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Sodium load and high affinity ouabain binding in rat and guinea-pig cardiac tissue

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1 An estimation of the actual Na/K-ATPase transport activity in intact cardiac cells was made by measuring the binding of [^{3}H]-ouabain to rat and guinea-pig ventricular strips. At the low [^{3}H]-ouabain concentration of 1 nM equilibrium binding was hardly obtained after an incubation time of five hours.

2 Different procedures known to alter the sodium load of the cardiac preparations influenced [^{3}H]-ouabain binding: the sodium ionophore monensin enhanced [^{3}H]-ouabain binding, the local anaesthetic dibucaine and a reduction of external sodium ion concentration diminished [^{3}H]-ouabain binding; [^{3}H]-ouabain binding was similarly affected by these procedures in the rat and guinea-pig.

3 Since [^{3}H]-ouabain binding occurred predominantly at the high-affinity binding sites of rat myocardium under the applied experimental conditions, it was concluded that these binding sites represent Na/K-ATPase molecules involved in sodium ion transport.

Introduction

The existence of two populations of ouabain binding sites in rat heart ventricular muscle, both related to positive inotropy, has been shown in recent studies (Erdmann *et al.*, 1980: Adams *et al.*, 1982; Finet *et al.*, 1983; Noel & Godfraind, 1984; Herzig & Mohr, 1984). The low-affinity, high-capacity ouabain binding sites are Na/K-ATPase molecules. It has been suggested that the high-affinity, low-capacity binding sites could be either (a) Na/K-ATPase molecules involved in Na/K-transport, (b) inactive isozymes, or (c) completely different membrane proteins (Adams *et al.*, 1982; Noel & Godfraind, 1984; Herzig & Mohr, 1984). Noel & Godfraind (1984) found that high-affinity ouabain binding corresponded to an inhibition of ATPase-activity in rat heart microsomal preparations. However, it seemed worthwhile to investigate whether ouabain gets bound with high affinity to the ATPase molecules actually involved in the transport of Na- and K-ions in intact rat ventricular tissue. In intact preparations from guinea-pig hearts it has been shown that under conditions of altered sodium load ouabain binding is modified (Dutta & Marks, 1969; Bentfeld *et al.*, 1977; Yamamoto *et al.*, 1979; Alsen *et al.*, 1982; Kennedy *et al.*, 1983; Temma & Akera, 1983). From this, it was concluded that an increased ion transport was due to an increased rate of transport cycles of Na/K-ATPase, which led to a more frequent appearance of the ouabain binding conformation of the enzyme, thus inducing an enhanced ouabain binding. Accordingly, if the high-affinity ouabain binding of rat heart preparations was influenced by an altered sodium load, then the high-affinity binding sites should be Na/K-ATPase molecules participating in ion transport. In other words, an attempt to compare the dependency of ouabain binding on sodium load in intact rat and guinea-pig ventricular tissue seemed worthwhile to see if high-affinity binding of ouabain displays the same properties in both species, which would indicate whether it occurs at binding sites with identical physiological function. [^{3}H]-ouabain binding was measured at a very low [^{3}H]-ouabain concentration of 1 nM, firstly, to avoid an ouabain effect on Na/K-homeostasis, and secondly, to label almost exclusively the high-affinity binding sites in rat ventricular strips.

Methods

Strips were prepared from rat and guinea-pig right ventricles as described previously (Herzig & Mohr, 1984). The preparations were equilibrated for 1 h in an organ bath containing 500 ml of a modified Tyrode solution (in mM: NaCl 136.8, KCl 5.4, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, $MgCl_2$ 1.05, NaH_2PO_4 0.21, glucose 5.5) gassed with carbogen (95% O_2; 5% CO_2) at a stimulation frequency of 1.5 Hz (rectangular pulse, duration 5 ms, intensity about 30% over threshold), and maintained at a constant temperature of 32°C.

Thereafter conditions were modified as described in the Results section; after 10 min 1 nM [^{3}H]-ouabain was added to the organ bath. At the indicated time intervals after addition of [^{3}H]-ouabain (Figure 1) two strips were removed, blotted between two sheets of filter paper, weighed and dissolved overnight in 2 ml Soluene 350 (Packard Instruments) at 40°C. After the addition of 10 ml Dimilume 30 (Packard) the radioactivity was determined by liquid scintillation counting (Packard Tri Carb 460 C) at a counting efficiency of about 35%. In each experiment control values of [^{3}H]-ouabain binding to ventricular strips under unchanged conditions were established.

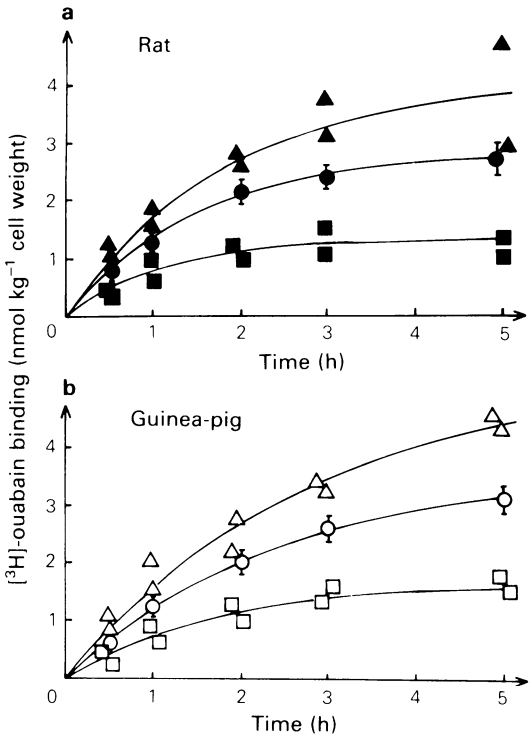

Figure 1 Specific [^{3}H]-ouabain binding as a function of time in rat (a; filled symbols) and guinea-pig (b; open symbols) ventricular strips in the presence of 1 nM [^{3}H]-ouabain. The data represent the difference between total [^{3}H]-ouabain binding and the amount of [^{3}H]-ouabain accumulated in the extracellular space, which has been assumed to amount to 30% of tissue wet weight in every experiment. In the control groups (circles) each point represents the mean value, and vertical lines s.e.means, of all control experiments (n = 50 in rat; n = 40 in guinea-pig). In the case of 3 μM monensin (triangles) and reduction of external sodium to 83.5 mM (squares) single data are depicted, i.e. each point represents the value of specific [^{3}H]-ouabain binding in one ventricular strip. The curves are calculated by means of a non-linear regression computer programme (see Results).

Results

The time course of specific [^{3}H]-ouabain binding to rat and guinea-pig ventricular strips under control conditions is shown in Figure 1a and b, respectively. Since equilibrium of binding was hardly obtained after 5 h, the equilibrium value was estimated by fitting an exponential function to the data. A computer programme of non-linear regression analysis according to Marquardt (1963) was applied. The correlation coefficient r was calculated according to Sachs (1984).

The sodium ionophore, monensin, was added in a concentration of 3 μM, which had a positive inotropic action only, i.e. neither an increment of diastolic tension nor arrhythmia occurred (data not shown). [^{3}H]-ouabain binding was increased in both species (Figure 1): rat + 37% (r = 0.89; control group r = 0.96), guinea pig − 36% (r = 0.98; control group r = 0.98).

When the sodium concentration in the bath was lowered to 83.5 mM (iso-osmolarity maintained by addition of sucrose) a marked positive inotropism occurred and [^{3}H]-ouabain binding was reduced (Figure 1): rat − 38% (r = 0.90; control group r = 0.96), guinea pig + 36% (r = 0.98; control group r = 0.97).

The local anaesthetic drug dibucaine was added in concentrations which exerted a marked negative inotropic effect but did not induce asystole; the appropriate concentrations were 1 μM in rat and 3 μM in guinea-pig. [^{3}H]-ouabain binding decreased in both species: rat − 16% (r = 0.94; control group r = 0.94); guinea-pig − 31% (r = 0.97; control group r = 0.94).

Reduction of the stimulation frequency to 0.3 Hz, which induced a positive inotropic effect in rat and a negative inotropic effect in guinea-pig, diminished [^{3}H]-ouabain binding by 15% in rat (r = 0.96; control group r = 0.96) and by 11.5% in guinea-pig (r = 0.97; control group r = 0.96). Increasing the beat frequency to 6 Hz enhanced the contractile force in guinea-pig and reduced contractile force in rat. While the [^{3}H]-ouabain binding was increased by 80% in guinea-pig (r = 0.95; control group r = 0.96), it remained unchanged in rat (r = 0.95; control group r = 0.98).

In order to make sure that in rat ventricular strips mainly the high-affinity binding sites were labelled with [^{3}H]-ouabain, [^{3}H]-ouabain binding was measured in the presence of 300 nM unlabelled ouabain; this concentration equals the K_D of high affinity ouabain binding determined by Erdmann *et al.* (1980) under nearly identical experimental conditions. Specific [^{3}H]-ouabain binding was reduced by 63% (r = 0.60; control group r = 0.98). The correlation coefficient was very low in this experiment because the experimental scatter was comparably high due to the small amount of [^{3}H]-ouabain being still specifically bound. However, the data clearly revealed that 300 nM

unlabelled ouabain induced about a half-maximal reduction of [^{3}H]-ouabain binding, indicating that [^{3}H]-ouabain at 1 nM labels almost exclusively the high-affinity binding sites with a K_D value of about 300 nM. In guinea-pig ventricular strips, 300 nM unlabelled ouabain decreased [^{3}H]-ouabain binding by 41% ($r = 0.92$; control group $r = 0.96$). Thus, ouabain binds with a lower affinity in guinea-pig ventricular strips than in rat ventricular strips; this finding is in accordance with the results from *in vitro* studies (Herzig & Mohr, 1984).

Discussion

A reduction of the external sodium concentration, the local anaesthetic drug dibucaine, and a decrease in beat frequency are all considered to lower the Na^+-influx into the heart muscle cells and hence the amount of Na^+ extruded per unit of time. These procedures reduced [^{3}H]-ouabain binding in rat and guinea-pig ventricular strips, probably because the reduced sodium load resulted in a lower Na/K-ATPase activity. Conversely, when the sodium load was increased in the presence of monensin, both in rat and in guinea-pig ventricular strips an enhanced [^{3}H]-ouabain binding indicated a higher Na/K-ATPase activity. Since these procedures similarly affected [^{3}H]-ouabain binding in rat and guinea-pig ventricular strips, it can be concluded that the high-affinity binding sites of rat depend on sodium load and hence are probably Na/K-ATPases, too.

However, in contrast to the guinea-pig, the increment in beat frequency failed to increase [^{3}H]-ouabain binding in rats. At present, this phenomenon has to remain unexplained; possible reasons may be: a more pronounced elevation of the potassium ion concentration in the extensively branched and tortuous T-tubular system of the rat (Forssmann & Girardier, 1970) leading to a potassium-induced reduction of ouabain affinity; or a decrease in the number of high affinity binding sites could occur at a high beat frequency, if high- and low-affinity binding sites were interconvertible (Mansier & Lelievre, 1982; Grupp *et al.*, 1984). In any case, the absence of a frequency-induced elevation of [^{3}H]-ouabain binding matches the observation that ouabain is less effective at high beat frequencies with regard to the positive inotropism in rats (Arletti & Bazzani, 1982).

In general, this comparative study revealed similar effects on [^{3}H]-ouabain binding in rat and in guinea-pig ventricular strips. The unexpected failure of an increased beat frequency to increase binding in rat heart was an exception, which should be kept in mind. However, the similarities obtained in this study between ouabain binding sites in guinea-pigs and high affinity ouabain binding sites in rats suggest that they have the same functional properties. Although no simultaneous measurements of K^+-uptake and Na^+-extrusion were made, binding of [^{3}H]-ouabain at low concentrations is considered to be appropriate to indicate the actual Na/K-ATPase transport activity in intact organs. Therefore, we conclude that the high-affinity ouabain binding sites of rat, similar to the binding sites of guinea-pig ventricular myocardium, are probably Na/K-ATPase molecules involved in ion transport of intact cells.

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