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The effect on the rat isolated atria of amiodarone in the presence of either ouabain or verapamil

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Amiodarone causes a decrease in the rate of contraction of the rat isolated atria and has a negative inotropic action in the paced preparation. Interactions occur between amiodarone and oubain and amiodarone and verapamil. It is possible that the clinically reported drug interaction with amiodarone may have a component of direct interactions on the myocardium rather than solely changes in plasma protein binding.

Amiodarone is a benzofuran derivative which has been used extensively for the treatment of angina pectoris (Barzin & Freson 1969). More recently it has been shown to be effective in the treatment of a wide variety of supraventricular and ventricular dysrhythmias (Rosenbaum et al 1976). Its antidysrhythmic activity differs from that of the other drugs and this has prompted a reclassification of antidysrhythmic agents (Vaughan Williams 1970) to include a class of drugs (Class III) which prolong the action potential.

Amiodarone is also unusual in that it has a very long half life (14 to 28 days) and its effects persist for 10 to 20 days after treatment is terminated (Rosenbaum et al 1976). In view of this, the administration of other drugs while amiodarone is still acting may be unavoidable. A number of potentially hazardous drug interactions with amiodarone have been reported, of which potentiation of the bradycardia induced by cardiac glycosides, β-blockers and calcium antagonists have been included in the company's data sheet and the British National Formulary. The mechanism of these interactions has not been identified but they may be related to the extensive protein binding of amiodarone (Moysey et al 1981). However, a direct effect of heart muscle must also be considered and therefore this study was designed to investigate interactions between amiodarone and ouabain a cardiac glycoside, verapamil a calcium antagonist and noradrenaline using the rat isolated atria preparation.

METHOD

Adult male Wistar rats were killed by a blow to the head. The atria were rapidly dissected out and suspended at a tension of 1 g in an organ bath containing 20 ml of Double Calcium Krebs-Henseleit solution maintained at 37 °C and gassed with 95% oxygen and 5% carbon dioxide. Tension changes were recorded via an Ether strain gauge transducer connected to a Grass Polygraph 79C. Atrial rate was recorded from a tachograph triggered from the tension output. In separate experiments preparations were paced at 4 Hz using a pulse width of 1 ms and a supramaximal stimulus using a Grass SD9 stimulator. The atria were allowed to stabilize before any test solutions were added. Cumulative concentration responses were obtained for each drug alone and in the presence of the modifying agents. Each concentration was in contact with the atria for 3 min before the next dose was added to the bath, starting with the initial dose of 10 ng ml^{-1}. The preparations were washed at least three times before any drug was added to the bath and 'pretreatment' concentrations of drug were added to the bath at least 10 min before each cumulative concentration response was measured. Statistical analysis was carried out using the Kruskal-Wallis 1-way analysis of variance test for the unpaced preparations and the Mann-Whitney U Test for the paced preparations (Siegal 1956).

Drugs

Serial dilutions of ouabain, verapamil and noradrenaline were made in double calcium Krebs-Henseleit solution to give concentrations of 1 mg ml^{-1}, and 100, 10 and 1 μg ml^{-1}. Amiodarone hydrochloride is a white crystalline powder which is insoluble in water. 10 mg of powder was mixed with 1 ml 5% Tween 80 in an ultrasonic mixer until the solution was clear. 9 ml of double calcium Krebs-Henseleit solution at 37 °C was then added. Serial dilutions of 100, 10 and 1 μg ml^{-1} in Double Calcium Krebs-

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Henseleit solution were then made and all kept at 37 °C. A fresh set of amiodarone solutions were made for each experiment. Tween 80 was also mixed with Double Krebs-Henseleit solution to give concentrations of 0·5, 0·05, 0·005 and 0·0005%.

RESULTS

Amiodarone, ouabain and verapamil all caused concentration-dependent decreases in rate of beating (Fig. 1) and increases in tension of the rat atrium preparations (Fig. 2). However, the relationship between fall in rate and increase in tension was dissimilar for the three drugs. Ouabain had more effect on tension than on rate, whilst amiodarone in the higher concentration had more effect on rate. Verapamil caused atrial arrest with doses greater than 2·5 μg ml^{-1}, but this was readily reversed by washing the preparations. Tween 80 had no effect on atrial rate or atrial tension. Noradrenaline caused a concentration dependent increase in atrial rate which was unaffected by the presence of 0·05, 0·05 or 0·5 μg ml^{-1} amiodarone.

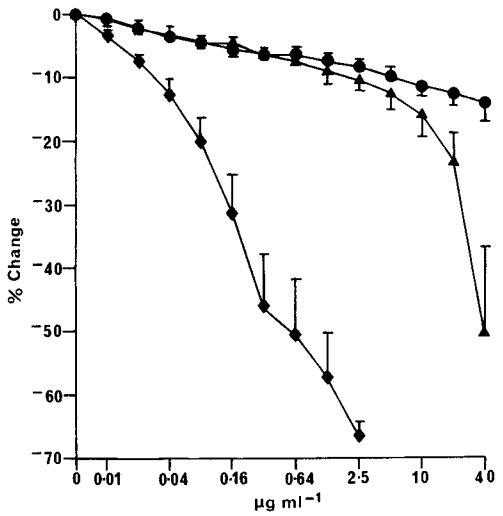

FIG. 1. The chronotropic effects of cumulative concentrations of amiodarone (▲), ouabain (●) and verapamil (◆). Each point represents the mean (n = 7) ± s.e.m.

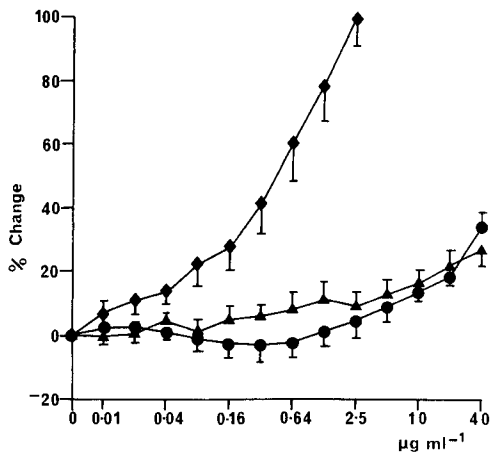

FIG. 2. The inotropic effects of cumulative concentrations of amiodarone (▲), ouabain (●) and verapamil (◆). Each point represents the mean (n = 7) ± s.e.m.

Interactions between amiodarone and ouabain

The mean atrial rate in preparations before treatment with amiodarone was 184 ± 12 beats min^{-1} (n = 7) and this was not significantly altered by pre-treatment with 5, 50 and 500 ng ml^{-1} ouabain. The inhibitory concentration 15% (IC15) for amiodarone was 12·3 ± 5·1 μg ml^{-1} and this was reduced to 5·3 ± 1·9 μg ml^{-1}, 2·1 ± 0·8 μg ml^{-1} ($P < 0·01$) and 0·9 ± 0·4 μg ml^{-1} ($P < 0·01$) by 5, 50 and 500 ng ml^{-1} ouabain respectively. These concentrations of ouabain converted the apparent positive inotropic action of amiodarone in the unpaced preparation to a negative inotropic action (Table 1).

Paced preparations produced a mean tension of 0·47 ± 0·06 g and this was unaffected by pre-treatment with 500 ng ml^{-1} ouabain. Amiodarone had a negative inotropic effect in paced preparations with an IC15 of 19·7 ± 7·3 μg ml^{-1}. Ouabain enhanced amiodarone's negative inotropic action; the IC15 for amiodarone in the presence of 500 ng ml^{-1} ouabain was 1·2 ± 0·6 μg ml^{-1} ($P < 0·01$) in the paced preparation.

The presence of amiodarone had little effect on the chronotropic response to ouabain except when the highest concentration of amiodarone (500 ng ml^{-1}) was tested. The IC15 for ouabain alone was 28·3 ± 7·4 μg ml^{-1} and this was reduced to 2·2 ± 1·6 μg ml^{-1} (n = 7; $P < 0·01$) in the presence of 500 ng ml^{-1} amiodarone. Lower doses of amiodarone had no significant effects upon the negative chronotropic action of ouabain. In contrast, the lower concentrations of amiodarone (5 and 50 ng ml^{-1}) did significantly decrease the positive inotropic response to ouabain in the spontaneously beating preparation (Table 2). The lack of effect of 500 ng ml^{-1} amiodarone on ouabain-induced increases in tension was probably due to the concurrent negative chronotropic action of this combination of agents, since 500 ng ml^{-1} amiodarone significantly decreased the positive inotropic effects of ouabain at all concentrations in the paced preparation ($P < 0·01$; Fig. 3).

Table 1. The inotropic response to cumulative concentrations of amiodarone alone and amiodarone in the presence of ouabain on atrial tension. Results are expressed as means of the percentage change in atrial tension ± s.e.m.

	% Change in atrial tension			
Amiodarone μg ml^{-1}	C n = 7	Ouabain 5 ng ml^{-1} n = 6	Ouabain 50 ng ml^{-1} n = 6	Ouabain 500 ng ml^{-1} n = 6
0·01	−0·28 ± 1·90	−1·36 ± 1·49	2·18 ± 1·11	−1·97 ± 1·24
0·02	0·33 ± 3·27	0·38 ± 2·24	1·43 ± 1·87	−7·12 ± 2·63
0·04	4·27 ± 3·11	−1·48 ± 2·37	5·35 ± 1·43	−9·55 ± 3·75
0·08	0·91 ± 2·69	−1·14 ± 3·40	3·19 ± 1·71	−11·05 ± 2·31
0·16	5·03 ± 3·85	−0·59 ± 3·03	−5·12 ± 1·27	*−11·98 ± 2·04
0·32	6·12 ± 3·75	−0·55 ± 4·81	**−11·14 ± 2·24	**−10·65 ± 2·08
0·64	8·21 ± 5·42	−2·17 ± 4·25	*−13·50 ± 1·58	**−13·29 ± 3·61
1·28	11·70 ± 4·52	−2·73 ± 4·20	**−9·42 ± 1·36	**−17·28 ± 8·59
2·56	9·70 ± 3·97	−4·80 ± 5·52	**−9·18 ± 2·71	**−10·72 ± 2·28
5·12	12·87 ± 4·36	−4·83 ± 5·00	4·01 ± 3·64	Arrests
10·24	16·35 ± 4·39	−3·28 ± 6·19	9·25 ± 2·75	
20·48	22·24 ± 4·53	2·34 ± 7·45	11·27 ± 1·73	
40·96	27·44 ± 5·50	8·81 ± 8·89	15·22 ± 2·05	

*$P < 0·05$, **$P < 0·01$.

Table 2. The inotropic effect of cumulative concentrations of ouabain alone and ouabain in the presence of amiodarone. Results are expressed as means of the percentage change in atrial tension ± s.e.m.

	% Change in atrial tension			
Ouabain μg ml^{-1}	C n = 7	Amiodarone 5 ng ml^{-1} n = 6	Amiodarone 50 ng ml^{-1} n = 6	Amiodarone 500 ng ml^{-1} n = 7
0·01	2·27 ± 1·09	−2·08 ± 2·08	−1·51 ± 2·18	5·00 ± 2·89
0·02	2·29 ± 1·09	−2·08 ± 2·08	−2·20 ± 2·44	−0·58 ± 3·11
0·04	1·16 ± 2·42	−4·32 ± 2·12	−2·43 ± 3·15	4·64 ± 3·21
0·08	−1·13 ± 3·86	−3·27 ± 1·47	−3·48 ± 1·59	3·53 ± 3·60
0·16	−2·32 ± 4·70	−4·79 ± 1·08	−7·04 ± 3·68	6·53 ± 2·83
0·32	−2·99 ± 5·49	−3·01 ± 2·10	−9·25 ± 2·10	5·04 ± 3·62
0·64	−2·02 ± 5·24	−3·34 ± 2·22	−11·34 ± 2·93	4·94 ± 3·26
1·28	1·79 ± 5·57	−1·82 ± 2·16	**−11·61 ± 3·88	7·71 ± 3·18
2·56	4·81 ± 5·59	1·57 ± 2·98	*−8·47 ± 6·33	9·64 ± 2·48
5·12	9·55 ± 4·32	3·87 ± 3·32	**−4·18 ± 3·19	11·45 ± 3·03
10·24	14·17 ± 2·40	*5·24 ± 4·07	**1·06 ± 4·65	14·96 ± 3·23
20·48	19·14 ± 2·99	**7·03 ± 4·79	**7·40 ± 5·20	21·90 ± 3·46
40·96	33·69 ± 5·18	8·07 ± 4·16	12·46 ± 4·55	31·77 ± 4·94

*$P < 0·05$, **$P < 0·01$.

Interaction between amiodarone and verapamil

In the spontaneously beating atria, verapamil 5 and 50 ng ml^{-1} reduced the IC15 for amiodarone's action on atrial rate from 12·3 ± 5·1 to 1·0 ± 0·3 μg ml^{-1} and 0·6 ± 0·4 μg ml^{-1} respectively ($P <$ 0·01). The effect of amiodarone on atrial tension was unaffected by verapamil pre-treatment. These concentrations of verapamil had no significant effects on heart rate or tension before the addition of amiodarone. The combination of verapamil and amiodarone tended to cause sudden atrial arrest which was not reversible by washing the preparation. For this reason, no data could be obtained for amiodarone in the presence of 500 ng ml^{-1} verapamil. The negative inotropic effect of amiodarone recorded using the paced preparations (n = 6, mean tension 0·47 ± 0·06 g; IC15 19·7 ± 7·3 μg ml^{-1}) was enhanced in the presence of 50 ng ml^{-1} verapamil (n = 6, mean tension 0·58 ± 0·07 g, IC15 0·12 ± 0·03 μg ml^{-1}, $P < 0·01$).

The presence of amiodarone caused significant reduction in the inotropic effect of verapamil ($P <$ 0·01) (Table 3), but there was no significant effect on the negative chronotropic effect of verapamil. Again, the combination of amiodarone and verapamil tended to cause irreversible atrial arrest. In the

Table 3. The inotropic effect of cumulative concentrations of verapamil alone and verapamil in the presence of amiodarone. Results are expressed as percentage change ± s.e.m.

	% Change in atrial tension			
Verapamil μg ml^{-1}	C n = 7	Amiodarone 5 ng ml^{-1} n = 6	Amiodarone 50 ng ml^{-1} n = 7	Amiodarone 500 ng ml^{-1} n = 6
0·01	7·35 ± 3·35	2·78 ± 2·79	−2·06 ± 2·09	−0·69 ± 3·57
0·02	10·59 ± 4·03	4·87 ± 2·68	2·26 ± 2·78	**−10·20 ± 4·70
0·04	13·24 ± 3·29	9·64 ± 1·76	7·01 ± 2·32	−13·83 ± 5·55
0·08	22·01 ± 6·59	13·03 ± 1·55	13·20 ± 3·24	**−19·19 ± 4·66
0·16	27·54 ± 7·70	18·65 ± 2·67	18·04 ± 3·89	**−21·50 ± 3·89
0·32	40·89 ± 9·10	29·43 ± 2·92	24·00 ± 4·00	**−18·51 ± 4·87
0·64	60·65 ± 12·15	35·14 ± 3·25	*17·97 ± 4·54	**−8·71 ± 5·41
1·28	78·35 ± 10·42	40·20 ± 4·70	**4·36 ± 3·12	**−0·09 ± 5·29
2·56	101·77 ± 12·88	50·34 ± 3·52	**−1·58 ± 3·23	*8·14 ± 4·13
5·12	All arrested	64·76 ± 3·92	All arrested	All arrested
10·24		All arrested		

*$P < 0·01$, **$P < 0·001$.

paced preparations, the presence of 500 ng ml^{-1} amiodarone had no effect on the negative inotropic action of verapamil.

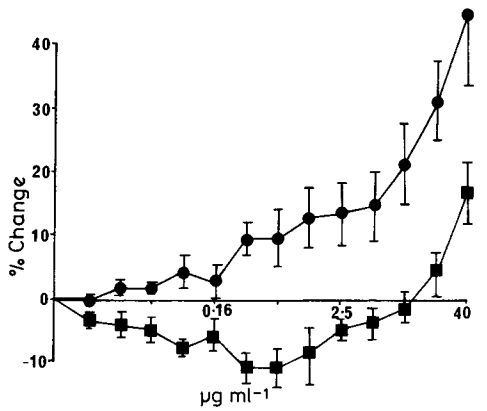

FIG. 3. The inotropic effect of cumulative concentrations of ouabain (●) (n = 6, initial tension 0·56 ± 0·07 g) and ouabain in the presence of 500 ng ml^{-1} amiodarone (■) (n = 6, initial tension 0·52 ± 0·06 g) in the paced preparations. Each point represents the mean ± s.e.m.

DISCUSSION

Previous studies of the effects of amiodarone on the rat isolated heart have involved intravenous injection of a 5% solution of amiodarone before excision of the heart (Lubbe et al 1979). This methodology reduces some of the advantages of isolated tissue studies as significant tissue binding of the drug can occur before it reaches the organ to be investigated. To overcome the problem of amiodarone's low solubility in physiological fluids, dilutions in Tween 80 were used in the present study. With the precautions described, no amiodarone precipitation was observed and control preparations were unaffected by the presence of Tween 80.

Amiodarone caused a concentration-dependent fall in the rate of contraction of the rat isolated atria which was readily reversed by washing. Dose-dependent falls in heart rate have been observed in the anaesthetized guinea-pig (Singh & Vaughan Williams 1970) and dog (Charlier 1970). The bradycardia reported by both these groups was not atropine-sensitive and the work of Charlier (1970) suggested that it was unlikely to involve β-receptors. Amiodarone, in the concentrations used, did not reduce the tachycardia due to noradrenaline. This finding does not support the view that the negative chronotropic effect of amiodarone depends on adrenergic blockade. High concentrations of amiodarone had a negative inotropic effect on the paced isolated rat atrial preparation. Reduced myocardial contractility with high doses of amiodarone have been reported with the intravenous use of amiodarone in man (Marcus et al 1981).

Electrophysiological studies have shown that the decrease in heart rate with amiodarone is due to a prolongation of the duration of the action potential and a reduction in the slope of diastolic depolarization (Singh & Vaughan Williams 1970; Olsson et al 1973; Goupil & Lenfant 1976). This action is at least in part a direct action of the drug on the sinus node, although the ionic mechanism which is the basis of these effects is unclear (Singh et al 1980).

Ouabain caused a concentration-dependent potentiation of both the negative chronotropic and inotropic effects of amiodarone and its negative inotropic effect in the paced preparations. However, both in this study and the work of others (Bigger &

Hoffman 1980) it has been shown that ouabain has relatively minor effects on atrial rate and a positive inotropic action when administered alone. Nevertheless in the absence of vagal tone cardiac glycosides increase the refractory period of atrial muscle, probably by a direct action (Bigger & Hoffman 1980). This property of ouabain, when coupled with the increased duration of the action potential produced by amiodarone might well enhance the negative chronotropic effect. The enhancement of the negative inotropic effects of amiodarone by ouabain requires further study. Singh & Vaughan Williams (1970) also investigated interactions between amiodarone and ouabain and found that amiodarone protected the anaesthetized guinea-pig against ouabain-induced dysrhythmias but not against cardiac arrest. In the present study the interaction observed was greater when ouabain was added to the tissue first rather than after the amiodarone. Furthermore, it is difficult to relate the doses used by Singh & Vaughan Williams (1970) to those used in the present study where a less beneficial interaction occurred. Toxicity has been reported in man when amiodarone was added to a digoxin therapeutic regimen (Moysey et al 1981). These authors ascribed this interaction to enhanced plasma digoxin concentrations, although this view has been challenged (Achilli & Serra 1981). The present study if applicable to man suggests that a direct interaction on cardiac tissue is also possible.

The combination of amiodarone and verapamil caused atrial arrest. Verapamil blocks the slow inward current carried principally but not solely by calcium ions in cardiac tissues (Shigenobu et al 1974). The negative chronotropic effect of verapamil may be explained by the involvement of slow channel activity in the generation of the pacemaker potential (Singh et al 1980). This effect of verapamil is partially hidden in-vivo by reflex sympathetic stimulation associated with the fall in blood pressure. Thus both verapamil and amiodarone appear to reduce atrial rate by a direct action on the sino-atrial node and an additive effect with combinations may be expected.

In summary, we have shown that direct interaction occurs between amiodarone and either the cardiac glycoside, ouabain or the calcium antagonist, verapamil.

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