Kinetics and inotropic action of probenecid in guinea-pig heart in vitro

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Summary. Probenecid (100–750 $\mu g \cdot ml^{-1}$) was found to inhibit cardiac contraction force in untreated and digoxin-treated (100 $\mu g \cdot ml^{-1}$) isolated right guinea-pig atrium in vitro by a reversible process, without influencing beating frequency. At low concentrations (1.5–60 $\mu g \cdot ml^{-1}$), ^{14}C-probenecid was accumulated into right atrium by an oxygen-dependent process. Correlation between uptake and negative inotropic action of probenecid could not be found.

Interactions of the uricosuric drug probenecid in the pharmacokinetics of cardiac glycosides are long known. It could be shown in vivo and in vitro that probenecid inhibits the intestinal transport of digitoxin[1,2]. Moreover, a striking inhibitory effect of probenecid on the hepatic uptake and the biliary excretion of ouabain in rats has been described[3–5]. Additionally, interactions between probenecid and digoxin in the plasma protein binding have been reported[6]. The metabolism of digitoxin in rats was also influenced by probenecid, leading to an inhibition of C_{12}-hydroxylation[7]. From these findings, the question arises whether probenecid also interacts with cardiac glycosides on their target organ, the heart. Moreover, it was of interest to know whether probenecid itself has any effect on heart muscle activity. Therefore the present in vitro study was performed to investigate inotropic effects and uptake of probenecid into heart tissue as well as its effect on the positive inotropic action of digoxin.

Methods. All experiments were performed on spontaneously beating isolated right atria ($160 \pm 10 \cdot min^{-1}$) of fed female guinea-pigs (400–600 g). Digoxin crist. was purchased from Merck Inc., Darmstadt. Labelled (sp. act. 24 $\mu Ci \cdot mg^{-1}$) and unlabelled probenecid (Benemid®) was a generous gift of Merck, Sharp and Dohme, Rahway, NY. For all experiments a modified Tyrode-solution of the following composition was used as incubation medium: NaCl 136.0 mM, KCl 2.67 mM, $CaCl_2$ 1.8 mM, $NaHCO_3$ 11.9 mM, KH_2PO_4 0.417 mM, $MgCl_2$ 0.507 mM, glucose 5.0 mM. The fluid was perfused with carbogen (O_2: CO_2 = 95:5). Temperature was 37 °C and pH 7.4. In some experiments, in which nitrogen was used as gas phase, the carbonate buffer was replaced by phosphate buffer.

Uptake of ^{14}C-probenecid. Tissue slices of right atria were made by a Stadie-Riggs microtome and incubated in the solution described for 90 min. At the beginning of the experiments, ^{14}C-probenecid was added, leading to concentrations of 1.5–2000 $\mu g \cdot ml^{-1}$ (6 μM–7.0 mM). Some experiments under anaerobic conditions were performed at a probenecid concentration of 3 $\mu g \cdot ml^{-1}$ (11 μM). ^{14}C-probenecid content was measured after combustion of tissue slices by a Tricarb Sample Oxidizer 305 and calculated from dpm after liquid scintillation counting of the developing $^{14}CO_2$.

Inotropic effects. Contration force and frequency of spontaneously beating right atrium were measured isometrically by a Hellige TF 19 after equilibration for 60 min in the solution described above. Resting tension was 1 p. Probenecid concentration varied from 100 to 750 $\mu g \cdot ml^{-1}$ (0.35–2.63 mM). Interaction with digoxin was studied at the same probenecid concentrations, which were applied 30 min after addition of digoxin (100 $ng \cdot ml^{-1} = 0.128$ μM). At this time, the maximal positive inotropic effect of the glycoside had reached equilibrium. For each probenecid concentration given, 5–7 atria were freshly prepared as described above.

Results and discussion. Dose dependence of ^{14}C-probenecid uptake into right atria of guinea-pigs is shown in figure 1. There is an accumulation of the drug at low concentrations leading to T/M (tissue/medium) ratios significantly higher than 1. These findings suggest participation of an oxygen-dependent uptake or binding process at low probenecid concentrations, which is abolished under anaerobic conditions. At high probenecid concentration, the T/M ratio dropped to values of nearly 1, indicating that binding of the

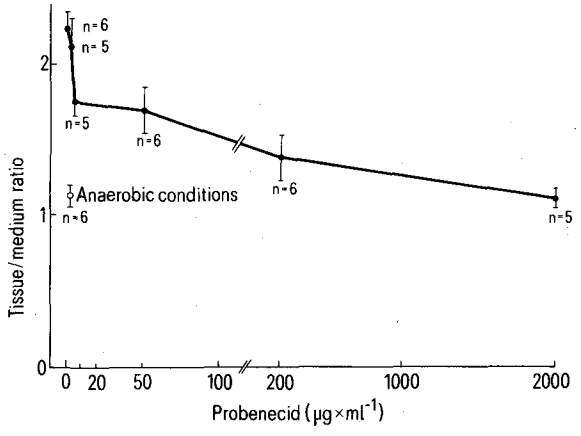

Fig. 1. Dose dependence of ^{14}C-probenecid uptake into isolated right atria of guinea-pigs. For experimental conditions refer to 'methods'. The results on content of ^{14}C-probenecid in heart tissue are figured as T/M (tissue/medium) ratios. All values are given as the means±SD of n experiments. 1 series of experiments was performed under anaerobic conditions by nitrogen instead of carbogen perfusion.

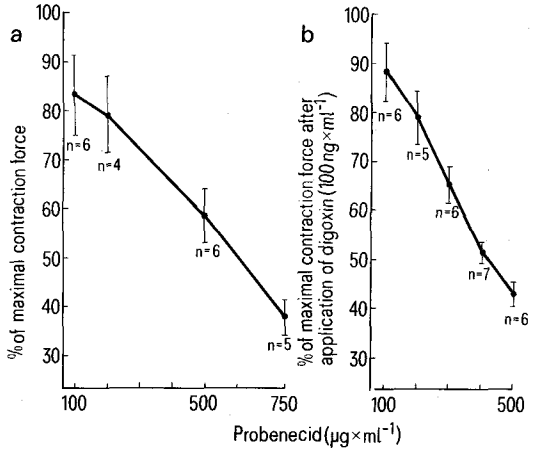

Fig. 2. *a* Negative inotropic effect of probenecid on isolated spontaneously beating guinea-pig atrium. *b* Negative inotropic effect of probenecid on isolated spontaneously beating guinea-pig atria which were treated with digoxin (100 $\mu g \cdot ml^{-1} = 0.128$ μM). The results are given as percentage of the maximal inotropic effect. For experimental conditions, refer to 'methods'. Each value is given as the mean±SD of n experiments.

drug is saturable in the concentration range investigated. Similar findings on liver tissue are reported by Gigon and Guarino[8]. Own experiments (not depicted), on the time dependence of the uptake process, showed saturation after 60 min incubation time with a $t^1/_2$ of 18 min.

Figure 2a shows the dose-dependence of the negative inotropic probenecid effect. The values are given as percentage of maximal contraction force after equilibration and before probenecid was administered in the concentrations described. The maximal inhibitory effect was reached after 11.3±4.2 min showing no correlation with the time course of uptake process. After washout twice, the negative inotropic effect of probenecid was completely reversible. Moreover, it should be mentioned that no influence on the beating frequency occurred under the conditions described. The probenecid concentration used in these experiments was 2–10fold higher than serum levels in man at doses used in clinical practice. Moreover, it must be taken into account that 90% of serum probenecid is bound to plasma albumin[9], whereas the incubation medium used in our experiments did not contain any protein.

Finally, the probenecid effect on digoxin pretreated right atria was investigated. As shown in figure 2b, probenecid inhibits the positive inotropic action of digoxin ($100\ ng \cdot ml^{-1} = 0.128\ \mu M$). The values are given as percentage of maximal inotropic action after digoxin application, which was about 130% of the contraction force of untreated right atria.

The effects of probenecid on digoxin-treated as well as on untreated heart tissue in vitro may be explained as metabolic inhibition. This suggestion is supported by Pakarinen et al.[10-12], who found inhibition of α-ketoglutarate- and succinate-oxydation in kidney mitochondria caused by probenecid. Oxygen consumption of guinea-pig kidney slices as well as of rat liver slices was also found to be decreased. A correlation of uptake and/or binding of probenecid to heart tissue and its negative inotropic action cannot be derived from the present findings, because any coincidence regarding the time course or the concentration dependence of both processes is lacking.

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Picrotoxin-diazepam interaction in a behavioural schedule of differential reinforcement of low rates[1]

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Summary. In rats working in a behavioural schedule of differential reinforcement of low rates (6 or 10 sec), picrotoxin (1 mg kg^{-1}) decreased the number of premature responses and increased (in DRL 10 sec only) the number of rewarded responses. The effect of picrotoxin was antagonized by diazepam (2 mg kg^{-1}). In contrast to picrotoxin, strychnine (1.5 mg kg^{-1}) increased the number of premature responses.

Recent studies suggest an involvement of gamma-aminobutyric acid (GABA) processes in the control of behavioural inhibition. Interruption of GABAergic transmission by picrotoxin may either enhance inhibition elicited by aversive events[2], or despite 1 contradictory report[3], reverse benzodiazepine-induced release of responding in conflictual schedule[4,5]. In addition it has been evidenced that benzodiazepines - drugs known to release response under aversive conditions - may facilitate GABAergic transmission[6,7].

Since benzodiazepines were reported to increase response maintained under differential reinforcement of low rates (DRL) procedures[8,9], the possibility of an involvement of a GABAergic component in the control of response under such procedures was investigated. In DRL, the reinforcement schedule requires that responses must be withheld for a certain length of time in order for the response to be rewarded. The effect of picrotoxin was studied in rats working in a DRL procedure and compared to that obtained with another convulsant, strychnine, a glycine antagonist devoid of activity on GABA receptors[10]. Furthermore the capacity of diazepam to reverse picrotoxin effects was investigated.

Material and methods. Experiments were conducted using 3 operant boxes (placed in ventilated sound insulating cubicles) with an automatic food dispenser delivering 45 mg Noyes pellets. The boxes were equipped with 2 levers (5.5 cm above the grid floor), activation of which required a vertical force of at least 12 g to operate the microswitch.

The rats (male Wistar A.F., 150–160 g at the beginning of the study), maintained at 80–85% of their normal body weight, were first trained (16 daily sessions of 15 min) to press the right lever of the Skinner-box to obtain pellets according to a continuously reinforced schedule.

Then, DRL-6-sec, or for some rats DRL-10-sec, was introduced. These schedules contain a 6-sec (or 10-sec) period during which all bar press responses must be withheld by the rat to ensure access to the reinforcing food. Responses which occur earlier than 6 sec (or 10 sec) after the preceding bar press, reset the clock and started a new 6-sec (or 10-sec) holding period. Furthermore these DRL schedules