Supplement to
"Basic Research in Cardiology", Vol. 79 (1984)
Editors: R. Jacob (Tübingen) and W. Schaper (Bad Nauheim)

79 (a.)

Cardiac Glycoside Receptors and Positive Inotropy

Evidence for more than one receptor?

Symposium, Munich, October 26–29, 1983

Editor: E. Erdmann (Munich)

With Contributions by L. Brown, W. H. Barry, D. Charlemagne, E. Erdmann, A. A. L. Fox, U. Fricke, W. H. van Gilst, T. Godfraind, K. Greeff, M. Heller, K.-O. Haustein, G. Isenberg, R. Jacob, T. Kazazoglou, D. Kim, K. Kochsiek, J. M. Koomen, W. Krawietz, M. Lazdunski, L. G. Lelievre, H. Lüllmann, P. Mansier, E. Noack, D. Noble, D. Palm, Th. Peters, H.-H. Prillwitz, J. F. Renaud, B. Rossi, J. A. M. Schevers, Th. W. Smith, W. Schmitz, H. Scholz, B. Swynghedauw, B. Wagenknecht, M. F. Wendt-Gallitelli, A. Wellstein, K. Werdan, J. Wilting, A. Ziegler, B. Zwißler

Steinkopff Verlag · Darmstadt

Contents

Cardiac glycoside binding sites in cultured heart muscle cells

K. Werdan, B. Zwißler, B. Wagenknecht, W. Krawietz and E. Erdmann

Medizinische Klinik I der Universität München (FRG)

Summary

Binding of (^{3}H)-ouabain to cultured cardiac muscle and non muscle cells from chicken embryos and neonatal rats has been characterized and correlated with ouabain-induced inhibition of the sodium pump, as well as with the positive inotropic action of the drug.

1. Cardiac muscle and non muscle cells from 10–12 day-old chicken embryos are characterized by a single class of ouabain binding sites (muscle cells: dissociation constant $K_D = 1.5 \times 10^{-7}$M; binding capacity B = 2.6 pmoles/mg cell protein).

2. Two classes of ouabain binding sites, however, have been found in cardiac muscle and non muscle cells from 1–3 day-old, neonatal rats (muscle cells: high affinity, low capacity sites: $K_D = 3.2 \times 10^{-8}$M, B = 0.2 pmoles/mg protein; low affinity, high capacity sites: $K_D = 7.1 \times 10^{-6}$M, B = 2.6 pmoles/mg protein).

3. Half maximal inhibition of active ($^{86}Rb^+ + K^+$)-influx occurs at 5.8×10^{-7}M ouabain in chicken heart muscle cells and at 1.3×10^{-5}M in rat heart muscle cells ([K^+] = 0,75 mM). Decreases in cell-K^+ (EC_{50} = 6.7×10^{-7}M and 1.9×10^{-5}M) and increases in cell-Na^+ (7.4×10^{-7} and 10^{-5}–10^{-4}M) parallel ouabain-induced inhibition of the sodium pump.

4. Up to 10^{-6}M, ouabain does not affect velocity of cell wall motion in cultured rat heart muscle cells. A concentration-dependent increase in cell wall motion is observed at concentrations between 5×10^{-6} and 5×10^{-5}M, being indicative of a positive inotropic effect. At 10^{-4}M ouabain, arrhythmias are present.

5. Our data demonstrate the existence of one single class of cardiac glycoside receptors in cultured cardiac muscle cells from chicken embryos. In rat heart muscle cells, inhibition of the sodium pump, as well as positive inotropy, is coupled to occupation of the low affinity, high capacity cardiac glycoside receptor. In contrast, occupation of the high affinity, low capacity ouabain binding site of rat heart cells does not produce any measurable inhibition of sodium pump activity nor positive inotropy. Nature and function of this high affinity site remain to be elucidated.

Key words: cardiac glycosides, receptor, heart muscle cells, sodium pump, inotropy

Introduction

The discovery of different classes of ouabain binding sites in cardiac cell membranes from adult rats (4) has again stimulated the – still controversial – discussion of the mechanism of the positive inotropic action of cardiac glycosides (15). Subsequently, further experimental evidence has been presented, questioning sodium pump inhibition as the only mechanism of positive inotropic action of this drug: characterization of a high affinity receptor for cardiac glycosides in rat heart, coupled to positive inotropy, but not involved in sodium pump inhibition (4); bimodal inotropic response of the rat heart to cardiac glycosides (1, 6, 8, 11); existence of different forms of ($Na^+ + K^+$)-ATPase molecules (12, 13).

However, when discussing different forms of cardiac glycoside receptors and ($Na^+ + K^+$)-ATPase molecules in the heart, the parallel existence of cardiac muscle and non muscle cells has to be taken into account; the latter mainly consisting of fibroblasts and endothelial cells. Neither in cardiac cell membranes nor in cardiac muscle preparations, binding of cardiac

glycosides to muscle cells can be distinguished from binding to cardiac non muscle cells. The demonstration of high and low affinity binding sites for ouabain in rat heart could therefore reflect different glycoside receptors in different classes of cardiac cells. For exclusion of this possibility, it is necessary to study separately cardiac glycoside binding to cardiac muscle as well as to cardiac non muscle cells.

From hearts of neonatal rats and chicken embryos, cultured cardiac muscle as well as non muscle cells can be obtained. We have used this established technique to characterize cardiac glycoside receptors in muscle and non muscle cells from both species (22, 23). The present paper concentrates on the question: does the intact rat heart muscle cell in culture indeed possess two different classes of cardiac glycoside receptors?

Materials and methods

Materials:

Chemicals were purchased from NEN Chemicals, D-6072 Dreieich, FRG ($^{22}Na^+$, carrier-free; $^{86}RbCl$, 0.9–4.6 mCi/mg; (^3H)-ouabain, 14–20 Ci/mmol); Biochrom, D-1000 Berlin, FRG (Collagenase "Worthington", 125–250 U/mg, CLS II; fetal calf serum; horse serum; CMRL 1415 ATM medium); Serva Biochemica, D-6900 Heidelberg, FRG (Trypsin 1 : 250, No 37 290). All other chemicals were of analytical grade and purchased from Merck, D-6100 Darmstadt, FRG and from Boehringer-Mannheim, D-6800 Mannheim, FRG.

All *methods* used have been described in detail elsewhere (19, 20, 22, 23). In brief, the procedures are: *Cell culture techniques:* Muscle and non muscle cells from 50–200 hearts of 10–12 day-old chicken embryos and 1–3 day-old neonatal rats have been prepared under sterile conditions and separately cultured (19, 22, 23): disaggregation of the hearts by repeated incubation (10 min periods at 37°) in trypsin (0.12%)-collagenase (0.03%)-salt solution (Ca^{2+}, Mg^{2+} free); separate cultivation of muscle cells (seeding density about 10^5 cells/cm^2) and non muscle cells in 25 cm^2 plastic culture flasks (Nunclon plastics, DK-Roskilde, Denmark) at 37° after application of differential attachment technique, in growth medium (CMRL 1415 ATM, bicarbonate free, pH 7.40), supplemented with 10% fetal calf serum and 0.05 mg/ml gentamycin. The medium for cultivation of cardiac muscle cells was additionally supplemented with 10% horse serum. Experiments have been carried out with muscle cells after 2–3 days in culture, with non muscle cells after 1 subcultivation (splitting ratio 1 : 2, detachment of the cells by 0.05% trypsin + 0.02% EDTA in Ca^{2+}, Mg^{2+} free salt solution (Biochrom, Berlin)). At that time, muscle cells had formed a synchronously beating monolayer, non muscle cells were at confluency. The term "non muscle cells" refers to heart cells in culture, lacking sarcomeres, mainly consisting of fibroblasts and endothelial cells (19, 22).

Measurement of ($^{86}Rb^+ + K^+$)-influx, (^3H)-ouabain binding and cellular contents of Na^+ and K^+ in heart cells:

Standard measurements have been carried out at 37° with cells (0.2–2.0 mg protein/flask) in 25 cm^2 plastic flasks (incubation volume 4.1 ml) in serum-supplemented (2.5% fetal calf serum, 2.5% horse serum), Hepes buffered (20 mM, pH 7.40) CMRL 1415 ATM medium with lowered K^+ concentration (0.75 mM). For measurement of the rate of active K^+ influx (20), cells have been incubated for 10 min with ($^{86}Rb^+$) in tracer amounts (2×10^6 cpm/flask); uptake linearly increased with time during this period; more than 90% of ($^{86}Rb^+ + K^+$)-influx could be inhibited by excess (10^{-3}M) of ouabain. Measurement of (^3H)-ouabain binding (about 3.5×10^6 cpm/flask) to the cells has been carried out in a similar manner (see also legend to fig. 1). Cellular K^+ contents have been measured by flame photometry, the exchangeable pool of intracellular Na^+ has been obtained by measurement of cellular $^{22}Na^+$ tracer under equilibrium conditions (20). For washing procedures, lysis of the cells, determination of cellular radioactivity and measurement of cellular protein according to the method of Lowry – using bovine serum albumin as standard – see (19, 20). The data presented in this report are means from closely correlating duplicates or triplicates. All experiments described have been carried out at least three times.

Results

Characterization of (^{3}H)-ouabain binding sites in cultured cardiac muscle cells from neonatal rats and chicken embryos

For comparison, binding experiments with cultured cardiac muscle cells have been carried out under identical conditions, both with cells from chicken embryos, as well as with cells from neonatal rats. In both cell types, (^{3}H)-ouabain binding is composed of specific and non specific binding. Three methods for measurement of non specific (^{3}H)-ouabain binding – which represents about 7 % (chicken cells) and 17 % (rat cells) of total binding at 10^{-7}M – have been applied, yielding identical results: binding to heat-denatured cells (60 min at 60°), binding in the presence of high K^+ (20 mM) or high ouabain (10^{-3}M) concentrations (22, 23). Specific (^{3}H)-ouabain binding to the cells is saturable within 30 min at 6×10^{-8}M, being stable for at least 6 hours. Concentration dependence of specific (^{3}H)-ouabain binding under equilibrium conditions is shown in the experiments of fig. 1 a:

in case of cardiac muscle cells from chicken embryos, saturation of binding is obtained at 10^{-6}M ouabain; in cardiac muscle cells from neonatal rats, however, specific binding increases up to 10^{-5}M ouabain, the highest concentration tested. Analysis of these binding data according to Scatchard (fig. 1 b) demonstrates a straight line in case of heart cells from chicken embryos, being indicative of a single class of ouabain binding sites. In case of heart cells from neonatal rats, on the other hand, a curved plot is obtained. As experimental evidence for negative cooperativity of binding is absent (see below), this finding indicates the presence of more than one class of ouabain binding sites in rat heart muscle cells: applying the program of Weidemann et al (18), binding data are compatible with the presence of two classes of ouabain binding sites: high affinity, low capacity sites, as well as low affinity, high capacity sites. Mean values for dissociation constants and binding capacities are given in table 1. Cardiac non muscle cells have fewer binding sites than cardiac muscle cells, but with similar binding affinities for ouabain: also one class of sites is present in chicken cells, but two classes in rat cells (22, 23).

The existence of one single class of binding sites in chicken heart cells, but of two classes in rat heart cells is further supported by different kinetics of ouabain binding process in these cells: in chicken heart cells, (^{3}H)-ouabain association follows second order, ouabain dissociation single first order reaction; the dissociation constant calculated from the rate constants being in good agreement with the one obtained from measurement of concentration-dependent ouabain binding under equilibrium conditions (22). In rat heart muscle cells, however, biphasic dissociation kinetics are present (table 1), the fast component being due to dissociation from the low affinity site ($t_{1/2} \leqq 12$ min at 37°). the slow component representing dissociation from the high affinity site ($t_{1/2} = 575$ min at 37°). High concentrations of ouabain (10^{-4}–10^{-3}M) do not alter kinetics of ouabain dissociation in rat heart muscle cells. Therefore, the existence of negative cooperativity of the binding sites can be excluded (3).

Further characterization of (^{3}H)-ouabain binding reveals a strong temperature dependency, as tested for binding to the sites in chicken heart cells and to the high affinity sites in rat heart muscle cells (table 1); binding to these sites is strongly suppressed by potassium ions, while ouabain dissociation from these sites is not altered by K^+ (22, 23).

Taking all experimental evidence together, our data demonstrate the existence of a single class of ouabain binding sites in cardiac muscle cells from chicken embryos, but of two classes of binding sites – about 10 % high affinity sites and about 90 % low affinity sites – in cardiac muscle cells from neonatal rats.

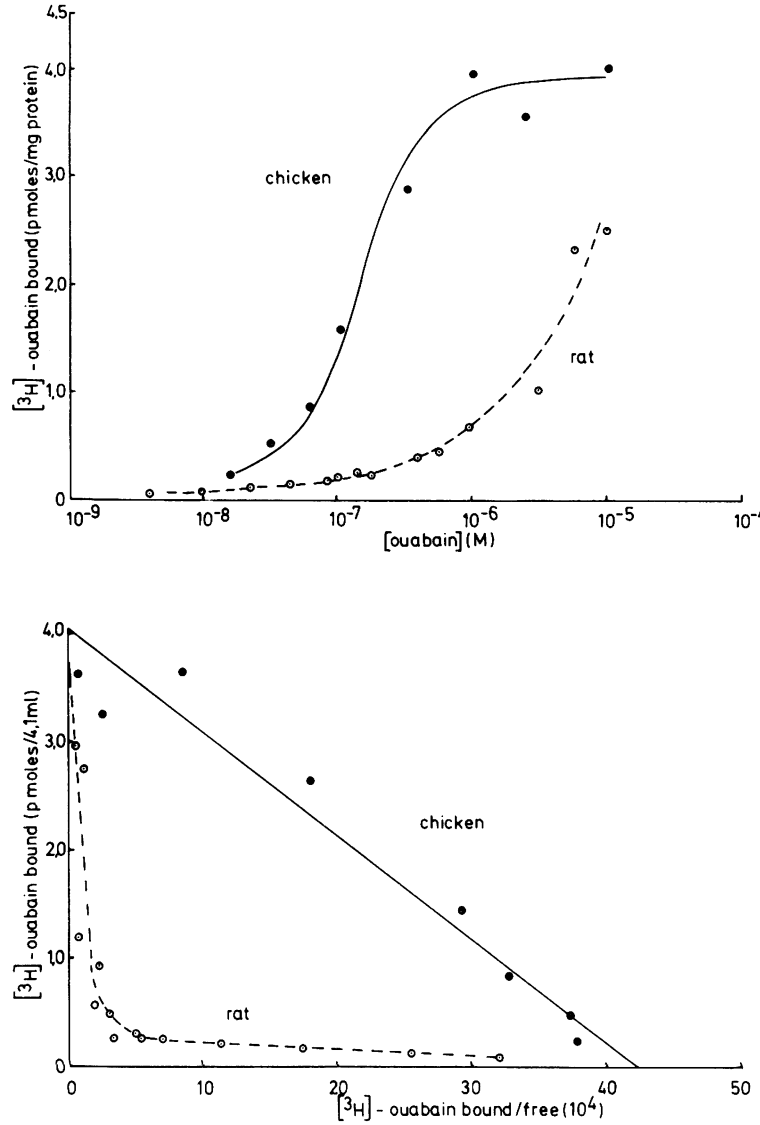

Fig. 1. Concentration dependence of specific (^{3}H)-ouabain binding to cultured heart muscle cells from chicken embryos and neonatal rats.

Fig. a. for measurement of (^{3}H)-ouabain binding, cells have been incubated for 4 hours at 37° in serum-supplemented, Hepes buffered CMRL medium ([K^+] = 0.75 mM) in the presence of (^{3}H)-ouabain concentrations as indicated (see abscissa). For calculation of specific (^{3}H)-ouabain binding, non specific binding at 10^{-3}M ouabain (about 10% (chicken) and 20% (rat) of maximal counts bound) has been subtracted from total (^{3}H)-ouabain binding.

Fig. b. Scatchard plot analysis (16) of binding data presented in fig. a. Chicken cells: linear regression analysis yields the following results: B = 4.3 pmoles/mg protein; $K_D = 2.2 \times 10^{-7}$M; r = 0.98. Rat cells: analysis according to Weidemann et al (18) for two classes of binding sites yields the following values: B_1 = 0.2 pmoles/mg protein, $K_{D1} = 1.6 \times 10^{-8}$M; B_2 = 4.6 pmoles/mg protein, $K_{D2} = 8.9 \times 10^{-6}$M.

Table 1. Characterization of specific (^{3}H)-ouabain binding and of ouabain-induced inhibition of the sodium pump in cultured cardiac muscle cells from chicken embryos and neonatal rats. Values are given as mean from at least 3 experiments each. For further details see (22, 23).

	Cardiac muscle cells from	
	Chicken embryos	Neonatal rats
Ouabain-induced inhibition of ($^{86}Rb^+ + K^+$)-influx (EC_{50},M)	5.8×10^{-7}	1.3×10^{-5}
Ouabain-induced decrease in cellular K^+(EC_{50},M)	6.7×10^{-7}	1.9×10^{-5}
Ouabain-induced increase in cellular Na^+(EC_{50},M)	7.4×10^{-7}	10^{-5}–10^{-4}
	(^{3}H)-ouabain binding sites	
	high affinity	low affinity
Dissociation constant K_D (M)	1.5×10^{-7}	3.2×10^{-8}	7×10^{-6}
Binding capacity			
pmoles/mg cell protein	2.6	0.2	2.6
sites/cell	8.6×10^5	8×10^4	10^6
Association rate constant k_{+1} ($M^{-1}s^{-1}$), 37°	3.2×10^4	$\sim1.5\times10^4$	
Dissociation rate constant k_{-1} (s^{-1}), 37°	4.5×10^{-3}	2×10^{-5}	$\geqq9\times10^{-4}$
Activation energy of			
association (kJ/mol), 9–36°	56	~70	
dissociation (kJ/mol), 9–36°	107	~90	
equilibrium binding (kJ/mol), 9–36°	–63		

Characterization of ouabain binding sites in heart muscle cells from chicken and rat as cardiac glycoside receptors

If ouabain binding sites – as characterized in the preceding section – represent cardiac glycoside receptors, then inhibition of the sodium pump and/or positive inotropic action should result from their occupation.

Sodium pump activity of cardiac muscle cells in culture has been monitored by measurement of ouabain-sensitive ($^{86}Rb^+ + K^+$)-influx (see methods). Concentration-dependent, progressive inhibition of ($^{86}Rb^+ + K^+$)-influx occurs at increasing ouabain concentrations in incubation medium, with EC_{50} values of 5.8×10^{-7}M (cardiac muscle cells from chicken embryos) and 1.3×10^{-5}M (cardiac muscle cells from neonatal rats), respectively. As consequence of sodium pump inhibition, cell-K^+ falls and cell-Na^+ rises (table 1). As can be seen from the experiments of fig. 2, occupation of the ouabain binding sites in chicken cells and occupation of the low affinity binding sites in rat cells are correlated with inhibition of the sodium pump. In contrast, binding of ouabain to the high affinity sites in rat cells does not inhibit ($^{86}Rb^+ + K^+$)-influx.

Increase in amplitude and velocity of cell wall motion represents the equivalent of positive inotropic action in beating cardiac muscle cells in culture (9, 17). In case of cultured cardiac muscle cells from chicken embryos, a concentration-dependent increase in amplitude of cell wall motion is observed between 10^{-7} and 10^{-6}M ouabain (2). The dissociation constant (1.5×10^{-7}M) for specific (^{3}H)-ouabain binding to these cells is within the same concentration range (figs. 1, 2, table 1), demonstrating close coupling of ouabain binding to its binding sites and positive inotropic action.

For beating heart muscle cells from neonatal rats, the concentration-dependent increase in velocity of cell wall motion by ouabain is shown in fig. 3: while 5×10^{-7} and 10^{-6}M ouabain are without any influence, clear-cut increases are shown at 5×10^{-6}-5×10^{-5}M ouabain. A further increase in ouabain concentration (10^{-4}M) leads to arrhythmias. The ouabain effect on contractility is readily reversible: it can be washed out within 5 min (23).

Discussion

High and low affinity ouabain binding sites were first demonstrated in cardiac cell membranes from adult rats (4, 5). This finding can now be extended to the intact rat heart muscle cell: with all experiments carried out under identical conditions, one single class of

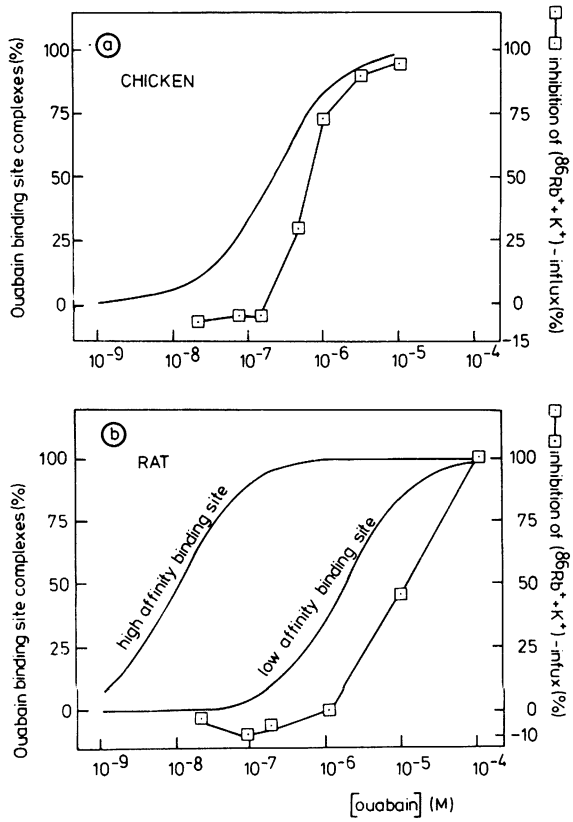

Fig. 2. Effect of (^{3}H)-ouabain binding to its binding sites on ouabain-sensitive ($^{86}Rb^+ + K^+$)-influx rate in cultured cardiac muscle cells from chicken embryos and neonatal rats. Concentration-dependent, specific (^{3}H)-ouabain binding has been carried out and analysed according to Scatchard as described in the experiments of fig. 1. Based on the measured K_D-values, application of law of mass action allows construction of the curves for binding site occupancy (–). Chicken cells: $K_D = 2.0 \times 10^{-7}$M; rat cells: K_{D1} (high affinity site) = 1.1×10^{-8}M, K_{D2} (low affinity site) = 1.7×10^{-6}M. ($^{86}Rb^+ + K^+$)-influx rates have been obtained by incubation of the cells for 5 min with ($^{86}Rb^+$), after a preincubation period of 4 hours at the appropriate ouabain concentration; [K^+] = 0.75 mM. From the amount of ($^{86}Rb^+ + K^+$) taken up, initial transport rates for ($^{86}Rb^+ + K^+$) have been calculated (20).

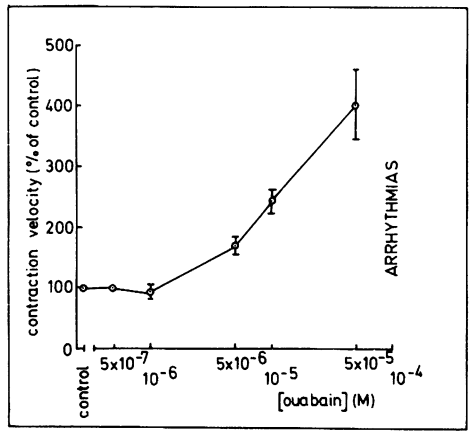

Fig. 3. Effect of ouabain on velocity of cell wall motion in rat heart muscle cells in culture. Beating of electrically driven (pacing rate 100/min; 100 V; pulse duration 10 msec) rat heart muscle cells – cultured on glass coverslips – has been monitored under a phase contrast microscope, using an electro-optical monitoring system ((19); 37°; superfusion of the cells (4 ml/min) with serum-free, Hepes buffered incubation medium (CMRL 1415 with modified Ca^{2+} (0.3 mM) and K^+ (1.5 mM) concentrations)). During course of observation, velocity of cell wall motion has been registered every 30 sec. After a control period of 5 min, ouabain at different concentrations has been added to the perfusion medium. After reaching a new steady state level of velocity of cell wall motion (10 min), ouabain has been washed out by superfusion of the cells with ouabain-free medium; the ouabain effect being readily reversible within 5 min. Velocity of cell wall motion is given as mean ± SEM (n = 3–10), as % of control. Only one ouabain concentration has been tested with each single cell.

ouabain binding sites has been found in cultured cardiac muscle cells from chicken embryos, but two classes of ouabain binding sites are present in rat heart muscle cells. This experimental evidence comes from both: measurement of (^{3}H)-ouabain binding under equilibrium conditions, as well as kinetic studies of (^{3}H)-ouabain binding processes. Results obtained with cardiac cell membranes – prepared from the same tissues as cultured heart cells – further support the existence of different classes of ouabain binding sites in hearts from neonatal rats (22, 23).

In presenting our results, it should be emphasized that work from other laboratories has shown the existence of a single class of saturable high affinity ouabain binding sites (14), as well as the presence of saturable high affinity and non saturable low affinity ouabain binding sites in cultured heart cells from neonatal rats (7); in cardiocytes from adult rats, also a high affinity binding site for ouabain has been described (1). In contrast to our results, high and low affinity binding sites for cardiac glycosides have been also found to occur in cultured cardiac muscle cells from chicken embryos (10); the reason for these contradictory results remains to be clarified.

The single class of ouabain binding sites (about 10^6 sites/cell) – as found under our experimental conditions – in cardiac muscle cells from chicken embryos (fig. 1) fulfills the criteria of a cardiac glycoside receptor: binding of ouabain to these sites results in a positive inotropic effect (2), as well as in inhibition of the sodium pump (fig. 2a). Though no linear coupling of receptor occupancy and sodium pump inhibition exists in chicken cardiac muscle

cells (fig. 2a; for discussion see ref. 21, 22), a linear correlation has been found in cardiac non muscle cells from this species (21, 22).

In case of cultured cardiac muscle cells from neonatal rats, both pharmacological actions of cardiac glycosides can be attributed to occupation of the low affinity, high capacity ouabain binding site (10^6 sites/cell): inhibition of the sodium pump (fig. 2b), as well as positive inotropic action (fig. 3).

The role of the high affinity, low capacity site of rat heart cells (8×10^4 sites/cell), on the other hand, remains to be elucidated: an involvement of this site in sodium pump inhibition can be clearly ruled out, as has been shown for cultured cells (fig. 2b, (23)), as well as for heart tissue of adult rats (4). It remains to be confirmed, whether this site indeed represents part of the ($Na^+ + K^+$)-ATPase molecule (12, 13). No experimental evidence exists at present, demonstrating that occupation of this high affinity site is coupled to positive inotropy in these cells, though this coupling has been clearly shown to occur in ventricular strips from adult rats (1, 4, 11). The reason for this discrepancy is unknown: it may result from methodological limitations of the monitoring system used to measure small increases in amplitude and velocity of cell wall motion (23); coupling of the high affinity receptor with the positive inotropic event may be development-dependent and not be fully established in the cardiocytes of neonatal rats; coupling may be destroyed by the trypsin-collagenase treatment of the heart and may not be fully repaired or renewed during the cell culture period.

At present, three methodological approaches indicate the presence of high and low affinity cardiac glycoside receptors in rat heart: work with cardiac cell membranes, with cardiac muscle preparations and with beating heart muscle cells in culture. Based on the results obtained with cultured rat heart cells, it can be ruled out that the different types of cardiac glycoside receptors only represent receptors of different cell types (e.g. muscle and non muscle cells) of the rat heart.

The expert technical assistance of B. Walther is gratefully acknowledged. This work was supported by the Wilhelm-Sander-Stiftung (78.014.3) and by Deutsche Forschungsgemeinschaft (Er 65/4–3).

References

1. Adams RJ, Schwartz A, Grupp G, Grupp I, Lee SW, Wallick ET (1982) High-affinity ouabain binding site and low-dose positive inotropic effect in rat myocardium. Nature 296:167–169
2. Biedert St, Barry WH, Smith TW (1979) Inotropic effects and changes in sodium and calcium contents associated with inhibition of monovalent cation active transport by ouabain in cultured myocardial cells. J Gen Physiol 74:479–494
3. De Meyts P, Roth J, Neville DM, Gavin JR, Lesniak MA (1973) Insulin interactions with its receptors: experimental evidence for negative cooperativity. Biochem Biophys Res Commun 55:154–161
4. Erdmann E, Philipp G, Scholz H (1980) Cardiac glycoside receptor, ($Na^+ + K^+$)-ATPase activity and force of contraction in rat heart. Biochem Pharmacol 29:3219–3229
5. Erdmann E (1981) Influence of cardiac glycosides on their receptor. In "Handbook of experimental pharmacology", Vol 56/I cardiac glycosides (ed. Greeff K), Springer Verlag Berlin, 337–380
6. Finet M, Noel F, Godfraind T (1982) Inotropic effect and binding sites of ouabain to rat heart. Arch int Pharmacodyn 256:168–170
7. Friedman I, Schwalb H, Hallaq H, Pinson A, Heller M (1980) Interaction of cardiac glycosides with cultured cardiac cells. II. Biochemical and electron microscopic studies on the effects of ouabain on muscle and non muscle cells. Biochim Biophys Acta 598:272–284
8. Grupp IL, Grupp G, Schwartz A (1981) Digitalis receptor desensitization in rat ventricle: ouabain produces two inotropic effects. Life Sciences 29:2789–2794

9. Kaufmann R, Tritthart H, Rodenroth S, Rost B (1969) Das mechanische und elektrische Verhalten isolierter embryonaler Herzmuskelzellen in Zellkulturen. Pflügers Arch 311:25–49
10. Kazazoglou T, Renaud JF, Rossi B, Lazdunski M (1983) Two classes of ouabain receptors in chick ventricular cardiac cells and their relation to (Na^+, K^+)-ATPase inhibition, intracellular Na^+ accumulation, Ca^{2+} influx, and cardiotonic effect. J Biol Chem 258:12163–12170
11. Koomen JM, van Gilst WH, Zimmerman ANE, van Nordwijk J (1982) A concentration-dependent biphasic positive inotropic action of ouabain on isolated hearts of rat and guinea pig. Arch int Pharmacodyn 255:212–219
12. Mansier P, Lelievre LG (1982) Ca^{2+}-free perfusion of rat heart reveals a (Na^+ +K^+)-ATPase form highly sensitive to ouabain. Nature 300, 535–537
13. Mansier P, Cassidy PS, Charlemagne D, Preteseille M, Lelievre LG (1983) Three Na^+, K^+-ATPase forms in rat heart as revealed by K^+/ouabain antagonism. FEBS Letters 153:357–360
14. McCall D (1979) Cation exchange and glycoside binding in cultured rat heart cells. Am J Physiol 236:C87–C95
15. Noble D (1980) Mechanism of action of therapeutic levels of cardiac glycosides. Cardiovascular Res 14:495–514
16. Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660–672
17. Schanne OF, Bkaily G (1981) Explanted cardiac cells: a model to study drug actions? Can J Physiol Pharmacol 59:443–467
18. Weidemann MJ, Erdelt H, Klingenberg M (1970) Adenine nucleotide translocation of mitochondria. Identification of carrier sites. Eur J Biochem 16:313–335
19. Werdan K, Bauriedel G, Bozsik M, Krawietz W, Erdmann E (1980) Effects of vanadate in cultured rat heart muscle cells. Vanadate transport, intracellular binding and vanadate-induced changes in beating and in active cation flux. Biochim Biophys Acta 597:364–383
20. Werdan K, Bauriedel G, Fischer B, Krawietz W, Erdmann, Schmitz W, Scholz H (1982) Stimulatory (insulin-mimetic) and inhibitory (ouabain-like) action of vanadate on potassium uptake and cellular sodium and potassium in heart cells in culture. Biochim Biophys Acta 687:79–93
21. Werdan K, Zwißler B, Wagenknecht B, Krawietz W, Erdmann E (1983) Quantitative correlation of cardiac glycoside binding to its receptor and inhibition of the sodium pump in chicken heart cells in culture. Biochem Pharmacol 32:757–760
22. Werdan K, Wagenknecht B, Zwißler B, Brown L, Krawietz W, Erdmann E (1984) Cardiac glycoside receptors in cultured heart cells: I. Characterization of one single class of high affinity receptors in heart muscle cells from chick embryos. Biochem Pharmacol, 33:55–70
23. Werdan K, Wagenknecht B, Zwißler B, Brown L, Krawietz W, Erdmann E Cardiac glycoside receptors in cultured heart cells. II. Characterization of a high affinity and a low affinity binding site in heart muscle cells from neonatal rats. Biochem Pharmacol, in press

Authors' address:

Dr. Karl Werdan, Medizinische Klinik I, Klinikum Großhadern, Marchioninisstr. 15, D-8000 München 70 (FRG)