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MYOCARDIAL DIGOXIN UPTAKE: DISSOCIATION BETWEEN DIGITALIS-INDUCED INOTROPISM AND MYOCARDIAL LOSS OF POTASSIUM

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1 The time course of myocardial uptake of digoxin, of increase in inotropic effect and of changes in myocardial potassium content were studied following a single intravenous dose of digoxin.

2 Nineteen dogs with intact circulation were investigated by the use of a biopsy technique which allowed samplings before and 10, 30, 60, and 90 min after administration of digoxin.

3 The myocardial concentration of digoxin was 196×10^{-9} mol/kg 10 min after administration of digoxin. Uptake continued at a slower rate, maximum concentration being 293×10^{-9} mol/kg at 60 minutes.

4 The inotropic effect increased parallel with the uptake of digoxin; 10 min after digoxin, contractility was 127% of the control value and this increased to 139% at 90 minutes.

5 Myocardial potassium content was slightly increased 10 min after digoxin, suggesting an initial stimulation of membrane Na^+-K^+ ATPase. A subsequent significant fall in the myocardial potassium content probably reflects ATPase inhibition.

6 The temporal dissociation between the early onset of the positive inotropic effect and the delayed inhibition of membrane Na^+-K^+ ATPase indicates that inotropism of digitalis glycosides is not mediated by the same binding site as that responsible for inhibition of Na^+-K^+ ATPase.

Introduction

It is well established that digitalis glycosides increase the force of myocardial contractility but the mechanism of this action is still unknown.

In 1953 Hajdu advanced the hypothesis that digitalis-induced inotropism is related to an inhibition of membrane Na^+-K^+ ATPase. Evidence supporting this concept was recently reviewed by Langer (1972). He concluded that the positive inotropic effect of digitalis is not seen, unless inhibition of the ATPase enzyme is also observed. However, his arguments were based on studies performed with relatively high concentrations of digitalis either on isolated, perfused heart preparations or on isolated myocardial muscle preparations. Studies of the isolated Na^+-K^+ ATPase enzyme have revealed that low concentrations of digitalis stimulate enzyme activity while higher concentrations inhibit it (Lee & Yu, 1963; Palmer & Nechay, 1964; Oppelt & Palmer, 1966). Stimulation of Na^+-K^+ ATPase increases intracellular potassium while enzyme inhibition leads to reduction in potassium content. This biphasic interaction between digitalis and Na^+-K^+ ATPase and its relation to myocardial performance and uptake of digoxin have not been studied in intact animals.

The present study, using a biopsy technique allowing serial samplings from the same myocardium was undertaken in order to follow the temporal uptake of digoxin by the intact canine heart and to correlate this with the inotropic effect and changes in myocardial potassium.

Methods

Nineteen dogs weighing 18.5–25 kg were divided into three groups. Group 1: Eight dogs were given digoxin and serial myocardial biopsies were taken. Group 2: Seven dogs were given digoxin but biopsies were taken only at the end of the study. The group served as a control for haemodynamic parameters. Group 3: Four dogs received no digoxin and served as a control for the entire experimental procedure.

Experimental procedure

Anaesthesia was induced with 300–500 mg of narcobarbital (Narcodorm). After relaxation with tubo-

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curarine, orotracheal intubation was performed and the animal was connected to a respirator (Bird Mark 10) via a ventimeter (Airshield V-2) to ensure constant ventilation. Anaesthesia was maintained with N_2O/O_2 (flow 3 and 1.5 l/min, respectively) in a to-and-fro system. The anaesthetic together with pethidine (10 mg/kg), given once or twice an hour when needed, abolished pain reflexes and only when absence of pain had been assured was (+)-tubocurarine (10 mg i.v.) given to relax the chest and so facilitate the operative procedure.

The heart was exposed through a left-sided anterior thoracotomy. The left atrium was catheterized through the auricle for pressure recording and blood sampling. Retrograde catheterization of the left ventricle was performed with a catheter of the pig tail type. Atrial and ventricular pressures were measured by the use of Elema Schönander transducers (type EMT 35) (resonant frequency (F_D) of the catheter transducer system = 20 Hz and damping factor (β) = 0.2). The first derivative of the ventricular pressure curve, was obtained by means of a differentiating unit with a cut-off frequency of 338 Hz (King & Searle, 1972). Since completing these studies, similar results were obtained using a catheter with an F_D of about 35 Hz, β of 0.7 and a cut off frequency of the differentiating unit of about 600 Hz (N.E. Bille-Brahe, E. Steiness & N. Lomholt, unpublished observations).

The haemodynamic parameters and a standard limb lead of the ECG were displayed simultaneously on an Elema Schönander multichannel recorder (Mingograf 81).

When haemodynamic stability was reestablished (usually after 10–15 min), pethidine and tubocurarine were discontinued and halothane was added to the anaesthetic mixture in a concentration (0.5–0.7%) sufficient to cause 20–50% depression of the initial dp/dt_{max}; 60 min were allowed for stabilization at this new level before digoxin, 0.05 mg/kg body weight, was given as an intravenous bolus injection.

Blood samples

Heparinized arterial blood samples were drawn before and 5, 10, 15, 20, 30, 45, 60, 75, and 90 min after the injection of digoxin for measurements of plasma concentrations of digoxin, sodium, and potassium.

Arterial P_{O_2}, P_{CO_2}, and pH were determined before and 10, 30, 60, and 90 min after the administration of digoxin.

Biopsies

A knife biopsy weighing 200–400 mg was taken from the left ventricular wall 15 min before the administration of digoxin in all dogs. Great care was taken to avoid cutting coronary vessels. The myocardial incision was closed with a continuous silk suture. In group 1 and 3, myocardial biopsies were again taken at 10, 30, 60, and 90 minutes. In group 2, a biopsy was taken again at 90 min only.

Any blood loss, including sampling, was replaced with an electrolyte-containing plasma expander (Haemaccel), and the estimated loss of water by evaporation was replaced with isotonic glucose.

All the animals were finally killed with intravenous narcobarbital.

Analytical procedures

Plasma digoxin concentrations were measured in duplicate by radioimmunoassay (Steiness, 1974).

Plasma potassium and sodium were determined in duplicate by flame photometry (Instrumentation Laboratory 143). Blood gases and pH were measured with a Radiometer.

The myocardial biopsies were divided into four pieces, one of which was used for determination of digoxin and the others for measurement of potassium.

The tissue content of digoxin was estimated by the cited radioimmunological method, following homogenization in 0.9% w/v NaCl solution (saline) and extraction with dichloromethane (Steiness, 1974). The sensitivity of the method used was 10×10^{-9} mol/kg tissue. The recovery of known amounts of digoxin added to the myocardial homogenates averaged 95% (s.d. = ±6%) between 50 and 1000 mol $\times 10^{-9}$/kg tissue.

Tissue content of potassium was determined in triplicate with a s.d. of 19 mmol/kg fat-free tissue according to Valentin & Olesen (1973). Briefly, the specimens were weighed before and after drying and after extraction of fat to determine the content of water and fat-free solids. The tissue content of potassium was measured following extraction with nitric acid and expressed in mmol/kg fat-free solids.

Statistics

The Wilcoxon test for paired data was used for the determination of statistical significance.

Results

Digoxin

After injection, similar plasma digoxin curves were obtained in groups 1 and 2 (Figure 1). Plasma concentrations continued to fall throughout the study.

Left ventricular digoxin concentration increased after intravenous injection and reached a median value of 67% of the maximum median value within 10 min (Table 1). A significant uptake continued beyond the first 10 min, since the median value was significantly higher after 60 min than after 10 min ($P < 0.02$,

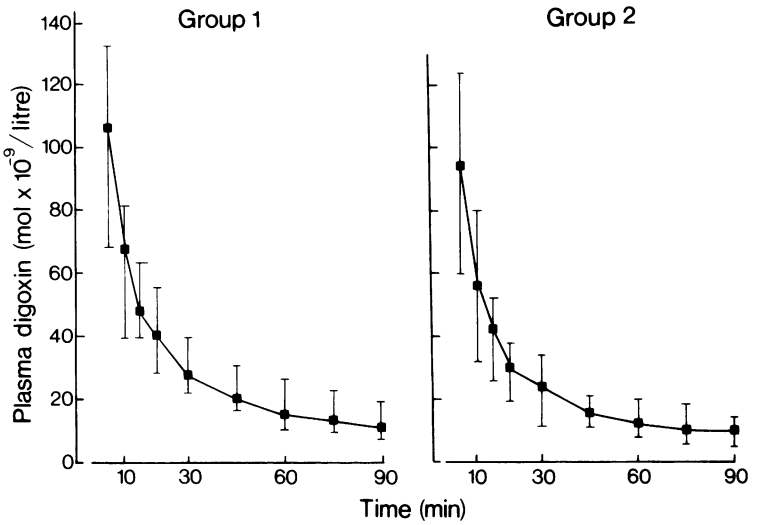

Figure 1 Plasma digoxin concentrations (median) in dogs following an intravenous dose of 0.05 mg digoxin/kg body weight. The vertical lines represent the ranges.

group 1). The peak myocardial digoxin concentration was reached between 30 and 90 min (Figure 2). No significant difference between groups 1 and 2 was found in the myocardial content of digoxin at 90 minutes.

Electrolytes

Only minor changes in plasma potassium were observed and plasma concentration of sodium remained unchanged throughout the study. The

Table 1 Myocardial digoxin concentration (mol x 10^{-9}/kg tissue and in parentheses μg/kg tissue) in dogs following an intravenous injection of 0.05 mg digoxin/kg body weight

		Minutes after digoxin				
Group		*0*	*10*	*30*	*60*	*90*
1	Median	0	196 (154)	228 (179)	293 (230)	237 (186)
n=8	Range		166–284	189–376	136–385	150–467
2	Median	—	—	—	—	226 (177)
n=7	Range					188–406

Table 2 Myocardial potassium content (mmol/kg fat-free solids) in dogs following intravenous administration of 0.05 mg digoxin/kg body weight

		Minutes after digoxin				
Group		*0*	*10*	*30*	*60*	*90*
1	Median	375	387	365	353	358*
n=8	Range	340–462	350–475	273–431	330–427	334–402
2	Median	383	—	—	—	357*
n=7	Range	351–429				321–408
3	Median	318	300	307	327	321
n=4	Range	250–341	277–300	266–396	266–361	266–394

* The pooled values for groups 1 and 2 were significantly lower at 90 min than at 0 min ($P<0.01$).

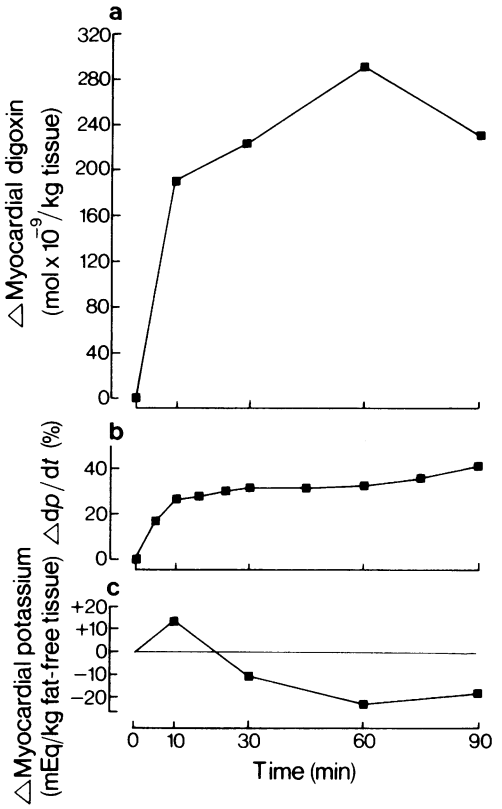

Figure 2 Median values of (a) myocardial digoxin concentration, (b) left ventricular contractility (dp/dt_{max}), and (c) myocardial potassium in dogs following an intravenous injection of digoxin (0.05 mg/kg body weight). The dp/dt values are from group 2 (no cardiac surgery between 0 and 90 min, the other data from group 1.

anaesthetic and experimental procedures themselves caused no change of the plasma concentrations of potassium and sodium.

Following a slight initial increase (insignificant) the median myocardial potassium concentrations decreased during the study period (Table 2). Myocardial potassium concentrations were similar in groups 1 and 2 before administration of digoxin and both groups showed a similar reduction at 90 minutes. By contrast the experimental procedure itself caused no change in the myocardial potassium, although the zero level of myocardial potassium was, for unknown reasons, significantly lower in group 3 than in groups 1 and 2.

Haemodynamic parameters

As shown in Table 3, left ventricular dp/dt_{max} increased during the first 10 min in both groups 1 and

Table 3 Left ventricular contractility (dp/dt_{max}) following intravenous administration of 0.05 mg digoxin/kg body weight

Group		*Minutes after digoxin*										
		0	*5*	*10*	*15*	*20*	*30*	*45*	*60*	*75*	*90*
1	Median	100	119	121	112	114	122	119	124	122	118
$n=8$	Range	—	105–150	110–135	100–130	100–136	105–155	89–143	95–150	84–154	89–147
2	Median	100	116	127	127	127	129	131	137	137	139
$n=7$	Range	—	100–142	104–167	104–167	108–150	108–163	113–175	113–208	113–192	100–200
3	Median	100	100	96	105	100	100	95	100	100	105
$n=4$	Range	—	90–107	90–110	96–107	90–110	96–105	90–105	95–107	95–106	90–110

2. In group 1 dp/dt_{max} tended to fall after each biopsy and the slight increase at 90 min was insignificant. In group 2 there was a similar rapid increase in contractility (Table 3), but in these animals in contrast to those of group 1, peak dp/dt_{max} remained stable after 10 minutes.

Other haemodynamic parameters, left ventricular systolic and end-diastolic pressures, left atrial mean pressure and heart rate remained unchanged during the study.

Blood gases

Arterial P_{O_2}, P_{CO_2}, and pH did not change significantly during the study.

Discussion

Previous studies of myocardial digoxin uptake have been performed either on isolated muscles (Prindle, Skelton, Epstein & Marcus, 1971) or using intact animals, which were killed at different times after the administration of digoxin (Cohn, Kleiger & Harrison, 1967; Deutscher, Harrison & Goldman, 1972; Goldman, Deutscher, Schweizer & Harrison, 1972; Hopkins, Lloyd & Taylor, 1974). Most measurements were performed at 30 to 60 min after the administration of digoxin, when a myocardial digoxin of about 200–300 mol $\times 10^{-9}$/kg tissue was found.

Deutscher *et al.* (1972), Goldman *et al.* (1972) and Hopkins *et al.* (1974) studied myocardial uptake as early as 5 to 15 min after administration of digoxin in a small series of intact dogs and found, even at these times, a high myocardial content of digoxin. However, individual variations in the digoxin content were large. These methods require one animal for each determination and are poorly suited for study of the temporal pattern of uptake.

With the sequential biopsy technique used in this study it was shown that the myocardial digoxin concentration was high 10 min after injection, confirming the results of Deutscher *et al.* (1972), Goldman *et al.* (1972), and Hopkins *et al.* (1974). In addition a significant uptake of digoxin was shown to continue for at least 60 min after administration. A tendency towards a fall in myocardial digoxin content between 60 and 90 min perhaps reflects the early phase of elimination from tissues (Figure 2).

Immediately after intravenous digoxin, plasma concentrations were high. However, even at 5 min the plasma concentration was lower than the total myocardial content at 10 minutes. Plasma protein binding in dogs is less than in humans, being 15 and 25%, respectively (Steiness & Rasmussen, unpublished data), and the total extracellular space of the myocardial tissue amounts to approximately 30% tissue weight (Glitsch, 1972). Consequently, less than 10% of the myocardial digoxin content at 10 min was present in the extracellular myocardial fluid. In conformity with other papers dealing with this subject, the total myocardial digoxin content has, therefore, been given without correction. The present investigation, in agreement with studies of the subcellular fractions of myocardial digoxin (Conrad & Baxter, 1964; Dutta, Goswami, Lindower & Marks, 1968) suggests that the myocardial uptake takes place by rapid active cellular transport.

A significant positive inotropic effect was demonstrated 5 min after intravenous administration of digoxin to dogs with a controlled and stable anaesthetic cardiodepression. The increase of the positive inotropic effect continued from 5 to 10 min and in accordance with the results of Deutscher *et al.* (1972) and Goldman *et al.* (1972) we were unable to demonstrate further significant increases of the contractility after 15 minutes.

A transient decrease of dp/dt_{max} was observed after a biopsy. The myocardial uptake of digoxin and the changes of myocardial potassium content (group 1) are therefore compared to the haemodynamic parameters from the series not subjected to repeated biopsy (group 2). The two groups are comparable since no significant difference was found between the plasma digoxin curves (Figure 1), the myocardial content of potassium before and 90 min after digoxin (Table 2), or the myocardial content of digoxin 90 min after digoxin (Table 1).

The development of the positive inotropic effect during the first 10 min seemed related to the myocardial uptake of digoxin. On the other hand, the myocardial digoxin content continued to increase up to 60 min without a corresponding increase of the positive inotropic effect (Figure 2). The digoxin uptake between 10 and 60 min may, therefore, not be correlated to further digoxin binding at the binding sites responsible for inotropic effect.

It is well established that digitalis glycosides inhibit the active Na^+-K^+ transport across the cell membrane, causing a decrease of the intracellular potassium content. In accordance with this well-described phenomenon, a loss of myocardial potassium was observed between 30 and 90 min after administration of digoxin, while no significant change was observed at 10 minutes. The results suggest a possibility of a change of the myocardial content of potassium following an intravenous digoxin dose. Such a possibility would accord with the results from studies of the effect of digitalis on the isolated Na^+-K^+ ATPase enzyme (Lee & Yu, 1963; Palmer & Nechay, 1964; Oppelt & Palmer, 1966). On the other hand, measurements of the myocardial cellular influx and efflux of potassium have revealed that whereas low doses of digoxin inhibit the potassium efflux more than the corresponding potassium influx and thereby increase the cellular potassium content, higher doses of digoxin inhibit the potassium influx more than the corresponding efflux and thereby reduce the cellular

content of potassium (Grupp & Charles, 1964). The initial minor increase (non-significant) of the myocardial content of potassium observed in the present study could be due to either a greater initial inhibition of the potassium efflux than that of potassium influx, or an initial stimulation of the ATPase.

The positive inotropic effect was demonstrated shortly after intravenous administration of digoxin, and a near maximum effect was reached by 10 min, when the myocardial concentration of digoxin was high. At the same time, evidence of low ATPase digoxin binding was demonstrated. Subsequent binding of digoxin at the 'ATPase binding site' was not followed by a major increase of the positive inotropic effect.

These observations suggest that the positive inotropic effect is independent of the digoxin effect on the Na^+-K^+ ATPase. This is in agreement with the results from wash-out studies in isolated heart preparations perfused with a constant concentration of digitalis (Okita, Richardson & Roth-Schechter, 1973; Peters, Raben & Wassermann, 1974). In these studies the ATPase inhibition was washed out more rapidly than the positive inotropic effect. In accordance with these investigations the present results lead to the assumption that at least two different binding sites for digitalis are present in the myocardium. One of these is responsible for the inhibition of the membrane ATPase and the other for development of the positive inotropic effect.

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