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How fast do cardioactive steroids act independently of diffusion in guinea-pig myocardium?

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1 The rate of onset of the inotropic effect of different cardioactive steroids (in order of increasing potency: dihydroouabain, digoxigenin, digoxin, ouabain, digitoxin) was studied in guinea-pig papillary muscles stimulated at 0.5 Hz. For an estimate of diffusion rate the time to defined levels of effect was evaluated in relation to the diameter of cylindrical muscles.

2 The dependence of onset rates on muscle diameter was more pronounced with a highly potent steroid, e.g., digitoxin, than with a less potent compound, e.g., dihydroouabain; that is, the apparent rate of diffusion was inversely correlated with the inotropic potency. Reduction of the ratio of receptor to steroid concentration with increase of ouabain or K^+ concentration enhanced the apparent rate of diffusion.

3 Extrapolation to negligibly short diffusion distance indicated that the effects of the various steroids develop faster in the absence of diffusion. The effect of 50 μmol l^{-1} of dihydroouabain appeared more quickly than with ouabain in the perfused heart. The time courses of the inotropic effect in perfused hearts and in papillary muscles of diameters < 0.75 mm were superimposable, indicating that the onset of the dihydroouabain effect was not controlled by diffusion.

4 After the interference of diffusion had been excluded, the rates of onset of action correlated inversely with the inotropic potencies of the steroids. The dissociation rate of the drug-receptor complex appeared to be related to the different receptor affinities.

Introduction

Both binding to receptors and diffusion appear to limit the onset and offset of the inotropic effect of some cardioactive steroids in guinea-pig papillary muscle (Ebner *et al.*, 1985). These results suggested that the reaction of the steroid with the receptors reduces the free drug concentration in the extracellular space, thereby retarding its diffusion and, subsequently, the occupation of the receptors in the muscle core.

Considering the proposed mechanism, the rates of diffusion of steroids of high and low potency may be expected to differ. In view of the different magnitudes of their equieffective concentrations the low extracellular concentration of a drug with high potency will be reduced to a greater extent than the higher concentration of a less potent steroid by the amount of drug reacting with the receptors. In consequence, diffusion of the more potent compounds should be delayed more effectively. Corresponding to the variable effectiveness of receptor binding as temporal 'site of loss', saturation of receptors with the increase of steroid concentration likewise should become evident in enhancement of diffusion rate. As shown previously (Ebner, 1981; Kenakin, 1984) saturable sites of loss most effectively reduce the free drug concentration in the extracellular space when it is far below saturation of uptake sites.

In view of the postulated, variable influence of diffusion, the rate of action of cardioactive steroids at controlled stimulation frequency and without interference from diffusion seems to be unknown. To estimate the role of diffusion, we determined the dependence of the rate of onset of the inotropic effect of different cardioactive steroids on diffusion distance, i.e., the diameter of cylindrical papillary muscles. For comparison the effects of ouabain and dihydroouabain were evaluated in regularly paced, perfused hearts. These studies have been published in part in preliminary form (Ebner, 1986).

Methods

Experiments on papillary muscles

Cylindrical papillary muscles of widely different diameters from the right ventricle of guinea pigs (250–

350 g) of either sex were connected to a force transducer and incubated in modified Krebs-Henseleit solution. The incubation medium was constantly gassed by 5% CO_2 in O_2; the temperature was 35°C and the pH 7.5. For control conditions the composition of the medium was (mmol l^{-1}): NaCl 115, $NaHCO_3$ 24.9, KH_2PO_4 1.2, $MgSO_4$ 1.2, KCl 4.7, $CaCl_2$ 3.2, and glucose 10. Different K^+ concentrations were adjusted with KCl. Isometric contractions were elicited from a resting force of 3.92 mN. During the initial equilibration period of 1 h, under control conditions, the muscles were stimulated at 1 Hz by square-wave pulses of 3 ms duration at an intensity slightly above stimulation threshold. After additional exchange of the bath medium in the experiments at different K^+ concentrations, stimulation frequency was lowered to 0.5 Hz. When the new steady state of force of contraction was established, the appropriate concentration of a cardioactive steroid was added and the development of its positive inotropic effect was followed. Only one concentration was tested on each muscle. At the end of the experiment the diameter of the muscle was determined in the original experimental set-up under a microscope as described previously (Ebner & Waud, 1978).

Experiments on perfused hearts

One hour before the guinea pigs (300–350 g weight) were killed, they were injected with 500 iu of heparin sulphate intraperitoneally. After dissection, Langendorff heart preparations were perfused at 35°C with the control medium. To suppress automaticity, atria, parts of both ventricles, and the interventricular septum were excised so that only the area perfused by the anterior descending left coronary artery remained. Some of these preparations could be paced regularly at 0.5 Hz via platinum electrodes. After inserting hooks the preparation was connected to a force transducer. Isometric contractions developed from a resting force of 58.8 mN. Dead-space volume of the apparatus was 10 ml; perfusion rate (in ml min^{-1}) was 19.0 ± 0.6 ($n = 4$) and 14.3 ± 0.7 ($n = 3$) in ouabain and dihydroouabain experiments, respectively, at a perfusion pressure of 6.8 kPa. Special care was taken to avoid any development of contracture by adequate perfusion and vigorous bubbling with 5% CO_2 in O_2. After 1 h incubation in control conditions and stimulation at 0.5 Hz, perfusion was changed to a medium containing the respective steroid. The development of the inotropic effect then was followed with time.

Evaluation of experiments

Force of contraction was evaluated from tracings on a pen recorder at chart speeds between 0.16 and 1 mm s^{-1}. The rate of onset was quantified with the time to 10, 25, 50, 75, or 90% of the steady-state effect. Effect refers to increase above pre-drug value; it is expressed as ΔF_c. The values are presented as individual data or arithmetic means ± s.e.mean. Linear regressions were calculated according to the method of least squares. Differences between samples were assessed by the two-tailed t test. Statistical significance was assumed at 5% probability of error.

Drugs and materials

Dihydroouabain was obtained from Hommel AG, Adliswil, Switzerland. Ouabain, digitoxin, digoxin, and digoxigenin were purchased from Serva, Heidelberg, Germany; heparin sulphate was from Nordmark Werke, Uetersen, Germany. Dihydroouabain and ouabain were added as aqueous solution. The stock solutions of digitoxin, digoxin and digoxigenin contained ethanol to give a final concentration of maximally 0.3% in the bath. Control experiments with ouabain indicated similar rates of onset of the inotropic effect, irrespective of the absence or presence of 0.3% ethanol.

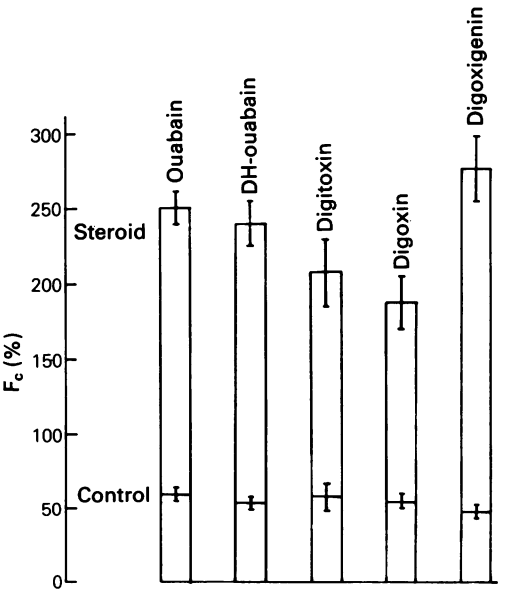

Figure 1 The force of contraction as influenced by some cardioactive steroids: force of contraction (F_c) of guinea-pig papillary muscles as developed at 0.5 Hz, 5.9 mmol l^{-1} K^+, in the absence (control) and presence (steroid) of one of the following steroids is shown (in μmol l^{-1}): ouabain, 0.8; dihydroouabain, 50; digitoxin, 0.4; digoxin, 1.3; digoxigenin, 30. The ordinate scale refers to percentages of the value after the initial equilibration period at 1 Hz (= 100%). The number of experiments ranged from 11 to 20.

Results

Dependence of rate of inotropic effect of some cardioactive steroids on the diameter of papillary muscles

In Figure 1 the force values have been normalized as percentage of the value after the initial equilibration period at 1 Hz (100% = 5.8 ± 0.5 mN; $n = 66$). When stimulation frequency was set at 0.5 Hz, the magnitude of force of contraction was halved. After the effects of the steroids had fully developed, force of contraction was more than twice its initial value. As compared with ouabain, the effects of the other steroids, with the exception of digoxin, were not significantly different.

The relationship between the rates of onset of the inotropic effect and the muscle diameter was determined from the experiments shown in Figure 1, by evaluating the time t_x required to produce a definite effect (10, 25, 50, 75, 90% of the steady-state effect). For demonstration digitoxin and dihydroouabain were selected (Figure 2, (a) and (b), respectively). With each steroid the respective onset time increased with muscle diameter. Its influence became more promin-

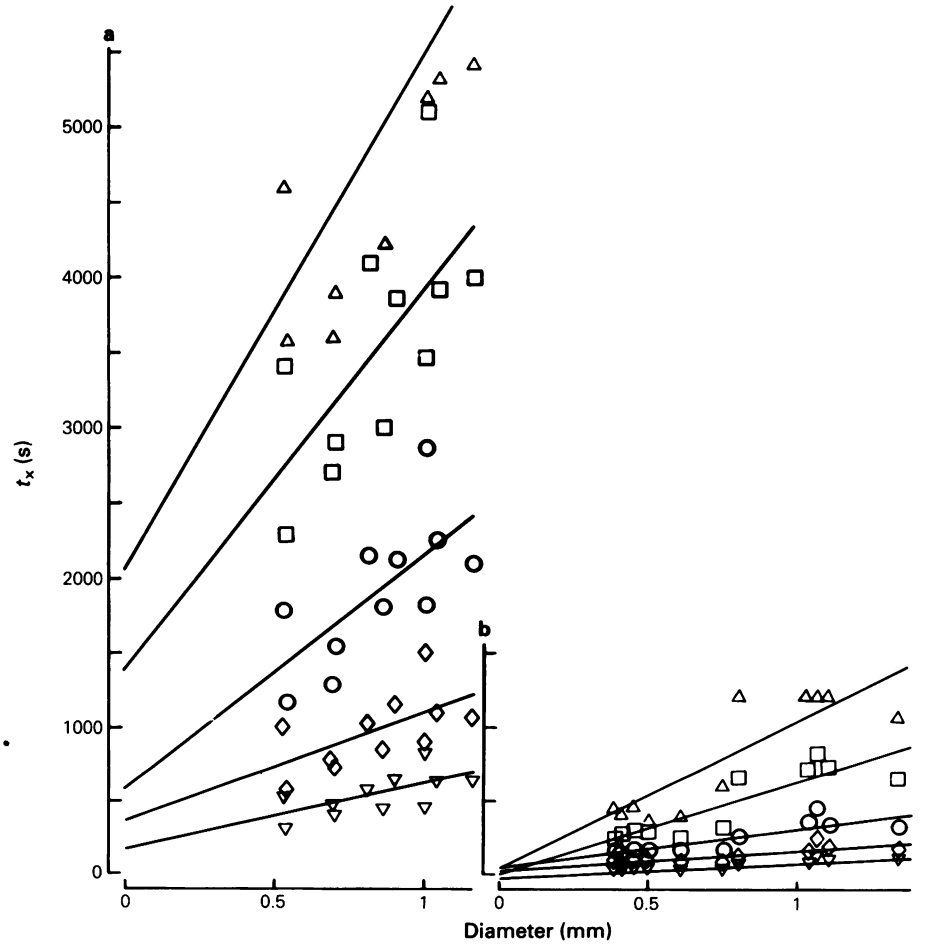

Figure 2 The rates of onset of the inotropic effect of digitoxin and dihydroouabain in relation to muscle diameter: the rates of onset of the digitoxin (a) and dihydroouabain (b) effect were determined by the times required to reach definite levels of effect (t_x, as defined by the different symbols); from top to bottom t_{90} (△), t_{75} (□), t_{50} (○), t_{25} (◇) and t_{10} (▽). t_x values were correlated with the corresponding muscle diameter. All correlation coefficients were significantly different from zero, (digitoxin, $P < 0.05$; dihydroouabain, $P < 0.001$). The concentrations were as indicated in legend to Figure 1. Ordinates: time to a definite level of effect as a percentage of the steady-state effect in s; the same scale was used in both panels; abscissa scale: muscle diameter in mm.

ent at the higher levels of effect; as compared with t_{10} the slopes of all other regressions were significantly steeper in the case of dihydroouabain; with digitoxin the regression at t_{90} was significantly different. At a fixed level of effect in Figure 2, the slopes obtained with digitoxin seemed to be steeper than with dihydroouabain. This difference was confirmed by the relationship between the half-times to steady-state effect (t_{50}) of several cardioactive steroids and the muscle diameter in a large number of experiments (Figure 3; ouabain data were taken from Ebner *et al.*, 1985). Slope and intercept of the dihydroouabain regression were significantly different from the respective parameters of other regressions with the exception of digoxigenin. Although the exact relationship between the time to attain a definite effect level and the muscle diameter is unknown, we tried to define the onset of the inotropic effect unrelated to diffusion by linear extrapolation to negligibly small muscle diameters. The intersections with the ordinate scale at zero diameter at the various levels of effect in Figure 2 and 3, therefore, were taken to construct the time courses of Figure 4a. It was then found that the inotropic effects of the five steroids developed at more similar, although not identical, rates. For comparison the time courses of the steroid effects were compiled from muscles of 0.75 to 1.1 mm diameter (Figure 4b).

Ignoring the factor of variation due to muscle geometry, the rates of onset of the effect of cardioac-

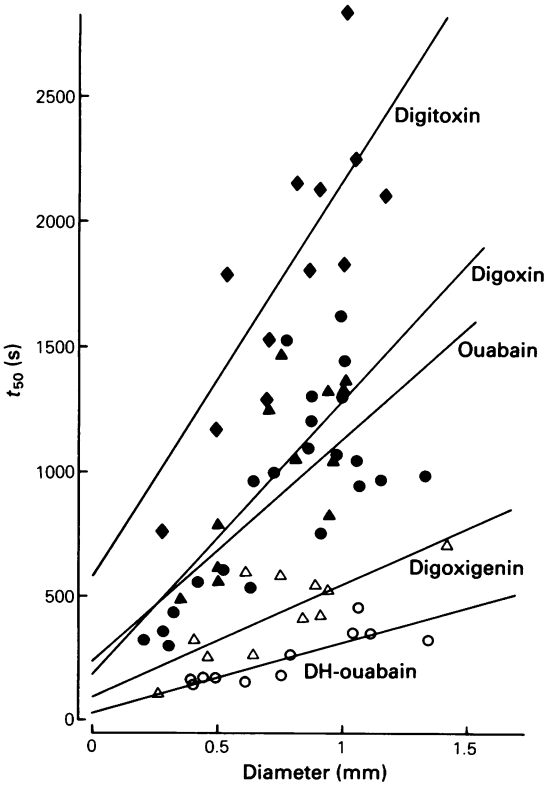

Figure 3 The half-time to steady-state effect (t_{50}) of some cardioactive steroids in relation to the muscle diameter: the half-time to steady-state effect was obtained at the steroid concentration indicated in the legend to Figure 1. Some data are shown in Figure 2. The symbols represent the following steroids: (◆) digitoxin, (▲) digoxin, (●) ouabain, (△) digoxigenin, (○) dihydroouabain. Correlation coefficients are significantly different from zero ($P<0.01$; except digitoxin, $P<0.05$, and dihydroouabain, $P<0.001$). The parameters of the regressions are plotted in Figure 5. Ordinate scale; half-time to steady-state effect in s, t_{50}; abscissa scale: muscle diameter in mm.

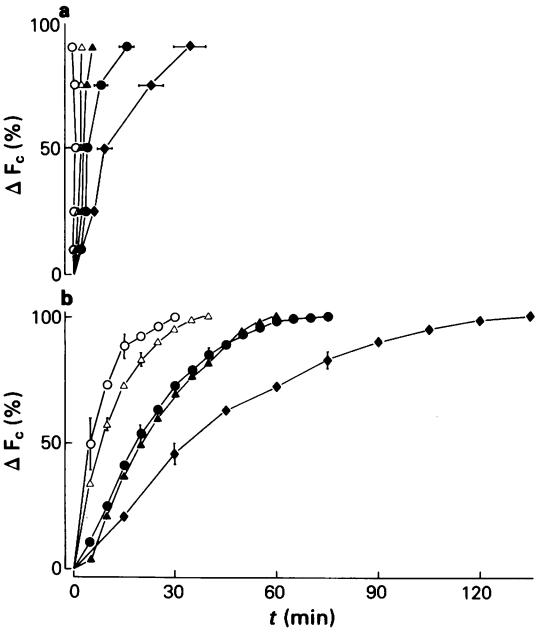

Figure 4 The onset of the inotropic effect after extrapolation to zero diameter (a) and in the presence of diffusion (b): the data were obtained from the experiments of Figures 1–3. In (a) the intersections of regressions, partially shown in Figure 2, with ordinate at zero diameter are plotted at the respective level of effect. The mean values with some s.e.mean of (b) were compiled from muscles of roughly similar diameters (0.86 to 0.95 mm; $n = 3-12$). Ordinates: inotropic effect, i.e., increase above pre-drug values as percentage of steady-state effect, Δ F_c%; abscissa scale: time in min. (◆) Digitoxin, (▲) digoxin, (△) digoxigenin, (●) ouabain, (○) dihydroouabain.

tive steroids correlated negatively with inotropic potencies, i.e., receptor affinities, in guinea-pig papillary muscle (Ebner *et al.*, 1985). The present findings enable us to distinguish two components of the previous observation. Firstly, even though the variation of onset rates of the various steroids was largely reduced after correction for the influence of diffusion, the dependence of the extrapolated half-times to steady-state effect $t_{50'}$ on inotropic potencies persisted (Figure 5a; the half-maximally effective concentrations EC_{50} were taken from Ebner *et al.*, 1985). Secondly, the rates of onset of the potent steroids were more effectively delayed by increase of diffusion distance according to the replot of the slopes between half-times to steady-state effect and muscle diameter in dependence on inotropic potencies (Figure 5b).

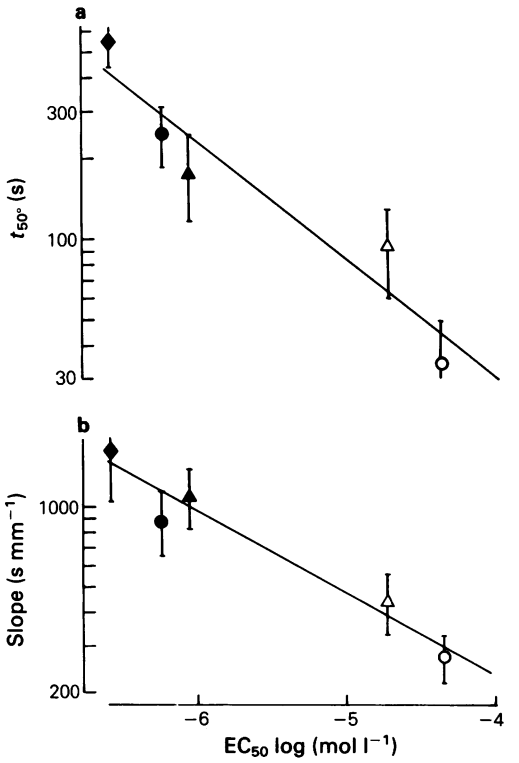

Figure 5 Rates of onset after extrapolation to zero diameter (a) and apparent rates of diffusion (b) in dependence on the inotropic potency of some cardioactive steroids: the parameters of the regressions of Figure 3, i.e., intersections with ordinate, $t_{50'}$, and slopes, were correlated with half-maximally effective concentrations (EC_{50}) obtained under identical conditions. Regressions: (a) log $t_{50'} = (-0.24 \pm 0.07) - (0.43 \pm 0.08)$ log EC_{50}, $r = -0.95$, $P = 0.01$; (b) log slope $= (1.17 \pm 0.03) - (0.30 \pm 0.04)$ log EC_{50}, $r = -0.97$, $P < 0.01$. Asymmetric s.e.means are due to the log scale of ordinates, s.e.means of EC_{50} were smaller than the size of symbols. Ordinates: (a) $t_{50'}$ in s, intersection of the t_{50} – diameter plot with ordinate at zero diameter; (b) slope of t_{50} – diameter plot in s mm^{-1}; log scales; common abscissa scales: log values of EC_{50} in mol l^{-1}. Symbols as in Figure 4.

Saturation of receptors accelerates the apparent rate of diffusion in papillary muscles

As shown in Figure 6 ouabain produced concentration-dependent increases in contraction at steady state (100% = 5.0 ± 0.4 mN, $n = 95$). When in these experiments half-times to steady-state effect (t_{50}) were correlated with muscle diameter according to the procedure of Figure 3 both the intercept with ordinate $t_{50'}$ and, above 1 μmol l^{-1} ouabain, the slopes of the regressions were significantly diminished with ouabain concentration (Figure 7). At high concentrations of ouabain, onset of toxicity could have depressed the magnitude of the steady-state effect. Hence t_{50} could have shortened, thereby altering the relation to muscle diameter. However, in three-dimensional lin-

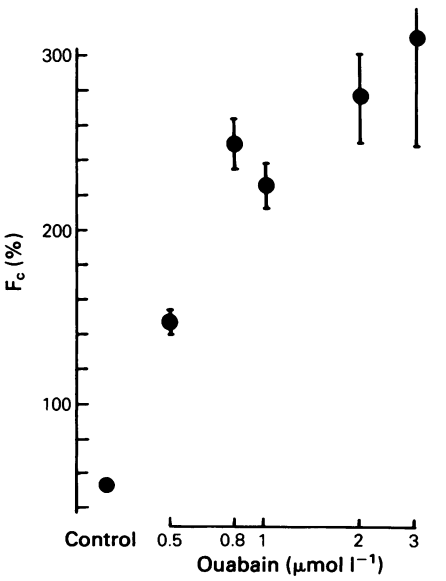

Figure 6 The force of contraction is influenced by different ouabain concentrations: the force of contraction (F_c) developed by papillary muscles at 0.5 Hz, 5.9 mmol l^{-1} K^+, in the absence (control) and presence of different ouabain concentrations are shown with different muscle groups ($n = 12$–27). Ordinate scale: force of contraction as percentage of the value after the initial equilibration period at 1 Hz (= 100%); abscissa scale: ouabain concentration in μmol l^{-1}, log scale.

ear regressions (t_{50} vs. diameter and steady-state force as a percentage of the initial control), except at 0.8 and 1 μmol l^{-1} ouabain ($r = 0.64$, $P < 0.01$ and $r = 0.43$, $P < 0.05$), t_{50} did not depend on steady-state force. In view of the lack of effect at the high concentrations toxicity-induced variation of force of contraction at steady state cannot explain the reduction of slope in Figure 7b. Rather the apparent rate of diffusion (Figure 7b) increased and the onset of the inotropic ouabain effect was accelerated after the influence of diffusion had been excluded (Figure 7a).

The different magnitudes of the ouabain effect caused t_{50} values to be determined at different levels of force of contraction, i.e., at the higher concentrations

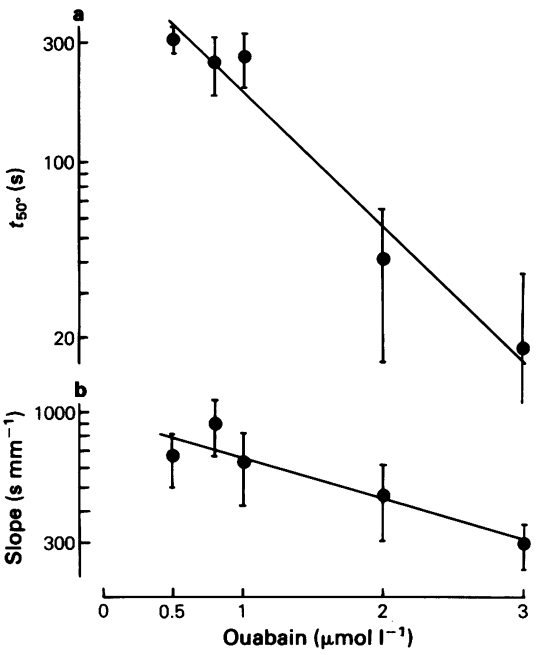

Figure 7 Rates of onset after extrapolation to zero diameter (a) and apparent rates of diffusion (b) in dependence on ouabain concentration: from the experiments of Figure 6, linear regressions relating the half-time to steady-state effect (t_{50}) to muscle diameter were calculated. The intersections with ordinate, $t_{50'}$, and slopes were correlated with the respective ouabain concentration. Regressions: (a) log $t_{50'} = (2.82 \pm 0.05) - (0.054 \pm 0.005)$ ouabain concentration, $r = -0.98$, $P < 0.01$; (b) log slope $= (2.979 \pm 0.034) - (0.016 \pm 0.003)$ ouabain concentration, $r = -0.93$, $P < 0.05$. Asymmetric s.e.means are due to the log scale of ordinates. Ordinates: (a), $t_{50'}$ in s, i.e., intersection of t_{50} – diameter plot with ordinate at zero diameter; (b) slope of t_{50} – diameter plot in s mm^{-1}; log scales; common abscissa scales: ouabain concentration in μmol l^{-1}, linear scale.

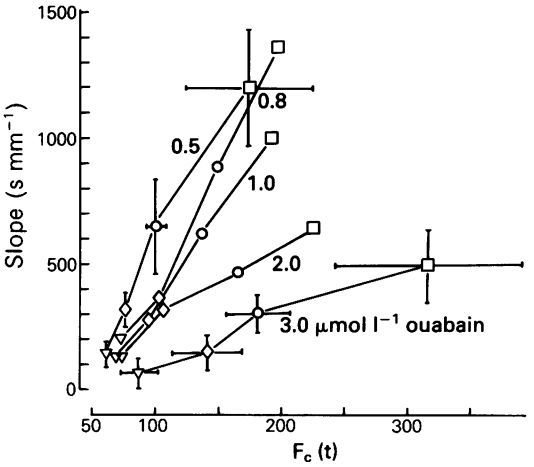

Figure 8 The relation between apparent rates of diffusion and force of contraction: dependence on ouabain concentration: from the experiments of Figure 6–7 regressions between the time to a definite effect (t_x), i.e., 10, 25, 50, 75% of steady-state effect, and muscle diameter were calculated according to Figure 2. Their slopes in s mm^{-1} were plotted on the ordinate scale in correlation with the corresponding force values F_c (t) as percentage of the value after the initial equilibration period (abscissa scale) under the respective ouabain concentrations, (□) t_{75}, (○) t_{50}, (◇) t_{25}, (▽) t_{10}.

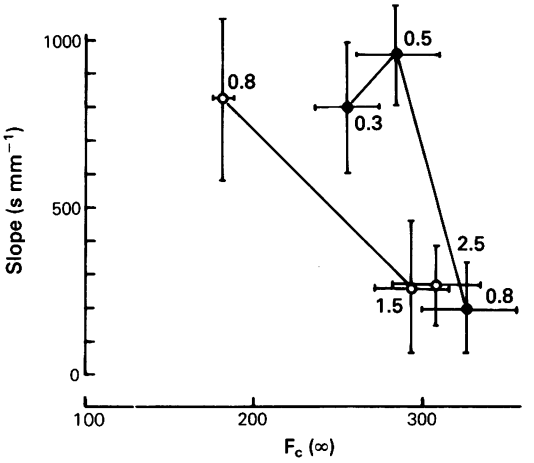

Figure 9 Dependence of apparent rates of diffusion of ouabain on K^+ concentration: the slopes of the relation of half-time to steady-state effect (t_{50}) to muscle diameter were determined at 2.4 (●) or 12 (○) mmol l^{-1} K^+ at various ouabain concentrations (slope in s mm^{-1}, ordinate scale). The slopes were correlated with the corresponding arithmetic means ± s.e.mean ($n = 15$ to 27) of force of contraction at steady state ($F_c(\infty)$) as a percentage of the value after the initial equilibration period (abscissa scale). Numbers beside points indicate ouabain concentration in μmol l^{-1}.

t_{50} corresponded to relatively greater force values. Considering the relevance of the level of evaluation as evident from Figure 2, it was attempted to estimate its influence. For this purpose the various slopes from the regressions of t_{10}, t_{25} etc. on diameter, were correlated with the force value at which the respective t_x had been determined. Figure 8 demonstrates that at a given value of force of contraction the slope of the t_x — diameter plot (ordinate scale of Figure 8) reduces with ouabain concentration. Thus it confirms the concentration-dependence of Figure 7.

When the relation of receptor to ouabain concentration altered with different K^+ concentrations, the equieffective concentrations of ouabain, as evidenced by the values of steady-state force on the abscissa scale (Figure 9), had to be increased in elevated K^+. Corresponding to the concentration-dependence of Figure 5 the slopes of the relation between t_{50} and diameter were significantly reduced (Figure 9) when after increase of K^+, equieffective concentrations are compared (0.5 vs. 2.5 μmol l^{-1} ouabain at 2.4 and 12.0 mmol l^{-1} K^+, respectively).

The onset of the inotropic effect of ouabain and dihydroouabain in the perfused heart

In the perfused heart, diffusion distance is short. Accordingly the inotropic effect of ouabain (0.8 μmol l^{-1}, 5.9 mmol l^{-1} K^+) developed from a basal force of contraction of 96.6 ± 3.2 mN ($n = 4$) with a half-time of 363 ± 27 s even faster than observed in very thin papillary muscles (Figure 10). The values

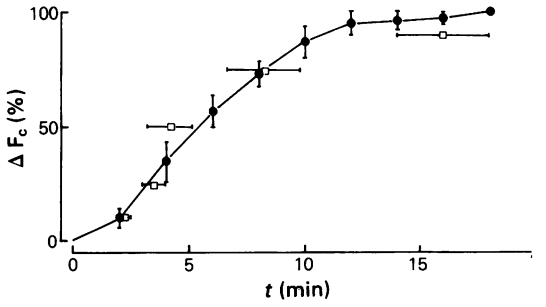

Figure 10 The development of the inotropic effect of ouabain in the perfused heart: in the perfused heart (●) the time course of the effect of 0.8 μmol l^{-1} ouabain is shown at 0.5 Hz, 5.9 mmol l^{-1} K^+, with arithmetic means ± s.e.mean of 4 preparations; (□) correspond to the times to definite levels of effect (t_x), linearly extrapolated to zero diameter of papillary muscles as obtained at equivalent experimental conditions (from Figure 4). Ordinate scale: positive inotropic effect of ouabain, i.e., increase above pre-drug value, as percentage of the maximal effect (100% ΔF_c in perfused hearts = 82.3 ± 13.3 mN, $n = 4$); abscissa scale: time in min.

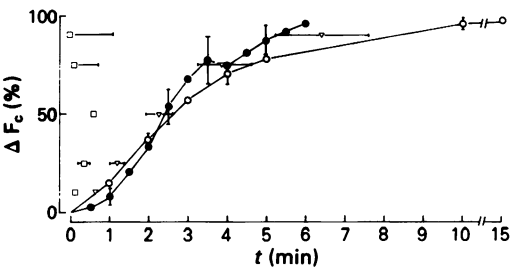

Figure 11 The development of the inotropic effect of dihydroouabain in the perfused heart: in the perfused heart (●) the time course of the effect of 50 μmol l^{-1} dihydroouabain is shown at 0.5 Hz, 5.9 mmol l^{-1} K^+, with arithmetic means ± s.e.mean. For comparison the times to definite levels of effect (t_x) are indicated after linear (□) or parabolic extrapolation (▽) to zero diameter of papillary muscles as obtained under equivalent experimental conditions in the experiments of Figure 4; (○) represent the time course in papillary muscles of diameters < 0.75 mm. Number of perfused hearts was 3, beyond the broken line $n = 2$. Ordinate scale: positive inotropic effect of dihydroouabain, i.e., increase above pre-drug value, as percentage of the maximal effect (100% ΔF_c in perfused hearts = 79.1 ± 21.6 mN, in the papillary muscles of diameters < 0.75 mm, 4.5 ± 0.1 mN); abscissa scale: time in min.

obtained by linear extrapolation of t_x to zero diameter of papillary muscles (open quadrangles, replotted from Figure 4) were superimposable on the data from perfused hearts. With 50 μmol l^{-1} dihydroouabain (control force of contraction 45.4 ± 21.6 mN, $n = 3$) the inotropic effect appeared at the same rate in the perfused heart (filled circles, Figure 11; $t_{50} = 159 \pm 5$ s; $P < 0.002$ vs. t_{50} of ouabain) and in papillary muscles of < 0.75 mm diameter (open circles, Figure 11). The result was similar when, instead of the effect ΔF_c, the time courses of force of contraction F_c were plotted as percentage of pre-drug value (not shown). Comparison with the time course extrapolated to zero diameter (open quadrangles) reveals that linear extrapolation overestimates the rate of action in papillary muscles. However, when a parabolic relationship ($t_{50} = a + bd^2$) was assumed, extrapolated values (open triangles) became superimposable.

Discussion

Receptor-controlled diffusion

The results show that muscle diameter is an important determining factor of the onset of the inotropic effect of cardioactive steroids in papillary muscles. To evaluate the role of geometry one may refer, in first approximation, to conventional, concentration-

independent diffusion. If the same geometric shape is considered, diffusion rate depends solely on distance and constant of diffusion (see Crank, 1975; Smith, 1969). The diffusion-controlled development of the inotropic effects should consequently be determined by the same parameters. Dependence on muscle diameter has been shown. The slope of this relationship in s per mm serves as an indirect and inverse measure of diffusion rate; i.e., great influence of diffusion distance indicates slow diffusion rate, since it takes so long to build up an equivalent concentration profile, and vice versa. This is in accordance with the relation to diffusion rate that diffusion distance became more effective with the approach to the steady state (Figure 2), since then diffusion rate is zero.

The slopes of the relation between half-times to steady-state effect and muscle diameter (Figure 3) differ in dependence with the steroids. Similar slopes of this plot up to 1 μmol l^{-1} ouabain (Figure7) exclude the slight deviations from equieffectivity as the cause of the observation of Figure 3. The replot of slopes in dependence on EC_{50} (Figure 5b) rather favours a role of receptor affinity. This behaviour is to be expected if occupation of the receptors reduces the low extracellular concentration of the highly potent steroid more effectively than the high, equieffective, concentration of the low potent compound. Half-maximal binding of ouabain and dihydroouabain at 40 nmol l^{-1} and 5 μmol l^{-1}, respectively, at comparable experimental conditions in resting preparations (Ebner *et al.*, 1986; Ebner & Siegl, unpublished results) confirms the different affinities. Na-K-ATPase inhibitory potencies of both steroids were also similarly different (Ebner *et al.*, 1985). Assuming Michaelis-Menten kinetics, maximal binding capacity was 0.2 and 0.24 μmol ouabain kg^{-1}. Obviously receptor capacity and ouabain concentration in the present experiments (0.8 μmol l^{-1}) are comparable. Moreover the gradient during the pre-steady state comprises concentrations which are well below that value; i.e., the reaction with the receptors is likely to reduce free ouabain concentration. However, few receptors also interfere with a low potent drug, if 'pure' diffusion, i.e., without interference from receptors, is slow. A comparison with the diffusion of steroids in water, however, is not feasible, since the slow onset of the inotropic ouabain effect in resting preparations (Ebner *et al.*, 1986) and the dependence of diffusion rate on stimulation frequency (Ebner & Siegl, 1986) suggest bulk diffusion, as effected by the contractions, to be involved. The role of steroid receptors in diffusion also was confirmed by its concentration-dependence (Figures 7b, 8 and 9). Saturation of receptors with increase of ouabain concentration, in particular at high K^+, diminished the extent of immobilized ouabain relative to its free concentration and consequently accelerated diffusion. The present results, therefore, confirm the validity of the previously proposed model in agreement with models designed to fit the rates of action of atropine (Thron & Waud, 1968) or of tetrodotoxin (Colquhoun & Ritchie, 1972).

The nearly identical molecular volumes of ouabain and dihydroouabain precluded the possibility that different volumes determine the difference between diffusion rates. Interference of a conceivable O_2 deficiency in the thicker muscles or of different lipophilicities of the compounds has been excluded previously (Ebner *et al.*, 1985). The complete lack of effect of lipophilicity in particular suggests accumulation of the steroids in the tissue via parallel pathways: firstly, lipophilic uptake across the myocytes without remarkable uptake from or influence on the concentration in the extracellular space; secondly, hydrophilic uptake via the extracellular space, exposed to the receptors and solely relevant for the development of the effect.

Diffusion-corrected rates of action

Superimposition of the time courses of the ouabain effect in the papillary muscles and in the perfused heart was achieved after extrapolation to zero diameter (Figure 10). With poorly potent dihydroouabain (Figure 11), the similar onset rates in perfused hearts and thin papillary muscles and the superimposition accomplished by a parabolic relationship between t_{50} and muscle diameter likewise demonstrate the development of the effect independent of diffusion even in the 'macroscopic' papillary muscles.

Although the variation of rates was largely reduced after correction for the influence of diffusion, the correlation with inotropic potencies (Figure 5a) and the rates of ouabain and dihydroouabain action in the perfused heart (Figures 10 and 11) confirm this difference. Fitting straight lines to the data for low potent steroids, however, may have underestimated diffusion rate in papillary muscles while exaggerating diffusion-corrected rate of action. The development of the effects of ouabain and dihydroouabain in spontaneously beating chick myocytes at similar rates (Barry *et al.*, 1985) seems to be at variance. Presumably the change of contraction frequency (from 79.8 to 93.5 and from 92.1 to 106.8 beats per min, respectively) obscured the slight, but significant difference. Similarly the high concentration of Na^+ which fully activates Na-K-ATPase could have accelerated inhibition by ouabain and dihydroouabain (Ebner *et al.*, 1985) to such an extent as to impede the detection of differences.

The present experiments at controlled contraction frequency indicate the relevance of receptor affinity for diffusion-corrected rates of action. Therefore, they favour the view that the binding of steroids to

receptors governs the onset rates at the cellular level. The shortening of diffusion-corrected half-times with ouabain concentration (Figure 7a) is in accordance with the bimolecular nature of the reaction with the receptors. If receptor occupation limits the rate of action $t_{50'}$ of identically acting drugs at equally effective concentrations corresponds to a constant, though unknown, fractional receptor occupancy. According to conventional receptor kinetics ($k_{+1}A + k_{-1}$) determines the time course of receptor occupation (see e.g., van Ginneken, 1977); where k_{+1} and k_{-1} are the rate constants of association and dissociation, respectively; A is the drug concentration. $t_{50'}$ therefore contains information concerning the association and dissociation rates of the drug-receptor complex.

The role of association and dissociation in receptor affinity of the various steroids becomes evident when we consider the change of reaction rate with affinity, i.e., the equilibrium dissociation constant ($k_{-1}\ k_{+1}^{-1}$) of the drug-receptor complex AR. Matching receptor occupancy ($A_1R = A_2R$) at equally effective concentrations defines $A_1\ A_2^{-1} = (k_{-1}\ k_{+1}^{-1})_1\ (k_{-1}\ k_{+1}^{-1})_2^{-1}$ (eq.1) at equilibrium. Suffixes refer to different drugs. If affinity changes exclusively via association rate (k_{-1} = const), $(A\ k_{+1})_1 = (A\ k_{+1})_2$ (eq.2); i.e., reaction rate of equieffective concentrations does not change with affinity. Conversely, if dissociation rate changes (k_{+1} = const), we obtain $A_1\ (k_{-1})_1^{-1} = A_2\ (k_{-1})_2^{-1}$ (eq.3). In the latter case, association rate also would be altered by the change of A necessitated to balance the change of k_{-1}. The overall rate would become different. Correspondingly with increase of affinity via reduction of k_{-1}, the equieffective concentration would have to be reduced, the reaction rate slow down, although k_{-1} *per se* is unlikely to become visible in overall rate, since the high affinity of all steroids suggest $k_{-1} \ll k_{+1}$. In consequence the retardation of diffusion-corrected rates of action with increase of receptor affinity (Figure 5, 10, 11) shows that reduction of dissociation rate must have been relevant. This conclusion agrees with observations on Na-K-ATPase (Clark *et al.*, 1975) where primarily increase in the dissociation rate constant determined the reduction in affinity of various steroids.

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