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Factors underlying the variable inotropic effect of ouabain on isolated rat atria. Changes in contractile frequency and adrenergic mechanisms

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The effects of concentrations of ouabain on the isometric developed tension (IDT) of isolated left auricles driven at 0·4, 0·8 and 3·3 Hz, were explored. With 0·4 or 0·8 Hz, increasing concentrations of ouabain (3·4 or 6·8 $\times 10^{-6}$; 1·0; 3·4 or 6·8 $\times 10^{-5}$ M) elicited a decreasing enhancement of atrial contractile peak tension, whereas with 3·3 Hz the effect was augmented with concentration. Ouabain (3·4 $\times 10^{-6}$ M) enhanced comparably the IDT at all the driving rates, but at higher concentrations the effect varied with the frequency. The positive inotropic action of ouabain at 3·4 $\times 10^{-5}$ M on preparations driven at faster frequencies was antagonized by sotalol (MJ-1999) and also after catecholamine depletion by in vivo reserpinization followed by addition of tyramine in vitro. Cocaine or U-0521 significantly enhanced the positive inotropic effect of ouabain (3·4 $\times 10^{-5}$ M) in atria stimulated at low rates. On the contrary, sotalol, cocaine, U-0521 or catecholamine depletion did not alter the positive inotropic influence of ouabain at 3·4 $\times 10^{-6}$ M at any of the frequencies used. It is suggested that ouabain influenced contractile peak tension of rat isolated atria through two mechanisms: one, seen after higher concentrations, which appeared to be associated with adrenergic factors and dependent on the frequency of stimulation; the other, observable with the lower concentrations, which did not appear to have a direct relationship with adrenergic processes and which was independent of the frequency of stimulation.

We have previously demonstrated the existence of clear interrelationships between the magnitude of myocardial inotropic effects of catecholamines and the frequency of contractions (Sterin-Borda et al 1974). It has been observed that the variable positive inotropic effect of noradrenaline (NA) and isoprenaline on rat isolated atria driven at slow and fast rates was associated with different catecholamine inactivating processes, either by presynaptic uptake or by metabolism by catechol-*O*-methyl transferase (COMT). Such effects appeared to be greater at low frequencies of stimulation and therefore possibly to account for the reduced contractile effects of NA and isoprenaline observed in preparations beating at slower rates. This notion was further supported by a lack of variable actions of phenylephrine and calcium chloride (Sterin-Borda et al 1977).

We have also recorded that the frequency-force relationship of rat isolated atria varied with the stimulating procedure (Sterin-Borda et al 1974). The negative staircase observed in atrial preparations driven at progressively increasing frequencies (0·4, 0·8, 1·6 and 3·3 Hz), disappeared when each atrium was stimulated at only one of these rates (Sterin-Borda et al 1974).

In the present study the effects of several concentrations of ouabain upon the isometric developed tension of rat isolated driven left auricles has been explored. The influence of: (a) blockade of β-adrenoceptors; (b) depletion of catecholamine stores and (c) inhibition of presynaptic adrenergic neuronal uptake$_1$ and metabolism by COMT have also been examined.

MATERIALS AND METHODS

Male albino rats, Wistar strain, 200–250 g, were decapitated and the entire heart quickly excised and placed in a modified Krebs Ringer bicarbonate (KRB) solution (Sterin-Borda et al 1974), at room temperature (20 °C), and gassed with 5% CO_2 in oxygen. The auricles were separated from the ventricles and dissected free from extraneous tissue and the left atrium was transferred to a double walled organ bath containing 20 ml of KRB solution with 5·5 mM glucose as the substrate gassed as above through sintered glass and kept at a constant 30 °C and pH 7·4. The isometric tension developed by the atrial contractions (peak tension) was recorded as

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reported by Lacuara et al (1971). Briefly, the atrium, anchored at one end to a glass holder the other end tied with a silk thread, was connected to a force transducer (Statham UC3; Gold Cell) and subjected to a constant resting tension of 750 mg by means of a micrometer attached to a transducer the output of which was amplified and recorded.

To avoid artifacts evoked by dissection, an equilibration period of 60 min was allowed before the control isometric developed tension (IDT), in mg, was determined. Atrial preparations were electrically stimulated by two pointed platinum electrodes with square-wave pulses of 0·5 ms a voltage not exceeding 10% above threshold and frequencies of 0·4, 0·8 and 3·3 Hz. Each auricle was tested at only one frequency. The tension developed by the preparations of all the groups, was similar, i.e. not influenced by the stimulating rate. This finding is in keeping with observations made by Sterin-Borda et al (1974).

Mean tension values (in mg) were recorded 10 min after equilibration (0 time) and before the addition of ouabain; these values were considered the absolute initial control tension. The effects elicited by the drug were then followed over 15 min. The maximal magnitude of IDT observed after ouabain was compared with that of initial controls (considered as 100%) and expressed as per cent changes. Each atrial preparation, driven at the frequencies indicated, was exposed to one concentration of glycoside. In other cases, cocaine, sotalol, tyramine or U-0521 was added to the bath 30 min before the ouabain, to see how they altered the positive inotropic response to the glycoside. The influence of tyramine was always explored in reserpinized preparations.

Freshly prepared solutions of the following agents, dissolved in KRB, were added in volumes not exceeding 0·2 ml: ouabain octahydrate (Sigma Chemical Co.) at 3·4 or 6·8 $\times 10^{-6}$; 1·0, 3·4, or 6·8 $\times 10^{-5}$ M; (sotalol MJ-1999, Mead Johnson) at 1·0 $\times 10^{-4}$ M; cocaine HCl (Soubeiran Chobet) at 1·0 $\times 10^{-5}$ M; tyramine HCl (Sigma Chemical Co.) at 1·7 $\times 10^{-7}$ M and U-0521 (3′,4′-dihydroxy-*O*-methyl propiophenone)* at 1·0 $\times 10^{-4}$ M. All these concentrations represent the final ones in the suspending media. Reserpine (Sigma Chemical Co.) (5 mg kg^{-1}, i.p.) was given 24 h before death.

Results were compared by Student's *t*-test. Differences between means were considered significant if $P = 0{\cdot}05$ or less.

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RESULTS

The positive inotropic effect of different concentrations of ouabain upon the isolated left atrium driven at different frequencies

The magnitude of the positive inotropic responses to oubain was both concentration- and frequency-dependent (Fig. 1). At 0·4 or 0·8 Hz, increasing concentrations of ouabain reduced its maximal stimulating effect on contractile tension, the opposite being observed on auricles driven at 3·3 Hz. Ouabain (3·4 $\times 10^{-6}$ M) comparably enhanced atrial IDT at all driving rates whereas above this concentration its actions varied with frequency. At 0·4 or 0·8 Hz, the positive inotropic effect was significantly less than at 3·3 Hz. The development of the inotropic response was always rapid and reached a plateau 10 min after addition of ouabain.

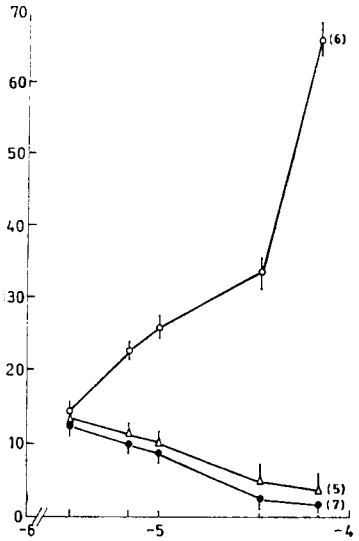

FIG. 1. Effect of several concentrations of ouabain (abscissa: M) upon the isometric developed tension (IDT) of isolated left rat atrium (ordinate: % change). Each point represents the mean values of 5 to 7 preparations expressed as per cent change against the initial control tension developed by auricles electrically stimulated at different frequencies. Vertical bars indicate the s.e.m. ● 0·4 Hz; △ 0·8 Hz; ○ 3·3 Hz.

The changes in isometrically-developed tension produced by the different concentrations of ouabain were not related to the levels of atrial contractility seen before addition of ouabain, i.e. the absolute control tension values before ouabain were similar for the three frequencies (see Table 1).

Table 1. Absolute magnitude of the isometric developed tension of rat auricles driven at different frequencies. Effects of sotalol, cocaine, U-0521, reserpinization and reserpinization with tyramine

	Isometric developed tension (mg)*					
Frequency	Controls without treatment**	Sotalol (1×10^{-4} M)***	Cocaine (1×10^{-5} M)***	U-0521 (1×10^{-4} M)***	Reserpine (5 ml kg^{-1}, i.p.)****	Reserpine (5 mg kg^{-1}, i.p.) plus tyramine ($1{\cdot}7 \times 10^{-7}$ M)*****
0·4 Hz	760·4 ± 58·8 (21)	682·0 ± 102·2 (10)	700·8 ± 40·1 (12)	701·0 ± 109·7 (10)	768·0 ± 70·5 (10)	792·2 ± 69·1 (10)
0·8 Hz	737·0 ± 44·2 (20)	703·6 ± 46·1 (11)	684·5 ± 69·7 (11)	717·2 ± 82·3 (11)	750·0 ± 65·6 (10)	785·5 ± 67·2 (10)
3·3 Hz	751·5 ± 43·3 (20)	742·4 ± 81·5 (10)	727·0 ± 96·7 (10)	706·0 ± 83·7 (12)	762·5 ± 92·6 (10)	765·0 ± 90·4 (10)

* Mean magnitudes ± s.e.m. Numbers between parentheses indicate the number of preparations.
• Initial values recorded at 10 min following equilibrium.
** Values taken at 30 min after drug addition.
**** Reserpine injected 24 h before death. Values on table obtained at 10 min following equilibrium.
***** Reserpine injected 24 h before death; tyramine in vitro. Values in Table taken 30 min after tyramine addition.

Effect of MJ-1999 on the positive inotropic action of ouabain upon isolated left atrium driven at different frequencies

Sotalol, a competitive β-adrenoceptor blocking drug, inhibited the contractile augmentation elicited by $3{\cdot}4 \times 10^{-5}$ M ouabain on atria stimulated at 0·8 and 3·3 Hz but failed to alter peak tension at 0·4 Hz (Fig. 2, panel A). On the other hand the positive inotropic effect of ouabain at $3{\cdot}4 \times 10^{-6}$ M was not modified by sotalol at any frequency (panel B).

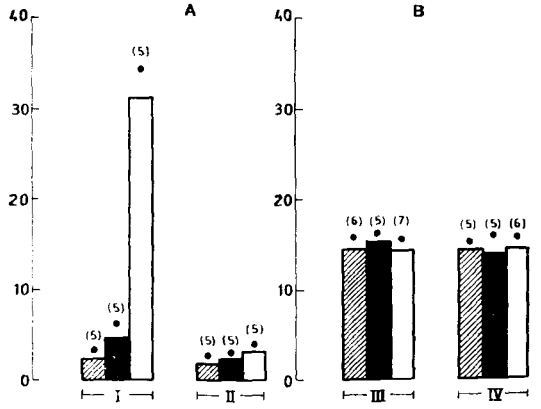

FIG. 2. Influence of sotalol on the positive inotropic action of two concentrations of ouabain on the isolated left rat atrium driven at different frequencies. Each column represents mean values expressed as per cent changes against the initial control tension values at various Hz. Hatched column 0·4 Hz; solid column 0·8 Hz; open column 3·3 Hz. Dots on top of columns indicate the s.e.m. Figures in the parentheses refer to the number of preparations. I = ouabain $3{\cdot}4 \times 10^{-5}$ M; II = sotalol $1{\cdot}0 \times 10^{-4}$ M plus ouabain $3{\cdot}4 \times 10^{-5}$ M; III = ouabain $3{\cdot}4 \times 10^{-6}$ M; IV = sotalol $1{\cdot}0 \times 10^{-4}$ M plus ouabain $3{\cdot}4 \times 10^{-6}$ M. In the presence of sotalol, a significant inhibition of the effect of ouabain $3{\cdot}4 \times 10^{-5}$ M at 0·8 and 3·3 Hz. Ordinate: I.D.T. (% change).

Table 1 shows the absolute control values of isometric contractile tension in the presence of sotalol, before addition of ouabain and that they were comparable at all frequencies.

Effect of tissue catecholamine depletion on the positive inotropic action of ouabain upon isolated left atrium driven at several frequencies

The probable depletion of myocardial catecholamines arising from reserpinization in vivo and the presence of tyramine in vitro is associated with a significant reduction in the inotropic action of $3{\cdot}4 \times 10^{-5}$ M ouabain on auricles driven at 0·8 or 3·3 Hz, but not at 0·4 Hz (Fig. 3, panel A). On the contrary, the influence of $3{\cdot}4 \times 10^{-6}$ M ouabain was similar at all frequencies both in the ouabain-treated controls and in the catecholamine-depleted preparations (panel B). Here again the isometric tension developed by "reserpinized tyraminized auricles" before ouabain was similar to the control at all rates (Table 1).

Influence of cocaine on the positive inotropic effect of ouabain upon the isolated left atrium driven at different frequencies

Cocaine, which inhibits presynaptic catecholamine uptake$_1$, clearly potentiated the enhancement of isometric developed tension elicited by $3{\cdot}4 \times 10^{-5}$ M ouabain on atria driven at 0·4 and 0·8 Hz, but failed to alter ouabain action in preparations driven at 3·3 Hz (Fig. 4, panel A). On the other hand, the positive inotropic effect of $3{\cdot}4 \times 10^{-6}$ M ouabain was similar in the presence and absence of cocaine (Fig. 4 panel B). As before, the absolute magnitude of the tension developed by atria beating at various rates

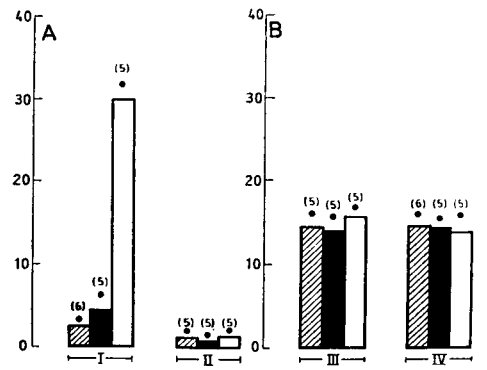

FIG. 3. Effect of tissue catecholamine depletion on the positive inotropic action of two concentrations of ouabain over isolated left rat atrium driven at several frequencies. Auricles were obtained from reserpinized rats. Tyramine was in vitro. Other conditions and details as described for Fig. 2. I = ouabain $3{\cdot}4 \times 10^{-5}$ M; II = reserpine 5 mg kg^{-1} plus tyramine $1{\cdot}7 \times 10^{-7}$ M plus ouabain $3{\cdot}4 \times 10^{-5}$ M; III = ouabain $3{\cdot}4 \times 10^{-6}$ M; IV = reserpine 5 mg kg^{-1} plus tyramine $1{\cdot}7 \times 10^{-7}$ M plus ouabain $3{\cdot}4 \times 10^{-6}$ M. Note the significant reduction of the stimulating action of $3{\cdot}4 \times 10^{-5}$ M ouabain upon reserpinized auricles driven with 0·8 and 3·3 Hz. Ordinate: I.D.T. (% change).

and exposed to cocaine before ouabain, was similar (Table 1).

Effect of U-0521 on the positive inotropic action of ouabain upon isolated left atrium stimulated at different frequencies

U-0521 enhanced the positive inotropic action of $3{\cdot}4 \times 10^{-5}$ M of ouabain at 0·4 and 0·8 Hz, but not at 3·3 Hz (Fig. 5 panel A). The positive contractile effect of $3{\cdot}4 \times 10^{-6}$ M ouabain was not altered on atria incubated with or without U-0521, at any of the frequencies (panel B).

The absolute isometric developed tension of preparations driven at 0·4, 0·8 or 3·3 Hz and exposed to U-0521 before ouabain had a similar amplitude (see Table 1).

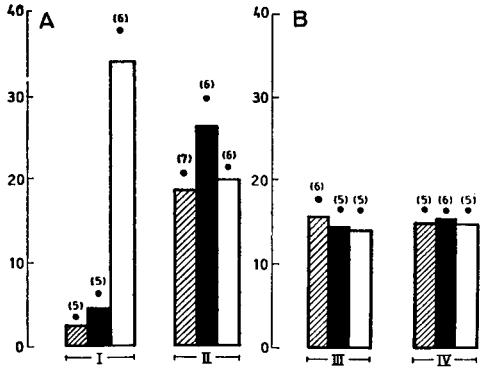

FIG. 4. Influence of cocaine on the positive inotropic action of two concentrations of ouabain over isolated left rat atrium driven at different frequencies. Other conditions and details as described for Fig. 2. I = ouabain $3{\cdot}4 \times 10^{-5}$ M; II = cocaine $1{\cdot}0 \times 10^{-5}$ M plus ouabain $3{\cdot}4 \times 10^{-5}$ M; III = ouabain $3{\cdot}4 \times 10^{-6}$ M; IV = cocaine $1{\cdot}0 \times 10^{-5}$ M plus ouabain $3{\cdot}4 \times 10^{-6}$ M. Note in presence of the cocaine a significant enhancement of the stimulating action of $3{\cdot}4 \times 10^{-5}$ M ouabain upon auricles driven with 0·4 and 0·8 Hz. Ordinate: I.D.T. (% change).

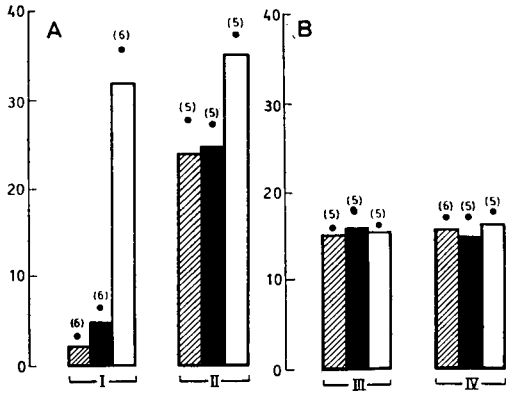

FIG. 5. Influence of U-0521 on the positive inotropic action of two concentrations of ouabain over isolated left rat atrium driven at different frequencies. Other conditions and details as described for Fig. 2. I = ouabain $3{\cdot}4 \times 10^{-5}$ M; II = U-0521 $1{\cdot}0 \times 10^{-5}$ M plus ouabain $3{\cdot}4 \times 10^{-5}$ M; III = ouabain $3{\cdot}4 \times 10^{-6}$ M; IV = U-0521 $1{\cdot}0 \times 10^{-5}$ M plus ouabain $3{\cdot}4 \times 10^{-6}$ M. Note in presence of U-0521 the significant augmentation of the stimulating action of $3{\cdot}4 \times 10^{-5}$ M ouabain upon auricles driven with 0·4 and 0·8 Hz. Ordinate: I.D.T. (% change).

DISCUSSION

The findings suggest that ouabain acts upon the peak tension developed in isolated atria by two mechanisms: one, affected by the frequency of stimulation, appears to be subserved by adrenergic factors and is found with the higher concentration of ouabain; the other, independent of the frequency of stimulation, does not have a direct relationship with adrenergic processes and occurs with lower concentrations of ouabain. The concentrations of ouabain were selected on the basis of its activity on the rat heart being less than that on the rabbit, cat or guinea-pig myocardium (Ku et al 1976).

The marked influence of the frequency of stimulation upon the magnitude of the positive inotropic effect of cardiac glycosides, has been repeatedly explored in guinea-pig atria (Furchgott & Gubareff 1958; Koch-Wesser & Blinks 1962; Vincenzi 1967; Koch-Wesser 1971) and ventricles (Sanyal & Saunders 1958), in cat papillary (Koch-Wesser & Blinks 1962; Koch-Wesser 1971) and auricular

muscle (Koch-Wesser & Blinks 1962), and in rat ventricle (Benforado 1958) as well as in rabbit atria (Koch-Wesser & Blinks 1962; Koch-Wesser 1971). In general, the prevailing opinion is that stimulating frequency modifies the concentration of glycosides required to obtain a maximal positive inotropic effect (Masuoka & Saunders 1950; Vincenzi 1967). The present findings show that the lower concentration of ouabain ($3{\cdot}4 \times 10^{-6}$ M) elicited a similar inotropic effect at all the frequencies used. This is in keeping with results in cat (Koch-Wesser & Blinks 1962) and guinea-pig atria (Gersmeyer & Holland 1963). With higher concentrations the enhancement of atrial peak tension varied with the frequency of stimulation, i.e. it was less at the lower and greater at the higher rate. Furthermore our results also indicate that at the lower frequencies (0·4 and 0·8 Hz) the inotropic action of the higher concentrations of ouabain is reduced whereas at 3·3 Hz it increases proportionally with concentration.

It is generally accepted that the positive inotropic influence of effective concentrations of cardiac glycosides varies with contractile frequency as a result of differences in the magnitude of contractile tension existing before the addition of glycoside (Koch-Wesser 1971). However our findings do not support this. Such discrepancy could arise from the species of animal and/or the stimulating procedure used. In any event our results are compatible with those relating to the influence of certain catecholamines and contractile frequency (Sterin-Borda et al 1977). Therefore the dissimilar magnitude of the contractile effects elicited by ouabain on atria driven with 0·4, 0·8 or 3·3 Hz, cannot be accounted for on the basis of a different amplitude of contraction before its addition to the bath (Ku et al 1976; Sterin-Borda et al 1977).

Our findings also suggest that the effects of ouabain not only changes with the frequency of stimulation but concentration is a factor in the mechanism by which the glycoside produces its positive inotropic influence.

Indeed, the action of high concentrations of ouabain over atrial peak tension appears to be associated with adrenergic mechanisms which apparently are not important with lower concentrations. The increased positive inotropic effect of $3{\cdot}4 \times 10^{-5}$ M ouabain over preparations driven at the fast frequency was antagonized by sotalol and after catecholamine depletion by reserpinization followed by tyramine in vitro. On the other hand, the presence of cocaine, an inhibitor of the presynaptic neuronal uptake$_1$ of catecholamines (Trendelenburg 1963) or U-0521, a blocker of catecholamine metabolism by COMT (Giles & Miller 1967), coincided with a significant enhancement of the positive inotropic effect of the same concentration of ouabain tested over auricles driven at low rates.

But sotalol, cocaine, U-0521 or catecholamine depletion, were not able to alter the positive contractile action of the lower concentration of ouabain tested at any frequency.

The existence of interrelationships between the positive inotropic effect of ouabain, adrenergic mechanisms and frequency of contractions, is supported by studies with rat isolated auricle documenting the influence of frequency on the inotropic action of catecholamines (Sterin-Borda et al 1977).

The small positive inotropic effect of noradrenaline at low frequencies appears to arise from catecholamine inactivating processes either by presynaptic neuronal uptake$_1$ or by metabolism via COMT. These mechanisms seem to be of greater importance at slower driving frequencies (Bloomquist & Angelakos 1970a, b; Bell & Grabsch 1976) and could explain the reduced positive inotropic influence of noradrenaline upon auricles driven at a slower rate (Sterin-Borda et al 1977).

The possibility of an adrenergic role underlying the action of glycosides on the myocardium has been postulated by several workers (Tanz 1964; Govier 1965; Seifen 1974; Shudo 1975; Fujimoto 1976); and denied by others ; Morrow 1963; Spann et al 1966a,b. Our results provide further evidence supporting the former view. Indeed, it would appear that the inotropic effects of ouabain on the isometric contractile tension of rat isolated atria seem to be produced through two different mechanisms each becoming evident at different concentrations of the drug. Only at the higher concentration was ouabain's actions related to the frequency of stimulation. Then its positive inotropic effect could be associated with the release of catecholamine from tissue stores. Finally, the relation between beating rate and magnitude of the processes of catecholamine inactivation, apparently greater at slower frequencies, might explain why the inotropic influences of the higher concentrations of ouabain, presumably catecholamine-dependent, are clearly less at 0·4 or 0·8 than at 3·3 Hz.

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