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Independence of the positive inotropic effect of ouabain from the inhibition of the heart Na^+/K^+ pump

(ouabain binding sites/low-K solutions/dihydroouabain)

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ABSTRACT **Isolated left atria from guinea pigs were stimulated at 3.3 Hz and bathed at 30°C in Tyrode's solution containing 6 mM KCl. After equilibration, this solution was replaced by a low-K solution or by Tyrode's solution containing ouabain or dihydroouabain. These treatments evoked an increase in the contractility of the atria. The time to peak increase was about 30 min, and the inotropic effect was sustained for at least 40 min. After 30 min, ^{42}K was added to the bathing solution in order to estimate the activity of the Na^+/K^+ pump. A linear relationship was observed between the degree of inhibition of the Na^+/K^+ pump and the increase in systolic tension. The regression line was the same for low-K solutions and dihydroouabain but not for ouabain. For a given degree of inhibition of the pump, ouabain evoked a higher increase in contractility. These findings indicate that inhibition of the Na^+/K^+ pump can be the only mechanism responsible for the positive inotropic effect of dihydroouabain but cannot be the sole mechanism for that of ouabain.**

The positive inotropic effect evoked by cardiac glycosides has been attributed to inhibition of the Na^+/K^+ pump (1–5). One of the main supports of this hypothesis is the observation that any intervention that increases intracellular Na^+ content enhances the twitch tension in cardiac muscle. Evidence has been presented for the existence of a membrane Na^+/Ca^{2+} exchange operating in such a way that an increase in cytosolic Ca^{2+} concentration occurs when intracellular Na^+ increases (5–7). Because the contractile state of the heart muscle is determined by the concentration of Ca^{2+} in the cytoplasm, the above hypothesis has received much credit.

However, several observations indicate that the mode of action of cardiac glycosides is more complex. It has been reported that low doses of ouabain stimulate the Na^+/K^+ pump and increase the contractility of the heart (8–14). This suggests that more than one mechanism could be responsible for the inotropic effect. In order to examine this possibility further, we investigated the relationship between the inhibition of the Na^+/K^+ pump and the positive inotropic effect evoked by low-potassium solutions, by ouabain, and by dihydroouabain; dihydroouabain is a glycoside with a saturated lactone ring which does not stimulate the Na^+/K^+ pump (11–14). The results show that inhibition of the Na^+/K^+ pump could be the only mechanism responsible for the positive inotropic effect of dihydroouabain but not for that of ouabain.

MATERIALS AND METHODS

Physiological Solutions. The composition of the Tyrode solution was (mM): NaCl, 137; KCl as indicated in the text; $CaCl_2$, 1.82; $MgCl_2$, 0.105 (10% of usual); NaH_2PO_4, 0.417; $NaHCO_3$, 11.9; glucose, 5.5. It was equilibrated at 30°C with 95% O_2/5% CO_2.

Ouabain or dihydroouabain (gift from SIMES, Milano, Italy) 0.01 M stock solutions were made in water and were added to Tyrode's solution to reach the final concentration.

Preparation. Albino guinea pigs weighing approximately 400 g were killed by a blow on the head and bled; the hearts were rapidly removed. The atria were dissected out in physiological solution containing 2.7 mM KCl. The left atria were suspended in an organ bath between platinum electrodes, under a resting tension of 500 mg. The atria were stimulated with rectangular 10-msec pulses (strength at least twice threshold) at a rate of 3.3 Hz. Recordings of the contractile activity were made by isometric lever using two strain gauges as part of a balanced bridge, the output of which was fed into a potentiometric recorder. The atria were first stimulated for 30 min in Tyrode's solution containing 2.7 mM KCl, which was thereafter changed for the physiological solution containing 6 mM KCl. The systolic tension was sensitive to the change in KCl. A time of 30 min was sufficient to reach the new level of contractility which, with 6 mM KCl, was constant for a further 90-min observation period. Expressed in g/100 mg, the mean (± SEM) systolic tension was equal to 1.13 ± 0.02 (n = 193) for 2.7 mM KCl and to 0.75 ± 0.05 (n = 109) for 6 mM KCl.

Ionic Content Determinations. At the end of the incubation, the atria were removed from the organ bath and each preparation was blotted on filter paper and was pressed three times with a roller weighing 350 g. After weighing, each atrium was placed in a quartz crucible, left overnight at 100°C, and then weighed again. To remove organic material, it was left at 500°C for 18 hr. The residue was dissolved in 1 ml of 1 M HCl and assayed by atomic absorption as described for another tissue (15).

Uptake of ^{42}K. At the end of the preincubation period in the presence or absence of the glycosides, ^{42}K (Amersham) was added to the solution (5–10 nCi/ml; 1 Ci = 3.7×10^{10} becquerels); 10 min later, the atria were dipped for 5 sec in a nonradioactive solution. The atria were blotted and weighed as described for ionic determination. Each atrium was dissolved in 0.2 ml of a solution composed of equal parts of perchloric acid (37%, wt/vol) and H_2O_2. This solution was heated at 75°C during 30 min; after cooling, 5 ml of Aqualuma (LUMAC) was added.

The radioactivity of the samples was measured in a liquid scintillation counter (Packard Tri-Carb 3375); the efficiency was the same for both tissue and solution. The uptakes were expressed as: mmol/kg wet weight = [cpm in muscle/wet weight (kg)] × (mmol/liter of medium/cpm/liter of medium).

Statistical Methods. Whenever possible, values are presented as mean ± SEM. Significance of differences between means was checked by Student's t test.

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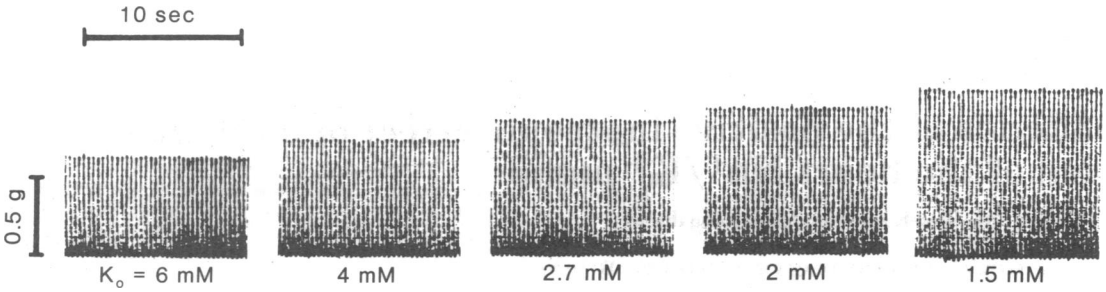

FIG. 1. Recordings of the contractile activity of one isolated guinea pig left atrium bathed at 30°C in Tyrode's solution in which the KCl concentration was progressively decreased as indicated. The time of incubation in each solution was 20 min.

RESULTS

The exposure of isolated atria to low-K media caused the systolic tension to increase. This inotropic effect was reversible and was inversely related to the KCl concentration (Fig. 1). The time to peak was 10–20 min, after which the contractility was sustained for at least 40 min. The diastolic tension remained unchanged.

^{42}K was added to the bath after an incubation of 30 min in Tyrode's solution of different K concentrations. The net ^{42}K uptake was dependent on the K concentration of the medium (Table 1). The net ^{42}K uptake is the sum of passive and active K exchanges. The passive exchange was estimated in atria treated with 0.1 mM ouabain, which blocked the Na^+/K^+ pump (11). The active exchange was obtained by subtracting ^{42}K passive exchange from ^{42}K net uptake. The turnover of the Na^+/K^+ pump in low-K solution was expressed as a percentage from the ratio between the active (ouabain-sensitive) ^{42}K uptake at a given KCl concentration of the medium and the active ^{42}K uptake at 6 mM KCl. A straight-line relationship was found between the inhibition of active ^{42}K uptake and the increase in the systolic tension of isolated atria immersed in low-K solutions (Fig. 2).

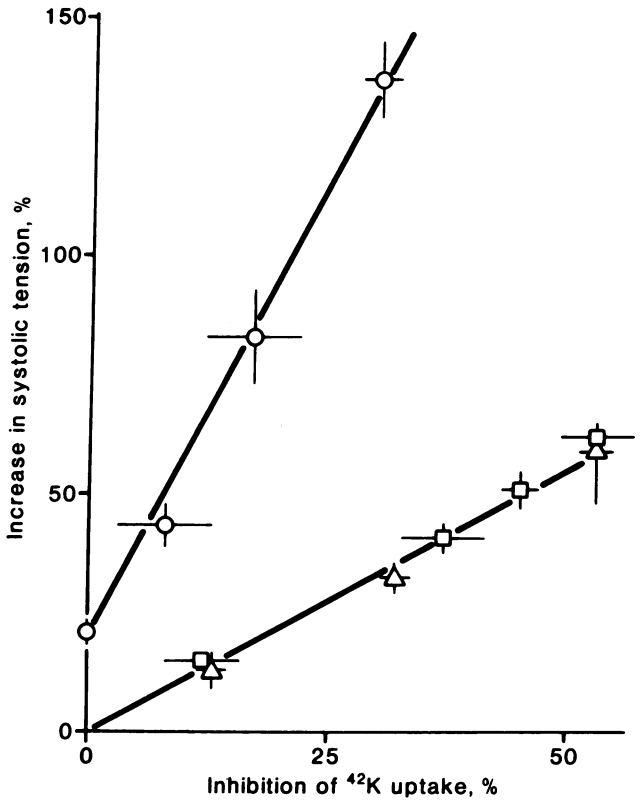

FIG. 2. Relationship between the inhibition of ^{42}K uptake and the increase in systolic tension. Inhibition of the ouabain-sensitive ^{42}K uptake was evoked by: □, low-K solutions containing, respectively, 4, 2.7, 2, and 1.5 mM KCl (n = 6); △, dihydroouabain at concentration 1, 3, and 10 μM (n = 8); and ○, ouabain at concentrations 10, 100, 300, and 600 nM (n = 8). The limits of SEM are shown when they exceed the diameter of the symbol.

In other experiments, the atria bathed in 6 mM KCl were treated with various concentrations of ouabain or dihydroouabain sufficient to inhibit the Na^+/K^+ pump. As reported (11), the lowest active concentrations evoked only an increase of the systolic tension. The highest active concentrations evoked, in addition, an increase of the diastolic tension that was accompanied by a progressive decrease of the systolic tension. With the lowest active concentrations, the time to maximal inotropic effect was 15–20 min, after which the tension was sustained for at least 45 min. ^{42}K was added to the bath 30 min after the addition of the glycosides in order to allow estimation of the active K uptake. Within the range of concentrations reported in Fig. 2 and which did not alter the diastolic tension, the increase in systolic tension was dependent on the inhibition of the Na^+/K^+ pump, but the dependence was not the same with ouabain and dihydroouabain. For dihydroouabain the relationship between the inhibition of the Na^+/K^+ pump and the increase in systolic tension was not different from the one found in the low-K solutions. With ouabain, the slope of this relationship was steeper.

Because the measurement of ^{42}K uptake showed that 600 nM ouabain exerted an inhibition similar to that brought about by a low-K solution containing 2.7 mM KCl, the ionic composition of atria incubated under those conditions for 45 min was estimated and compared with that of control atria incubated in physiological solution containing 6 mM KCl. Addition of ouabain and lowering of the KCl concentration to 2.7 mM caused the same significant ($P < 0.001$) gain in Na^+ and Ca^{2+} and loss in K^+ (Table 2).

Table 1. Influence of extracellular KCl concentration on the uptake of ^{42}K by isolated guinea pig atria

KCl, mM	^{42}K uptake, mmol/kg wet wt: Net	Uptake in presence of 0.1 mM ouabain	Ouabain-sensitive ^{42}K uptake, %
6	5.84 ± 0.14 (16)	4.60 ± 0.16 (16)	100
4	4.46 ± 0.18 (4)	3.36 ± 0.24 (4)	88
2.7	3.11 ± 0.14 (8)	2.33 ± 0.14 (8)	63
2	2.69 ± 0.05 (4)	2.01 ± 0.13 (4)	55
1.5	1.89 ± 0.10 (4)	1.31 ± 0.05 (4)	47

Electrically stimulated left guinea pig atria were incubated in the indicated solutions for 30 min; then, ^{42}K was added to the solution for a further period of 10 min. The number of experiments is given in parentheses. Data are mean ± SEM.

Table 2. Influence of extracellular concentration of KCl or ouabain on the ionic composition of isolated guinea pig atria

Solution	KCl, mM	Ionic content, mmol/kg wet wt		
		Na^+	K^+	Ca^{2+}
Control (20)	6	74.8 ± 1.0	72.1 ± 1.1	2.5 ± 0.09
Low K (10)	2.7	83.9 ± 1.2	61.1 ± 1.8	2.9 ± 0.09
Ouabain at 600 nM (10)	6	85.0 ± 1.6	59.2 ± 1.1	3.0 ± 0.09

Electrically stimulated left guinea pig atria were incubated in the indicated solution for 45 min. The number of experiments is given in parentheses. Data are expressed as mean ± SEM.

DISCUSSION

The present experiments were undertaken in order to analyze the relationship between the inhibition of the Na^+/K^+ pump and the contractility of isolated guinea pig atria. Low-K and dihydroouabain solutions that exerted the same degree of inhibition of the pump evoked the same inotropic effect. Furthermore, the gain in Na^+ was accompanied by a gain in Ca^{2+}. This strongly supports the hypothesis that the contractility of the heart is dependent on the turnover of the Na^+/K^+ pump which regulates indirectly the transmembrane Na^+/Ca^{2+} exchange (6, 7).

It has been reported that the inotropic effect of low-K solutions could be due solely to their action on the Na^+/K^+ pump (16). Accordingly, it can be proposed that the inotropic effect of dihydroouabain is due only to the inhibition of this pump.

As far as ouabain is concerned, the observed increase in contractility was much higher than could have been predicted from the experiments with dihydroouabain and low K. It therefore is likely that the inotropic effect of ouabain is the sum of more than one process: one related to the inhibition of the Na^+/K^+ pump and another related to a still unknown mechanism. It has been reported that, at low concentration, ouabain stimulates the Na^+/K^+ pump through an interaction with high-affinity binding sites different from the low-affinity binding sites responsible for the inhibition of the pump. The interaction with these sites also evokes a positive inotropic effect (11). Because dihydroouabain does not stimulate the Na^+/K^+ pump (11, 13, 14), it is likely that the ouabain inotropic effect that is not accounted for by the inhibition of the Na^+/K^+ pump is due to an interaction with these high-affinity sites.

It has been reported that cardiac glycosides increase an inward Ca^{2+} current in heart tissue (17). This observation was made with a glycoside with an unsaturated lactone ring; there is at present no available information on the action of dihydro derivatives. One must therefore be cautious in postulating that the inotropic effect of ouabain which is not accounted for by the inhibition of the Na^+/K^+ pump is due to an enhanced Ca^{2+} inward current because an increase of Ca_i might also be caused by the release of intracellularly sequestered calcium (18).

It has been postulated that the binding of cardiac glycosides through their lactone ring to the receptors is a two-point attachment resulting from resonance in the ring (19). Such resonance does not occur when the lactone ring is saturated. An electrostatic binding between the unsaturated lactone and a putative negative charge of the receptor would not occur with dihydroouabain. The qualitative difference in the biological properties of ouabain and dihydroouabain is consistent with these chemical differences.

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