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QUANTITATIVE CORRELATION OF CARDIAC GLYCOSIDE BINDING TO ITS RECEPTOR AND INHIBITION OF THE SODIUM PUMP IN CHICKEN HEART CELLS IN CULTURE

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

Medizinische Klinik I, Universität München, Klinikum Großhadern, D-8 München 70, Germany

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The mechanism of positive inotropic action of cardiac glycosides is still a matter of controversy (1). The disagreement mainly relates to the question whether increased force of contraction is caused by, and therefore necessarily accompanied by, an inhibition of the sodium pump. At therapeutic levels of the drug, sodium pump has been described as not influenced, inhibited or even stimulated, depending on the species investigated, the drug applied and the method used (1,2). The problem is further complicated by the presence of two different binding sites for cardiac glycosides. In rat heart, occupation of the high affinity site being linked to positive inotropic effect without inhibition of $(Na^+ + K^+)$-ATPase activity or active $(^{86}Rb^+)$-uptake as measure of sodium pump activity (3). In few of the experiments described, however, has inhibition or stimulation of the sodium pump by cardiac glycosides been correlated quantitatively with the percentage of binding sites occupied by cardiac glycoside molecules. We therefore have measured simultaneously (^3H)-ouabain binding, active $(^{86}Rb^+ + K^+)$-uptake and cellular K^+ in myocardial beating muscle cells as well as in myocardial non muscle cells in culture, derived from chick embryos.

Methods: Muscle and non muscle cells from hearts of 9–12 day-old chick embryos have been prepared and separately cultured as described in detail for rat heart cells (4,5). 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). At that time, muscle cells had formed a synchronously beating monolayer, non muscle cells were at confluency. For measurement of (^3H)-ouabain binding, $(^{86}Rb^+ + K^+)$-uptake and cellular K^+, cells (0.2–2.0 mg protein/flask) were incubated in Hepes buffered (20 mM, pH 7.40)

CMRL 1415 ATM medium (K^+ free), supplemented with 2.5 % fetal calf serum, 2.5 % horse serum and the appropriate ouabain concentration, for 240 min at 37°C, the K^+ concentration adjusted to 0.75 mM. For measurement of (^3H)-ouabain binding under equilibrium conditions, about 5 µCi (^3H)-ouabain/flask (14 Ci/mmol, NEN, Dreieich, Germany) have been added to the medium at the beginning of the incubation period. For measurement of ($^{86}Rb^+$+ K^+)-influx rate (measuring time 10 min), about 1 µCi $^{86}Rb^+$/flask (specific activity 1 mCi/mg; NEN, Dreieich, Germany) has been added to the cells 240 min after starting incubation period with ouabain. 8-10 increasing ouabain concentrations have been applied, to characterize - always within the same experiment - ouabain binding to the cells, as well as the concentration dependent inhibition of ($^{86}Rb^+$+ K^+)-influx and decrease in cellular K^+ by ouabain. Each value is based on triplicate measurements; unspecific (^3H)-ouabain binding is defined as (^3H)-binding to the cells in the presence of 10^{-2} M ouabain. For further experimental details - including measurement of cell protein according to Lowry (6), cellular K^+ by flame photometry, (^3H)-ouabain and ($^{86}Rb^+$)-radioactivity - and also for materials see (4,5). For calculation of the number of ouabain binding sites/cell, cell protein has been determined: heart muscle cells 0.55 mg/10^6 cells (n=6), heart non muscle cells 0.24 mg/10^6 cells (n=12). The term "non muscle cells" refers to heart cells lacking sarcomeres, mainly consisting of fibroblasts and endothelial cells.

Table 1: (^3H)-OUABAIN BINDING SITES, ($^{86}Rb^+$+ K^+)-INFLUX RATES AND CELLULAR K^+ OF CULTURED HEART MUSCLE AND NON MUSCLE CELLS FROM CHICK EMBRYOS (Mean ± SEM, n = 5-17)

$[K^+]$ = 0.75 mM:	HEART MUSCLE CELLS	HEART NON MUSCLE CELLS
(^3H)-OUABAIN BINDING SITES		
dissociation constant K_D (M)	(1.5 ± 0.2) x 10^{-7}	(1.9 ± 0.2) x 10^{-7}
number of binding sites		
pmoles/mg cell protein:	2.6 ± 0.3	2.1 ± 0.1
sites/cell :	860.000 ± 100.000	300.000 ± 15.000
($^{86}Rb^+$+ K^+)-INFLUX RATE		
(nmoles/mg protein x min)	15.4 ± 1.2	7.5 ± 0.8
CELLULAR K^+		
(nmoles/mg protein)	568 ± 39	261 ± 31

Results: (^3H)-ouabain binding experiments demonstrate saturable binding to cultured myocardial muscle and non muscle cells derived from chick embryos. Scatchard plot analysis (7) reveals a single class of binding sites, whose affinity for ouabain is similar both in muscle and non muscle cells (table 1). In both cell types, ($^{86}Rb^+$+ K^+)-influx rate (table 1) can be inhibited, and cellular K^+ pool (table 1) can be decrea-

sed by ouabain in a concentration dependent manner to less than 10 % of control values. According to law of mass action, the percentage of ouabain binding sites occupied by ouabain can be calculated for every ouabain concentration chosen (fig. 1, abscissa). This percentage can then be correlated with the rate of ouabain-sensitive ($^{86}Rb^+$+ K^+)-uptake and with the amount of ouabain-sensitive cellular K^+ at the very same ouabain concentration (see fig. 1, ordinates given as % of control values): in non muscle cells, a stoichiometric relationship exists over the whole ouabain concentration range between glycoside binding sites occupied by ouabain, and reduction of ouabain-sensitive ($^{86}Rb^+$+ K^+)-influx or cellular K^+. Muscle cells, however, behave differently: in these cells up to 40 % of glycoside binding sites can bind ouabain with only minor reduction in ($^{86}Rb^+$+ K^+)-influx and cellular K^+. Further increases in the percentage of binding sites occupied by ouabain then decrease ($^{86}Rb^+$+ K^+)-uptake and cellular K^+ as expected (fig. 1).

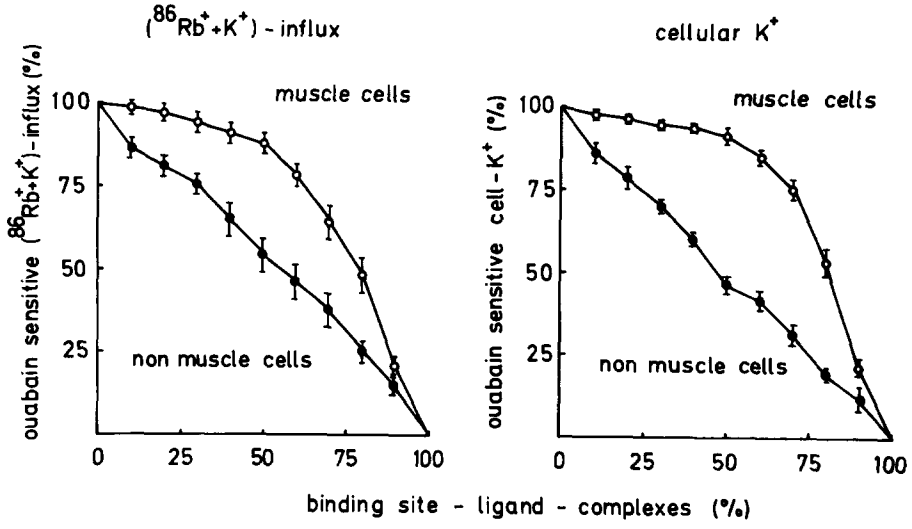

Fig. 1: Effect of (^3H)-ouabain binding to its binding site on ouabain-sensitive ($^{86}Rb^+$)-influx rate and cellular K^+ pool in cultured muscle (o—o) and non muscle cells (•—•) from chick heart. According to K_D= ([O] x [B]) / [BO], the percentage of ouabain binding sites occupied by ouabain BO can be calculated for every ouabain concentration [O] chosen; [B] = concentration of free ouabain binding sites = concentration of total binding sites - [BO]. The calculation is based on the assumption that one ouabain binding site binds one molecule of ouabain. From concentration-effect curves, the values for ouabain-sensitive ($^{86}Rb^+$+ K^+)-influx and cellular K^+ (in % of control without ouabain) have been obtained for the ouabain concentrations producing 10,20,30....90 % of binding site-ligand-complexes. Data are given as mean±SEM; n = 9 for heart muscle cells, n = 5 for heart non muscle cells. For experimental details see "methods".

Discussion: The stoichiometric relationship between (^{3}H)-ouabain binding and ($^{86}Rb^{+}$+ K^{+})-influx inhibition in heart non muscle cells (fig. 1) is in agreement with the inactivation of the (Na^{+}+ K^{+})-ATPase molecule due to binding of an ouabain molecule in vitro (8,9). Similar results have been obtained with erythrocytes (10). The correlation of glycoside receptor occupation and sodium pump inhibition in heart muscle cells, however, does not fit this simple concept at all: only minor effects on ($^{86}Rb^{+}$+ K^{+})-influx and cellular K^{+} are observable even when about 40 % of all glycoside receptors are occupied by oubain molecules (fig. 1). This either means that ouabain binding is not strictly coupled to sodium pump inhibition, or the non-inhibited portion of sodium pump molecules can in part compensate - by increased activity - for the ouabain-inactivated pump molecules (12). Sodium pump activity in chicken heart muscle cells is sustained despite the formation of a considerable amount of glycoside receptor - ouabain - complexes. This could explain the experimental finding that no or disproportionally small inhibition of sodium pump activity and change of cellular Na^{+} and K^{+} have been measured at therapeutic levels of cardiac glycosides (2,12,13).

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