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MYOCARDIAL UPTAKE OF DIGOXIN IN CHRONICALLY DIGITALIZED DOGS

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1 The time course of myocardial uptake of digoxin, increase in contractility and changes in myocardial potassium concentration was studied for 90 min following an intravenous digoxin dose to long-term digitalized dogs.

2 Nineteen dogs were investigated by the use of a biopsy technique which allowed sampling before and after administration of digoxin.

3 Ten minutes after administration of digoxin the myocardial concentration increased from 60 to 306 nmol/kg tissue; the myocardial concentration of digoxin was significantly lower (250 nmol/kg tissue) after 30 min and then increased again.

4 The transmural myocardial distribution of digoxin was uniform before and 90 min after administration of digoxin in long-term digitalized dogs but at 10 min after administration, both the subepicardial and the subendocardial concentration of digoxin were significantly lower than that of the mesocardial layer.

5 During the first 10 min the dp/dt_{max} increased to 135% of the control level. The increase remained unchanged during the rest of the study.

6 Myocardial potassium decreased throughout the study.

7 The M-configuration of the myocardial uptake curve and the non-uniformity of myocardial distribution of digoxin observed at 10 min after administrating digoxin to long-term digitalized dogs indicate that the distribution of myocardial blood flow may be changed during chronic digitalization.

Introduction

Studies of the myocardial uptake of digoxin in dogs with intact circulation have demonstrated a very fast myocardial uptake of digoxin and an initial correspondence between the myocardial digoxin concentration and the positive inotropic effect (Doherty & Perkins, 1966; Goldman, Deutscher, Schweizer & Harrison, 1972; Deutscher, Harrison & Goldman, 1972; Steiness & Valentin, 1976). In previous work it was indicated that the positive inotropic effect is secondary to changes in the intracellular concentrations of sodium and potassium induced by digitalis (Langer, 1972).

On the other hand, both *in vitro* and *in vivo* studies have demonstrated digitalis-induced positive inotropic effect without simultaneous inhibition of the Na-K membrane ATPase (Klaus, Kuschinsky & Lüllmann, 1961; Grupp & Charles, 1964; Lüllmann & Ravens, 1973; Okita, Richardson & Roth-Schechter, 1973; Steiness & Valentin, 1976). These observations, therefore, suggested the existence of at least two different myocardial receptor sites for digitalis. One of these, localized on the cell membrane, is responsible for the inhibition of the Na-K membrane ATPase (Schwartz, Matsui & Laughter, 1968). The localization of the other receptor is unknown, but could be correlated to the liberation and the rebinding of ionized calcium during the contractile cycle and thus be responsible for the positive inotropic effect.

These problems have been investigated in animals without previous long-term digitalization. The purpose of the present study was to see whether long-term treatment with digoxin influences the rate of the myocardial digoxin uptake, the digoxin-induced positive inotropic effect and the digoxin-induced myocardial loss of potassium produced by intravenous digoxin.

Methods

Nineteen dogs, ranging in weight from 17.5 to 26 kg (median 21 kg), were studied. Eleven of the dogs

received 0.25 mg digoxin twice daily during the last 8 days before the study. The last dose was given not later than 12 h before the study to ensure an equilibrium between the plasma concentration of digoxin and the myocardium (steady state). These dogs were divided into two groups. Group 1: In eight dogs series of myocardial biopsies were taken before and after an intravenous dose of digoxin (0.05 mg/kg body weight). Group 2: In three dogs myocardial biopsies were only taken before and 90 min after administration of digoxin; this group served as control for the haemodynamic parameters. Further, in two dogs (Group 3) without previous long-term digitalization and in three dogs (Group 4) pretreated with digoxin as previously described transmural biopsies were taken from the left ventricular wall 10 min after an intravenous dose of digoxin. Group 5 comprised three chronically digitalized dogs from which transmural biopsies were taken 90 min after administration of digoxin. All the dogs received a normal diet and no medication apart from digoxin was given. Four of the animals, those with the highest plasma concentrations of digoxin, lost their appetite after five to six days of digitalization, but no vomiting or diarrhoea was observed. No electrocardiographic sign of digitalis toxicity was found.

Experimental procedure

Anaesthesia was induced with narcobarbitone (Narcodorm) and following tubocurarine and pethidine, orotracheal intubation was performed and a respirator connected. Anaesthesia and relaxation were maintained with pethidine, tubocurarine, and N_2O/O_2, 3 + 1.5 litres/min in a to-and-fro system.

Left-sided thoracotomy was performed and the left atrium was catheterized through the left auricle. Retrograde catheterization of the left ventricle was performed from the left femoral artery. In three dogs the aorta was catheterized instead of the left atrium. Atrial and left ventricular or aortic pressures were measured by Elema Schönander transducers (type EMT 35). The pressures and a standard limb lead of the ECG were recorded simultaneously. The first derivative of the ventricular pressure curve, i.e. left ventricular dp/dt_{max}, was obtained by means of an R/C differentiating unit.

When haemodynamic stability was established, pethidine and tubocurarine were discontinued and halothane was added to the anaesthetic mixture in a concentration of about 0.7%, sufficient to reduce the dp/dt_{max} by about 25–50%. Sixty minutes were allowed for stabilization at this level before the intravenous administration of 0.05 mg digoxin/kg body weight. This digoxin dose is known to cause a positive inotropic effect but no toxic symptoms in dogs without previous long-term digitalization (Steiness & Valentin, 1976).

Blood samples

Heparinized arterial blood was drawn for measurement of plasma concentrations of digoxin, potassium, and sodium just before and 5, 10, 15, 20, 30, 45, 60, 75, and 90 min after the intravenous injection of digoxin. Arterial P_{O_2}, P_{CO_2}, and pH were measured before and 10, 30, 60, and 90 min after administration of digoxin in Group 1.

Biopsies

In Groups 1 and 2 biopsy specimens weighing 200–400 mg were taken from the left ventricular wall, about one quarter to one third of the walls thickness, 15 min before the intravenous administration of digoxin. In Group 1 myocardial biopsies were again taken after 10, 30, 60, and 90 minutes. In Group 2 an additional biopsy was taken after 90 min only.

Transmural biopsy specimens were taken from two different parts of the left ventricle of the animals in Groups 3, 4, and 5. Each of these specimens was divided into three layers of the wall, i.e. the subendocardial, the mesocardial, and the subepicardial.

Any blood loss, including sampling, was replaced with an electrolyte containing plasma expander (Haemaccel), and water loss with isotonic glucose.

All animals were killed at the end of the experiment.

Analytical procedure

Plasma and myocardial concentrations of digoxin were measured in duplicate by radioimmunoassay as previously described (Steiness, 1974; Steiness & Valentin, 1976). The sensitivity of the method is 0.25 nmol/l plasma and 10 nmol/kg tissue; the precision is 0.06 nmol/l plasma and 5 nmol/kg tissue. The small size of the single myocardial biopsy specimens did not allow measurements of digoxin in subcellular fractions.

Plasma potassium and sodium were determined in duplicate by flame-photometry. Tissue potassium was determined in triplicate according to Valentin & Olesen (1973) and expressed in mmol/kg fat-free solids.

Blood gases and pH were measured with a Radiometer.

Statistics

All the statistical tests used were non-parametric and based on the ranking of results. The Wilcoxon test for paired data was used for the determination of statistical significance. For comparison between different groups, the Wilcoxon test for non-paired data was used. When more than two sets of data from the same group were compared, the Friedman two-way analysis of variance was used (for methods see Geigy Scientific Tables, 1970).

Results

As shown in Table 1 there was no correlation between the digoxin concentration in the plasma and in the myocardium at steady state.

After the intravenous administration of digoxin, a rapid increase of plasma digoxin concentration was followed by a gradual decrease (Figure 1). The myocardial digoxin concentration increased sigficantly and 10 min after the injection it was four to five times higher than the steady state level ($P < 0.01$) (Table 1, Group 1). Later the myocardial digoxin concentration decreased, and after 30 min it was significantly lower than the 10 min value ($P < 0.02$) but still significantly higher than the initial level ($P < 0.01$). This decrease was followed by a secondary increase ($P < 0.02$) and the 10 min level was reached about 60 min after the digoxin administration. Between 60 and 90 min a minor decrease in myocardial digoxin concentration was observed. The myocardial digoxin concentrations before and 90 min after digoxin administration in Group 2 were within the ranges of those observed in Group 1 (Table 1).

In dogs without previous long-term digitalization the digoxin concentrations found in the subepicardial, subendocardial and mesocardial layers 10 min after intravenous administration of 0.05 mg digoxin/kg body weight did not differ significantly from one another (Table 2, Group 3). Similar results were observed 90 min after digoxin administration in the chronically digitalized dogs (Table 2, Group 5). However, in the chronically digitalized dogs the digoxin concentrations found in the mesocardial layers of the myocardium after 10 min were significantly higher than in the other layers ($P < 0.05$).

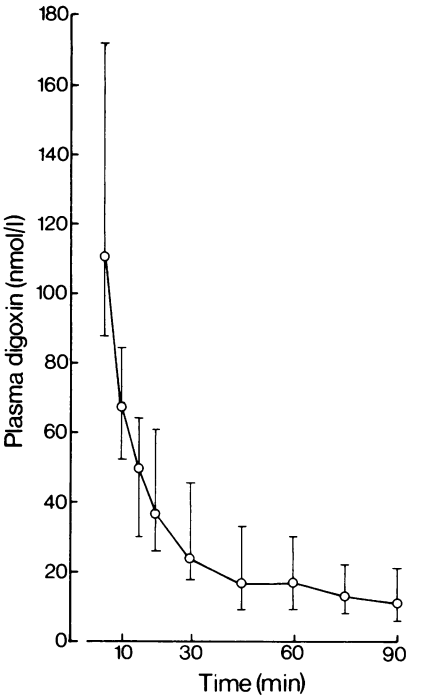

Figure 1 Median plasma digoxin concentrations after intravenous administration of 0.05 mg digoxin/kg body weight to chronically digitalized dogs.

Table 1 Steady state plasma digoxin concentrations and myocardial concentrations of digoxin before and after intravenous administration of digoxin to chronically digitalized dogs. (Figures in parentheses are μg/l plasma or μg/kg tissue)

	Dog no.	*Plasma digoxin in nmol/l (or μg/l)*	*Myocardial digoxin in nmol/kg (or μg/kg) Minutes after digoxin*				
			0	10	30	60	90
Group 1	1	1.4 (1.1)	56 (44)	189 (148)	159 (125)	230 (180)	218 (171)
	2	1.2 (0.9)	78 (61)	302 (237)	238 (187)	—	206 (162)
	3	0.6 (0.5)	149 (117)	509 (399)	337 (264)	570 (447)	457 (358)
	4	1.8 (1.4)	13 (10)	310 (243)	262 (205)	300 (235)	202 (158)
	5	2.3 (1.8)	40 (31)	134 (105)	164 (129)	158 (124)	187 (147)
	6	0.8 (0.6)	33 (26)	216 (169)	103 (80)	175 (137)	245 (192)
	7	0.6 (0.5)	137 (107)	375 (294)	302 (237)	384 (301)	321 (252)
	8	1.4 (1.1)	66 (52)	430 (337)	379 (297)	429 (336)	349 (274)
	Median	1.3 (1.0)	60 (47)	306 (245)	250 (196)	300 (235)	232 (182)
Group 2	9	2.6 (2.0)	96 (75)	—	—	—	323 (253)
	10	1.7 (1.3)	49 (38)	—	—	—	244 (191)
	11	2.3 (1.8)	135 (106)	—	—	—	358 (281)

Electrolytes

Only minor changes in plasma concentrations of potassium and sodium were observed. The myocardial concentration of potassium decreased rapidly after digoxin administration (Table 3, Group 1) and after 30 min was significantly lower than the initial content ($P < 0.02$). The decrease of the myocardial potassium concentration continued, although not significantly, during the remaining part of the study.

Haemodynamics

The contractility, dp/dt_{max}, increased within a few minutes after the administration of digoxin. Ten minutes after the administration, dp/dt_{max} had increased by a mean of 35% (Table 4). After repeated biopsy sampling in Group 1 some reduction in dp/dt_{max} was seen. By contrast, the control animals of Group 2 which were biopsied only at 0 and 90 min maintained steady dp/dt_{max} (Table 4). Other

Table 2 The transmural myocardial content of digoxin (nmol/kg tissue and in parentheses μg/kg tissue) 10 or 90 min after an intravenous dose of 0.05 mg digoxin/kg body weight

Treatment	*Group*	*10 min after digoxin*			*90 min after digoxin*		
		Subepi-cardial	*Meso-cardial*	*Subendo-cardial*	*Subepi-cardial*	*Meso-cardial*	*Subendo-cardial*
No long-term digitalization	3	289	265	322	—	—	—
		322	338	321	—	—	—
		260	242	275	—	—	—
		297	310	335	—	—	—
	Median	293(229)	276(216)	322(252)			
Chronic digitalization	4	313	378	312	—	—	—
		483	710	600	—	—	—
		656	750	550	—	—	—
		362	486	403	—	—	—
		439	562	467	—	—	—
	Median	439(344)*	562(441)	467(366)*			
Chronic digitalization	5	—	—	—	439	455	486
		—	—	—	420	443	467
		—	—	—	151	184	135
		—	—	—	170	169	184
		—	—	—	512	493	580
	Median				420(329)	443(347)	467(366)

* $P < 0.05$.

Table 3 Myocardial concentration of potassium (mol/kg fat free tissue) before and after an intravenous digoxin dose given to chronically digitalized dogs ($n = 8$)

	Minutes after digoxin				
	0	10	30	60	90
Median	369	354	350*	348*	328*
Range	336–381	291–390	299–378	310–379	301–377

* $P < 0.02$; ** $P < 0.01$.

Table 4 Left ventricular contractility (dp/dt_{max}) before and after intravenous administration of digoxin 0.05 mg/kg body weight to chronically digitalized dogs (% of initial value)

Group			Minutes after digoxin										
			0	5	10	15	20	30	45	60	75	90	
1 ($n=8$)	dp/dt_{max} % change	Median	100	117**	135**	115	110	120	120	118	118	118	
		Range		5–55	5–100	0–75	0–90	5–90	0–110	0–100	0–100	0–100	
	dp/dt_{max}	Dog 9	100	119	120	122	125	125	119	125	125	130	
2	dp/dt_{max}	Dog 10	100	130	130	130	140	140	140	140	150	140	
	dp/dt_{max}	Dog 11	100	120	120	125	124	130	130	130	124	—	

** $P<0.01$.

haemodynamic parameters, left ventricular systolic and end-diastolic pressures and ventricular rate, remained unchanged, and the ECG demonstrated sinus rhythm during the study. No change in pH, Po_2, and Pco_2 was observed.

Discussion

Since it became possible to measure plasma concentrations of digoxin (Smith, Butler & Haber, 1969), many investigations have revealed fairly good correlation between the steady state digoxin concentration in plasma and its therapeutic effect (Chamberlain, White, Howard & Smith, 1970; Beller, Smith, Abelmann, Haber & Hood, 1971; Evered & Chapman, 1971; Bertler, Gustafson, Ohlin, Monti & Redfors, 1973). Therefore, it has been argued that the plasma digoxin concentration is related to the myocardial digoxin concentration. Indeed Coltart, Howard & Chamberlain (1972) and Redfors, Bertler & Schuller (1973) found correlation between the concentration of digoxin in plasma and atrial tissue in man. On the other hand, it has been difficult to demonstrate a similar correlation between the digitalis concentration in plasma and in the ventricle, possibly because of a varying degree of fibrosis in the human material (Storstein, 1973; Coltart, Gullner, Billingham, Goldman, Stinson, Kalman & Harrison, 1974). Nevertheless, no correlation between the plasma and the left ventricular concentration of digoxin was found in the present study of non-fibrotic dog hearts (Table 1).

Recent studies suggested the existence of at least two different myocardial receptor sites for digoxin (Okita *et al.*, 1973; Steiness & Valentin, 1976). The supposition is made that by measuring the decrease in myocardial potassium content as a term of the inhibition of the Na-K APTase, the binding of digoxin to one of these kind of receptors can be estimated from the effect of the binding. Binding to the 'inotropic' receptor might similarly be estimated from the development of the positive inotropic effect (dp/dt_{max}) (Klaus *et al.*, 1961; Grupp & Charles, 1964; Okita, Richardson, Roth-Schechter & Thomas, 1969; Roth-Schechter, Okita, Thomas & Richardson, 1970; Lüllmann & Ravens, 1973; Okita *et al.*, 1973; Steiness & Valentin, 1976). It was not possible, however, to evaluate the digoxin bound to the 'inotropic' receptor at steady state. Our technique for measurement of contractility (dp/dt_{max}) did not permit comparison between the absolute values of dp/dt_{max} from one series to another, but allowed only the following of changes of dp/dt_{max} in the same animal during the study.

The myocardial uptake of digoxin after intravenous digoxin in dogs without long-term digitalization was recently demonstrated to be very fast initially and to

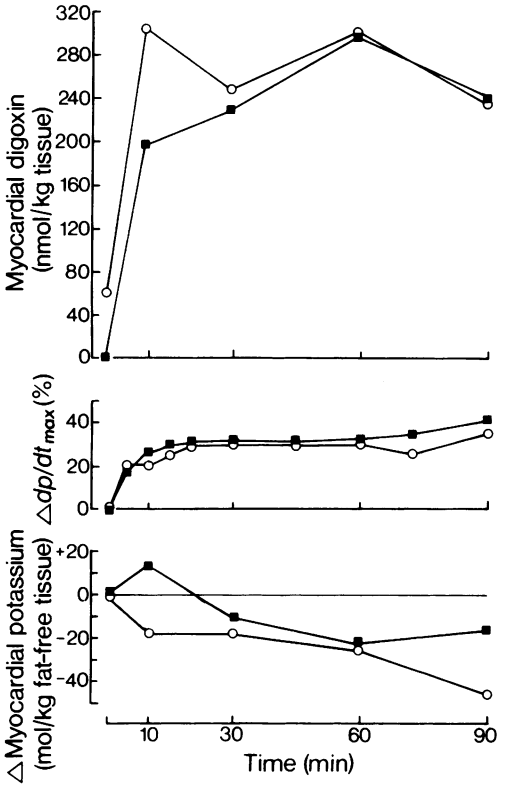

Figure 2 The myocardial concentrations of digoxin (median), the inotropic effect (median), and the myocardial potassium content (median) at intervals after an intravenous digoxin dose (0.05 mg/kg body weight) in chronically digitalized dogs (O) and in dogs without previous long-term digitalization (■); (Steiness & Valentin, 1976).

continue for 60 min (Figure 2 and Steiness & Valentin, 1976). Chronically digitalized dogs also had a very rapid initial uptake of digoxin in the myocardium but the subsequent shape of the uptake curves differed significantly (Figure 2). This difference could be due to a change of the digoxin affinity for one or both of the digoxin receptor sites. However, the positive inotropic effect, which is postulated to express the amount of digoxin bound to the 'inotropic' receptor, was quite similar to that produced by the same intravenous dose of digoxin in dogs without previous long-term digitalization (Figure 2). If this receptor had a changed affinity for digoxin, a parallel change in the inotropic response would be expected. It is therefore suggested that the 'inotropic' receptor has an unchanged affinity for digoxin after long-term digitalization with the doses given in the present investigation.

The affinity of digoxin to the Na-K APTase correlated receptor was also unchanged. As seen from Figure 2 the loss of myocardial potassium per unit time in the chronically digitalized dogs was equal to that in non-digitalized dogs, except during the first 10 minutes.

The loss of myocardial potassium following intravenous digoxin in non-digitalized dogs begins after about 15 min (Steiness & Valentin, 1976). This was interpreted as a slow binding of digoxin to the Na-K ATPase correlated receptor. In chronically digitalized dogs, however, the myocardial content of potassium under steady state conditions was not appreciably lower than in dogs without previous long-term digitalization (Steiness & Valentin, 1976). An additional increase of the digoxin receptor concentration must, therefore, produce further inhibition of the ATPase with a decrease of the myocardial content of potassium within 10 minutes.

The significant change in myocardial uptake of digoxin (Table 1 and Figure 2) may therefore be due to changes in the myocardial distribution of non-specifically bound digoxin rather than to changes in the digoxin uptake by one of the specific receptor sites. The myocardial biopsies in Group 1 were taken from the surface of the ventricular wall and were about one quarter to one third of the walls thickness. The biopsy involved the subepicardial layer and different amounts of the mesocardial layer of the ventricular wall. To elucidate the hypothesis that the myocardial distribution of digoxin is changed in chronically digitalized dogs, the transmural distribution of digoxin in the myocardium was investigated. Uniform concentrations of digoxin were found in different layers of the left ventricular wall 10 min after the intravenous administration of digoxin to non-digitalized dogs. On the other hand, in the chronically digitalized dogs both the subendocardial and the subepicardial concentrations were found to be significantly lower than the mesocardial concentration at 10 min but not at 90 min after digoxin administration. The difference between the non-uniform distribution of digoxin in chronically digitalized dogs and the uniform distribution in dogs without previous long-term digitalization suggests an initial slower rate of digoxin uptake into the subepicardial and the subendocardial layer in chronically digitalized dogs, that could be due to a change in myocardial blood flow. Our results do not permit evaluation of this suggestion.

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