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The inotropic effect of ouabain and its antagonism by dihydroouabain in rat isolated atria and ventricles in relation to specific binding sites

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1 The inotropic effect of ouabain has been studied in rat ventricles and atria.

2 The concentration-effect curve of ouabain may be fitted by a model assuming the existence of two saturable components. The component with the higher sensitivity to ouabain accounted for 30% of the maximal increase in systolic tension in ventricles and for only 5% in atria. Increase in diastolic tension was only apparent at ouabain concentrations required to observe the low sensitivity component.

3 [^{3}H]-ouabain binding has been examined in microsomes prepared from atria and ventricles. High and low affinity binding sites have been observed. The ratio of high and low affinity ouabain binding sites was 4 fold lower in microsomes from rat atria than from rat ventricles. This could account for the difference in the response of these two tissues to the inotropic action of ouabain.

4 In ventricular strips the high sensitivity component was much less apparent in the presence of dihydroouabain than with ouabain.

5 When ventricular strips were preincubated in the presence of dihydroouabain 3 μM, the increase in systolic tension evoked by ouabain 1 μM was significantly reduced. Cumulative concentration-effect curve studies showed dihydroouabain antagonism to the high sensitivity component.

Introduction

Recently, it has been demonstrated that the inotropic action of ouabain in rat ventricular strips shows a concentration-effect curve characterized by two components (Adams, Schwartz, Grupp, Shin-Woong, Wallick, Powell, Twist & Gathiram, 1982; Finet, Noël & Godfraind, 1982). However, such an observation has not been shown with rat atria (Ku, Akera, Tobin & Brody, 1976). Therefore, we have re-examined the inotropic effect of ouabain on rat heart. We have compared the contractile response of ventricles and atria to various concentrations of the glycoside. We have also studied [^{3}H]-ouabain binding to microsomes prepared from both ventricles and atria.

In guinea-pig isolated atria, it has been proposed that the inotropic effect of ouabain is the sum of two processes: one related to the inhibition of the Na^+, K^+-pump and another related to a still unknown mechanism (Ghysel-Burton & Godfraind, 1979; Noble, 1980). In this preparation, Na^+, K^+-pump inhibition seems to be the only mechanism responsible for the positive inotropic effect of dihydroouabain, the derivative of ouabain with a saturated lactone ring (Godfraind & Ghysel-Burton, 1980). Furthermore, dihydroouabain has been shown to act as an antagonist to the positive inotropic effect of ouabain that is independent of Na^+, K^+-pump inhibition (Godfraind, Ghysel-Burton & De Pover, 1982). Therefore, we have studied the inotropic action of dihydroouabain on rat ventricular strips and examined how this drug interfered with the action of ouabain.

The results show that the concentration-effect curve of ouabain is different in ventricles and in atria. This could be due to a difference in the relative distribution of high and low affinity ouabain binding sites within these two tissues. The results also indicate that competition between dihydroouabain and ouabain occurs at high affinity sites.

Methods

Protocol for contractile experiments

Female Wistar rats weighing approximately 250 g were killed and the hearts rapidly removed. Whole left atria and ventricular strips (approximate length 10 mm, width 1.5 mm and weight 25 mg) cut close to the right atria, perpendicularly to the axis of the heart were dissected and suspended in an organ bath under an initial resting tension (diastolic) of 500 mg in Tyrode solution (composition (mM): NaCl 137, KCl 6, $CaCl_2$ 1.82, $MgCl_2$ 1.05, NaH_2PO_4 0.417, $NaHCO_3$ 11.5, glucose 5.5) bubbled with a mixture of 95% O_2 and 5% CO_2 at 30°C.

Ventricular strips were stimulated at 1 Hz by needle electrodes with rectangular pulses of 10 ms duration (voltage twice threshold). Atria were stimulated at 1 Hz either by needle or field electrodes. The contractile activity was recorded with an isometric transducer fed into a potentiometric recorder. The cardiac glycosides were added after an initial equilibration period of 90 min (the bathing solution being changed about every 15 min during this period). The contractility of these preparations was compared to that of controls. For each glycoside concentration, the inotropic action was estimated when the peak effect was reached. In one set of experiments non cumulative concentration-effect curves were obtained by exposing each strip to a single concentration of ouabain or dihydroouabain. In another set of experiments the cardiac glycosides were added cumulatively to the bath in order to obtain cumulative concentration-effect curves. These protocols were also used to study the interaction of dihydroouabain and ouabain. Ventricular strips of rat were preincubated for 30 min in the presence of dihydroouabain 3 μM before the addition of a single concentration of ouabain. Other ventricular strips were preincubated for 30 min in the presence of dihydroouabain (1 or 3 μM) before the cumulative addition of ouabain.

Determination of [^{3}H]-ouabain binding

Preparation of microsomes enriched in sodium-potassium activated adenosinetriphosphatase ($(Na^+ + K^+)$-ATP-ase) and determination of enzyme activities were performed as described previously for guinea-pig heart (Godfraind, De Pover & Verbeke, 1977) with slight modifications. Each microsomal preparation was obtained from homogenate prepared either from 50 rats (ventricles) or from 150 rats (atria). [^{3}H]-ouabain binding was determined by a filtration technique described previously for rat heart ventricles (Noël & Godfraind, 1983). Briefly, microsomal proteins (about 150 μg) were incubated at 37°C in a medium containing (mM): $MgCl_2$ 3, P_i-Tris 3, EGTA 1, maleate-Tris 20 (pH 7.4) and various concentrations of [^{3}H]-ouabain. After 10 min incubation, samples of 200 μl were filtered on Whatman glass fibre filters (GF/F). After washing three times with 20 ml of chilled solution (0.25 M sucrose, 5 mM Tris-HCl, pH 7.4 at 0°C) the filters were added to a scintillation solution and the radioactivity counted. Non-specific binding was estimated from samples incubated in the absence of Mg^{2+} and P_i or in the presence of 4 mM unlabelled ouabain. Specific binding was calculated as the difference between the total and the non-specific binding.

One enzymatic unit corresponds to the amount of enzyme that hydrolyzes 1 μmol of ATP per min.

Analysis of the results

The experimental data from both contractile and binding studies were analysed assuming that the relation between the glycoside concentration and the effect (or the binding) could be considered either as being due to one saturable component or to the sum of two saturable components.

In the case of one saturable component

$$v = \frac{V_M \cdot F}{K + F} \qquad (1)$$

In the case of two saturable components

$$v = \frac{V_{M1} \cdot F}{K_1 + F} + \frac{V_{M2} \cdot F}{K_2 + F} \qquad (2)$$

Where v = ^{3}H-glycoside specifically bound (binding experiments) or increase in tension (contractile experiments).

V_M = capacity (B_{max} binding experiments) or maximal increase in systolic tension (E_{max} contractile experiments).

K = dissociation constant (K_d, binding experiments) or concentration producing a half maximal effect (EC_{50} contractile experiments).

F = binding experiments: free concentration of glycoside calculated by subtracting the amount of ouabain bound from the total amount of ouabain; contractile experiments: concentration of glycoside

Data were analysed using a computerized non-linear regression program based on the steepest descent technique which adjusts the parameters to minimize the sum of relative or absolute squared errors (Cumps, 1977). This approach involves the use of untransformed data in order to avoid statistical bias (Cornish-Bowden, 1976) and provides an estimate of the standard deviations of each of the calculated parameters so that the goodness of fit can be

assessed. The validity of the model was tested by verifying that all the computed parameters were significantly different from zero (Student's *t* test, $P < 0.05$). This allowed one to choose which, of model (1) or (2), was the most representative of the experimental observations. In binding studies, MLAB, a non-linear least-squares curve fitting program (Knott, 1979) was also used to fit models of one and two saturable components to the data transformed according to Scatchard (i.e. bound ouabain/free ouabain as a function of free ouabain).

Whenever possible, values are presented as means ± s.e.mean. Significance of differences between means or between parameters (see Table 1) were tested by Student's *t* test, a level of probability of less than 0.05 being considered as significant.

Drugs

Dihydroouabain was a gift from SIMES, Milano, Italy. Ouabain was purchased from Boehringer GmbH, Mannheim, Germany and [^{3}H]-ouabain from New England Nuclear, Boston, USA. All other chemicals were of analytical grade.

Results

Inotropic and tonotropic effects of ouabain and dihydroouabain in atria and in ventricular strips

The contractility of electrically driven rat left atria and right ventricular strips was monitored in Tyrode solution containing various concentrations of ouabain ranging from 10^{-7} M to 10^{-3} M. The isometric contractile force (systolic tension) developed in the absence of drug was 400 ± 40 mg ($n = 20$) for ventricular strips and 264 ± 30 mg ($n = 22$) for atria.

Time-courses of the inotropic and tonotropic effects of ouabain were studied at various concentrations. As illustrated in Figure 1, the time to peak

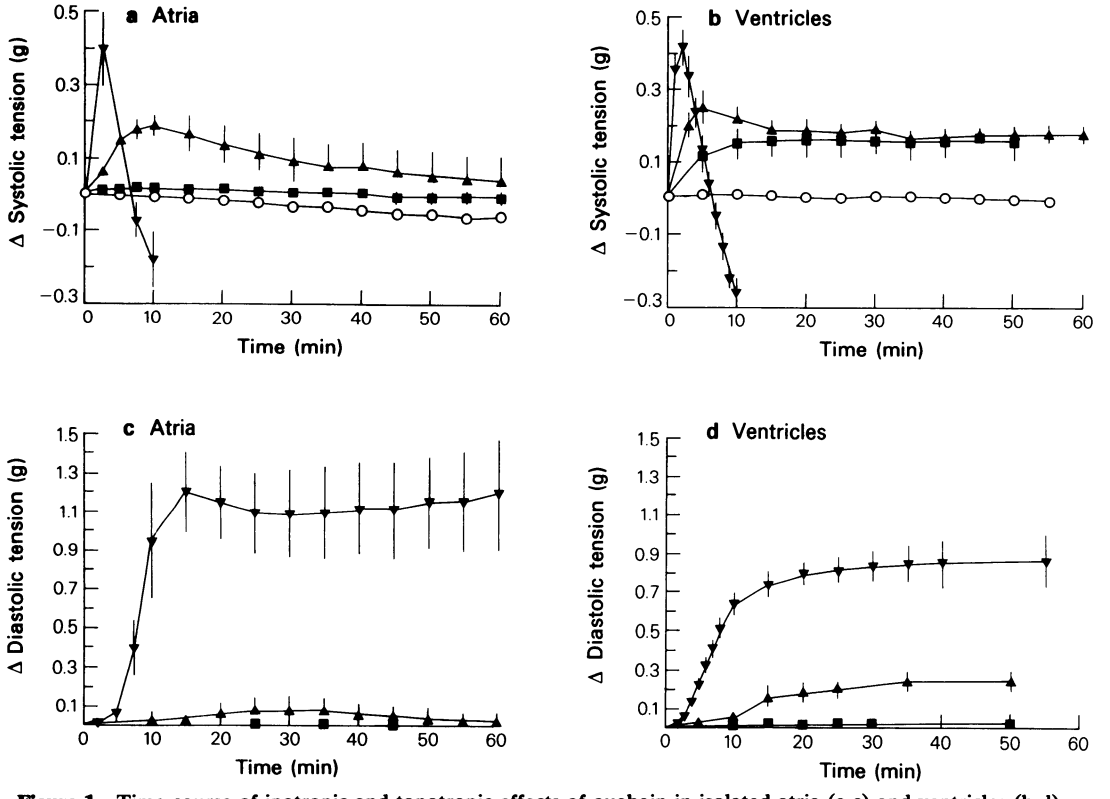

Figure 1 Time course of inotropic and tonotropic effects of ouabain in isolated atria (a,c) and ventricles (b,d). Ventricular strips and left atria were incubated at 30°C in a physiological solution (Tyrode, $K^+ = 6$ mM) and electrically driven at a rate of 1 Hz. The systolic tension in absence of drug was 0.4 ± 0.04 g ($n = 20$) for ventricular strips and 0.26 ± 0.03 g ($n = 22$) for atria. The initial diastolic tension was 0.5 g for both tissues. The increases (Δ) in systolic and diastolic tension were measured in both atria and ventricles in the absence of glycoside (○) and in the presence of ouabain 10 μM (■), 100 μM (▲) and 1000 μM (▼). Each point is the mean ± s.e.mean of at least 4 experiments.

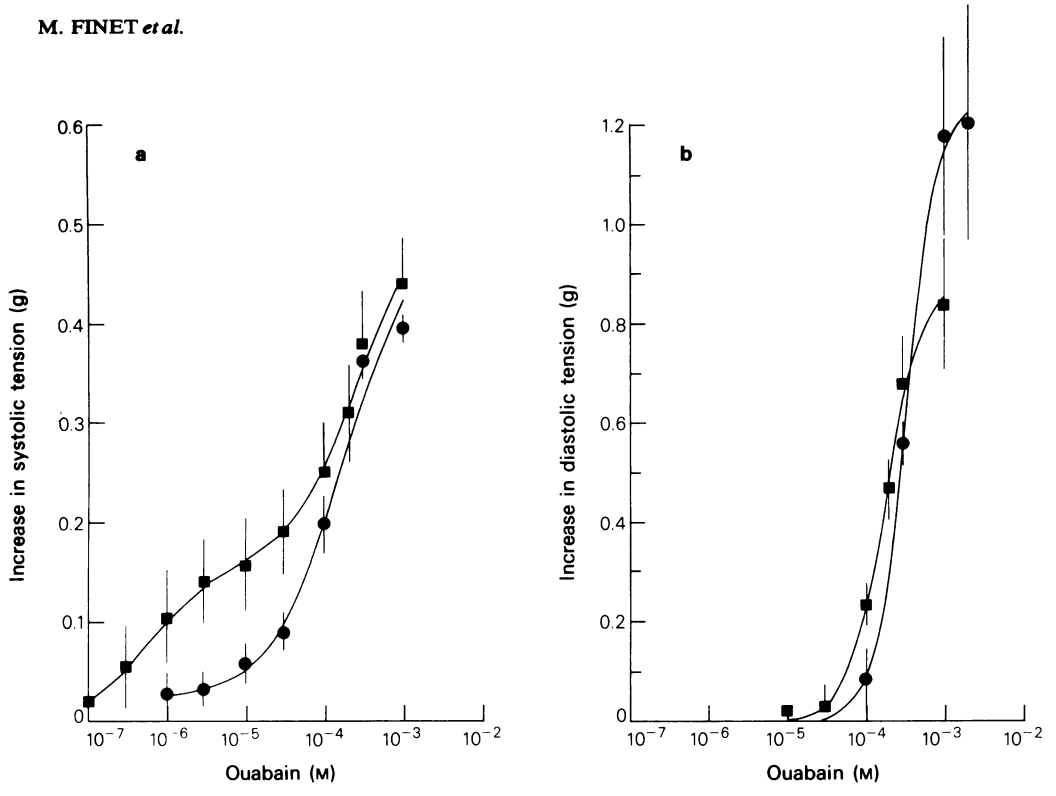

Figure 2 Inotropic (a) and tonotropic (b) effects of ouabain in isolated atria and ventricles. Each ventricular strip (■) and left atrium (●) was exposed to a single concentration of ouabain. Each point is the mean ± s.e.mean of at least 4 experiments. The theoretical curves for the inotropic effects were drawn using the model and parameters reported in Table 1. For the tonotropic effects, curves were drawn by eye.

inotropic effect decreased in a concentration-dependent manner in both atria (Figure 1a) and ventricles (Figure 1b). For concentrations lower than 10^{-5} M, the time to peak effect was about 30 min, whereas it was less than 10 min for concentrations higher than 3×10^{-5} M.

The increase in systolic tension was sustained at concentrations lower than 3×10^{-5} M, whereas in the presence of higher concentrations it declined once peak tension was attained.

The time to onset and to peak of tonotropic effects also decreased in a concentration-dependent manner

Table 1 Computed parameters of the inotropic and tonotropic effects of ouabain and dihydroouabain in atria and ventricular strips

	Ventricles				*Atria*	
	Ouabain		*Dihydroouabain*		*Ouabain*	
	High sensitivity component	*Low sensitivity component*	*High sensitivity component*	*Low sensitivity component*	*High sensitivity component*	*Low sensitivity component*
Inotropic effect	($n = 10$)		($n = 11$)		($n = 7$)	
E_{max} (g)	0.16 ± 0.01	0.37 ± 0.04	0.051 ± 0.010**	0.36 ± 0.03	0.026 ± 0.006*	0.47 ± 0.05
EC_{50} (μM)	0.61 ± 0.06	270 ± 64	4.0 ± 1.4*	652 ± 176	0.05 ± 0.33	169 ± 44
Tonotropic effect	($n = 5$)				($n = 25$)	
E_{max} (g)	—	0.85 ± 0.13			—	121 ± 0.24
EC_{50} (μM)	—	170			—	310

Inotropic data of Figures 1 and 3 were analysed by a non-linear regression program using the model of the sum of two saturable components (see methods). The computed parameters are given with their standard deviation representing the goodness of fit. Tonotropic data of Figure 1 were analysed using a graphical method. E_{max} corresponds to the maximal increase of the basal tension. n = number of different concentrations used.
*$P < 0.05$; **$P < 0.001$ compared ouabain (ventricles).

in both atria (Figure 1c) and ventricles (Figure 1d). When present, that increase in diastolic tension always followed the inotropic effect with some delay (compare Figure 1a and 1c; Figure 1b and 1d). Figure 2a shows the relation between ouabain concentration and systolic tension in rat ventricular strips and in left atria.

In ventricular strips, the log concentration-effect curve was complex with a shoulder around 10^{-5} M. The experimental data were fitted by model (2) (sum of two saturable components, see methods). This indicates that the inotropic effect was the sum of two inotropic components. The computed parameters are given in Table 1.

The component of the inotropic effect observed at low concentrations of ouabain (high sensitivity component) accounted for 30% of the maximal increase in systolic tension evoked by the glycoside. The low sensitivity component was observed at much higher concentration; the ouabain EC_{50} value was in this case 400 fold greater than for the high sensitivity component (Table 1).

In atria, the log concentration-effect curve appeared also to be different from a single sigmoid curve (Figure 2a). However, the analysis according to the same model as the one used for ventricles did not allow a significant EC_{50} value for the high sensitivity component to be obtained although it allowed a significant estimate of E_{max} which accounts for only 5% of the maximum increase in force. Therefore, if it exists in atria, the high sensitivity inotropic component is likely to be of much less importance than in ventricles. The ouabain EC_{50} value for the low sensitivity inotropic component in atria was 169 ± 44 μM, a value not significantly different from the one estimated for the low sensitivity component in ventricles (Figure 2a and Table 1). In both atria and ventricles, the log concentration-effect curves for the tonotropic effect evoked by ouabain 3×10^{-5} M to 3×10^{-3} M were nearly superimposed, with EC_{50} values of 170 μM in ventricles and of 310 μM in atria. The maximal increase in diastolic tension was equal to 0.85 ± 0.13 g in ventricles and to 1.21 ± 0.24 g in atria. Comparison of left and right panels of Figure 2 shows that an inotropic effect was produced by low ouabain concentrations (from 10^{-7} M to 10^{-5} M) without any observable increase in diastolic tension. This was much more evident in ventricular strips than in atria.

The action of dihydroouabain has been compared to that of ouabain in ventricular strips. In agreement with previous observations (Ghysel-Burton & Godfraind, 1979) the dihydroderivative appeared to be less potent than ouabain but the concentration-effect curve was not simply shifted to the right (Figure 3). Although the maximum increase in systolic tension was not different with the two glycosides, the relative important of the two components of the inotropic action was not the same (Table 1). This was due to the fact that the maximal increase in systolic tension corresponding to the high sensitivity component was 3 fold higher with ouabain than with dihydroouabain. EC_{50} values of dihydroouabain and ouabain were in a ratio of 2.4 for the low sensitivity component and of 6.5 for the high sensitivity one. As observed with ouabain, dihydroouabain evoked an increase in the diastolic tension at the high concentrations corresponding to the low sensitivity component of the inotropic effect.

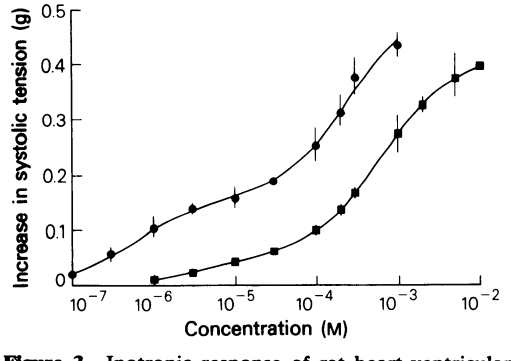

Figure 3 Inotropic response of rat heart ventricular strips to single concentrations of ouabain (●) and dihydroouabain (■). The systolic tension in absence of drug was 0.38 ± 0.03 g ($n = 31$). Solid lines are the theoretical curves derived from the model of two saturable components using the parameters reported in Table 1 (see methods). Each point is the mean ± s.e.mean of at least 3 experiments.

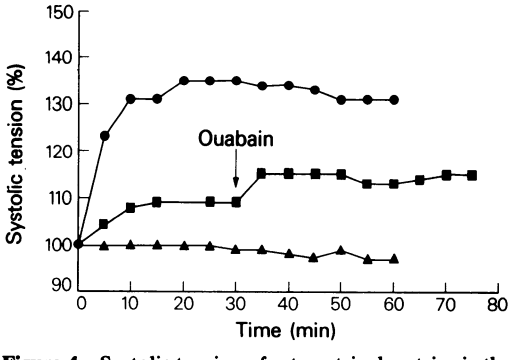

Figure 4 Systolic tension of rat ventricular strips in the absence of glycoside (▲) and in the presence of ouabain 1 μM (●) or dihydroouabain 3 μM (■), as a function of time. After an incubation period of 30 min in the presence of dihydroouabain 3 μM, ouabain 1 μM was added (arrow). The increase in systolic tension is expressed as a percentage of the initial systolic tension. Each curve corresponds to a representative experiment.

Antagonism by dihydroouabain of the inotropic effect of ouabain

Recently, it has been shown that the inotropic action of ouabain on guinea-pig atria was antagonized by dihydroouabain (Godfraind *et al.*, 1982); we, therefore, studied this effect in rat heart. Dihydroouabain 3 μM was used because at this concentration it exerted only a weak inotropic effect equal to 4.5 ± 1.5% of the maximal effect. As Figure 4 illustrates, when ouabain 1 μM was added to rat ventricular strips pretreated with dihydroouabain 3 μM, the contractile tension developed by the ventricular strips was much lower than that observed when ouabain 1 μM alone was added.

This observation was confirmed with ouabain 3 μM, a concentration that also evoked the high sensitivity inotropic component. At a higher concentration of ouabain (10 μM), the antagonism of dihydroouabain was overcome, indicating that this effect of dihydroouabain was reversible (Figure 5).

Figure 6 shows cumulative concentration-effect curves of ouabain on rat ventricular strips in control conditions and after a 30 min preincubation period in the presence of dihydroouabain 1 μM or 3 μM. Dihydroouabain 1 μM significantly depressed inotropic responses evoked by the low concentrations of ouabain (10^{-7} M to 3×10^{-6} M). Dihydroouabain 3 μM significantly reduced the responses to ouabain 10^{-7} M to 10^{-5} M ($P < 0.05$), but did not affect the responses to higher concentrations of ouabain.

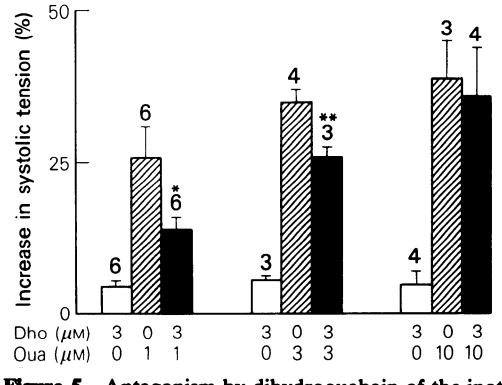

Figure 5 Antagonism by dihydroouabain of the inotropic effect of ouabain. Ventricular strips were incubated for 30 min in the absence or presence of dihydroouabain 3 μM. Ouabain was then added at the indicated concentrations and the increase in systolic tension measured when the peak effect was obtained. Columns are means with s.e.mean bars and number of experiments above each column. Open columns are diydroouabain (Dho) alone, diagonally hatched columns ouabain (Oua) alone and solid columns dihydroouabain + ouabain. Student's *t* test was used to compare mean values obtained in the presence and absence of dihydroouabain. $*P < 0.05$; $**P < 0.02$.

Ouabain binding sites in microsomes prepared from rat ventricles and atria

Microsomal preparations enriched in Na, K-ATPase were prepared from rat ventricles and rat atria. In both tissues (Table 2), the Na, K-ATPase activity accounted for 73 to 85% of the total ATPase activity. For any concentrations so far examined, [^{3}H]-ouabain binding to atrial and ventricular microsomes reached a maximum after 10 min incubation in the Mg^{2+}, P_i-medium. When data were expressed according to Scatchard (1949), curvilinear plots were obtained for the two tissues (Figure 7).

Experimental data have been analysed assuming that the binding was the sum of two saturable components (equation 2, see methods). Mean values ± s.e.mean of computed parameters (see methods) obtained in three different preparations of each tissue are shown in Table 3. The dissociation constants of ouabain for high and low affinity binding sites were similar in microsomes prepared from atria and from

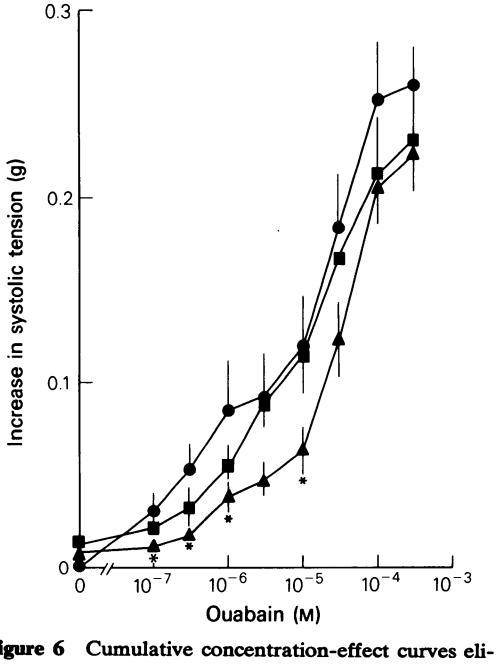

Figure 6 Cumulative concentration-effect curves elicited by ouabain alone (●) or ouabain and dihydroouabain (1 μM ■; 3 μM ▲). Ventricular strips were preincubated for 30 min in the presence of dihydroouabain before addition of ouabain. Each point is the mean ± s.e.mean of 6 observations. A significant difference from control responses in the absence of dihydroouabain is shown by $*P < 0.05$.

Table 2 Characterization of microsomal preparations from ventricles and atria

	Specific activity (μmol Pi mg protein^{-1} h^{-1})		*Yield* (units g^{-1} wet weight)	
	(Na + K)-ATPase	*Mg-ATPase*	*(Na + K)-ATPase*	*Mg-ATPase*
Ventricles ($n = 3$)	31 ± 2.1	5,2 ± 0.8	0.43 ± 0.04	0.07 ± 0.002
Atria ($n = 3$)	24 ± 4	9.1 ± 0.9*	0.45 ± 0.27	0.17 ± 0.09

Yield values are expressed in enzymatic units per gram of wet weight. One enzymatic unit is the amount of enzyme necessary to hydrolyse 1 μmol of ATP per min. n = number of preparations.
*$P < 0.05$ compared to ventricles.

ventricles. However, the capacity of the high affinity ouabain binding sites appeared to be four times lower in microsomes from atria (2.3 ± 0.5 pmol unit^{-1}) than from ventricles (9.4 ± 1.4 pmol unit^{-1}). This difference was significant when analysed using Student's $t$test ($P < 0.01$). Treatment of experimental data by a different curve fitting program (MLAB, see methods) has given similar parameters (data not shown).

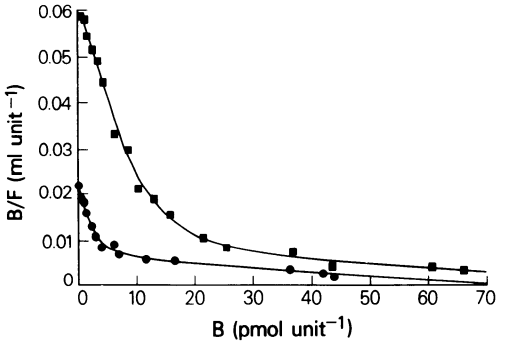

Figure 7 Scatchard plot for [^{3}H]-ouabain binding to microsomes from ventricles (■) and atria (●). The microsomes were incubated at 37°C for 10 min in the presence of 3 mM $MgCl_2$ 3 mM P_i-Tris, 1 mM EGTA, 20 mM maleate-Tris (pH 7.4) and various concentrations of (tritiated + cold)-ouabain (from 10^{-8} M to 2×10^{-5} M). The non-specific binding measured in the presence of 4 mM unlabelled ouabain was subtracted from corresponding values of total binding, to obtain the specific binding. Each point is the mean of triplicate determinations for a typical experiment. The theoretical curves were drawn using the computed parameters obtained when the untransformed data were analysed according to equation 2 (sum of two saturable components, see methods). B = ouabain specifically bound. F = free concentration of ouabain (calculated).

Discussion

The present study shows that the inotropic effect of ouabain is different in ventricles and atria of rat heart.

In agreement with previous observations, the positive inotropic action of ouabain in ventricles appears to be the sum of two components (see Adams *et al.*, 1982; Finet *et al.*, 1982). The component of the inotropic effect with the higher sensitivity to ouabain was sensitive to antagonism by dihydroouabain. It was observed at concentrations of ouabain that specifically interacted with high affinity binding sites in microsomes. The other component of the inotropic effect was observed at higher concentrations of ouabain, which are required to saturate the low affinity binding sites.

Table 3 Ouabain binding sites in microsomes from ventricles and atria

	Ventricles ($n = 3$)	*Atria* ($n = 3$)
High affinity sites		
B_{max} (pmol unit^{-1})	9.4 ± 1.4	2.3 ± 0.5*
K_d (μM)	0.21 ± 0.01	0.16 ± 0.05
Low affinity sites		
B_{max} (pmol unit^{-1})	87 ± 15	69 ± 4
K_d (μM)	13 ± 3	12 ± 3

Microsomal preparations from atria and ventricles were incubated for 10 min at 37°C in a (Mg-Pi)-medium containing (mM): $MgCl_2$ 3, P_i-Tris 3, EGTA 1, maleate-Tris 20 (pH 7.4) and various concentrations of [^{3}H]-ouabain (from 10^{-8} M to 2×10^{-5} M). Binding data were analysed using a computerized model for the sum of 2 saturable components (see methods, eg. 2). Four parameters were calculated for each experiment. The mean parameters of 3 experiments, ± s.e.mean, are given for both tissues and were compared by Student's $t$test. *$P < 0.01$.

The high sensitivity inotropic component was much lower in atria than in ventricles. This may be related to the very low amount of high affinity binding sites found in microsomes prepared from this tissue.

The analysis of the concentration-effect curve describing the increase in diastolic tension shows that both in ventricles and atria, it can be represented by one single sigmoid. This suggests that only one family of binding sites was responsible for this action. For both atria and ventricles, this was likely to be the low affinity sites, as indicated by the EC_{50} values shown in Table 1.

In guinea-pig atria, dihydroouabain is not only less potent than ouabain but also presents different qualitative properties. In the concentrations ranging below those producing a positive inotropic effect, it antagonizes the inotropic action of ouabain (Godfraind *et al.*, 1982).

In rat ventricles, dihydroouabain evoked a maximum increase in systolic response similar to the response to ouabain but in the lower range of concentrations, its interaction with the high affinity inotropic receptors evoked a much lower increase in contractility than ouabain. This observation indicates that the intrinsic activity of dihydroouabain is lower than the intrinsic activity of ouabain at activating the inotropic process at high affinity sites. This is in agreement with the existence of an antagonism exerted by dihydroouabain to the ouabain inotropic effect. It has been proposed that the ouabain inotropic effect is due to the inhibition of the Na^+, K^+-pump (Repke, 1963; Langer, 1977), but this theory is not universally accepted (Ghysel-Burton & Godfraind, 1980; Noble, 1980; Godfraind, 1982). In rat ventricles, the range of concentrations at which ouabain inhibits the pump appears to be higher than 10^{-5} M according to Erdmann, Philipp & Scholz (1980). These inhibitory concentrations are similar to those required to interact with the low affinity binding sites and to evoke the low affinity component of the inotropic effect as well as the increase in diastolic tension. Therefore, it is likely that the low affinity component is due to pump inhibition.

The high sensitivity component is probably not related to Na^+, K^+-pump inhibition which would produce changes in ionic gradients that can interfere with the Na^+, Ca^{2+} exchange, a mechanism proposed to be responsible for the inotropic effect associated with pump inhibition (Langer, 1977). However, in other tissues, such as sheep Purkinje fibres (Hart, Noble & Shimoni, 1983) and guinea-pig atria (Godfraind & Ghysel-Burton, 1980), it has been proposed that the inotropic effect of ouabain is due to more than one mechanism and the present observations on rat heart are in agreement with this theory.

One of the difficulties encountered in the study of the mechanism of the inotropic action of ouabain is the absence of a hypothesis allowing an analysis of the cellular mechanism responsible for this inotropic action that operates at a low concentration, independently of the interaction with the Na^+, Ca^{2+} exchange mechanism observed at high concentrations. Some hypotheses have recently been proposed by Weingard, Koss & Tsien (1978) and Marban & Tsien (1982), based on electrophysiological studies. These authors have suggested that low doses of cardiac glycoside could increase the amplitude of the slow Na^+, Ca^{2+} current in heart muscle. In addition, it has been postulated (Godfraind, 1982) that at low concentrations ouabain could augment the importance of the Ca^{2+}-induced Ca^{2+}-release mechanism operating physiologically in the heart. This mechanism has been shown to be extremely prominent in the rat heart as compared to other mammals (Fabiato, 1982).

The high and low affinity binding sites for ouabain that have been demonstrated in this work have been proposed by us to be located in two $(Na^+ + K^+)$-ATP-ases isozymes (Finet *et al.*, 1982, Noël & Godfrained, 1983). From a morphological point of view a main difference between atria and ventricles is the development of T-tubules associated with sarcoplasmic reticulum involved in Ca^{2+}-induced Ca^{2+}-release (Sommer & Johnson, 1979, Fabiato & Fabiato, 1975). If high affinity binding sites are located close to T-tubules or associated with them, their interaction with ouabain could contribute to an increase in contraction without a reduction of whole cell ionic gradient. The biochemical properties of the two forms of $(Na^+ + K^+)$-ATP-ase that have been described by Noël & Godfraind (1983) are consistent with the present observation that the antagonism between ouabain and dihydroouabain takes place on high affinity sites.

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