Br. J. Pharmac. (1981), **73**, 333–340

DOWN-REGULATION OF THE SODIUM PUMP FOLLOWING CHRONIC EXPOSURE OF HeLa CELLS AND CHICK EMBRYO HEART CELLS TO OUABAIN

J.F. AITON, J.F. LAMB & P. OGDEN

Department of Physiology and Pharmacology, Bute Medical Buildings, University of St Andrews, St Andrews, Fife KY16 9TS

1 HeLa cells and primary cultures of embryonic chick heart cells were grown in medium containing low concentrations of ouabain for 24 h.

2 Compared with normal cells, cells grown in ouabain have fewer free sodium pump sites, an increased intracellular sodium concentration and a decreased intracellular potassium concentration. The cells are able to maintain their intracellular ion contents because the remaining pump sites have an increased turnover rate.

3 When cells that have been chronically exposed to ouabain are returned to normal growth medium, the sodium pump site numbers increase; the recovery process begins within 6 to 8 h and is complete within 24 h. Recovery of pump site numbers is primarily dependent upon *de novo* protein synthesis since the protein synthesis inhibitor, cycloheximide, prevents recovery.

Introduction

Although the digitalis group of compounds is widely used in the clinical treatment of cardiac disorders, relatively little is known about the cellular mechanisms underlying the therapeutic actions of digitalis. One reason for this is that in most experiments only the acute effects of the drug have been examined, whereas in clinical practice, digitalis is routinely prescribed over prolonged periods.

In a previous paper we demonstrated that prolonged treatment of an established human cell line with therapeutic concentrations of ouabain caused a large decrease in sodium pump site numbers (Lamb & McCall, 1972; Boardman, Lamb & McCall, 1972). The present paper extends these studies to another human cell line (HeLa) and also to primary cultures of spontaneously contracting chick embryo heart cells.

The results obtained confirm that chronic exposure to low concentrations of ouabain causes a decrease in sodium pump site numbers of both HeLa and chick heart cells. The response appears to be analogous to receptor site 'down-regulation' which is a feature of many different ligand-receptor interactions (see Catt, Harwood, Aguilera & Dufau, 1979 for a review).

Methods

Cell culture

HeLa cells were grown as previously described in Eagle's Basal Medium supplemented with 10% calf serum (Lamb & McCall, 1972; Aiton & Lamb, 1980). For experimental purposes, monolayers of cells were grown to confluency in 60 mm 'Sterilin' plastic petri dishes (approximately 2×10^6 cells/plate).

Monolayer cultures of spontaneously contracting myocardial cells were derived from 11 day-old embryonic chick hearts subjected to multiple periods of tryptic digestion (Horres, Lieberman & Purdy, 1977; Horres, Aiton & Lieberman, 1979). Non-muscle cell overgrowth was minimised by using the cell cultures within 3 days of plating. The myocardial cells were grown on 35 mm 'Nunclon' petri dishes in Medium 199 supplemented with 10% foetal bovine serum and 2% chick embryo extract. Myocardial cell cultures were incubated at 37°C in a humidified environment of 96% air and 4% CO_2.

Chronic ouabain treatment Twenty four hours before carrying out an experiment, the original growth medium was discarded and replaced with fresh medium (control cells) or medium containing ouabain. Appropriate ouabain concentrations were obtained by dilution from a sterile, 10^{-3}M stock solution of ouabain made up in 136 mM NaCl. Before any experimental measurements were made, the growth medium was discarded and the cells rinsed four times with warm (37°C) Krebs solution in order to remove unbound, extracellular ouabain.

0007-1188/81/060333-008 $01.00

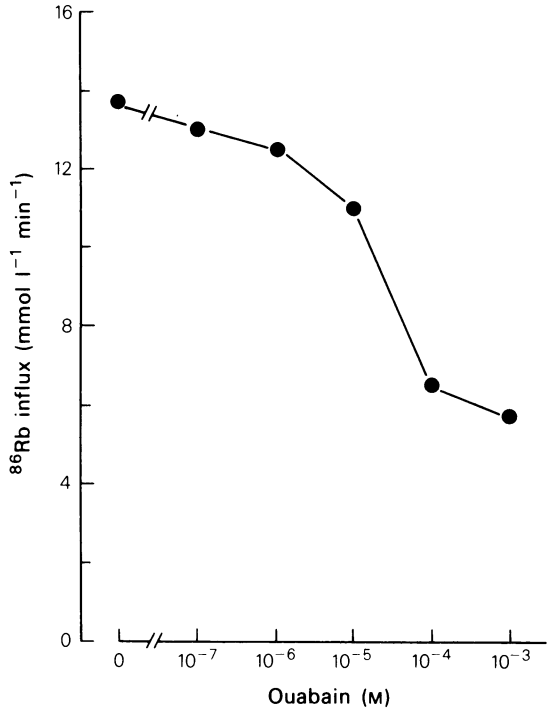

Figure 1 Dose-response curve for the effect of ouabain on the ^{86}Rb influx into monolayer cultures of spontaneously contracting embryonic chick heart cells. Each point is the mean of three observations; standard error < symbol size.

Experimental measurements

The techniques used in this study have been described in detail elsewhere (Lamb & McCall, 1972; Boardman, *et al.*, 1972; Boardman, Huett, Lamb, Newton & Polson, 1974; Aiton & Lamb, 1980) and are summarised below. All experiments were carried out at 37°C.

^{86}Rb influx ^{86}Rb was used as an isotopic tracer to indicate potassium movements, since work in our own, and in other laboratories (Cuff & Lichtman, 1975) has shown that the cells handle ^{86}Rb tracer in the same way as potassium. The ^{86}Rb influx into cell monolayers was measured during a 10 min (HeLa) or 1 min (chick) incubation in Krebs solution containing 0.2 μCi/ml ^{86}Rb. At the end of the influx period, the monolayer was rapidly rinsed four times with ice-cold Krebs solution to remove extracellular isotope and then trypsinised to form a single cell suspension. After trypsinisation, the cell number and mean cell volume of each experimental plate was determined on a Coulter Counter (Model zF) fitted with a Channelyzer attachment (Model C1000). The ^{86}Rb content of the samples was measured in a liquid scintillation spectrophotometer (Packard, model 3320) by the Cerenkov method.

Although we had previously established the ouabain sensitivity of cultured human cells (10^{-7}M ouabain required for half-maximal inhibition of the ^{86}Rb influx; Lamb & McCall, 1972), we lacked comparable data on embryonic chick heart cells.

A preliminary series of experiments established that chick heart cells were some 200 times less sensitive to ouabain than HeLa cells, with half-maximal inhibition of the ^{86}Rb influx being obtained with 2×10^{-5}M ouabain (Figure 1).

With both cell types, maximal inhibition of the ^{86}Rb influx was obtained with 10^{-3}M ouabain. The fraction of the ^{86}Rb influx catalysed by the sodium pump (the ouabain sensitive ^{86}Rb influx) was taken as the difference between the ^{86}Rb influxes measured in the presence and absence of 10^{-3}M ouabain.

[^{3}H]-ouabain binding Sodium pump site numbers were estimated by determining the specific binding of [^{3}H]-ouabain, i.e. the difference between the total [^{3}H]-ouabain binding, measured in K-free Krebs solution, and the non-specific [^{3}H]-ouabain binding, measured in 15 mM-K Krebs solution (Boardman *et al.*, 1972; Baker & Willis, 1972). Monolayers of cells were rinsed four times with Krebs solution at 37°C (either K-free or 15 mM K) and incubated for 15 min in Krebs containing 2×10^{-7}M [^{3}H]-ouabain (0.4 μCi/ml [^{3}H]-ouabain). At the end of the incubation period the cells were rinsed four times with ice-cold Krebs solution and the cell number, mean cell volume and ^{3}H content determined in the usual manner.

Double label technique In some experiments with HeLa cells, a double label technique was used to measure (a) the specific binding of [^{3}H]-ouabain; (b) the ouabain-sensitive ^{86}Rb influx and (c) the rate constant of ^{86}Rb loss, using only two experimental plates. For each plate the sequence was as follows: cells were exposed to ^{86}Rb uptake medium for 10 min; washed for 20 s; treated with K-free medium containing 2×10^{-7}M [^{3}H]-ouabain for 20 min; washed again and trypsinised to form a single cell suspension. The cell number, cell volume, ^{86}Rb and ^{3}H content of this suspension was then determined. The ^{86}Rb influx was calculated from that remaining in the cells plus the amount which appeared in the various washing and loading solutions. The rate constant of ^{86}Rb loss was calculated by dividing the amount of Rb lost during the ouabain labelling period by the initial ^{86}Rb content. The [^{3}H]-ouabain binding was determined in the usual way. The second plate of each pair was treated with 10^{-3}M ouabain during the initial ^{86}Rb influx determination and so gave values for the ouabain-insensitive ^{86}Rb influx and the non-specific [^{3}H]-ouabain binding. The affin-

ity for ouabain was determined by pretreating (for 10 min) the cells with different concentrations of ouabain in K-free medium and then carrying out a double label experiment.

Intracellular ion contents Cell monolayers were rinsed four times with ice-cold, isotonic choline chloride and the cell Na and K extracted during a 3 h incubation in de-ionised water. Na and K content of the extract was determined on an EEL 450 flame photometer. Estimates of cell number and mean cell volume were obtained from plates run in parallel to those used for ion analysis.

Solutions Flux experiments were carried out at 37°C in Krebs solution containing (mmol/1): NaCl 137, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$. $7H_2O$ 1.2, NaH_2PO_4 0.3, KH_2PO_4 0.3, HCl 12, Tris base 14, and glucose 11. Calf serum was added (1% v/v) and the pH adjusted to 7.36. [^{3}H]-ouabain binding experiments were carried out in K-free or 15 mM K Krebs solution. K-free Krebs solution was prepared by omitting KCl, substituting NaH_2PO_4 for KH_2PO_4 and dialysed calf serum for normal serum; 15 mM K Krebs was prepared by adding the appropriate amount of 1 M KCl to K-free Krebs solution.

Materials Whenever possible, inorganic salts of Analar quality were used. Drugs used were ouabain and cycloheximide (Sigma (Lond.) Chemical Co.). ^{86}Rb and [^{3}H]-ouabain were purchased from the Radiochemical Centre, Amersham. Tissue culture supplies were obtained from Flow Laboratories, Irvine, Scotland. New laid fertilised Hubbard hatching eggs were purchased from Buxted Poultry Ltd., Kinglassie Hatchery, Fife.

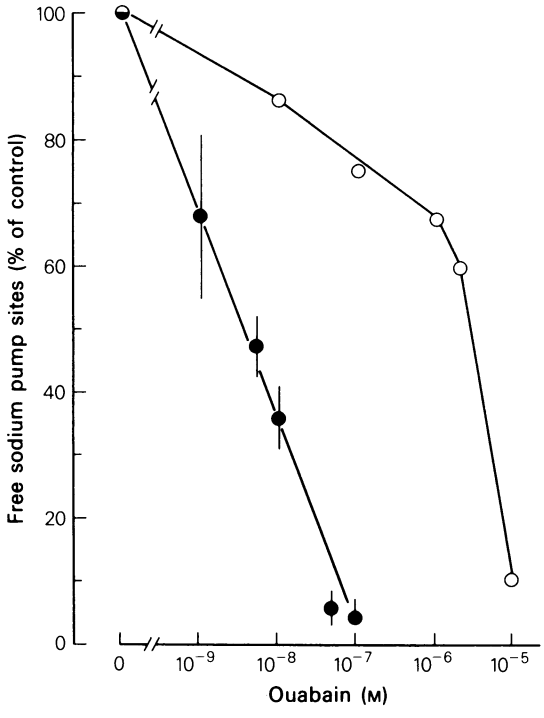

Figure 2 The effect of 24 h growth in different concentrations of ouabain on the sodium pump site numbers of HeLa cells (●) and embryonic chick heart cells (○). The free pump sites remaining after chronic exposure to ouabain are expressed as a % of control values. (HeLa; 0.87×10^6 sites/cell, s.e.±0.03; n=3: chick heart cells; 0.54×10^6 sites/cell, s.e.±0.01; n=4). Vertical bars indicate s.e. mean, in some cases the error < symbol size. Lines fitted by eye.

Results

The number of [^{3}H]-ouabain binding sites present in both HeLa and chick heart cells previously grown for 24 h in media containing different concentrations of ouabain is shown in Figure 2.

In HeLa cells, growth in medium containing approximately 5×10^{-9}M ouabain caused a 50% reduction in the number of free (unblocked) binding sites as measured by a subsequent exposure to [^{3}H]-ouabain. Embryonic chick heart cells were less sensitive and a ouabain concentration of approximately 2×10^{-6}M was required to reduce the number of free binding sites by 50%. This difference in sensitivity shown by HeLa and chick cells during chronic exposure to ouabain paralleled the ouabain sensitivity of the two cell types observed during acute ouabain treatment. Although the results of the previous experiment suggested that prolonged growth of both HeLa cells and embryonic chick heart cells in low concentrations of ouabain reduced the number of free sodium pump sites, we had no evidence to indicate whether the sites which remained after chronic exposure to ouabain had similar characteristics to the sodium pump sites normally present in these cells. We examined this point in a number of ways and, in each case, were unable to distinguish between the sodium pump sites of normal cells and the free sites remaining in cells that had been chronically treated with low concentrations of ouabain.

For example, the acute effects of ouabain on both the ^{86}Rb influx (Figure 3a) and ouabain binding (Figure 3b) were compared in both normal cells and cells grown for 24 h in 10^{-8}M ouabain (HeLa). In each group of cells, both the ^{86}Rb influx and the [^{3}H]-ouabain binding were half-blocked by the acute application of 3.8×10^{-8}M ouabain s.e. 0.44; (n=8). These results showed that (a) the affinity of the free sites remaining in the treated cells is the same as those present in the original population and (b) the same stoichiometric relationship between ouabain binding and sodium pump inhibition holds in normal cells and

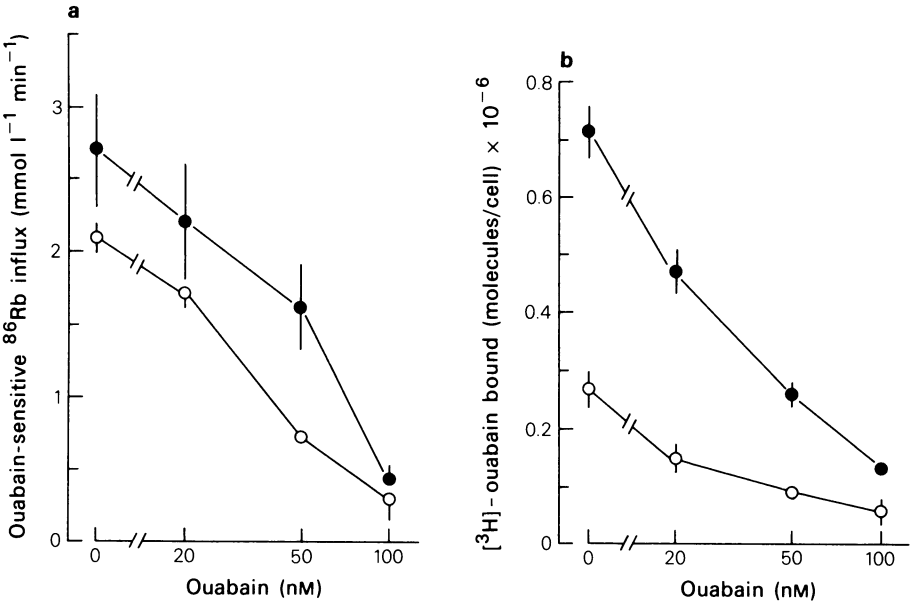

Figure 3 The effect of chronic exposure to 10^{-8}M ouabain on the ouabain affinity of HeLa cells. Control cells (●) or cells which had been grown for 24 h in 10^{-8}M ouabain (○) were pretreated for 10 min with K-free Krebs solution containing the ouabain concentrations shown on the abscissa scale. (a) The ouabain-sensitive ^{86}Rb influx and (b) the free [^{3}H]-ouabain binding sites were measured by the double label experimental technique. Note that growth in ouabain has no effect on the ouabain affinity of the sodium pump. Vertical bars indicate s.e. mean (n=8); in some cases error < symbol size.

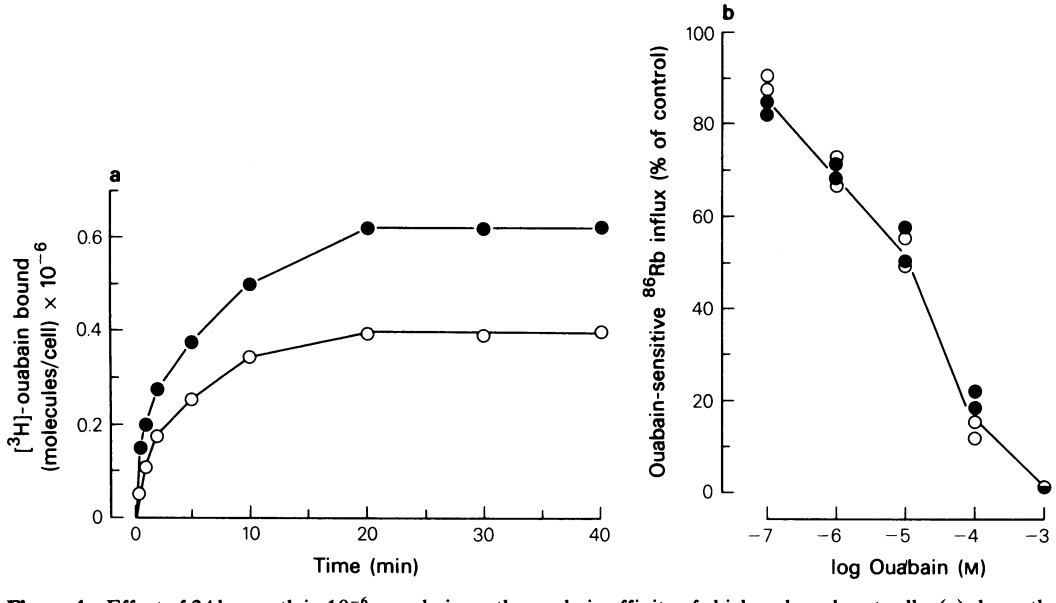

Figure 4 Effect of 24 h growth in 10^{-6}M ouabain on the ouabain affinity of chick embryo heart cells: (a) shows the time course of [^{3}H]-ouabain binding (1×10^{-7}M) to control cells (●) and cells grown from 24 h in 10^{-6}M ouabain (○). In both groups of cells, the half time of binding is approximately 3 min. (b) The ouabain-sensitive ^{86}Rb influx was measured in the presence of the different concentrations of ouabain shown on the abscissa scale. The concentration of ouabain required to half-maximally inhibit the ^{86}R influx is similar in both control (●) and treated cells (○) at 1×10^{-5}M ouabain. Each point is a single observation made with cells from the same culture.

Table 1 The effects of chronic exposure of HeLa cells to 10^{-8}M ouabain

	Control	*Ouabain (10^{-8}M) for 24 h*	P *value*
[Na]	29 ± 1 (14)	44 ± 2 (13)	<0.001
[K]	230 ± 8 (14)	203 ± 6 (13)	<0.02
[^{3}H]-ouabain bound (molecules/cell × 10^{-6})	0.79 ± .01 (7)	0.37 ± .02 (7)	<0.001
Flux/site (^{86}Rb ions site^{-1}s^{-1})	106 ± 9 (12)	178 ± 8 (12)	<0.001
Rate constant of ^{86}Rb efflux (min^{-1})	0.0147 ± .0002 (36)	0.0122 ± .0003 (40)	<0.001
Total ^{86}Rb influx (mmol l^{-1} min^{-1})	3.6 ± 0.2 (5)	3.2 ± 0.2 (5)	<0.2
Total ^{86}Rb efflux (mmol l^{-1} min^{-1})	3.4 ± 0.2 (14)	2.5 ± 0.2 (13)	<0.01

Collected data from four experiments on HeLa cells, comparing control cells and cells grown for 24 h in medium containing 10^{-8}M ouabain. Figures in parentheses indicate the number of observations. The ^{86}Rb influxes and effluxes of control cells (and also ouabain-treated cells) were not significantly different ($P>0.5$ for control cells and $P>0.05$ for ouabain-treated cells) indicating that the cells were in steady state with respect to their ^{86}Rb fluxes.

cells grown for 24 h in 10^{-8}M ouabain. Other experiments with HeLa cells indicated that the affinity of the sodium pump for extracellular potassium was unaffected by chronic exposure to low concentrations of ouabain; in both normal and treated cells, half-maximal activation of sodium pump activity was obtained with 2 mM $[K]_o$ (data not shown).

Comparable results were obtained in a similar series of experiments with embryonic chick heart cells. For example, neither the rate of [^{3}H]-ouabain binding (Figure 4a) nor the ouabain sensitivity of the ^{86}Rb influx (Figure 4b) were affected by 24 h growth in medium containing 10^{-6}M ouabain.

Physiology of chronically treated cells

In a previous series of experiments we noted that cells which had been grown in low concentrations of ouabain for up to 5 days were still capable of maintaining their intracellular sodium and potassium contents in a

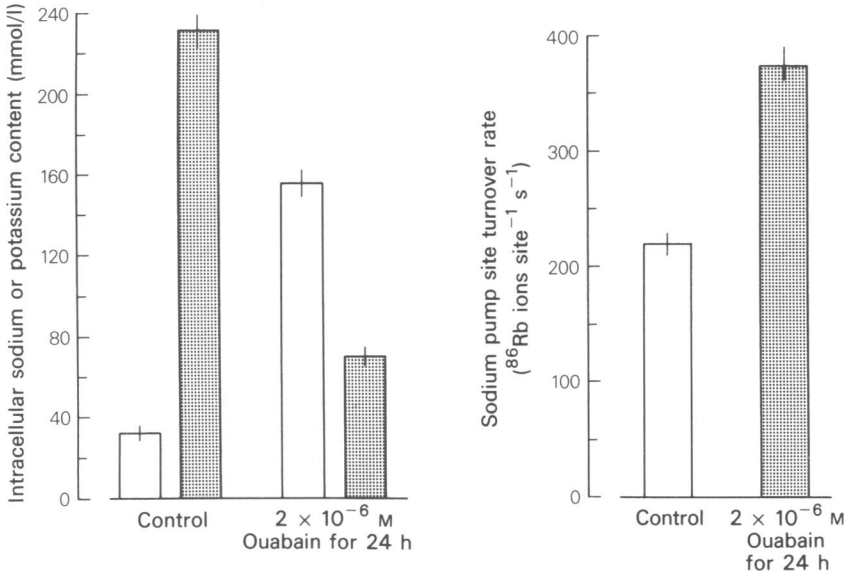

Figure 5 Effect of chronic exposure to 2×10^{-6}M ouabain on the (a) intracellular ion contents and (b) sodium pump turnover rate of embryonic chick heart cells. The intracellular ion contents, ouabain-sensitive ^{86}Rb influx and specific binding of [^{3}H]-ouabain were determined in control cells and cells which had been grown for 24 h in 2×10^{-6}M ouabain. The turnover rate of the sodium pump was calculated by dividing the ouabain-sensitive ^{86}Rb influx by the specific ouabain binding. Chronic exposure to 2×10^{-6}M ouabain causes a 5 fold increase in intracellular sodium concentration (open columns) and a concomitant fall in intracellular potassium concentration (stippled columns). The turnover rate of the sodium pump is 70% greater in ouabain-treated cells (stippled column) than in normal cells (open column). Vertical bars indicate s.e. mean ($n=4$ for ion content; $n=8$ for turnover rate measurement).

steady-state (Lamb & McCall, 1972). In order to establish what mechanisms might be involved in maintaining this steady state, we compared sodium pump site activity in normal cells and in cells chronically exposed to low concentrations of ouabain. Table 1 shows data obtained from HeLa cells grown in the presence or absence of 10^{-8}M ouabain for 24 h.

Cells which had been chronically treated with ouabain had fewer pump sites, an increased intracellular Na concentration and a decreased intracellular K concentration. The cells were able to maintain an ionic balance because the remaining free sodium pump sites had a higher turnover rate. Therefore, although chronic exposure to ouabain caused a decrease in sodium pump site numbers, the cells partially compensated through an increase in the activity of the remaining sites.

Similar results were obtained with embryonic chick heart cells. After 24 h growth in 2×10^{-6}M ouabain, both the intracellular sodium concentration and sodium pump site turnover rate were increased (Figure 5). It is of interest to note that spontaneous contractile activity was still present in cells that had been chronically exposed to ouabain concentrations of 2×10^{-6}M or less. No contractile activity could be observed in cells grown in ouabain concentrations of 10^{-5}M or more.

Recovery

The decrease in the number of free sodium pump sites seen in chronically treated cells is a result of both the prior occupancy of the binding sites by ouabain and sodium pump site internalisation (Lamb & Ogden, 1977; Will, Longworth, Brake & Cook, 1977). During internalisation, the 'sodium pump-ouabain' complex relocates from the plasma membrane to an intracellular compartment before degradation. We might expect, therefore, that two separate mechanisms would be involved in the recovery of sodium pump site number after cells are transferred from medium containing ouabain to control medium. First, part of the recovery would be a consequence of the dissociation of ouabain previously bound to the pump site. Secondly, there is likely to be a component of recovery which is dependent upon the synthesis and insertion of new sodium pump sites in the plasma membrane. This point was examined by growing HeLa cells for 24 h in 10^{-8}M ouabain and then allowing them to recover in normal medium, either in the presence or absence of cycloheximide (100 μg/ml). This concentration of cycloheximide is sufficient to inhibit protein synthesis by > 95% (Boardman *et al.*, 1974). Because the half time of dissociation of ouabain from the pump site is very slow (approximately 20 h, Boardman *et al.*, 1972) and independent of protein synthesis, the cycloheximide-sensitive component of recovery indicates the appearance of newly synthesized sodium pump sites. The time course of recovery shows that new sites first appeared within 7 h whereas cell numbers did not increase until later (Figure 6); recovery of sodium pump site numbers was complete within 24 h. Since cycloheximide blocks this increase in [^{3}H]-ouabain binding, we conclude that recovery of sodium pump site numbers was primarily dependent upon *de novo* protein synthesis. The recovery process in embryonic chick heart cells was essentially similar to that seen in HeLa cells; newly synthesized sodium pump sites were first detected some 8 h after the ouabain-treated cells were transferred to normal medium. Recovery of pump site numbers was, again, blocked by cycloheximide.

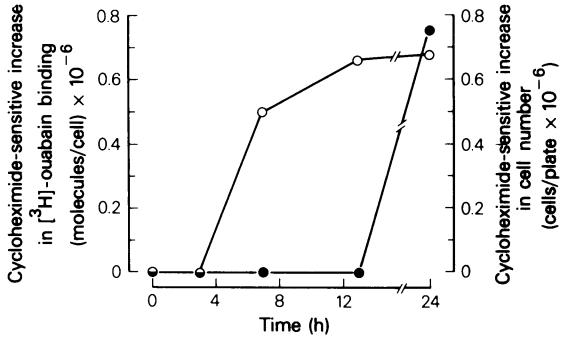

Figure 6 Recovery of sodium pump site number in HeLa cells previously grown for 24 h in 10^{-8}M ouabain. At time zero, cells were transferred to fresh medium or medium containing 100 μg/ml cycloheximide; plates were taken at 0, 3, 7, 13 and 24 h to determine cell numbers and [^{3}H]-ouabain binding. The figure shows the recovery of sodium pump sites (○) and cell numbers (●) expressed as the difference between cells grown in fresh medium and cells grown in medium containing cycloheximide. Standard error < symbol size (n=8 for cell numbers and n=4 for sites).

Discussion

These results show that there is a decrease in the number of sodium pump sites in the plasma membrane of HeLa cells and embryonic chick heart cells chronically exposed to low concentrations of ouabain. The magnitude of this response is dependent upon the concentration of ouabain in the growth medium while recovery of sodium pump site numbers is primarily dependent upon *de novo* protein synthesis.

These features of the interaction between ouabain and the sodium pump are similar to the process of receptor down-regulation which is a frequent consequence of the interaction of hormonal ligands with

their target cell receptors (e.g. the insulin receptor; Soll, Kahn, Neville & Roth, 1975) or drugs with their membrane receptors (e.g. the β-adrenoceptor; Lefkowitz, Limbird, Mukherjee & Caron, 1976).

A source of error that could arise during the estimation of the number of free sodium pump sites would be interference between the ouabain bound during the period of chronic exposure and the [^{3}H]-ouabain used for the subsequent estimation of pump site number. We think this error is negligible for two reasons. First, the 15 min incubation time used in determining the [^{3}H]-ouabain binding is negligible compared to the 20 h half time for the release of ouabain bound to the pump site (Boardman *et al.*, 1972); less than 1% of the ouabain molecules would be released during the incubation period. Secondly, even if all the bound ouabain molecules were released, the maximum increase in the ouabain concentration of the incubation medium would be of the order of 10^{-9}M, again a change of less than 1%.

In the present study, the primary effect of chronic ouabain exposure is greatly to increase the efficacy of the drug; comparable ouabain concentrations have relatively small effects when applied acutely. A direct comparison between the acute and chronic effects of ouabain can be made as follows (data taken from the experiment shown in Figure 3); HeLa cells treated chronically for 24 h with 10^{-8}M ouabain in normal Krebs solution have the same number of free sites (0.27×10^6 sites/cell; s.e.$\pm$0.02; n=3) as those treated acutely for 10 min with 5×10^{-8}M ouabain in K-free Krebs (0.26×10^6 sites/cell; s.e.$\pm$0.01; n=4). The increase in efficacy is equal to the concentration difference (times 5) times the inhibitory effects of potassium on the ouabain binding (times 10; Baker & Willis, 1970; 1972). At a first approximation, therefore, chronic exposure to low concentrations of ouabain leads to a 50 fold increase in drug efficacy.

Various conflicting hypotheses have been advanced to account for the therapeutic effectiveness of the cardiac glycosides. The conventional view would be that there is a causal relationship between the increase in myocardial contractility and sodium pump inhibition (Schwartz, Lindemeyer & Allen, 1975). In this scheme, the positive inotropic effect of ouabain is a consequence of an increased intracellular calcium concentration, which is secondary to an increased intracellular sodium concentration, (Langer & Serena, 1970). This hypothesis is not universally accepted and evidence has been presented that is consistent with other interpretations of glycoside action. For example, some argue for a glycoside effect independent of sodium pump inhibition (Okita, Richardson & Roth-Schechter, 1973; Blood & Noble, 1978) or, in some cases, as a consequence of sodium pump stimulation by low concentrations of ouabain (Blood, 1975; 1978). In these alternative hypotheses, inhibition of sodium pump activity is only associated with the toxic effects of glycoside action (Weingart, 1977).

Two findings in the present study may be pertinent to an understanding of the cellular mode of glycoside action. First, chronic exposure greatly enhances the efficacy of low concentrations of ouabain; ouabain concentrations that produce relatively small effects when applied acutely, cause a marked reduction in the number of free sodium pump sites when applied chronically.

Secondly, although the turnover rate of the remaining free sites is increased in cells chronically exposed to ouabain, because the total number of sites is reduced, the cells maintain a new intracellular ionic steady state with an elevated intracellular sodium concentration. This would reduce the transmembrane sodium gradient, inhibit sodium-calcium exchange and the subsequent increase in the intracellular calcium concentration would be responsible for the positive inotropic response (Blaustein, 1974).

The authors would like to thank Mr J. Taylor, St Andrews University Works Department and Kinglassie Hatchery for help in obtaining eggs; Mr I. Laurie, Mr R. Adam and Mrs M. Falls for technical assistance; Mrs M. Noble and Mrs J. Davidson for preparing the manuscript. We would also like to acknowledge the financial support given by the Wellcome Trust.

References

AITON, J.F. & LAMB, J.F. (1980). The effect of exogenous adenosine triphosphate on potassium movements in HeLa cells. *Q.J. exp. Physiol.*, **65**, 47–62.

BAKER, P.F. & WILLIS, J.S. (1970). Potassium ions and the binding of cardiac glycosides to mammalian cells. *Nature*, **226**, 521–523.

BAKER, P.F. & WILLIS, J.S. (1972). Binding of the cardiac glycoside ouabain to intact cells. *J. Physiol.*, **224**, 441–462.

BLAUSTEIN, M.P. (1974). The interrelationship between sodium and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmac.*, **70**, 33–82.

BLOOD, B.E. (1975). The influence of low doses of ouabain and potassium ions on sheep Purkinje fibre contractility. *J. Physiol.*, **251**, 69–70P.

BLOOD, B.E. Glycoside induced stimulation of membrane Na-K ATPase – fact or artifact. In *Biophysical Aspects of Cardiac Muscle*. ed. Morad, M. pp. 379–383. New York & London: Academic Press.

BLOOD, B.E. & NOBLE, D. (1978). Two mechanisms for the inotropic action of ouabain on sheep cardiac Purkinje fiber contractility. In *Biophysical Aspects of Cardiac Muscle*. ed. Morad, M. pp. 369–378. New York & London: Academic Press.

BOARDMAN, L., HUETT, M., LAMB, J.F., NEWTON, J.P. & POLSON, J.M. (1974). Evidence for the genetic control of the sodium pump density in HeLa cells. *J. Physiol.*, **241,** 771–794.

BOARDMAN, L.J., LAMB, J.F. & McCALL, D. (1972). Uptake of [^{3}H]-ouabain and Na pump turnover rates in cells cultured in ouabain. *J. Physiol.*, **225,** 619–635.

CATT, K.J., HARWOOD, J.P., AGUILERA, G. & DUFAU, M.L. (1979). Hormonal regulation of peptide receptors and target cell responses. *Nature*, **280,** 109–116.

CUFF, J.M. & LICHTMAN, M.A. (1975). Adaptation of potassium metabolism and restoration of mitosis during prolonged treatment of mouse lymphoblasts with ouabain. *J. cell. Physiol.*, **85,** 217–226.

HORRES, C.R., AITON, J.F., & LIEBERMAN, M. (1979). Potassium permeability of embryonic avian heart cells in tissue culture. *Am. J. Physiol.*, **236,** 163–170.

HORRES, C.R., LIEBERMAN, M. & PURDY, J.E. (1977). Growth orientation of heart cells on nylon monofilament: determination of the volume to surface area ratio and intracellular potassium. *J. mem. Biol.*, **34,** 313–329.

LAMB, J.F. & McCALL, D. (1972). Effect of prolonged ouabain treatment on Na, K, Cl and Ca concentration and fluxes in cultured human cells. *J. Physiol.*, **225,** 599–617.

LAMB, J.F. & OGDEN, P. (1977). The turnover of sodium pumps in HeLa cells. *J. Physiol.*, **269,** 79P.

LANGER, G.A. & SERENA, S.D. (1970). Effects of strophanthidin upon contraction and ionic exchange in rabbit ventricular myocardium: relation to control of active state. *J. mol. cell. Cardiol.*, **1,** 65–90.

LEFKOWITZ, R.J., LIMBIRD, L.E., MUKHERJEE, C. & CARON, M.G. (1976). The β adrenergic receptor and adenylate cyclase. *Biochim. biophys. Acta*, **457,** 1–39.

OKITA, G.T., RICHARDSON, F. & ROTH-SCHECHTER, B.F. (1973). Dissociation of the positive inotropic action of digitalis from inhibition of sodium-and-potassium-activated adenosine triphosphatase. *J. Pharmac. exp. Ther.*, **185,** 1–11.

SCHWARTZ, A., LINDENMAYER, G.E. & ALLEN, J.C. (1975). The sodium potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmac. Rev.*, **27,** 3–134.

SOLL, A.J., KAHN, C.R., NEVILLE, D.M. & ROTH, J. (1975). Insulin receptor deficiency in genetic and acquired obesity. *J. clin. Invest.*, **56,** 769–780.

WEINGART, R. (1977). The actions of ouabain on intercellular coupling and conduction velocity in mammalian ventricular muscle. *J. Physiol.*, **264,** 341–365.

WILL, P.E., LONGWORTH, J.W., BRAKE, E.T. & COOK, J.S. (1977). Analysis of intracellular drug (ouabain) sequestration as a mechanism of detoxification. *Mol. Pharmacol.*, **13,** 161–171.

(Received April 17, 1980. Revised August 8, 1980.)