Biochemical Pharmacology, Vol. 33, No. 7, pp. 1161–1164, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

CHRONIC EXPOSURE TO LOW K^+ INCREASES CARDIAC GLYCOSIDE RECEPTORS IN CULTURED CARDIAC CELLS: DIFFERENT RESPONSES OF CARDIAC MUSCLE AND NON MUSCLE CELLS FROM CHICKEN EMBRYOS

Karl Werdan, Gertrud Schneider, Wolfgang Krawietz and Erland Erdmann

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

(Received: 23 December 1983; accepted: 30 January 1984)

Chronic hypokalaemia increases the number of cardiac glycoside receptors in human erythrocytes (4), indicating an increased number of sodium pump molecules. If this adaptation mechanism also occurs in the heart, then patients with chronic K^+ depletion could have an attenuated positive inotropic response to cardiac glycosides, in comparison with acutely K^+-depleted patients (for discussion see (2); 5). However, this adaptation mechanism seems to be cell- and/or species-specific: increased $(Na^+ + K^+)$-ATPase activity in guinea pig myocardial tissue (1,3) and in rabbit heart (7), but decreased number of ouabain binding sites in rat soleus muscle (6). Recently we have characterized the properties of cardiac glycoside receptors in cultured cardiac muscle and non muscle cells from chicken embryos (8,9). These cells provide a useful model to quantitatively study the effect of different degrees of chronic K^+ depletion on the number of cardiac glycoside receptors and sodium pump molecules in cardiac muscle and non muscle cells.

Methods: All materials and methods used have been described in detail previously (8,9): preparation and separate cultivation of cardiac muscle and non muscle cells from 12-13 day-old chicken embryos (disaggregation of heart tissue at 37^o with trypsin(0.12%)-collagenase(0.03%)-salt solution(Ca^{2+},Mg^{2+}free); seeding of the cells($(1.1\text{-}1.8)\times10^5$cells/$cm^2$) in 25 cm^2 Nunclon plastic flasks in CMRL medium supplemented with 0.02 mg(muscle cells) or 0.05 mg(non muscle cells) gentamycin/ml and 10% fetal calf serum (non muscle cells) or 10% fetal calf serum and 10% horse serum in case of muscle cells; measurement of specific (^3H)-ouabain binding under equilibrium conditions (4×10^6 cpm/flask, 14-20 Ci/mmol, NEN Chemicals, D-6072 Dreieich, F.R.G.; incubation period 2 hours; $[K^+]$=0.75

mM, 37°, incubation volume 4.1 ml; measurement of unspecific (^{3}H)-ouabain binding at 10^{-4}M ouabain: 5 % of maximal counts bound); measurement of ouabain-sensitive ($^{86}Rb^{+}$+ K^{+})-influx rates (incubation period with ($^{86}Rb^{+}$): 5 min; $[K^{+}]$=0.75 mM; measurements represent initial velocities of active K^{+}-influx; 37°; $1.6x10^{6}$ cpm ($^{86}Rb^{+}$)/flask; incubation volume 4.1 ml; measurement of ouabain-insensitive influx at 10^{-4}M ouabain: 5 % of total influx); determination of cell protein according to the method of Lowry. After growing the cells for two days in culture (synchronously beating monolayers of muscle cells; confluent cultures of non muscle cells; about 1 mg of cell protein per flask), medium (see above) has been replaced by medium with the desired K^{+} concentrations (5% calf serum (fetal); in case of muscle cells: plus 5% dialysed horse serum; daily medium change). Determination of cell/protein ratio (8) after cultivation of cardiac muscle cells for three days at 5.4 and 0.7 mM K^{+} yielded the following results: $(4.7 \pm 0.8)x10^{6}$ and $(4.8 \pm 1.0)x10^{6}$ cells/mg protein (mean ± SD, n=6). The term "non muscle cells" refers to heart cells lacking sarcomeres, mainly consisting of fibroblasts and endothelial cells (8). The data presented in this report are mean values from closely correlating triplicates. All experiments have been carried out at least three times.

Results: In the presence of low K^{+} (0.7 mM), the capability of cardiac muscle cells from chicken embryos to bind ouabain steadily increases within 20-73 hours of incubation period (fig.1a), reaching a new equilibrium (about 150 % of control (5.4mM K^{+})) after 73 hours. The increased ouabain binding capacity of the cells grown at low K^{+} is due to an increased number of cardiac glycoside receptors: while the receptor affinity -as shown for cardiac non muscle cells in fig.2 - is unaltered by exposure of the cells to low K^{+} (1.0 mM) for three days, binding capacity per mg cell protein increases by 25 ± 8 % (cardiac muscle cells; mean ± SEM, n=5) and 66 ± 15 % (cardiac non muscle cells; n=7) above control (cells grown for 3 days at $[K^{+}]$=5.4 mM). As the cell/protein ratio is not

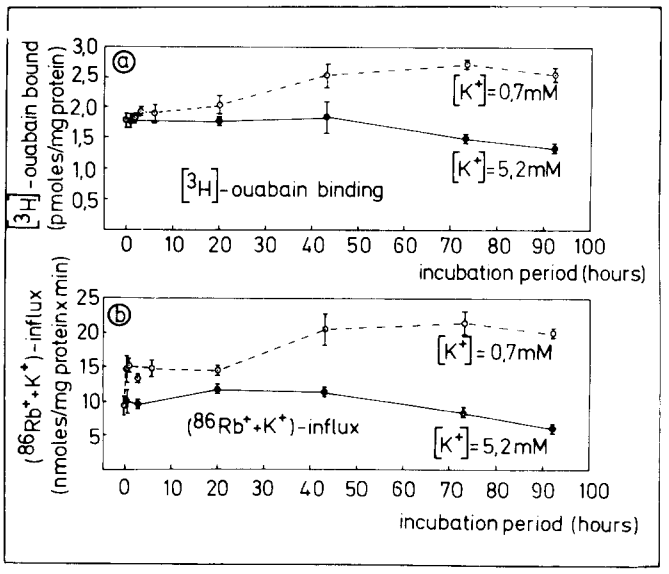

Fig.1: Chronic exposure of cardiac muscle cells from chicken embryos to low K^{+}. Influence on specific (^{3}H)-ouabain binding ($6.8x10^{-8}$M) and on active ($^{86}Rb^{+}$+K^{+})-influx.

altered by low K^+ (see methods), the increase in ouabain binding/ mg cell protein reflects an increase in ouabain binding sites per cell. These additional receptors represent additional, functioning sodium pump molecules (fig. 1b): when measured under identical K^+ concentrations (0.75 mM), a bimodal stimulation of active ($^{86}Rb^+$+K^+)-influx is observed in cardiac muscle cells grown at low K^+: an initial rise - observable within minutes after exposure of the cells to low K^+- is followed by an additional rise of the transport rate, the latter running in parallel with the increase in ouabain binding (fig.1a). Similar results as described in fig. 1 for cardiac muscle cells have been also obtained with cardiac non muscle cells (experiments not shown). With respect to the rise in cardiac glycoside receptor density induced by low K^+, quantitatively different responses have been found in cardiac muscle and non muscle cells (fig.3): in cardiac muscle cells, a significant rise in receptor density only occurs at very low (about 1 mM) K^+ concentrations.

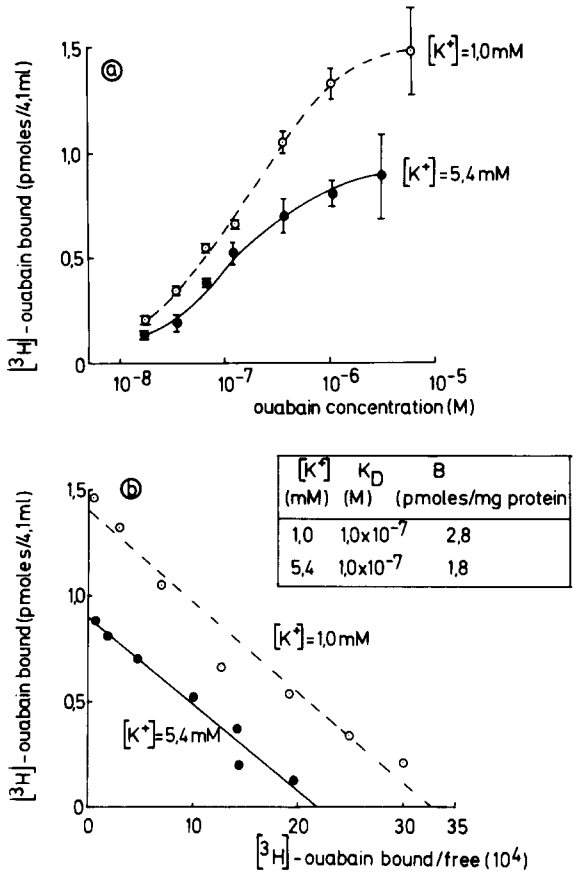

Fig.2: a) Concentration dependence of specific (^3H)-ouabain binding to cultured cardiac non muscle cells from chicken embryos, grown for 3 days at $[K^+]$ = 1.0 and 5.4 mM respectively. Fig. b) Scatchard plot analysis of binding curves presented in fig. a). Linear regression analysis (r = 0.98) yields the data given in the inset.

Discussion: Chronic exposure to low K^+ increases the number of sodium pump molecules and thereby of cardiac glycoside receptors in cardiac muscle and non muscle cells from chicken embryos (fig. 1-3, (5)), without alteration of the receptor affinity for ouabain (fig.2). Two mechanisms are available in these cells, to compensate - at least in part- for the reduction in sodium pump activity, induced by low K^+ (fig.1): a) enhancement of the activity of the sodium pump molecule, observable within minutes after low K^+ exposure, probably due to the increased intracellular Na^+ concentration (10); b) induction of additional sodium pump molecules. The latter process decreases cardiac glycoside sensitivity of chicken heart muscle cells (5), thereby counteracting the

low K^+-induced rise in cardiac glycoside receptor affinity (2,9). The degree of this adaptation mechanism, however, seems to be strongly cell-specific (fig.3): in case of cardiac muscle cells from chicken embryos, only very low external K^+ concentrations (about 1 mM) induce additional sodium pump molecules.

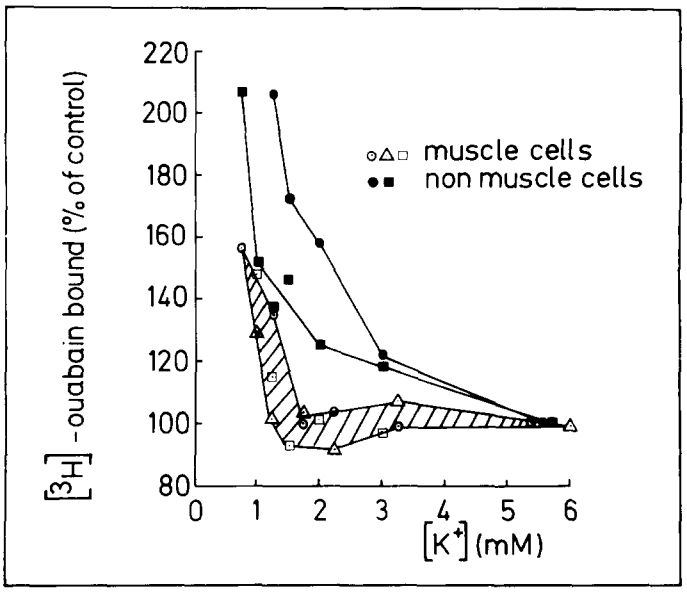

Fig. 3: Specific (^{3}H)-ouabain binding (6.8×10^{-8} M) in cardiac muscle and non muscle cells from chicken embryos: influence of different degrees of chronic (3 day period) K^+ depletion. Control values: muscle cells: 0.9 (o), 1.4 (△) and 1.2 (□) pmoles/mg protein; non muscle cells: 0.5 (•) and 1.0 (■) pmoles/mg protein.

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This work was supported by Wilhelm Sander-Stiftung (78.014.3) and by DFG (Er 65/4-3). The expert technical assistance of Miss Angelika Thomschke is gratefully acknowledged.