Biochimica et Biophysica Acta 939 (1988) 595–602
Elsevier

BBA 73981

Interactions of cardiac glycosides with cells and membranes. IV. Effects of ouabain and bumetanide on $^{86}Rb^+$ influx in cultured cardiac myocytes from neonatal rats *

Michael Heller [a], Haifa Hallaq [a] and Rivka Panet [b]

[a] *Institute of Biochemistry, Hebrew University – Hadassah Medical School and* [b] *Department of Medical Biophysics – Hadassah University Hospital, Jerusalem (Israel)*

(Received 17 November 1987)

Key words: Ouabain; Cardiac glycoside; Rubidium ion influx; Sodium/potassium ion cotransport; Bumetanide; (Rat heart)

Ouabain at nanomolar concentrations stimulates total Rb^+ influx by 20 ± 2% in monolayer cultures of myocytes which were either in physiologic ionic steady-state conditions ('control') or 'loaded with Na^+' following exposure to K^+-free medium. The ouabain-stimulated Rb^+ influx was completely abolished by 0.1 mM bumetanide both in 'control' and in 'Na^+-loaded' myocytes. Thus, addition of nanomolar concentrations of ouabain to myocytes markedly stimulate the bumetanide-sensitive Rb^+ influx. This influx was increased up to 3- and 4-fold in 'control' and 'Na^+-loaded' myocytes, respectively. Ouabain at nanomolar concentrations had no significant effect on the component of $^{86}Rb^+$ influx which is inhibited by millimolar concentrations of ouabain (the so called 'ouabain-sensitive' or 'pump-mediated' Rb^+ influx) in 'control' and 'Na^+-loaded' cells. It is proposed that the increased rates of bumetanide-sensitive Rb^+ influx are accompanied by an increased bumetanide-sensitive Na^+ influx through the Na^+/K^+ cotransporter and thus to a transient increase in intracellular Na^+ concentrations $[Na^+]_i$. The increase in $[Na^+]_i$, subsequently causes a transient elevation in $[Ca^{2+}]_i$ via the Na^+/Ca^{2+} exchanger and may be involved in the regulation of cardiac cells' contractility.

Introduction

It is commonly accepted that positive inotropic actions and toxic effects of cardiac glycosides are intimately related to their binding to the sodium/potassium pump [1–3]. Therapeutic doses of cardiac glycosides are those concentrations of the drug which elicit positive inotropic effects in the heart. In patients treated with digitalis, serum glycosides' levels in vivo, and positive inotropy occur in the nanomolar concentration range [4].

It has been proposed that cardiac glycosides partially inhibit active extrusion of Na^+ from cells causing its intracellular retention. Increased contractility is observed following transient elevation of $[Ca^{2+}]_i$. This could be obtained by a number of ways [5], one of which is by the Na^+/Ca^{2+} exchanger [1–3].

However, it has also been shown that therapeutic (i.e., nanomolar) concentrations of cardiac glycosides cause stimulation rather than inhibition

* These studies are included in the thesis of H.H. as partial fulfilment of her Ph.D. thesis.

Abbreviations: $[Na^+]_i$ and $[Ca^{2+}]_i$, intracellular concentrations of these ions.

Correspondence: M. Heller, Institute of Biochemistry, Hebrew University – Hadassah Medical School, P.O. Box 1172, Jerusalem, Israel 91010.

of the influx of K^+ [4]. This stimulation was attributed to the sodium pump.

Another transporter has been described in many eukaryotic cells which cotransports electroneutrally, Na^+, K^+ and Cl^- contributing significantly to the cellular fluxes of ions [6,7].

In the present communication we have studied the effects of 'therapeutic' and 'toxic' doses of ouabain on the $^{86}Rb^+$ influx in cultured cardiac muscle cells from postnatal rat hearts in the absence and presence of bumetanide.

Materials and Methods

Materials

Postnatal rats, 1–3-days old, were of local strain; Ham F-10 with or without Ca^{2+} and Mg^{2+} was purchased from Kibbutz Beth Ha'Emek, Israel; fetal calf and horse sera, from Gibco, U.S.A., trypsin and ouabain from Sigma, Tel-Aviv; antibiotics from Teva Pharamceuticals, Jerusalem; sterile petri dishes, pipettes, etc. from Falcon, U.S.A.

Bumetanide was provided by Laboratoire Leo, B.P. 9-28500 Verouillet, Denmark; $^{86}RbCl$, was purchased from New England Nuclear.

Methods

Cultures of postnatal rat heart cells were prepared as was previously described [8], briefly as follows: Heart ventricles, aseptically removed from 1–3-day-old rats, were minced into small pieces in a Ca^{2+} plus Mg^{2+}-free Ham F-10 medium, and the suspension of minced tissue was incubated with 0.1% (w/v) trypsin solution stirred at 100 r.p.m. at 37 °C, seven times for 20 min each time. After completion, the supernatant was decanted into a complete medium which contains serum to stop the action of trypsin on cells (Ham F-10 supplemented with Ca^{2+}, Mg^{2+}, 10% fetal calf serum, 10% horse serum, and per liter 200 000 units penicillin, 0.2 g streptomycin and 0.2 g gentamycin), and the entire cell suspension filtered through a 0.200 μm mesh sieve.

Cells were counted, diluted to a concentration of $7.5 \cdot 10^5$ cells/ml. Plates, 35 mm in diameter, contained $(1–1.5) \cdot 10^6$ cells, incubated in a complete medium for 60 min at 37 °C. This process causes a faster adherence of non muscle (fibroblasts) cells to the culture dish, leaving a highly enriched supernatant of muscle cells (myocytes – over 90% pure). Cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The medium was replenished every two days, and cells were used on the 4th or 5th day. At the time of experiment, the cells were confluent and synchronously contracting. Protein concentrations were determined by the method of Lowry et al. [9], including dodecylsulfate (0.0075% w/v) to dissolve membranes.

$^{86}Rb^+$ influx measurements

Rb^+ influx was conducted according to Panet et al. [10,11], in brief.

(a) Total Rb^+ influx. Plates were rinsed with 1 ml of the reaction mixture (in mM final concentrations): 150 NaCl, 5 RbCl, 10 Hepes-Tris buffer (pH 7.0), 0.5 $CaCl_2$, 5 $MgCl_2$, 10 glucose. The uptake reaction was started by adding 1 ml of the reaction mixture containing 2 to 5 μCi $^{86}Rb^+$ and continued for up to 20 min at 37 °C – the linear portion of the curve (cf. Fig. 1 and Results). The uptake was terminated by aspiration of the medium, the cells were rinsed rapidly twice, with 5 ml ice cold solution of 125 mM $MgCl_2$ and twice with 5 ml ice cold solution of 165 mM NaCl. The washing process did not affect intracellularly trapped $^{86}Rb^+$. The cells were lysed with 0.6 ml of 0.1 M NaOH containing 0.1% (w/v) sodium dodecyl sulfate, and radioactivity was counted in toluene-Triton scintillation medium.

(b) Ouabain-resistant Rb^+ influx. The reaction was the same as for total Rb^+ influx (a), in the presence of 1–1.3 mM ouabain.

(c) Ouabain and bumetanide resistant Rb^+ influx. Incubation mixture as in (b) with 0.1 mM bumetanide.

Ouabain-sensitive Rb^+ influx was obtained by subtracting ouabain-resistant from total Rb^+ influx. Bumetanide-sensitive Rb^+ influx was obtained by subtracting ouabain and bumetanide-resistant from ouabain-resistant Rb^+ influx, or by direct effect of bumetanide on total Rb^+ influx.

Rb^+ influx rates are expressed as nmol/mg protein per min.

Elevation of $[Na^+]_i$ in mycocytes and $^{86}Rb^+$ influx. The plates were rinsed and then incubated with the total influx medium in which RbCl (KCl)

was replaced by choline chloride for 1 to 30 min at 37° C. At the end of incubation, the medium was aspired and replaced by assay mixture for 1 to 10 min in the absence or presence of ouabain or bumetanide and the uptake measured as given above. Each experiment was done with three plates and was repeated 3–5 times.

Values presented in this study are means ± S.E. Significance of differences between means was checked by Student's *t*-test, with values of significance less than 0.05.

Results

Influx of $^{86}Rb^+$ in cultured heart cells

Cultured postnatal heart cells provide a useful model for studying effects of cardiac glycosides on the transport of K^+ [12,13]. Mycocytes were separated from fibroblasts as described in Methods and Rb^+ uptake was measured in both cells. $^{86}Rb^+$ uptake for both types of cells was linear during the first 20 min despite statistical fluctuations (Fig. 1). Accordingly, total influx rates were 45.9 ± 2 and 19.9 ± 1 nmol $^{86}Rb^+$/mg protein per min for myocytes and fibroblasts, respectively. Higher rates could be obtained if only the initial portion of these curves (i.e., 2–3 min) were used for calculation of rates. Since the rates of $^{86}Rb^+$ influx in fibroblasts are considerably lower than in myocytes (cf. Ref. 14), slight contamination of myocytes by fibroblasts is not expected to affect the measurements.

Despite quantitative differences in rates between the two types of cells, they displayed a similarity in the distribution Rb^+ influx components. Two components, i.e., ouabain sensitive and ouabain resistant comprise 70 ± 2% and 30 ± 2%, respectively, of the total $^{86}Rb^+$ influx in both cell types (data are not shown here). Similar results were obtained by McCall [15] in neonatal rat heart myocytes.

As shown in Fig. 2, part of the Rb^+ influx which is resistant to ouabain, was inhibited by bumetanide with a $K_{0.5}$ of approximately 10^{-7} M. Thus, total Rb^+ influx may therefore be divided into the following components: (a) ouabain-sensitive Rb^+ influx comprises about 70% of the total; (b) bumetanide-sensitive of about 10% of the total,

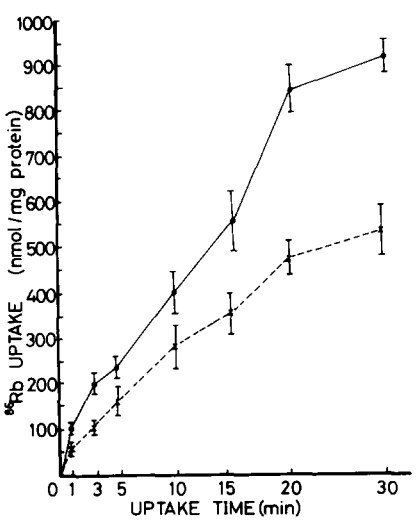

Fig. 1. Total $^{86}Rb^+$ uptake in myocytes and fibroblasts from postnatal rat hearts. $^{86}Rb^+$ uptake was measured as described in Methods using separate cultures of myocytes (●) and fibroblasts (×).

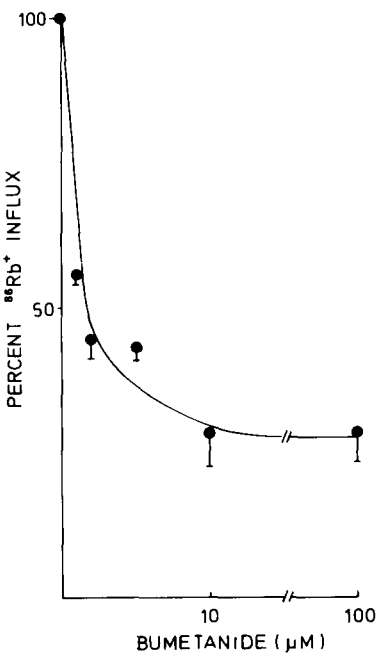

Fig. 2. Inhibition of ouabain resistant $^{86}Rb^+$ influx by bumetanide. The results are expressed as a percentage of the ouabain-resistant influx rate. Ouabain-resistant Rb^+ influx was measured in the presence of 1 mM ouabain and the indicated concentrations of bumetanide for 20 min as described in Methods. Ouabain-resistant Rb^+ influx (100%) was 10 nmol/mg protein per min.

and (c) a residual influx resistant to ouabain and bumetanide.

Effects of ouabain on total influx of $^{86}Rb^+$

The effect of different doses of ouabain, ranging from $5 \cdot 10^{-10}$ M to $1 \cdot 10^{-3}$ M, on the $^{86}Rb^+$ influx was determined in myocyte cultures. A biphasic response was observed. In the range of low concentrations of ouabain a stimulation of $20 \pm 2\%$ of the total $^{86}Rb^+$ influx could be seen. In the range of higher concentrations of ouabain, inhibition prevailed with an IC_{50} value of about $4 \cdot 10^{-5}$ M (Fig. 3a). At $1 \cdot 10^{-3}$ M ouabain, the Na^+/K^+ pump was completely blocked, leaving the ouabain-resistant Rb^+ influx.

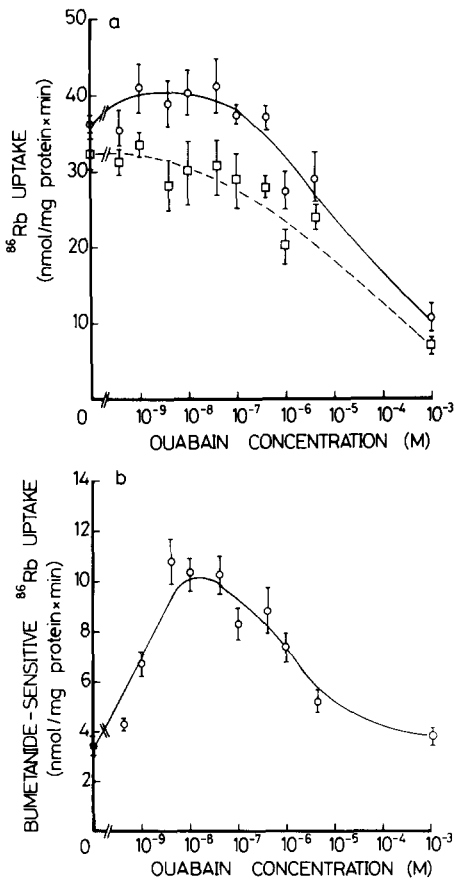

Fig. 3. Effect of ouabain $^{86}Rb^+$ on influx in the absence and presence of bumetanide. Myocytes were prepared, and $^{86}Rb^+$ influx measured for 20 min as described in Methods. Ouabain at the indicated concentrations was added to the assay mixture. (a) Total Rb^+ influx in the absence (○) and presence of 0.1 mM bumetanide (□). (b) Bumetamide-sensitive Rb^+ influx was calculate dby subtracting Rb^+ influx measured in the presence and absence of 0.1 mM bumetanide at the indicated concentration of ouabain.

We have measured the effect of different ouabain concentrations in the absence and presence of 0.1 mM bumetanide on total $^{86}Rb^+$ influx. As Fig. 3b shows, 0.1 mM bumetanide completely abolished the stimulatory effects of low concentrations of ouabain. The bumetanide-sensitive Rb^+ influx (calculated by subtracting Rb^+ influx in the presence of bumetanide from total Rb^+ influx) was stimulated from 3.4 to more than 10 nmol/mg protein per min by nanomolar concentrations of ouabain (Fig. 3b). On the other hand, the ouabain-sensitive influx was not affected. Similar results are also seen in Table I: 10 nM ouabain stimulated the bumetanide-sensitive Rb^+ influx by 244% whereas the ouabain-sensitive influx was stimulated only by 7%! The result that only the bumetanide-sensitive influx was stimulated by nanomolar concentrations of ouabain was unexpected. Therefore, we have measured the effects of ouabain also in myocytes which were preincubated in K^+-free medium. This process inhibits the Na^+/K^+ pump, elevates $[Na^+]_i$ causing the pump to turnover at rates approaching V_{max} [16–20]. The actual concentrations of $[Na^+]_i$ were not measured in these experiments (cf. however, Ref. 19 for chick embryo myocytes), but their consequences were clearly reflected by the corresponding increased rates of the $^{86}Rb^+$ influx (cf. Fig. 4 and Table I).

Pre-incubating myocytes for 5 or 10 min in a K^+-free medium, activated the Na^+/K^+ pump almost maximally and raised influx rates, when measured at optimal conditions, by 220% of their control values which represent values approaching V_{max}.

In the experiments described in Fig. 4, similar conditions raised the inward flow by 167% (i.e., from 56 to 92 nmol/mg protein per min).

As could be seen in Fig. 4, both the 'control' and the 'Na^+-loaded' cells exhibited similar response to ouabain:

(1) The range of ouabain concentrations causing stimulation of influx was from $5 \cdot 10^{-10}$ to $1 \cdot 10^{-6}$ M, in both cells.

(2) 5–10 nM ouabain caused a 12–14% stimulation of total influx, in both cells.

TABLE I

EFFECT OF THERAPEUTIC DOSES OF OUABAIN ON OUABAIN-SENSITIVE AND BUMETANIDE-SENSITIVE Rb^+ INFLUXES

Myocytes, 'Control' and 'Na^+-loaded' were prepared as described in Methods. The 'Na^+ loaded' cells were obtained following 5 min preincubation in K^+-free medium. Bumetanide (0.1 mM) was added in absence and presence of ouabain and the cells were incubated with $^{86}Rb^+$ for 10 min. $^{86}Rb^+$ influx was measured as described in Methods; Ouabain-sensitive influx was calculated by subtracting the bumetanide-sensitive and the residual (which is resistant to bumetanide and ouabain) influxes from total Rb^+ influx and not by the usual ouabain sensitivity since opposite effects of ouabain were measured here.

Myocytes	Additions	$^{86}Rb^+$ influx rate					
		total		ouabain-sensitive		bumetanide-sensitive	
		activity [a]	%	activity [a]	%	activity [a]	%
Control	none	38.1±1	100	28.3±2	100	3.4±0.2	100
	ouabain (10 nM)	45.9±1	118	30.5±2	107	8.3±0.3	244
'Na^+-loaded'	none	78.0±2	100	65.6±2	100	3.8±0.3	100
	ouabain (10 nM)	82.2±1	105	60.9±1	93	15.0±0.4	395

[a] Activity is expressed as nmol/mg protein per min.

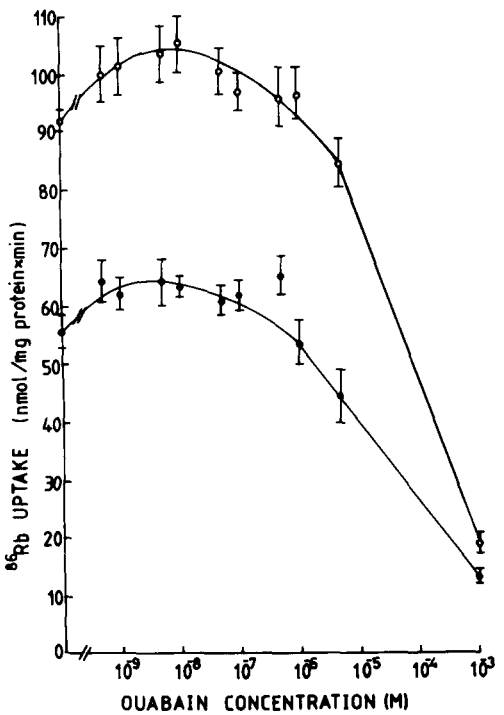

Fig. 4. Effect of ouabain on 'control' and 'Na^+-loaded' myocytes. $^{86}Rb^+$ influx was measured in ('control' myocytes (●)) and 'Na^+-loaded' myocytes preincubated for 5 min in $[K^+]_c$-free medium (○). Rb^+ influx was measured at 20°C for 5 min, in presence of the indicated ouabain concentrations.

(3) The IC_{50} value for inhibiting the influx was about $1 \cdot 10^{-4}$ M.

Since we could observe a similar stimulation by ouabain at nM concentrations in the 'Na^+-loaded' cells, we measured the effect of bumetanide on it (Table I). 10 nM ouabain stimulated the bumetanide-sensitive Rb^+ by 244% in the 'control' cells and surprisingly, by 395% in the 'Na^+-loaded' cells. Since the ouabain-sensitive Rb^+ influx was only very slightly stimulated (i.e., by 7%) in the 'control' cells, one cannot totally exclude any stimulation of the ouabain-sensitive influx under these circumstances. However, in 'Na^+-loaded' cells (cf. Table I), no stimulation of ouabain-sensitive Rb^+ influx was observed, perhaps a slight inhibition (i.e., by 7%). It may be concluded that these differences (i.e., ±7%) are not statistically significant. Neither could stimulation be seen in Rb^+ influx of 'control' cells in the presence of bumetanide (cf. Fig. 3a).

Discussion

Ample evidence supporting Na^+/K^+-ATPase as the receptor for positive inotropic effects of cardiac glycosides has been reviewed [1–3,21,22]. However, direct evidence to establish a causal link between glycoside induced inotropy and inhibition of active transport of Na^+ and K^+ across myocardial cell sarcolemma, is not always clear.

A major point of controversy has been the issue

of whether significant pump inhibition occurs at positive inotropic doses of cardiac glycosides. Various in-vitro cardiac preparations responded inotropically to concentrations of digitalis around 10^{-7} M [23–27]. However, plasma levels of free digoxin,digitoxin or ouabain in therapeutic conditions in humans are lower than 10^{-8} M [2,4]. On the other hand, in quiescent atrial preparations from guinea pig hearts, and other preparations, nanomolar concentrations of ouabain stimulated by 25% or more $^{86}Rb^+$ uptake. This stimulatory effect on transport was abolished either by pretreatment with propranolol or atropine [21,27,28].

Because of complexity in interpreting data from experiments in intact myocardial preparations, we have also decided to pursue flux studies in spontaneously beating cultured heart muscle cells from postnatal rat [22,29–31]. They bear certain resemblance to muscle cells from human hearts, i.e., both types of cells contain two ouabain binding sites having similar affinities for ouabain [22,25].

Nanomolar concentrations of cardiac glycosides, have only rarely been shown to inhibit the sodium pump, in a variety of cardiac preparations. Alternatively, mechanisms for a transient elevation of $[Na^+]_i$, other than inhibition of the Na^+/K^+ pump, could also be operative. Indeed, a Na^+/H^+ exchange mechanism has been proposed as a major uptake pathway for Na^+ in quiescent chick cardiac cells [32], and may play an important role in the positive inotropic as well as the toxic effects of cardiac glycosides [33].

In the present communication, we have shown that Rb^+ influx in myocytes is stimulated by nanomolar concentrations of ouabain which are considered therapeutic in patients maintained on digitalis therapy [4]. The influx was inhibited by higher concentrations with an IC_{50} value of $4 \cdot 10^{-5}$ M of ouabain but not completely even at toxic, millimolar concentrations. It was therefore postulated that perhaps other components of the Rb^+ influx, which are resistant to ouabain, could participate in the stimulation. The bumetanide-sensitive K^+ transporter has recently been characterized in several eukaryotic cells including chick embryo heart cells [6,7,10,11,34–41], but its physiological role is not clear. This K^+ transporter has been shown to be coupled to influxes of Na^+ and Cl^- with a 1 : 1 : 2 molar ratio [34,36,39].

The bumetanide-sensitive K^+ influx comprises between 10 and 40% of total Rb^+ influx in different cells. The activity of this transporter has been shown to change during cell differentiation and cell division or under the influence of different stimuli [11,34,40–42]. In this communication we show that therapeutic concentrations of ouabain stimulated the bumetanide-sensitive K^+ influx by 2–4-fold in growing myoblasts. Since the bumetanide-sensitive K^+ influx has been shown in a number of cells to cotransport Na^+ with K^+, it is proposed that the therapeutic effect of ouabain is initially to increase Na^+ influx via the bumetanide-sensitive transporter.

At concentrations of ouabain lower than $5 \cdot 10^{-6}$ M, which cause a stimulation of total influx of $^{86}Rb^+$, inclusion of $1 \cdot 10^{-4}$ M bumetanide abolished this stimulation almost completely (Fig. 3a and Table I). If the sodium pump is not activated by therapeutic doses of ouabain under physiological conditions, it must be ensured that this is also the case for other turnover rates of the pump. Rates approaching V_{max} were achieved by elevating $[Na^+]_i$ to saturate the internal sites for Na^+ ('Na^+ loading'). Temporarily inhibiting the Na^+/K^+ pump by exposure of the cells to a K^+-free solution caused a transient increase in $[Na^+]_i$ [16–18], the Na^+/K^+-transport was then reactivated by restoring K^+ to the medium. Murphy et al. [19] have shown in cultured heart muscle cells from chick embryo hearts that this technique caused an increase in $[Na^+]_i$ and also a linear loading of $[Ca^{2+}]_i$.

Although 'Na^+ loading' accelerated total $^{86}Rb^+$ uptake only moderately, it did not affect the bumetanide-sensitive transporter per se (Table I). On the other hand, as expected, the uptake due to the ouabain-sensitive pump increased considerably. The unexpected effect of nanomolar concentrations of ouabain was on the bumetanide-sensitive transporter, either under physiological conditions or even more prominent, at elevated concentrations of intracellular Na^+ (Table I). It seems that lowering or eliminating extracellular K^+ or the rise in intracellular Na^+ caused either a faster turnover of ouabain binding (Hallaq, Heller and Eylam, unpublished observations) or alternatively, it increased the occupancy of the high affinity sites for ouabain [31], or both. This in turn

may have caused a slight reduction in the activity of the ouabain-sensitive pump whereas the bumetanide-sensitive transporter became highly activated, perhaps by a so called 'membrane coupling', as explained below [6].

Stimulation of total $^{86}Rb^+$ influx by nanomolar concentrations of ouabain in 'control' or in 'Na^+-loaded' cells was abolished by bumetanide. The data in Fig. 3 and Table I enable us to exclude the sodium pump as the major responding partner to the low, therapeutic doses of ouabain. However, the sodium pump is the only known cellular binding site for cardiac glycosides. Postnatal rat myocytes possess high and low affinity receptor sites for these drugs [22,29,30]. The high-affinity sites bind ouabain primarily at nanomolar concentrations. This in turn may affect the receptor but indirectly modifies the activity of the Na^+/K^+ cotransporter and stimulates it in its 'influx mode' (i.e., activates influx). Toxic doses of ouabain (1 mM) which bind primarily to the low-affinity sites [22,29,30], have been shown to stimulate the cotransporter operating in the opposite direction (i.e., the 'efflux mode'). This has been demonstrated in certain cell cultures such as J774.2 mouse macrophage-like cell line and two of its variants: CT2 (a variant deficient in adenylate kinase) and J7H1 (deficient in the cAMP-dependent protein kinase). It was proposed that high concentrations of ouabain indirectly act on the cotransporter by a so-called 'membrane coupling' [6].

Stimulation of the influx of Rb^+ by the bumetanide-sensitive cotransporter and not by the sodium pump means also stimulation of Na^+ influx, causing a transient elevation of intracellular concentrations of Na^+. Indeed, a bumetanide-sensitive $^{22}Na^+$ uptake, which represents 17% of the total Na^+ uptake component was described in chick cardiac cells in culture [43]. Although the Na^+/H^+ exchange system is considered a major Na^+ uptake pathway in these cells [32], the $Na^+/K^+/Cl^-$ cotransporter represents $69 \pm 15\%$ of the activity of the Na^+/H^+ antiporter [43]. Hence this transient increase in $[Na^+]_i$ could affect the Na^+/Ca^{2+} exchanger of the sarcolemma [44,45]. Therefore, the transient increase in $[Na^+]_i$ by low, nanomolar concentrations of ouabain acting via the bumetanide-sensitive cotransporter, could provide an explanation for the inotropic effects of therapeutic doses of cardiac glycosides. Thus, following the transient rise in $[Na^+]_i$, an independent or perhaps simultaneous activation of two systems which extrude excessive Na^+, i.e., the Na^+/Ca^{2+} exchanger and the Na^+/K^+-ATPase could occur, depending upon their relative activities and their $K_m[Na^+]_i$ ratio. This may, perhaps, be the explanation for inconsistent observations of positive inotropy elicited by nanomolar concentrations of ouabain in different in vitro preparations.

More studies are needed to further pursue the proposed hypothesis on the stimulatory effect of nanomolar concentrations of ouabain on the $Na^+/K^+/2Cl^-$ cotransporter and its inotropic effects. The stimulation of bumetanide-sensitive Na^+ influx coupled to the bumetanide-sensitive K^+ influx which leads to an entry of Ca^{2+} via the Na^+/Ca^{2+} exchanger has to be further explored.

Acknowledgements

These studies were supported in part by grants obtained from the Chief Scientist, Ministry of Health.

References

1 Akera, T. (1981) in Handbook of Experimental Pharmacology (Greeff, K., ed.), Vol. 56/1, pp. 287–336, Springer, New York.
2 Erdmann, E. (1981) in Handbook of Experimental Pharmacology (Greeff, K., ed.), Vol. 56/1, pp. 337–380, Springer, New York.
3 Smith, T.W. and Barry, W.H. (1983) Curr. Topics Membr. Transp. 19, 843–884.
4 Noble, D. (1980) Cardiovasc. Res. 14, 495–514.
5 Scholz, H. (1986) in Cardiac Glycosides 1785–1985 (Erdmann, E., Greeff, K. and Skou, J.C., eds.), pp. 181–188, Springer, New York.
6 Bourrit, A., Atlan, H., Fromer, I., Melmed, R.N. and Lichtstein, D. (1985) Biochim. Biophys. Acta 817, 85–94.
7 Aiton, J.F., Chipperfield, A.R., Lamb, J.F., Ogden, P. and Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 389–398.
8 Yagev, S., Heller, M. and Pinson, A. (1984) In Vitro 20, 893–898.
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
10 Panet, R. and Atlan, H. (1980) J. Membr. Biol. 52, 273–280.
11 Panet, R., Fromer, I. and Atlan, H. (1982) J. Membr. Biol. 70, 165–169.
12 Horres, C.R., Wheeler, D.M., Piwnica-Worms, D. and Lieberman, M. (1987) in The Heart Cell in Culture (Pinson, A., ed.), Vol. 3, C.R.C. Press, Boca Raton.

13 Biedert, S., Barry, W.H. and Smith, T.W. (1979) J. Gen. Physiol. 74, 479–494.
14 Werdan, K., Bauriedel, G., Fischer, B., Krawietz, W., Erdmann, E., Schmitz, W. and Scholz, H. (1982) Biochim. Biophys. Acta 687, 79–93.
15 McCall, D. (1979) Am. J. Physiol. 936, C87–C95.
16 Page, E., Goerke, R.J. and Storm, S.R. (1964) J. Gen. Physiol. 47, 531–543.
17 Horres, C.R., Aiton, J.F., Lieberman, M. and Johnson, E.A. (1979) J. Mol. Cell. Cardiol. 11, 1201–1205.
18 Barry, W.H., Liechty, L., Beaudoin, D. and Smith, T.W. (1982) Trans. Assoc. Am. Phys. 95, 12–21.
19 Murphy, E., Aiton, J.F., Horres, R. and Lieberman, M. (1983) Am. J. Physiol. 14, C316–C321.
20 Eisner, D.A., Vaughan-Jones, R.D. and Lederer, W.J. (1983) Circ. Res. 53, 834–835.
21 Smith, T.W., Antman, E.M., Friedman, P.L., Blatt, C.M. and Marsh, J.D. (1984) Prog. Cardiovasc. Dis. 26, 413–441.
22 Erdmann, E., Werdan, K. and Brown, L. (1985) Trends Pharmacol. Sci. 6, 293–295.
23 Erdmann, E., Philipp, G. and Scholz, H. (1980) Biochem. Pharmacol. 29, 3219–3229.
24 Brown, L., Werdan, K. and Erdmann, E. (1983) Biochem. Pharmacol. 32, 423–431.
25 Hamlyn, J.M., Cohen, N. and Blaustein, M.P. (1983) Circulation (Supp. III), 249.
25 Brown, L. and Erdmann, E. (1984) Basic Res. Cardiol. 79, 50–55.
26 Michael, L.H., Schwartz, A. and Wallick, E.T. (1979) Mol. Pharmacol. 16, 136–146.
27 Hougen, T.J., Spicer, N. and Smith, T.W. (1981) J. Clin. Invest. 68, 1207–1214.
28 Smith, T.W., Kim, D. and Barry, W.H. (1984) Basic Res. Cardiol. 79 (Supp.) 140–146.
29 Friedman, I., Schwalb, H., Hallaq, H., Pinson, A. and Heller, M. (1980) Biochim. Biophys. Acta 598, 272–284.
30 Heller, M. (1987) Interactions of cardiac glycosides with cultured heart cells, in The Heart Cell in Culture (Pinson, A., ed.), Vol. 3, Chap. 20, C.R.C. Press, Boca Raton.
31 Werdan, K., Wagenknecht, B., Zwissler, B., Brown, L., Krawietz, M. and Erdman, E. (1984) Biochem. Pharmacol. 33, 1873–1886.
32 Frelin, C., Vigne, P. and Lazdunski, M. (1986) in Cardiac Glycosides 1785–1985 (Erdmann, E., Greef, K. and Skou, J.C., eds.), pp. 207–213, Springer, New York.
33 Kim, D., Cragoe, D. and Smith, T.W. (1987) Circ. Res. 60, 185–193.
34 Palfrey, H.C. and Greengard, P. (1981) Ann. N.Y. Acad. Sci. 372, 291–308.
35 Tupper, J.T. (1975) Biochim. Biophys. Acta 394, 586–596.
36 Geck, P., Pietrzyk, C., Burkhardt, B.C., Pfeiffer, B. and Heinz, E. (1980) Biochim. Biophys. Acta 600, 432–447.
37 McRoberts, J.A., Erlingen, S., Rindler, M.J. and Saier, M.H. (1982) J. Biol. Chem. 257, 2260–2262.
38 Atlan, H., Snyder, D. and Panet, R. (1984) J. Membr. Biol. 81, 181–188.
39 Panet, R., Snyder, D. and Panet, R. (1985) Biochim. Biophys. Acta 816, 278–282.
40 Panet, R. (1985) Biochim. Biophys. Acta 813, 141–144.
41 Panet, R., Amir, I. and Atlan, H. (1986) Biochim. Biophys. Acta 859, 117–121.
42 Panet, R., Digregorio, D.M. and Brown, R.H. (1987) J. Cell Physiol. 132, 57–64.
43 Frelin, C., Chassande, D. and Lazdunski, M. (1986) Biochem. Biophys. Res. Commun. 134, 326–331.
44 Reuter, H. and Seitz, N. (1968) J. Physiol. 195, 45–70.
45 Philipson, K.D. (1985) Biochim. Biophys. Acta 821, 367–376.