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EVIDENCE FOR PARTICIPATION OF CATECHOLAMINES IN CARDIAC ACTION OF OUABAIN: POSITIVE CHRONOTROPIC EFFECT

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1 The shortening of cycle length (=positive chronotropic effect) by ouabain produced in isolated spontaneously beating atria of the guinea-pig was analyzed.

2 The action of ouabain was dose-dependent; threshold response was seen at 1×10^{-7} M, and maximal response occurred at 4×10^{-7} M. The half-time of the ouabain effect was about 20 minutes.

3 The positive chronotropic effect of ouabain was reduced to 40% by β-adrenoceptor blockade (3.3×10^{-9} M propranolol) or by reserpine-depletion of catecholamines. Incubation of reserpine-treated atria with noradrenaline partially restored the action of ouabain.

4 The effect of ouabain was greatly dependent upon the calcium concentration. The optimal calcium level was 2.5×10^{-3} M. Calcium and ouabain acted synergistically.

5 Increasing calcium concentrations inhibited the positive chronotropic effect of noradrenaline in a manner similar to increasing ouabain concentrations.

6 A hypothesis is proposed which explains the chronotropic effect of ouabain on the basis of two mechanisms: (1) increase of the catecholamine concentration affecting the pacemaker; (2) mobilization of calcium, i.e. increase of the biologically effective intracellular calcium level.

Introduction

Ever since William Withering's classical report on the systemic effects of digitalis in man (Withering, 1785), the chronotropic action of cardiac glycosides has remained an issue of considerable controversy. Increases in heart rate as well as bradycardia have been reported repeatedly. Much of the conflicting evidence can now be ascribed to differences in the methodical approach: it seems that in the intact organism the direct cardiac action of digitalis is considerably altered by indirect actions, such as enhancement of cholinergic effects (Moe & Farah, 1970) or activation of reflex mechanisms (Gillis, 1969; Gillis, Quest & Standaert, 1969; Quest & Gillis, 1971).

Even in the isolated heart preparation, no uniform picture developed as to the chronotropic effects of digitalis. How much of this can be blamed on differences in preparations used, let alone species, can only be decided by further systematic investigation. However, there seems to be no doubt that doses of digitalis approaching 'toxic' levels very generally produce an increase in cardiac pacemaker activity, *in vivo* and *in vitro* alike. It is this type of chronotropic effect of cardiac glycosides with which this paper deals.

Several abstracts have been published which are concerned with the positive chronotropic effect of ouabain in the guinea-pig isolated atrium (Seifen & Wilbert, 1966; Seifen, 1968a; 1969). These data will be discussed here, and a hypothesis will be presented by which the action of ouabain on the cardiac pacemaker can be explained. The results clearly indicate that the chronotropic effect, to a major extent, is attributable to the interaction of ouabain with an adrenergic mechanism and thus present a strong argument in favour of the presently much discussed participation of catecholamines in the cardiac action of digitalis (Tanz, 1967; Gillis, 1971; Koch-Weser, 1971; Seifen, 1974).

Methods

Isolated, spontaneously beating right atria were taken from guinea-pigs (weight 180-250 g; either sex) which had been killed by a blow on the neck. Immediately after removal, the atria were fixed to platinum electrodes and suspended in a water-jacketed glass vessel containing 10 ml carbonate-buffered salt solution of the following standard composition (in mM): Na^+ 143.0, K^+ 4.7, Ca^{++} 2.5, Mg^{++} 1.2, Cl^- 130.1, HCO_3^- 25.0, and glucose 11.1. Phosphate was omitted to avoid

precipitation when high calcium concentrations were to be used. The bath fluid was equilibrated with 95% O_2 plus 5% CO_2, resulting in a pH of 7.35, and was kept between 37.07° C and 37.13° C by the thermostatically-controlled water jacket.

Before responses to drugs were tested, the atria were allowed to equilibrate in the bath fluid for 1 h and were washed every 5 to 10 minutes. After this period the spontaneous pacemaker activity had reached a steady value which, under control conditions, changed only slightly in the next 160 min as shown in Table 1.

Concentration-response curves were obtained by cumulatively adding drugs to the bath fluid. Single doses were contained in volumes of 0.01 to to 0.013 ml of an appropriate isotonic solution. The total volume added to the 10 ml bath never exceeded 0.15 ml. The time interval between additional doses was determined by the time needed for the individual dose of a drug to produce a maximal effect and maintain it for at least 3 min, and averaged for noradrenaline 5 min, for calcium 12 min, and for ouabain 40 minutes.

Atrial electrograms were recorded on a polygraph (Grass Model 5) at 1 min intervals and mean cycle length was derived from the electrogram as an average of measurements during a 10 s period. Changes in cycle length, if not stated otherwise, refer to differences between individual cycle lengths and the cycle length determined immediately after the 1 h pre-incubation period.

With rising ouabain concentrations some atria developed irregular pacemaker activity (on the average 30% at 4×10^{-7} and 85% at 8×10^{-7} M) alternating with periods of regular activity. It was necessary to ascertain whether these changes in cycle length were based on changes in the activity of the sinoatrial node, i.e., the original pacemaker, or whether they were due to interference from ectopic pacemaker activity induced by ouabain.

In the set-up used here, in which appreciable distortion of the geometrical relation between the atrium and the bipolar recording electrodes could not occur, the electrogram was a reasonable indicator for normotopic or heterotopic pacemaker activity: If electrogram signals showed identical configuration throughout, it seemed safe to assume that they originated from the same pacemaker area. A change in configuration of the signals, however, strongly suggested a different site of origin for this signal.

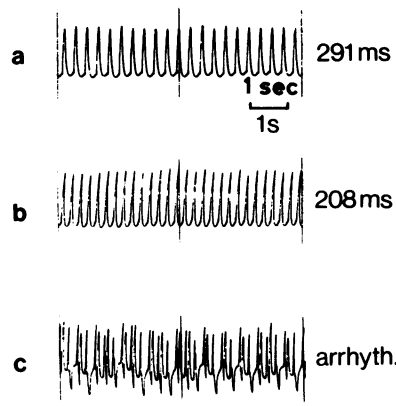

Fig. 1 Electrogram of guinea-pig isolated, spontaneously beating right atrium (a) before (b) 40 min after administration of ouabain 4×10^{-7} M, and (c) 40 min after increase of ouabain to 1×10^{-6} M. Note: there is no change in the configuration of the electrogram signals with 4×10^{-7} M ouabain, whereas, with 1×10^{-6} M, the electrogram signals show a change in configuration, which is indicative of ectopic pacemaker activity.

The electrogram signals obtained during ouabain-induced irregular pacemaker activity almost invariably had the same configuration as those obtained during regular activity (Figure 1). Hence, it was assumed that they originated from the same site, i.e., the sinoatrial node. In addition, the average cycle length determined during a period of irregular pacemaker activity did not differ from the cycle length observed during the preceding or the following period of regular pacemaker activity. Therefore, values obtained during this type of irregular pacemaker activity were thought fit for evaluation.

Table 1 Change of cycle length (ms) in spontaneously beating guinea-pig right atria following 1 h pre-incubation

	Minutes after 1 h pre-incubation				
	0	*40*	*80*	*120*	*140*
CL (ms)	308	305	302	299	300
ΔCL (ms)		−3.2 ± 2.3	−6.1 ± 3.3	−9.0* ± 3.5	−8.2* ± 2.9

Bath fluid of standard composition; CL = cycle length; ΔCL = shortening of CL; $n = 14$; means with s.e. mean.
* Different from zero at $P < 0.01$.

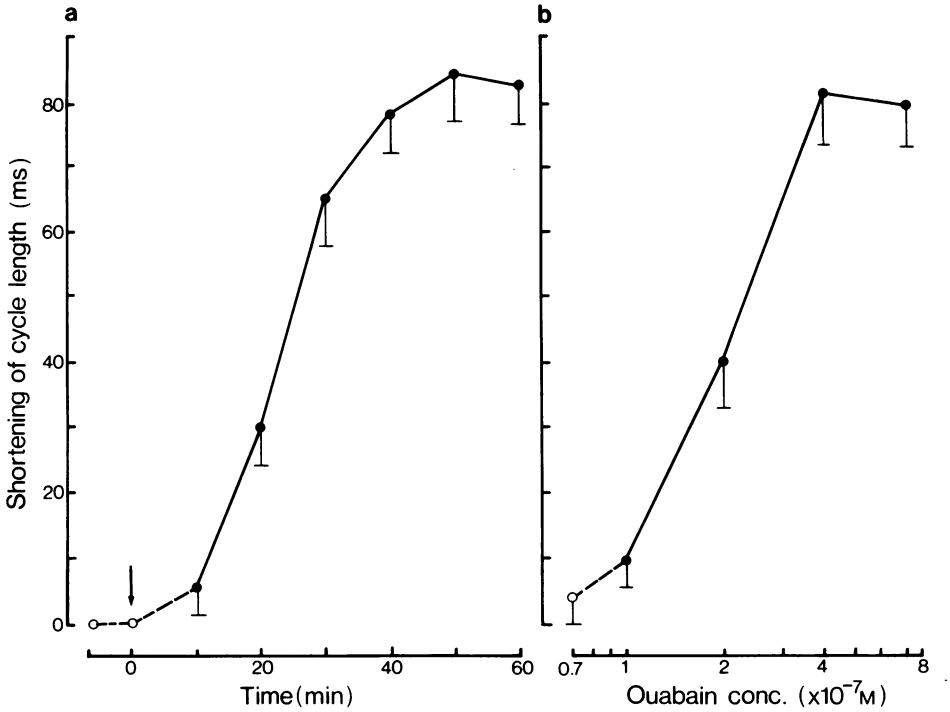

Fig. 2 (a) Time course of effect of ouabain (4×10^{-7} M at arrow) upon cycle length of guinea-pig isolated atria (single dose). Initial cycle length 298 ± 6.7 ms; $n = 26$; vertical bars indicate s.e. mean.

(b) Concentration-response curve for effect of ouabain on cycle length of guinea-pig isolated atria (cumulative doses). Initial cycle length 300 ± 5.3 ms; $n = 22$; vertical bars indicate s.e. mean. Open symbols indicate values which are not significantly different from zero ($P > 0.05$).

However, when the configuration of the electrogram signals differed from those seen during regular pacemaker activity which usually happened at ouabain concentrations of 1×10^{-6} M and higher, the data were excluded from evaluation (Figure 1).

When contractile force of the atrial muscle was determined, both left and right atrium of the same guinea-pig were mounted together in one organ bath. The left atrium was paced supramaximally (usually 3-4 V) by square stimuli of 0.3 ms duration at a rate of 3.33 Hz through punctate electrodes. Developed tension was recorded with a force-displacement transducer (Sanborn FTA-3-1) under isometric conditions and with a preload of 1 g (corresponding to half maximal tension output in the guinea-pig atria). Drugs used in this study were: (−)-noradrenaline hydrochloride (Arterenol, LaRoche, Basel), ouabain (g-strophanthin, Merck, Darmstadt), propranolol hydrochloride (Inderal, ICI, Alderly Park), and reserpine (Serpasil, Ciba, Basel). Reserpine (1 mg/kg) was administered twice subcutaneously 72 and 48 h before removal of the atria.

Statistical evaluation of the results was done by Student's t test.

Results

In the isolated spontaneously beating atrium of the guinea-pig, ouabain shortened the cycle length of the pacemaker rate, i.e., it produced a positive chronotropic effect. At 4×10^{-7} M, the average decrease in cycle length was 85 ms (Figure 2a). The effect of a single dose of ouabain attained its peak within about 40 min, was maintained for 15 to 20 min, and then declined slowly. The average half-time to peak effect was 22 min and did not depend on the concentration of ouabain. The magnitude of the effect, on the other hand, changed with the ouabain concentration (Figure 2b). The threshold concentration appeared to be 1×10^{-7} M. With cumulative doses of ouabain, a maximal shortening occurred at 4×10^{-7} M and averaged 84 ms, a value identical with that seen when the same ouabain concentration was attained by the application of a

single dose of ouabain (see Figure 2a). The steepness of the concentration-response curve is typical for cardiac glycosides. Concentrations above 1×10^{-6} M regularly induced ectopic impulse generation (see Fig. 1) and frequently blocked pacemaker activity completely.

The maximal change of cycle length produced by ouabain was in magnitude between that of calcium and that of noradrenaline (Table 2).

It is generally assumed that tachycardia in the presence of cardiac glycosides is indicative of 'toxic' action. In order to find out whether the positive chronotropic response of the atria to ouabain is associated with therapeutic or toxic concentrations, we compared the inotropic and chronotropic effect of ouabain in paired atria (right and left from same animal) in the same organ bath. The maximal inotropic action of the left atrium was seen at a ouabain concentration which was threshold for the chronotropic effect (Figure 3). In addition, at the ouabain concentration which produced a maximal chronotropic response in the right atrium the classical signs of digitalis toxicity were seen, that is: a decrease in tension development associated with an increase in the resting tension (=contracture) or complete inexcitability for externally applied stimuli combined with high spontaneous activity (ectopic pacemakers). It appears, therefore, that the occurrence of the positive chronotropic effect in the guinea-pig atrium, according to the generally used definition, falls in the range of pretoxic to toxic ouabain concentrations.

Changes in calcium concentration markedly influenced the response of the pacemaker to ouabain (Figure 4). The maximal effect of ouabain (4×10^{-9} M) occurred at calcium (2.5×10^{-3} M), the shortening in cycle length averaged 84 milliseconds. Lower as well as higher calcium concentrations diminished the maximal chronotropic effect of ouabain. The maximal shortening in cycle length averaged only 30 ms at calcium (8×10^{-3} M) and 21 ms at calcium (0.4×10^{-3} M).

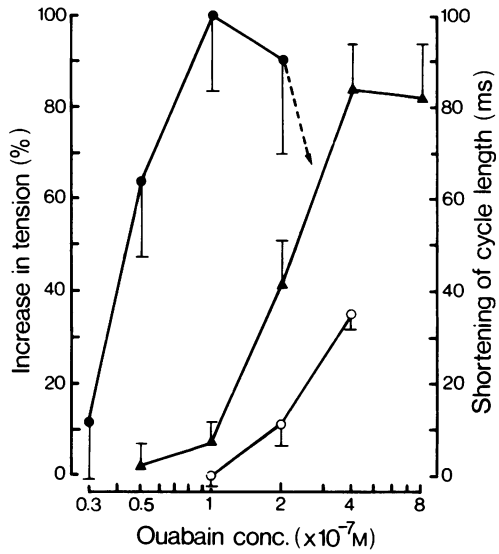

Fig. 3 Comparison between inotropic and chronotropic effects of ouabain in guinea-pig isolated atria. Abscissae at left: increase in tension expressed in % of maximal twitch tension (●) or in % of initial resting tension (○); abscissae at right: shortening in cycle length in ms (▲). Initial values: twitch tension 485 ±35 mg, resting tension 1000 mg; $n = 4$; cycle length 301 ± 6.4 ms; $n = 4$. Cycle length was determined in spontaneously beating right atria; tension was measured in paced (3.3 Hz, 0.3 ms, 3-4 V) left atria from same animals. Vertical bars indicate s.e. mean.

Table 2 Shortening of cycle length in spontaneously beating guinea-pig right atria produced by maximally effective concentrations of calcium, ouabain, and noradrenaline

	CL (ms)	*ΔCL (ms)*	n
Calcium (8×10^{-3} M)			
before (1.2×10^{-7} M)	323 ± 4.1	56 ± 6.4	15
12 min after	267		
Ouabain (4×10^{-7} M)			
before	302 ± 3.6	85* ± 6.4	20
40 min after	217		
Noradrenaline (2×10^{-5} M)			
before	296 ± 5.3	104 ± 5.3	20
5 min after	192		

CL = cycle length; ΔCL = shortening of CL; means with s.e. mean.
* Different from two other values in same column at $P < 0.01$.

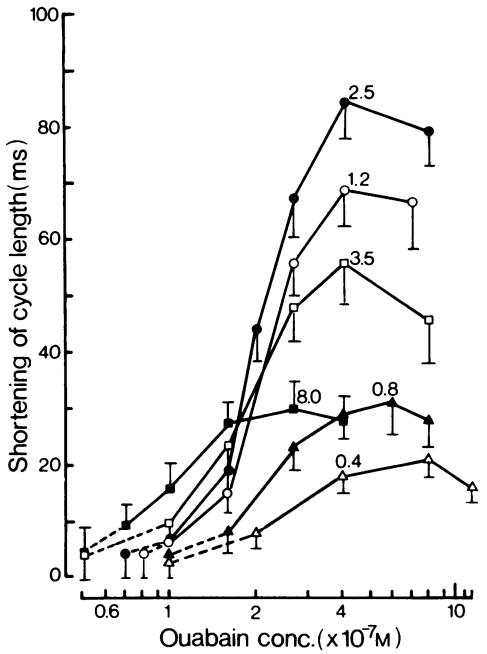

Fig. 4 Concentration-response curves for effect of ouabain on cycle length in the presence of different calcium concentrations. The initial cycle lengths (in ms) at the different calcium concentrations (x 10^{-3} M) and the number of experiments (*n*) were: 353 ± 15.9 at 0.4; *n* = 9; 343 ± 14.4 at 0.8; *n* = 8; 328 ± 6.3 at 1.2; *n* = 11; 300 ± 5.3 at 2.5; *n* = 22; 272 ± 6.6 at 3.5; *n* = 10; 257 ± 4.6 at 8.0; *n* = 10. Vertical bars indicate s.e. mean.

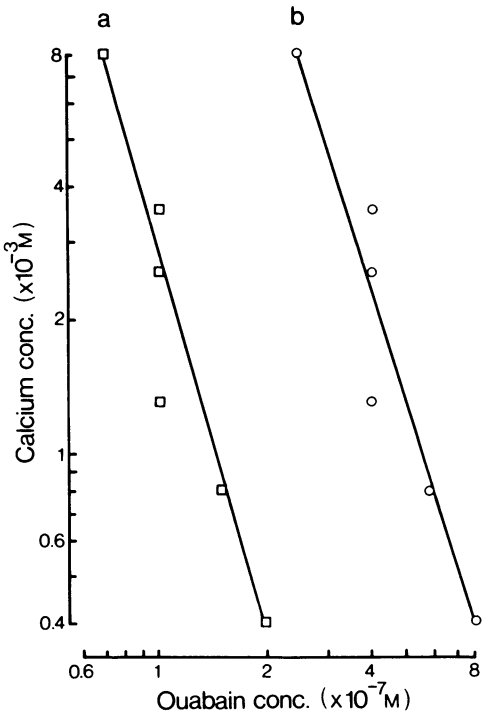

Fig. 5 Correlation between calcium concentration and ouabain concentration (a) at which first significant shortening of cycle length (threshold concentration) was observed or (b) at which maximal shortening of cycle length was seen. Data used in this graph are taken from results illustrated in Figure 4.

The ouabain concentrations which induced a maximal effect at each individual calcium concentration decreased with rising calcium levels from 8 x 10^{-7} M at calcium (0.4 x 10^{-3} M) to 2.5 x 10^{-7} M at calcium (8 x 10^{-3} M). Similarly, the threshold concentration decreased from 2 x 10^{-7} M at 0.4 x 10^{-3} M calcium to 0.7 x 10^{-7} M at 8 x 10^{-3} M calcium. The relationship between threshold concentrations as well as maximally effective concentrations of ouabain and the corresponding calcium concentrations was linear (Figure 5). The linearity together with the negative slopes indicate strongly that calcium and ouabain acted synergistically, i.e., in order to maintain a given response (threshold or maximum) a certain amount of calcium could be substituted by ouabain, or *vice versa*.

Several authors have claimed that cardiac effects of digitalis compounds can be diminished by inhibition of adrenergic mechanisms. Others failed to confirm these findings (for references see Koch-Weser, 1971). This controversy led to the study of the interaction of antiadrenergic substances with the chronotropic action of ouabain. Two different approaches were chosen: blockade of the β-adrenoceptors by propranolol, and depletion of the catecholamine stores by reserpine.

With a concentration of propranolol as low as 3.3 x 10^{-9} M a significant inhibition of the positive chronotropic effect of ouabain was seen as indicated by the shift of the concentration-response curve to the right (Figure 6). The maximal shortening in cycle length induced by ouabain (4 x 10^{-7} M) was reduced from 84 to 32 ms, that is by 62%. A similar reduction was seen for the effect of ouabain (2 x 10^{-7} M). No significant inhibition was seen at ouabain (7 x 10^{-7} M). However, with a 100-fold increase of the propranolol concentration (2 x 10^{-7} M) the chronotropic effect of ouabain (7 x 10^{-7} M) was reduced by 43%. The low concentration of

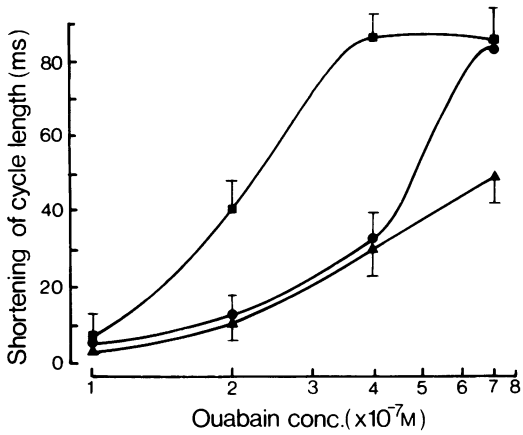

Fig. 6 Inhibition by propranolol of effect of ouabain on cycle length of guinea-pig isolated atria. Initial cycle length (in ms) and number of experiments (*n*) were: control, 300 ± 5.3, *n* = 22 (■); 3.3×10^{-9} M propranolol 296 ± 6.1, *n* = 11 (●); 3.3×10^{-7} M propranolol 302 ± 6.9, *n* = 11 (▲). Vertical bars indicate s.e. mean.

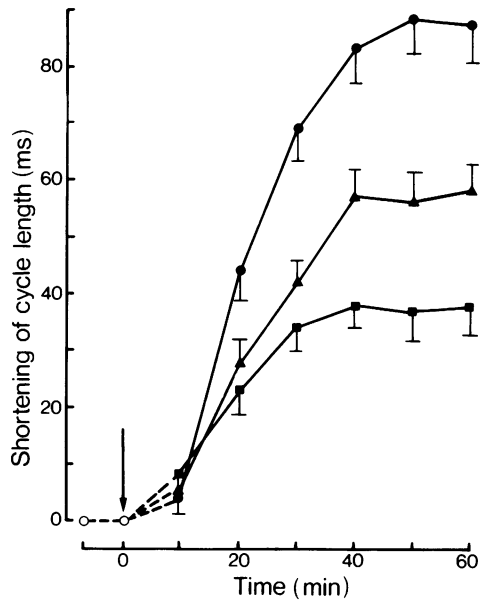

Fig. 7 Inhibition by reserpine of effect of ouabain (4×10^{-7} M at arrow) on cycle length of guinea-pig isolated atria. Initial cycle length (in ms) and number of experiments (*n*) were: control 298 ± 6.7, *n* = 26 (●); reserpine 290 ± 10.4, *n* = 20 (■); reserpine + noradrenaline 291 ± 7.9, *n* = 7 (▲). Vertical bars indicate s.e. mean.

propranolol used can be considered to display merely the 'specific' β-adrenoceptor blocking effect, and no 'unspecific' membrane activity (Fitzgerald, 1969). The fact that the inhibitory action was not enhanced by the 100-fold increase in concentration at the lower ouabain concentrations (2×10^{-7} and 4×10^{-7} M) is a further indication for the 'specific' action. One would have expected the non-specific inhibition of the ouabain effect to be more pronounced at the higher propranolol concentration. With propranolol (5×10^{-6} M), the effect of ouabain was completely abolished.

Pretreatment of the guinea-pigs from which the atria were taken with reserpine in a dose sufficient to inhibit the cardiac effects of sympathetic stimulation (Blinks & Waud, 1961), resulted in an inhibition of the positive chronotropic effect of ouabain to the same degree as that produced by β-adrenoceptor blockade (61% at ouabain (4×10^{-7} M), Figure 7). Incubation of the atria from reserpine-pretreated guinea-pigs for 0.5 h in a high concentration of noradrenaline (5×10^{-6} M) followed by complete washout of unbound noradrenaline, restored the shortening of cycle length, previously reduced from 84 to 34 ms, to 58 milliseconds.

These data strongly suggest that catecholamines participate in the production of the positive chronotropic effect of ouabain in the guinea-pig atrium. However, the fact that β-adrenoceptor blockade and depletion of the catecholamine stores do not completely inhibit the positive chronotropic effect suggests that some other mechanism must be involved.

It has been mentioned above (Fig. 5) that calcium and ouabain act synergistically: i.e., calcium and ouabain can, to some extent, substitute for each other. The question therefore was, whether calcium possibly participates in the production of the chronotropic effect of ouabain. Cardiac glycosides have been reported to increase the intracellular, freely exchangeable calcium, i.e., 'mobilize' calcium (Klaus & Kuschinsky, 1962; Lüllmann & Holland, 1962). It is also known that calcium shortens the cycle length in guinea-pig atria (Schaer, 1968; Seifen, 1968b), and in other species (Reiter & Noe, 1959; Seifen, Flacke & Alper, 1964a; Seifen, Schaer & Marshall, 1964b). Indirect evidence for the participation of calcium can be obtained from experiments designed on the basis of the following considerations: the positive chronotropic effect of calcium is maximal at a concentration of 8×10^{-3} M (Seifen, 1968b). Above this level, an increase in calcium prolongs rather than shortens cycle length. If mobilization

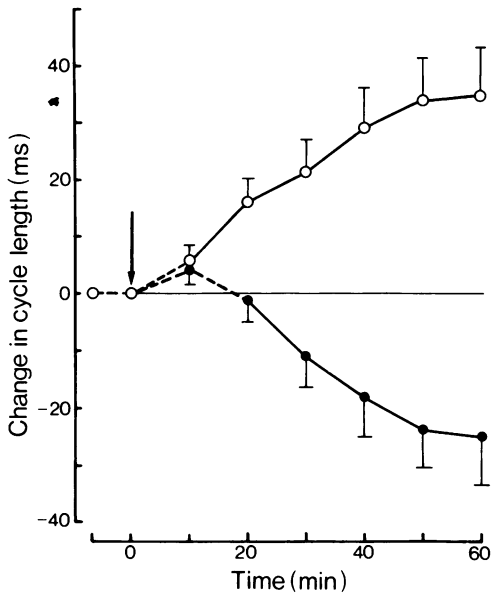

Fig. 8 Inhibition by propranolol of effect of ouabain (4×10^{-7} M at arrow) on cycle length of guinea-pig isolated atria in the presence of high calcium concentration. Initial cycle length (in ms) and number of experiments (n) were: 8×10^{-3} M calcium + 3.3×10^{-7} M propranolol 262 ± 5.9; $n = 10$ (•). Calcium (8×10^{-3} M) alone (○). Vertical bars indicate s.e. mean.

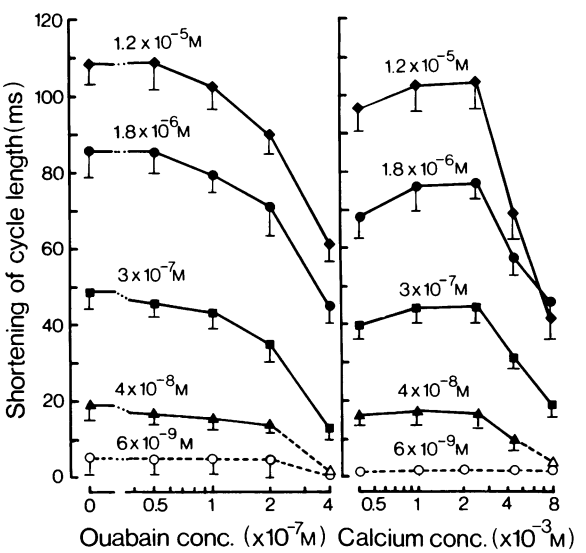

Fig. 9 (a) Inhibition by ouabain of effect of noradrenaline upon cycle length of guinea-pig isolated atria. Noradrenaline concentrations are indicated by figures associated with individual curves. Initial cycle length (ms) before addition of noradrenaline: no ouabain, 297 ± 4.5; 0.5×10^{-7} M ouabain, 296 ± 6.1; 1×10^{-7} M ouabain, 284 ± 5.4; 2×10^{-7} M ouabain, 273 ± 5.3; 4×10^{-7} M ouabain, 252 ± 7.2. Each point represents the mean of 14 experiments.

(b) Inhibition by calcium of effect of noradrenaline upon cycle length of guinea-pig isolated atria. Initial cycle length (ms) before addition of noradrenaline: 0.5×10^{-3} M Ca, 361 ± 8.7; 1×10^{-3} M Ca, 333 ± 12.9; 2×10^{-3} M Ca, 297 ± 6.5; 4×10^{-3} M Ca, 267 ± 8.0; 8×10^{-3} M Ca, 260 ± 8.8. Each point represents the mean of 10 experiments.

of calcium were involved in the chronotropic action of ouabain, one would expect that, in the presence of a high concentration of calcium, ouabain should prolong the cycle length instead of shorten it. The results shown in Fig. 4 indicated that at calcium (8×10^{-3} M) the chronotropic effect of ouabain was greatly reduced but not reversed. Yet, reversal of the calcium effect might be masked by catecholamine effects induced simultaneously by ouabain. Therefore, the experiments were repeated in the presence of a β-adrenoceptor blocking agent (Figure 8). In the presence of propranolol (3.3×10^{-7} M) the shortening of cycle length was reversed into a prolongation in cycle length of 24 ms, whereas, with calcium (8×10^{-3} M), ouabain still shortened cycle length, when propranolol was not present.

Another indirect test to obtain evidence for the participation of calcium in the action of ouabain was based on the observation that cardiac glycosides reduce cardiac effects of noradrenaline (Mendez, Aceves & Mendez, 1961). These results were confirmed in this preparation (Figure 9a). The chronotropic response to different concentrations of noradrenaline became increasingly smaller with rising ouabain concentrations. At ouabain (4×10^{-7} M), the inhibition of the noradrenaline effect amounted to an average of 60%. This inhibition of the effect of noradrenaline by ouabain might reflect mobilization of calcium by ouabain which, in turn, interfered with the action of noradrenaline. If this consideration is correct, one would expect calcium to inhibit the action of noradrenaline in the same way as did ouabain. As shown in Fig. 9b, elevation of the calcium concentration from 0.4×10^{-3} M to 8×10^{-3} M increasingly depressed the action of noradrenaline. Calcium (8×10^{-3} M) reduced the chronotropic effect of all noradrenaline concentrations by 40 to 80%. Thus, calcium, indeed, behaved very similarly to ouabain in attentuating the action of noradrenaline.

Discussion

The positive chronotropic effect of ouabain in guinea-pig isolated atria can be best explained by the hypothesis that ouabain acts through two different mechanisms: (1) an increase of the catecholamine concentration in the vicinity of the Pacemaker; (2) an increase in the biologically effective intracellular calcium concentration. The former mechanism is strongly supported by the observation that both blockade of β-adrenoceptors and depletion of catecholamine stores reduced the effect of ouabain to the same degree (by 60%). The hypothesis is strengthened by the finding that replenishment of the catecholamine stores of atria previously depleted by reserpine partially restored the action of ouabain. These data indicate that ouabain increased the active catecholamine level in the vicinity of the pacemaker.

However, little can be said as to how this was achieved. Digitalis glycosides diminish the catecholamine concentration in the heart (Loubatières, Bouyard, Chapal, Klein & Rondot, 1965; Förster & Rösler, 1967; Leitz & Stefano, 1970; Göthert, 1971), and other investigators showed that cardiac glycosides inhibit the uptake of catecholamines by heart muscle (Dengler, Michaelson, Spiegel & Titus, 1962; Leitz & Stefano, 1970). Also, digitalis compounds may interfere with catecholamine synthesis or metabolism. All of these possibilities require additional exploration.

Catecholamines alone could easily account for the magnitude of the effect ouabain on cycle length. However, the fact that antiadrenergic measures abolished only 60% of the action of ouabain suggests that another factor is involved. This factor could be that cardiac glycosides increase the readily exchangeable intracellular calcium fraction (Klaus & Kuschinsky, 1962; Lüllmann & Holland, 1962). Calcium has been shown to enhance pacemaker activity in isolated heart preparations (Reiter & Noe, 1959; Seifen *et al.*, 1964a; Seifen *et al.*, 1964b; Schaer, 1968; Seifen, 1968b). These observations make it very tempting to consider calcium mobilization as the second factor contributing to the positive chronotropic effect of ouabain in the guinea-pig isolated atrium.

Calcium alone cannot account for the ouabain effect. The positive chronotropic action of calcium is not inhibited by anti-adrenergic drugs (Seifen *et al.*, 1964a; Schaer, 1968), and is, at its maximum, significantly smaller than that elicited by ouabain (Table 2).

If calcium constitutes the second proposed mechanism of the hypothesis, one would expect ouabain to mimic the action of calcium, provided this effect is not altered by the adrenergic component. It is well established that cardiac glycosides and calcium act synergistically, and the linear relationship between equi-effective combinations of ouabain and calcium shown in Fig. 5 is good evidence for this synergism; for a given effect on the pacemaker, a decrease in calcium concentration can be compensated for by an adequate increase in ouabain, and *vice versa.* Furthermore, it was found that the dose-response curve to calcium is bell-shaped. Addition of calcium at the top of the dose-response curve reduces pacemaker activity (Seifen, 1968b). If ouabain acts via mobilization of calcium, one would have to postulate the same behaviour for ouabain. This, indeed, was seen; however, only after blockade of the concomitant adrenergic mechanism. (This, incidentally, is another piece of evidence for participation of catecholamines).

Finally, it has been demonstrated that the cardiac action of catecholamines can be greatly diminished by ouabain (Mendez *et al.*, 1961). The results presented here confirm this observation. If part of the effect of ouabain were due to intracellular mobilization of calcium, similar results would be expected when catecholamines are combined with calcium instead of ouabain. The observations presented in Fig. 9b are evidence for this. All of these results support the assumption that calcium is involved in the positive chronotropic response to ouabain of the guinea-pig atrium.

The data now available do not allow a decision as to whether the postulated effects of ouabain, i.e. the interaction with catecholamines and the mobilization of calcium, are merely occurring simultaneously or whether one triggers the other. The pursuit of this idea is of interest because, on one hand, calcium is able to enhance the release of catecholamines (Schümann & Philippu, 1963; Katz & Kopin, 1969; Philippu, Heyd & Burger, 1970), and, on the other, catecholamines increase the transmembrane exchange of calcium (Reuter, 1967). The former seems unlikely since chronotropic changes produced by calcium are not influenced by antiadrenergic drugs (Seifen *et al.*, 1964a; Schaer, 1968).

Although it seems unlikely that catecholamines participate in the positive inotropic action of cardiac glycosides (Tanz, 1967), more and more information is accumulating which supports the idea that catecholamines may be involved in causing arrhythmia. There are, however, contradictory reports from different investigators (for references see Gillis, 1971; Koch-Weser, 1971). In view of the hypothesis proposed in this paper, the overall response of cardiac tissue to pretoxic and toxic doses of digitalis compounds would be due

to the combined effect of mobilized calcium and catecholamines. It is obvious that the relationship between the biologically effective concentrations of catecholamines and calcium will determine, in any given species and/or at any given glycoside level, how effective countermeasures will be, and thus give us a key to explain the contradictory reports from different investigators. Also, it might give us a lead to reconsider the clinical approach to the treatment of digitalis-induced arrhythmias.

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