malmberg & Häggendal, 1970).

The question arises, therefore, whether *in vivo* MJ 1999 is truly more active, or if it is the propranolol which is relatively weaker. It has been suggested that the difference between the *in vitro* and *in vivo* results may be attributed to the fact that MJ 1999 is much less lipid soluble (Lish, Weikel & Dungan, 1965) than propranolol, but Levy (1968a) demonstrated that there is no correlation between lipid solubility and potency for β-adrenoceptor blockade. A more recent explanation (Barrett, 1970) is that propranolol loses 95% of its *in vitro* activity when it reaches the blood by becoming bound to plasma protein.

Although the reduction of the activity of propranolol by blood may provide an adequate explanation for the difference between *in vivo* and *in vitro* relative potencies, it does not exclude the possibility that MJ 1999 may have additional actions *in vivo* which propranolol has not. Aramendía & Kaumann (1967) provided evidence that MJ 1999 blocked the release of transmitter from sympathetic nerves, and a similar conclusion was reached by Bartlett & Hassan (1969). We have found that MJ 1999 greatly prolonged the duration of the intracellularly recorded action potential, an effect which had already been observed after thyroidectomy (Freedberg *et al.*, 1970), and after prolonged treatment with the drug amiodarone (Singh & Vaughan Williams, 1970b). It is suggested, therefore, that prolongation of the action potential may be considered as a separate class of anti-arrhythmic action. The main interest of the delay in repolarization produced by MJ 1999 is that it is an immediate effect, apparent after a few minutes' exposure to the drug *in vitro*, whereas the effect produced by thyroidectomy and amiodarone takes several weeks to develop. This property of prolonging the cardiac action potential was not possessed by practolol, which also has an electrophilic group in the *para* position, nor by AH 3474, with an electrophilic side-chain in the *meta* position.

Inotropic action

With regard to the inotropic actions of MJ 1999 there has been some controversy. Blinks (1967) and Kaumann & Blinks (1967) reported that MJ 1999 had a positive inotropic action, not due to β-adrenoceptor stimulation, on cat papillary muscle, but

had a negative inotropic action on atrial muscle. This would be consistent with our finding that MJ 1999 prolonged the action potential in both tissues, because, whereas prolonged depolarization increases contractions in ventricular muscle (Kavaler, 1959), in atrial muscle it does not (Kavaler, 1960). Hoffmann & Grupp (1969), however, reported that MJ 1999 reduced ventricular contractile force in anaesthetized dogs, both reserpinized and non-reserpinized. Negative inotropic effects of MJ 1999 on myocardial contractions in animals were also reported by Folle & Aviado (1965) and by Stanton, Kirchgessner & Parmenter (1965), and a hypotensive effect was reported by Farmer & Levy (1968b) in dogs, but not in hypertensive rats (Farmer & Levy, 1968a). MJ 1999 depressed contractions in isolated human atrial muscle (Levy, 1968b), and in man Puri & Bing (1969) observed that MJ 1999 reduced not only heart rate, but also the force and velocity of contraction. There was, however, no change in stroke volume or in left ventricular end-diastolic pressure. Frankl & Soloff (1968), Brooks, Banas, Meister, Szucs, Dalen & Dexter (1969), and Svedmyr, Malmberg & Häggendal (1970) concluded that MJ 1999 had no depressant effect on cardiac function in man.

Evidence concerning the inotropic actions of AH 3474 is more scanty. Blackburn *et al.* (1970) reported that the drug was devoid of β-adrenoceptor stimulant action. We have found a minor positive chronotropic and inotropic action, which is interesting from the structure-action point of view in that the presence of the phenolic OH in the *para* position might have been expected to confer some stimulant activity (Ariëns, 1967). It has usually been assumed that the β-adrenoceptors subserving inotropic and chronotropic functions are similar, but there are theoretical considerations which permit the hope that it would be possible to find a drug which could stimulate the one and not the other (Vaughan Williams, 1970a). A compound with pure chronotropic β-adrenoceptor blocking properties might be a safer anti-arrhythmic for patients with myocardial infarction, in whom the negative inotropic action of drugs is to be feared. In this context, although the specificity is in the opposite sense, it is of interest that Cohen, Vastagh, McLaughlin & Mitchell (1969) reported that lower concentrations of MJ 1999 blocked the inotropic action of isoprenaline than were required to reduce its chronotropic action.

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