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Action of ouabain on rat heart: comparison with its effect on guinea-pig heart

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1 The inotropic dose-response curve of ouabain in rat cardiac ventricular strips exceeded a concentration range of two decades (1×10^{-7} M to 3×10^{-5} M) displaying an intermediate plateau phase. In guinea-pig ventricular strips the inotropic ouabain concentrations spanned only one decade (1×10^{-7} M–1×10^{-6} M).

2 Ouabain-intoxication in guinea-pig ventricular strips occurring at 3×10^{-6} M consisted of arrhythmia and contracture, while in rat ventricular strips at the toxic concentration of 1×10^{-4} M only a progressive increase in diastolic tension was observed.

3 By means of atomic absorption spectroscopy the ouabain-induced loss of cellular potassium and gain of sodium in rat ventricular strips was detected only at concentrations of ouabain higher than 10^{-4} M.

4 Ouabain reduced the activity of Na/K-ATPase prepared from rat and guinea-pig cardiac ventricles to half of its maximum at 6.5×10^{-5} M in rat and 1.0×10^{-6} M in guinea-pig, rat heart Na/K-ATPase thus being about 60 fold less sensitive towards ouabain.

5 Specific [^{3}H]-ouabain binding to membrane suspensions prepared from rat and guinea-pig ventricles was characterized by a similar affinity in rat ($K_D = 4 \times 10^{-8}$ M) and guinea-pig ($K_D = 13 \times 10^{-8}$ M). The number of ouabain binding sites in rat membranes was only about 10% of the number found in guinea-pig membranes. In rat the presence of additional ouabain-binding with low affinity and high capacity seemed possible, but could not be verified for methodological reasons.

6 In the light of the biochemical results and binding data, the wider range of ouabain concentration exerting a positive inotropic effect in the rat may be attributed to the existence in the latter of two populations of receptors with different affinities for ouabain and different capacities. In contrast, in the guinea-pig, there is a single population. Nevertheless it is probable that all the receptors in both species are part of the Na/K-ATPase complex and mediate a positive inotropic effect after ouabain-binding in an identical manner.

Introduction

Rat heart was classified as an organ insensitive to cardiac glycosides, until Erdmann *et al.* (1980) demonstrated that in rat heart ventricular strips the dose-effect curve for positive inotropism began at a ouabain concentration as low as 10^{-7} M. This finding was confirmed by Adams *et al.* (1982), Koomen *et al.* (1982) and Finet *et al.* (1982), who additionally pointed out the biphasic shape of the dose-response curve displaying an intermediate plateau phase. Concerning the inhibitory action of ouabain on the activity of rat heart Na/K-ATPase the term 'insensitivity' is still justified, since enzyme inhibition starts at concentrations of about 10^{-5} M (Repke *et al.*, 1965; Allen & Schwartz, 1969; Akera *et al.*, 1969; Borsch-Galetke *et al.*, 1972; Ku *et al.*, 1976; Erdmann *et al.*, 1980). Since Na/K-ATPase is generally considered to represent the receptor for cardiac glycosides (Repke & Portius, 1963; Lee & Klaus, 1971; Schwartz *et al.*, 1975; Akera & Brody, 1978), the reasons for and implications of the discrepancy between the dose-ranges of positive inotropism and enzyme inhibition are a matter of debate. Furthermore, saturable ouabain-binding to rat cardiac membranes or to intact tissue has been shown to occur at low concentrations (Sharma & Banarjee, 1978; Erdmann *et al.*, 1980; Gheyouche *et al.*, 1981; Adams *et al.*, 1982). These match the concentrations, in which ouabain binds specifically to the hearts of guinea-pigs (e.g. Erdmann, 1981). Based on results concerning ouabain binding and the effects of ouabain on con-

tractility and Na/K-ATPase activity the guinea-pig heart is regarded as an organ with medium sensitivity towards cardiac glycosides (e.g. Bentfeld *et al.*, 1977; Busse *et al.*, 1979; Brown *et al.*, 1983). The present study was intended to investigate the effects of ouabain in rat and in guinea-pig cardiac ventricles. It was hoped that by comparison the extraordinary features of ouabain-action in rat heart could be elucidated. A preliminary account of this work has been presented at the Spring Meeting of the German Pharmacological Society (Herzig & Mohr, 1983).

Methods

Force of contraction

Strips (length 10–15 mm, weight 10–20 mg) were prepared from the right cardiac ventricles of rats (male, weighing about 250 g) and guinea-pigs (either sex, weighing about 300 g). They were mounted in organ baths containing 20 ml of a modified Tyrode solution (mM: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 12.0, NaH_2PO_4 0.21 and glucose 5.5), which was continuously gassed with 95% O_2/5% CO_2 and kept at 35°C. The strips were preloaded with 0.75 g and electrically stimulated at 1 Hz (rectangular pulses, duration 3 ms, intensity about 50% over threshold). The force of contraction was recorded isometrically. After an equilibration period of at least 1 h, each preparation was exposed to a single dose of ouabain; the effect was observed for 1 h; within this period the ouabain-reduced increment of contractility had attained an equilibrium.

Cellular content of Na and K

In separate experiments rat ventricular strips were prepared as described in the preceding paragraph and then exposed to ouabain for 30 min. Thereafter the strips were removed and processed as described previously (Bentfeld *et al.*, 1977). Tissue electrolyte content was determined by means of atomic absorption spectroscopy using calibration curves for Na and K, which were established in each experiment. Cellular Na- and K-contents were calculated by subtracting the content in the extracellular space (ECS) from the total content. The ECS represented about 30% of the total tissue weight as determined by the uptake of [^{14}C]-EDTA under control conditions and in the presence of 10^{-4} M ouabain (results not shown).

Na/K-ATPase activity

Rat and guinea-pig cardiac ventricles, which had been frozen and stored at −20°C, were subjected to the Na/K-ATPase preparation procedure described by Matsui & Schwartz (1966). ATPase activity was determined spectrophotometrically by means of the coupled optical assay at 35°C in a medium containing (mM) NaCl 100, KCl 10, $MgCl_2$ 5, EDTA 1, Tris HCl 50, $ATPNa_2$ 1; pH 7.4; an appropriate aliquot of the enzyme preparation; pyruvate-kinase; lactate-dehydrogenase; phosphoenolpyruvate; NADH and the indicated ouabain concentrations. Na/K-ATPase activity was the difference between total ATPase activity and the unspecific activity determined in the presence of ouabain 10^{-3} M.

[^{3}H]- ouabain binding

The procedures used to prepare a crude suspension of cardiac membranes from ventricles and to measure ouabain binding have been described elsewhere in detail (Dunst *et al.*, 1983) and were applied in the same way for both rat and guinea-pig hearts. Briefly, [^{3}H]-ouabain was incubated together with the membranes at 37°C for 90 min in a medium composed of (mM): NaCl 80, $MgCl_2$ 16, Tris HCl 50, $ATPNa_2$ 2.5; pH 7.3 and various concentrations of ouabain. Membranes were separated by a rapid filtration procedure and membrane-bound radioactivity was determined by liquid sintillation counting. Unspecific binding of [^{3}H]-ouabain was taken as its binding in the presence of 5×10^{-4} M unlabelled ouabain; it amounted to less than 5% of total binding. Specific binding, i.e. the difference between total and unspecific binding, was quantified relative to 1 µl of assay volume or relative to 1 mg of membrane protein. In order to investigate the association of [^{3}H]-ouabain, specific binding was initiated by addition of ATP to the reaction medium composed as described above and then 1 ml aliquots were withdrawn at the appropriate points of time and rapidly filtered under reduced pressure; when the equilibrium of [^{3}H]-ouabain binding was attained, dissociation of [^{3}H]-ouabain was measured after addition of an excess of unlabelled ouabain (for details see Dunst *et al.*, 1983).

The cardiac ventricles used in the biochemical experiments were collected from the remnants of the ventricles from which strips had been cut and from the hearts of additional animals. Protein content was determined according to the procedure of Lowry *et al.* (1951).

Results

Effect of ouabain on the force of contraction

The force of contraction of rat ventricular strips was increased by ouabain in the concentration range from 10^{-7} M to 3×10^{-5} M attaining a maximum of 1.6 fold above the control value (Figure 1a). Up to 10^{-5} M

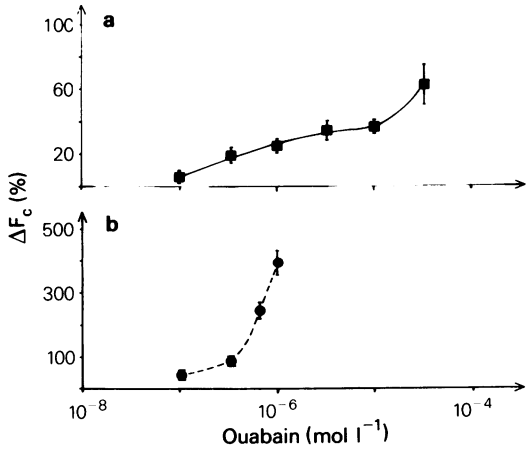

Figure 1 Effect of ouabain on the force of isometric contraction of ventricular strips of rat (a) and guinea-pig (b) hearts. Abscissa scale: ouabain concentration; ordinate scale: increase of the force of contraction as a percentage of the control value. Note the different scales. Indicated are the means and standard errors of 4–17 experiments; each strip was exposed to a single ouabain-concentration.

ouabain the dose-response curve was remarkably flat, only 3×10^{-5} M ouabain inducing a greater increment. Still higher concentrations of ouabain provoked a progressive rise in diastolic tension but no arrhythmia, thus indicating intoxication (Figure 2a).

In guinea-pig ventricular strips a positive inotropic effect of ouabain was also first detectable at 10^{-7} M, 10^{-6} M inducing a 4 fold increment of contractility. However, it has to be pointed out that in one of five preparations arrhythmia occurred; this muscle was excluded from the evaluation of the indicated mean value. At 3×10^{-6} M, ouabain was always toxic. In Figure 2b. two representative mechanograms obtained at this concentration are depicted, illustrating that arrhythmia preceded and then accompanied the toxic contracture.

Effect of ouabain on the cellular K- and Na-content

Ouabain intoxication in guinea-pig hearts has been shown to be parallelled by deterioration of the cellular electrolyte homeostasis (Bentfeld *et al.*, 1977; Busse *et al.*, 1979).

In order to evaluate the relationship between ouabain-induced intoxication and the cellular electrolyte content in rat heart tissue, the K- and Na-content was determined at different ouabain concentrations in rat ventricular strips. As shown in Figure 3 the ouabain-induced loss of potassium and gain of sodium became apparent only at concentrations higher than 10^{-4} M. It should be mentioned that smaller changes in the cellular ionic content at lower concentrations could not be excluded due to the limited sensitivity of the method of atomic absorption spectroscopy. However, in the present context it is important to note that major alterations of the cellu-

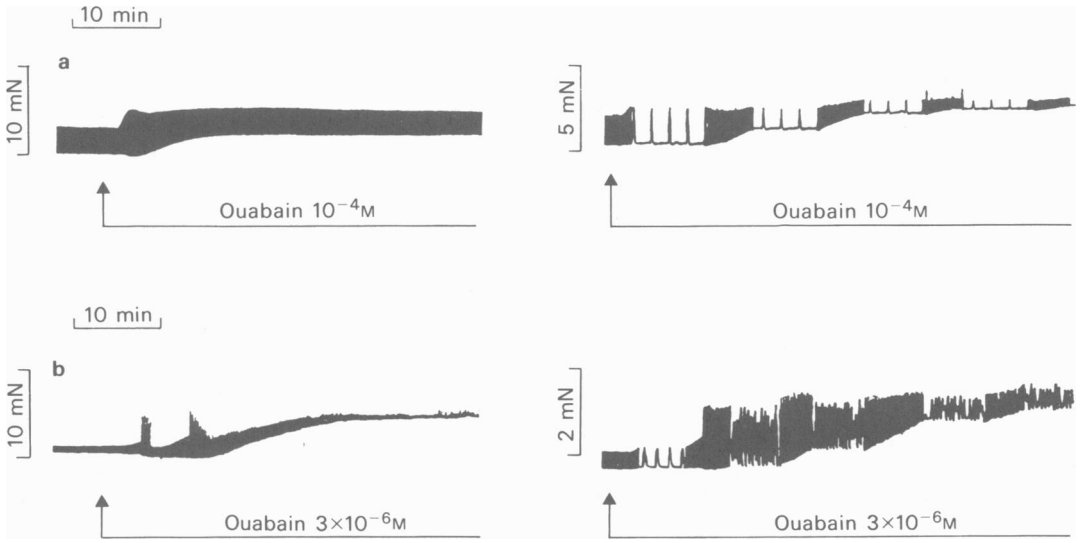

Figure 2 Representative mechanograms of four rat (a) and guinea-pig (b) ventricular strips exposed to toxic concentrations of ouabain. In the mechanograms on the right hand side the speed of the recording was temporarily accelerated 100 fold in order to illustrate that rat ventricular strips followed electrical pacing, while guinea-pig ventricular strips developed tachyarrhythmic spontaneous activity.

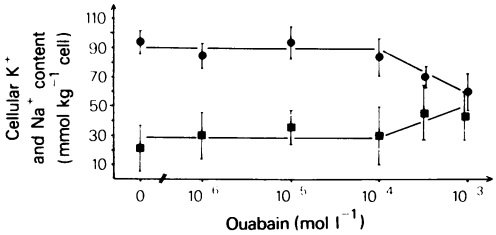

Figure 3 Effect of ouabain on the cellular contents of potassium (●) and sodium (■) in rat ventricular strips measured by the method of atomic absorption spectroscopy. Indicated are the mean values and the standard deviations of at least 8 preparations.

lar electrolyte balance did not occur at ouabain concentrations up to 10^{-4} M, this finding being in accordance with the observation that the toxic concentration of 10^{-4} M did not cause arrhythmia.

Effect of ouabain on the activity of the cardiac Na/K-ATPase

The inhibitory effect of ouabain on cardiac Na/K-ATPase activity in rat or guinea-pig heart has been described in a number of papers but for this comparative study it was considered necessary to measure this effect of ouabain under identical conditions.

About one fourth of both the total rat ATPase activity and the total guinea-pig ATPase activity was not inhibited by 10^{-3} M ouabain. The ouabain-sensitive specific ATPase activity amounted to 70 nmol mg^{-1} protein min^{-1} in the rat heart enzyme preparation and to 77 nmol mg^{-1} min^{-1} in the guinea-pig heart enzyme preparation, respectively. As shown in Figure 4 the dose-effect curves ran in parallel. The ouabain concentration inhibiting enzyme activity by 50% (IC_{50}) was 1.0×10^{-6} M for guinea-pig heart Na/K-ATPase. For rat heart Na/K-ATPase, a more than 60 times higher ouabain concentration was required, the IC_{50} being 6.5×10^{-5} M.

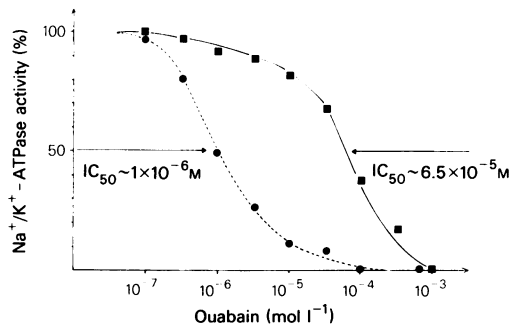

Figure 4 Effect of ouabain on the activity of Na/K-ATPase preparations obtained from rat (■) and guinea pig (●) cardiac ventricles. Na/K-ATPase activity was determined as the fraction of total ATPase activity, which was inhibited by 10^{-3} M ouabain. The Na/K-ATPase activity was plotted on the ordinate scale as percentage of control values. Points indicate the mean values of 2–9 determinations.

Binding of ouabain to cardiac membranes

In rat ventricular membranes, Na,Mg,ATP-dependent [^{3}H]-ouabain binding reached equilibrium after about 20 min of incubation; it remained stable for at least 2 h. The association proceeded with a rate constant $k_{+1} = (7.3 \pm 1.1) \times 10^4 M^{-1} s^{-1}$; the dissociation process followed a first order kinetic and could be described by a rate constant of dissociation $k_{-1} = (2.3 \pm 0.5) \times 10^{-3} s^{-1}$; the equilibrium dissociation constant calculated from these data amounted to $K_D = 3.1 \times 10^{-8}$ M. The indicated values represent the mean and the standard deviation of 4 experiments. The respective kinetic data obtained for guinea-pig cardiac membranes were (Dunst *et al.*, 1983): $k_{+1} = (2.5 \pm 0.6) \times 10^4 M^{-1} s^{-1}$, $k_{-1} = (3.9 \pm 0.5) \times 10^{-3} s^{-1}$, $K_D = 1.6 \times 10^{-7}$ M ($n = 7$). Thus the association of [^{3}H]-ouabain proceeded 3 fold faster in rat cardiac membranes than in guinea-pig membranes and the dissociation was half as fast; accordingly the affinity of ouabain binding to rat cardiac membranes exceeded the affinity of ouabain binding to guinea-pig membranes by a factor of about 5.

Representative experiments concerning the equilibrium binding of ouabain to rat and guinea-pig cardiac membranes are shown in Figure 5. Total [^{3}H]-ouabain binding in the presence of 10 nM [^{3}H]-ouabain was three times as high in guinea-pig membranes as in rat membranes (Figure 5a). When unlabelled ouabain was added in increasing concentrations up to 5×10^{-4} M, [^{3}H]-ouabain binding was reduced, indicating a saturable binding of ouabain. If the [^{3}H]-ouabain binding in the presence of 5×10^{-4} M unlabelled ouabain is assumed to represent the concentration-proportional unspecific ouabain binding, it is possible to calculate the saturable specific [^{3}H]-ouabain binding. Specific binding of [^{3}H]-ouabain was reduced in rat membranes to half of its maximum at $IC_{50} = 5 \times 10^{-8}$ M and in guinea-pig membranes at $IC_{50} = 14 \times 10^{-8}$ M. Taking into account the dilution of the specific activity by addition of unlabelled ouabain, the specific binding of ouabain (= [^{3}H]-ouabain + ouabain) was calculated (Figure 5b). While in guinea-pig membranes a saturation of specific ouabain binding is obvious, in rat membranes ouabain binding seems to increase considerably at higher ouabain concentrations. However, these 'high concentration data' have to be taken with reserve due to the limitations of the method arising from the very small difference between total

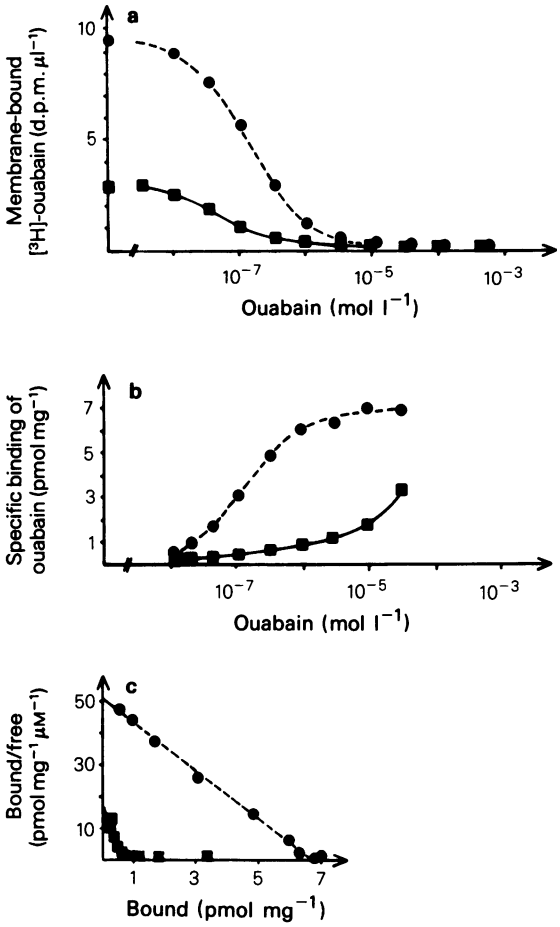

Figure 5 Binding of ouabain to cardiac membranes from rat (■) and guinea-pig (●) ventricles. (a) Reduction of the binding of [^{3}H]-ouabain present in the concentration of 10 nM by increasing concentrations of unlabelled ouabain. (b) Concentration-dependent specific binding of ouabain ([^{3}H]-ouabain + ouabain) per mg membrane protein. (c) Scatchard-plot of the data shown in Figure 5b. The values indicated depict the results of two representative experiments which were performed in a triplicate determination.

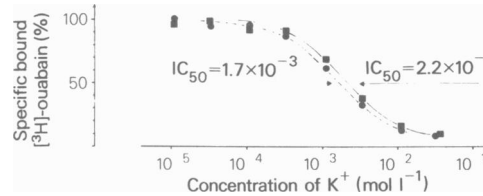

Figure 6 Inhibition of equilibrium specific [^{3}H]-ouabain binding to rat (■) and guinea pig (●) cardiac membranes by potassium ions. [^{3}H]- ouabain was added in a concentration of 9 nM. Specific binding in the absence of potassium ions was taken as 100%. Points represent the mean values of triplicate determinations.

and unspecific [^{3}H]-ouabain binding at these concentrations.

In the case of guinea-pig membranes a Scatchard-plot of the binding data (Figure 5c) revealed saturable binding to a single class of non-cooperative binding sites, yielding a dissociation constant $K_D = 13 \times 10^{-8}$ M and a maximum number of binding sites $B_{max} = 6.7$ pmol mg^{-1} membrane protein. In rat membranes the 'high-concentration' data deviated from the straight line connecting the low-concentration data. When the methodologically unreliable high-concentration data were not taken into account, the ouabain binding at low concentrations could be described by a $K_D = 4 \times 10^{-8}$ M and a $B_{max} = 0.7$ pmol mg^{-1} membrane protein.

In this context it should be mentioned that Erdmann *et al.*, (1980) identified high-affinity [^{3}H]-ouabain binding sites also in the intact cardiac tissue of rat ventricular strips.

Summarizing the findings, both in rat and in guinea-pig cardiac membranes ouabain bound with similar affinity of about 10^{-7} M. The number of binding sites in rat membranes amounted, however, only to about 10% of the number found in guinea-pig membranes. The presence of additional low affinity binding sites in rat membranes could not be excluded.

In an attempt to compare further the properties of this ouabain binding with similar affinity, the influence of potassium ions on the specific binding of [^{3}H]-ouabain (concentration of 9 nM) was measured in both species (Figure 6). The potassium concentrations which reduced [^{3}H]-ouabain binding by 50% were similar: IC_{50} in rat membranes 2.2×10^{-3} M K^+, IC_{50} in guinea-pig membranes 1.7×10^{-3} M K^+. Potassium diminished the equilibrium binding of ouabain by retarding the rate of association more than retarding the rate of dissociation; B_{max} remained unchanged (data not shown).

Discussion

For comparison, the characteristic features obtained with the two species are included in Figure 7.

The increase of force of contraction induced by ouabain in rat ventricular strips was markedly smaller than in guinea-pig ventricular strips. This may in part be due to the different force-frequency relationship: in contrast to guinea-pig ventricular muscle, rat ventricular muscle works near the maximum of contractility at low stimulation frequencies such as 1 Hz (McDowall *et al.*, 1955; Benforado, 1958; Hoffmann & Kelly, 1959; Koch-Weser & Blinks, 1963). Start-

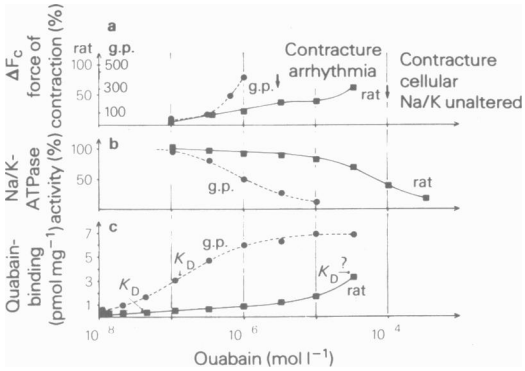

Figure 7 Synopsis of the data on the action of ouabain on cardiac tissue of rats and guinea pigs (g.p.) in order to facilitate comparison of the experimental results. Note, however, that conditions differed among experiments concerning force of contraction (a), Na/K-ATPase activity (b) and ouabain binding (c) with respect to the ionic environment of the Na/K-ATPase.

ing at 10^{-7} M the range of ouabain concentrations having positive inotropic effects was strikingly different in rat and guinea-pig heart: while the dose-response curve spanned only 1 decade in guinea-pig, it clearly exceeded 2 decades in rat. Which of these dose-effect curves has to be considered as an atypical cardiac glycoside dose-effect curve? Assuming that the interaction between ouabain and its pharmacological receptor is not influenced by negative cooperativity, the binding curve will cover approximately 2 decades (Clark, 1933). The inotropic dose-response curve, however, has to be smaller than 2 decades, since at higher receptor occupancy the inhibition of Na/K-ATPase pump capacity and the resulting deterioration of cellular Na/K-homeostasis will impair myocardial cell function, being apparent as ouabain-intoxication (for review see Lüllmann & Peters, 1979). According to this argument the dose-effect curve in guinea-pig hearts behaves as expected, whereas the dose-effect curve of rats is extraordinarily broad.

Rat and guinea-pig heart revealed different types of ouabain intoxication: in guinea-pig, arrhythmia preceded and then accompanied the development of contracture, while in rat hearts only a contracture was observed. Arrhythmia in guinea-pig heart has been demonstrated to occur as the result of a severe disturbance of cellular Na/K-homeostasis induced by toxic ouabain concentrations (Bentfeld *et al.*, 1977).

Since in rat ventricular strips the cellular electrolyte balance was found to be essentially unaltered at the toxic ouabain concentration of 10^{-4} M (Figure 3), the absence of arrhythmia is not surprising. The 'insensitivity' of the Na/K-homeostasis of rat ventricular muscle corresponds to the low sensitivity of rat heart Na/K-ATPase towards ouabain found *in vitro* (cf. Figure 4). In guinea-pig heart the comparably low toxic concentrations of ouabain are in accordance with the 60 fold lower concentrations inhibiting guinea-pig Na/K-ATPase as compared with rat heart Na/K-ATPase (cf. Figure 4). In spite of the limited usefulness of comparing results obtained by different methods the ouabain concentrations for guinea-pig Na/K-ATPase inhibition were rather similar to those at which specific binding of ouabain occurred in guinea-pig cardiac membranes. The difference between the IC_{50} for ATPase inhibition of 1.0×10^{-6} M and the K_D of binding of 1.3×10^{-7} M could be regarded as reflecting the potassium-sensitivity of ouabain binding, as measured in the binding experiment (cf. Figure 6): in the presence of 10 mM K^+ (the concentration also used in the ATPase assay) the affinity of ouabain binding was reduced to 1/10 of the affinity obtained in the absence of K^+.

In rat cardiac membranes the K_D of ouabain binding was 4×10^{-8} M. The 1000 fold higher IC_{50} of ATPase inhibition of 6.5×10^{-5} M cannot be explained as the result of the potassium-induced decrease of ouabain-affinity, since the sensitivity of ouabain binding towards K^+ is almost identical in rat and guinea-pig cardiac membranes (Figure 6). These results are in accordance with findings described by Erdmann *et al.* (1980), who measured within the same experiment [^{3}H]-ouabain binding and ouabain-induced inhibition of Na/K-ATPase and found a dissociation between high affinity binding and low affinity enzyme inhibition.

The inhibition of rat Na/K-ATPase induced by high ouabain concentrations is considered to be the consequence of ouabain-binding in this concentration range. Indeed, the binding experiments did not permit exclusion of the presence of additional low affinity binding sites. Furthermore, it has to be pointed out in this context that the number of high affinity ouabain binding sites in rat cardiac membranes was only 10% of the number found in guinea-pig cardiac membranes. Regarding the higher physiological beat frequency of rat heart compared with that of guinea-pig (Spector, 1956), the Na^+-load and accordingly the Na/K-ATPase transport capacity of rat myocardial cells should at least be similar to guinea-pig heart cells. In agreement with this, enzyme activity in rat and guinea-pig ATPase was found to be similar in our *in vitro* experiments. Since it seems unlikely that rat Na/K-ATPase operates with a 10 fold higher transport velocity, it has to be assumed that 90% of the Na/K-ATPase molecules escaped unambiguous identification as binding sites for ouabain in the binding assay, probably because of their low affinity towards ouabain.

Indeed, the assumption of two populations of

Na/K-ATPase molecules with different ouabain affinity offers the most probable explanation of the atypical effects of ouabain in rat cardiac muscle, as has been also suggested by Adams *et al.* (1982), Finet *et al.* (1982) and Koomen *et al.* (1982). Firstly, there is a small fraction of receptors of high affinity towards ouabain. These receptors are detectable in [^{3}H]-ouabain binding experiments and are characterized by an affinity, which is similar to the affinity of ouabain-receptors in guinea-pig cardiac membranes. Accordingly, the positive inotropic action of ouabain begins at similar concentrations in rat and guinea-pig ventricular strips. However, both the number of the high affinity binding sites and the extent of inotropic responses are considerably smaller in rat than in guinea-pig heart. Secondly, in contrast to guinea-pig there is a major fraction of low-affinity ouabain-receptors. They mediate the additional positive inotropic effect induced by high concentrations of ouabain. These receptors are Na/K-ATPase-molecules, since in the ATPase activity measurements, ouabain-induced inhibition occurred in that high concentration range. Occupancy of these receptors finally leads to the deterioration of cellular Na/K-homeostasis detected in rat ventricular strips at ouabain-concentrations exceeding 10^{-4} M.

In contrast to guinea-pig heart, however, the alteration of Na/K-gradients did not limit ouabain-induced positive inotropism in rat ventricular strips. Since intoxication became apparent as an increase of diastolic tension indicating an elevated diastolic concentration of free Ca-ions in the cytosol, ouabain intoxication was probably caused by a deterioration of the cellular Ca^{2+}-homeostasis. This suggestion is supported by the findings of Olbrich & Preuner (1982), who demonstrated (a) that the capacity of the plasmalemmal Ca-pump to extrude Ca^{2+} from the cells is considerably lower in rat heart than in guinea-pig heart and (b) that this Ca-pump can be completely inhibited by ouabain.

Whereas the low-affinity receptors certainly represent Na/K-ATPase molecules, it cannot be decided from the present results whether the high-affinity receptors are also Na/K-ATPase molecules. In principle, these receptors could consist of catalytically inactive Na/K-ATPase isozymes as suggested by Adams *et al.* (1982), or even of entirely different membrant proteins. The results of the present comparative study, however, suggest similarities between high-affinity ouabain action in rat and guinea-pig heart: (a) the [^{3}H]-ouabain binding experiments revealed a similar affinity of the binding sites in rat and guinea-pig cardiac membranes towards ouabain and towards other cardiac glycosides (data not shown) and indicated a nearly identical sensitivity of these sites towards potassium; (b) in both species a positive inotropic effect occurred in a similar range of ouabain concentration. These findings favour the idea that in rat heart as well as in guinea-pig heart the positive inotropic effect of ouabain is mediated by its binding to active Na/K-ATPase molecules. The fact that in rat an enzyme inhibition could not clearly be demonstrated at low ouabain concentrations in the *in vitro* Na/K-ATPase assay might result from the small proportion of ATPase activity of high affinity receptors contributing to the total activity, which is mainly related to the low-affinity receptors. Measuring the activity of a rat heart Na/K-ATPase, Finet *et al.* (1982) observed a ouabain-induced inhibition in the range from 3×10^{-8} M to 10^{-3} M and suggested the existence of two isozymes with high and low ouabain affinity. Furthermore, Mansier & Lelievre (1982) demonstrated that, under certain experimental conditions, Na/K-ATPase being highly sensitive towards ouabain coexisted together with Na/K-ATPase with low affinity in rat hearts.

The absence of a disturbance of cellular Na/K-contents at medium ouabain concentrations leading to a complete occupation of the small high-affinity Na/K-ATPase fraction is not surprising: Lüllmann & Peters (1979) and recently Werdan *et al.* (1983) showed that remaining unoccupied Na/K-ATPase molecules of myocardial cells can compensate for the inhibition of a certain amount of Na/K-ATPase activity induced by ouabain, probably because the maximum Na/K-ATPase transport capacity is in excess of the activity actually required under the experimental conditions. Due to the limited sensitivity of the method of atomic absorption spectroscopy the possibility cannot be excluded that there might have been a slight increment of the cytosolic Na^+-activity $[Na]_i$, which drove the unoccupied low-affinity Na/K-ATPase molecules at a higher turnover rate, thereby preventing a deleterious Na-accumulation. Recently Philipson & Nishimoto using isolated cardiac sarcolemmal vesicles found the relationship between Na^+-concentration and Na/K-ATPase-activity characterized by an ED_{50} of about 10 mM Na^+ and a Hill coefficient of nearly 3. According to these data, the increase of $[Na]_i$ required for a sufficient pump stimulation would probably have been too small to be detected by means of atomic absorption spectroscopy.

In conclusion, we postulate that the mechanism involved in the transformation of ouabain-binding into its effects is principally identical in rat and guinea-pig hearts. The atypical inotropic dose-effect curve in rat hearts results from the coexistance of two populations of ouabain-receptors with different affinities.

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