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Digoxin-specific antibody fragments and a calcium antagonist for reversal of digoxin-induced mesenteric vasoconstriction

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The effect of digoxin-specific antibody fragments on glycoside-induced mesenteric vasoconstriction were investigated. Digoxin caused a sustained contraction of strips of isolated feline mesenteric artery lasting for several hours, while in anaesthetized cats it produced a significant decrease in blood flow and increase in resistance in the mesenteric artery. In-vitro, digoxin's contractile effect was inhibited by 'prophylactic' addition of antibody to the organ bath, but the clinical use for prophylaxis is not a practical proposition. When the antibodies were added with the contraction of the arterial strip in response to digoxin already established, the tone of the preparation decreased significantly over 3 h, but the effect of the glycoside was not fully reversible. In-vivo, control animals not treated with antibodies developed arrhythmias, mesenteric blood flow fell by more than 50% and resistance increased by more than 80% relative to the initial values. These animals died of ventricular fibrillation before the end of the experiment. Animals treated with digoxin-specific antibody fragments after receiving digoxin injections showed no further decrease in mesenteric blood flow and 90 min after the last dose of digoxin, the flow was recovering and mesenteric resistance decreasing. Furthermore, all the animals that had received antibodies remained in sinus rhythm to the end of the experiment. In view of the latent period to onset of action of the antibodies, valuable time may be lost in impaired mesenteric blood flow. To bridge the gap or, indeed, as primary treatment, calcium antagonists merit consideration; in our experiments mesenteric vasoconstriction was abolished within a few minutes by application of the dihydropyridine calcium antagonist 4-(2,1,3-benzo-oxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic aid, diethyl ester (PY 108-068).

Cardiac glycosides give rise to mesenteric vasoconstriction which may lead in man to signs of intestinal ischaemia (Feinroth et al 1980), intestinal necroses and, to a non-occlusive mesenteric infarction (Gazes et al 1961; Muggia 1967; Hess & Stucki 1975). In this rare, but normally fatal complication of digitalis therapy, angiography reveals marked spasms, but no signs of embolic or thrombotic occlusion of the mesenteric arteries are seen either in the angiogram, at exploratory laparotomy or at autopsy (Hess & Stucki 1975). The condition cannot, therefore, be remedied by surgery. In the light of experimental studies, a number of treatments have been recommended, including treatment with papaverine, phenoxybenzamine, glucagon and, latterly, calcium antagonists (Williams et al 1968; Danford 1971; Brobmann et al 1976).

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Animal experiments have demonstrated that antibodies against cardiac glycosides are capable of abolishing pharmacological and toxic effects of digitalis in-vitro and in-vivo. Ovine digoxin-specific Fab and F(ab')2 antibody fragments have been used clinically to treat suicidal and accidental digitalis poisoning associated with life-threatening arrhythmias (Smith et al 1976; Hess 1981). Antibody fragments have a number of advantages over intact IgG antibodies, being more rapidly distributed throughout the body compartments with a more rapid onset of action and a shorter half-life ($t_{1/2}$); furthermore, since they are devoid of the Fc component, they are less immunogenic (Lloyd & Smith 1978; Smith et al 1979). Their effect on cardiac glycoside-induced mesenteric vasoconstriction in digitalis poisoning had not hitherto been investigated. This study describes the effect of digoxin-specific antibody fragments on the mesenteric arteries of the cat in-vitro and in-vivo.

METHODS

Production of digoxin-specific antibody fragments

As described by Butler & Chen (1967) sheep were immunized with a digoxin-albumin conjugate in Freund's complete adjuvant and were given booster doses at regular intervals (Hess 1981). The ovine antidigoxin serum prepared from repeated blood samples was pooled, and gamma globulin was precipitated from it with ammonium sulphate. After cleavage with pepsin or papain (Nisonoff et al 1960), the digoxin-specific F(ab')2 or Fab fragments were isolated by immuno-adsorption (Curd et al 1971) and stored at −20 °C.

In-vitro experiments on feline isolated mesenteric arteries

Cats were anaesthetized by a chloralose-urethane mixture and the main trunk of the superior mesenteric arteries was removed and cut into spiral strips 15 mm long and 1 mm wide. The preparations were suspended under a load of 1 g in 10 ml organ baths containing Krebs-Henseleit nutrient solution with the following composition (mmol litre^{-1}): KCl 4·7; NaCl 118; $MgSO_4 \cdot 7H_2O$ 1·2; KH_2PO_4 $CaCl_2 \cdot 2H_2O$ 2·4; $NaHCO_3$ 24·9; glucose 10·1. The bath was aerated with oxygen (95%) and carbon dioxide (5%). When a stable resting tension was attained, digoxin was added to give a bath concentration of 1·56 μg ml^{-1} in all the experiments (n = 10) and the changes in tension were recorded continuously for 6 h. All tonic contractions throughout the study were expressed as % of the maximum effect (ie. maximum increase in tension in response to digoxin = 100%). In two experiments, digoxin-specific Fab antibody fragments were added, together with the digoxin at the start of the experiments at a concentration of 200 μg ml^{-1}, this providing a 2 mol litre^{-1} excess of antibody. In four experiments the same dose of antibodies was added 3 h after the digoxin. In another two experiments the digoxin-treated strips were washed repeatedly with glycoside-free Krebs-Henseleit nutrient solution for 10 s at 5-min intervals, the washout period starting 3 h after addition of digoxin.

In-vivo experiments on anaesthetized cats

The experiments were carried out on spontaneously breathing cats of either sex ca 1·8–2·4 kg. The cats were anaesthetized by i.m. injection of chloralose (43 mg kg^{-1}) and urethane (430 mg kg^{-1}) and the trachea and a femoral vein cannulated. The heart rate and mean arterial pressure were continuously recorded on a polygraph (Texas Instruments) via a catheter in a femoral artery using a Statham-P23 Db transducer. The e.c.g. from a limb lead was monitored on an oscilloscope. After median laparatomy, the superior mesenteric artery was freed below its origin in the aorta and an electromagnetic measuring head (diameter 2·0 to 2·5 mm) was fitted around the main trunk proximal to the first branch. Blood flow in the mesenteric artery was continuously recorded with an electromagnetic flowmeter (Narcomatik). The resistance was obtained by dividing the mean arterial pressure (mm Hg) by the flow rate (ml min^{-1}). Drug effects were expressed as percentage change of initial pretreatment values shown in Table 1.

Table 1. Initial pretreatment values (mean ± s.d.) from cat experiments. These are the absolute values from which % changes due to drug application are calculated and presented in Fig. 2.

	Before treatment with	
	digoxin (controls, n = 3)	digoxin + F(ab')2 (n = 5)
Body weight (kg)	2·4 ± 0·1	1·9 ± 0·2
Heart rate	225 ± 35	204 ± 19
Mean arterial pressure mmHg	134 ± 27	137 ± 12
Mesenteric flow ml min^{-1}	55 ± 13	29 ± 7
Mesenteric resistance mmHg ml^{-1} min^{-1}	2·5 ± 0·7	4·9 ± 1·5

As soon as the measured variables were stable, all the animals (n = 8) were given three doses of 50 μg kg^{-1} digoxin by i.v. injection at 10-min intervals. All animals, except for the three controls, were given 25 mg kg^{-1} digoxin-specific F(ab')2 antibody fragments intravenously 10 min after the third dose of digoxin and then observed during 90 min. Towards the end of the experiments in 4 cats previously treated with digoxin plus F(ab')2 the effect of PY 108-068, a recently developed benzoxadiazolyl dihydropyridine derivative with calcium slow channel blocking properties comparable to nifedipine (Hof et al 1982), in doses of 50 ng kg^{-1} given sublingually was observed for 15 min. The sublingual route of application was chosen since that route could be a possibility in emergency situations in man.

RESULTS

In-vitro experiments on isolated mesenteric arteries (Fig. 1)

Digoxin increased the tone of the artery strips within about 3 h, and where no antibodies were added to

the bath the state of contraction persisted almost unchanged until the end of the experiments, i.e. for 6 h. When each preparation was washed with digoxin-free solution, the extent of glycoside-induced contraction attained after 3 h was slightly increased during the first 30 min of the washout period, probably due to the replacement of the nutrient solution, and then the tissue gradually relaxed over the next 3 h, demonstrating that withdrawal of digoxin from the vessel strips is slow and detectable only after a period of time. When digoxin and specific Fab antibody fragments were given together at the start of the experiment, no digoxin-induced contraction occurred. However, when the Fab antibody fragments were added to the organ bath after digoxin had been allowed to act for 3 h, the contracted strip preparations showed some inconsistent relaxation, the digoxin response being reduced on average by 50%. In one of these experiments no significant antibody effect was discernible, while in another experiment Fab completely abolished the contractile state induced by the glycoside. In the other two experiments the antibody inhibited the digoxin response by 55 and 45%.

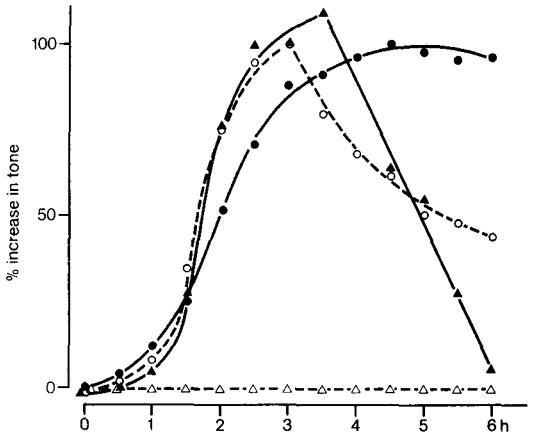

FIG. 1. Contraction of strips of mesenteric artery expressed as a % of the maximum response to 1·56 μg ml^{-1} digoxin (0 h), ●——● controls (n = 2); ○- - -○ Fab 200 μg ml^{-1} 3 h after digoxin (n = 4); △- - -△ Fab 200 μg ml^{-1} together with digoxin at 0 h (n = 2); ▲——▲ preparation washed from 3 h after digoxin onwards (n = 2).

In-vivo experiments in anaesthetized cats (Fig. 2)

Ten minutes after the third injection of digoxin, there was a fall in heart rate of about 10%, while the mean arterial pressure increased on average by 5%. Relative to the pretreatment value, blood flow in the mesenteric artery fell by 4% after the first injection of digoxin, by 10% after the second and by 32% after the third. The resistance increased 12% after the first, 25% after the second and 52% after the third injection of digoxin. Up to this time (30 min) there were no significant differences between the animals treated with F(ab')2 and the controls.

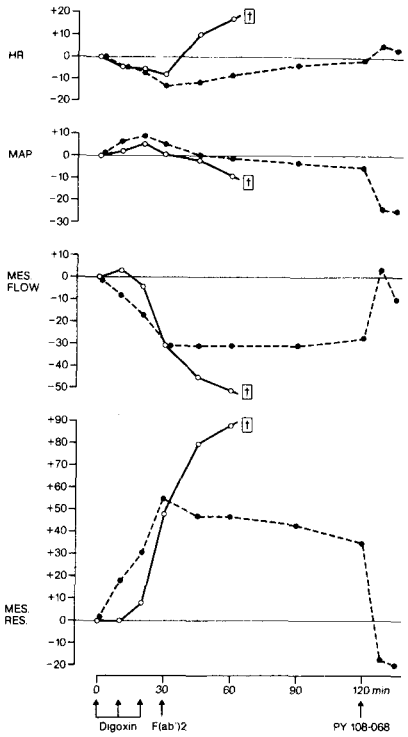

FIG. 2. Trend of heart rate (HR), mean arterial pressure (MAP), blood flow (MES.FLOW) and resistance (MES.RES.) in the mesenteric artery. The results (means) are the differences as a % of the initial values presented in Tab. 1. ○——○ controls (n = 3); ●- - -● F(ab')2 treatment (n = 5); arrows, indicate digoxin 50 μg kg^{-1} i.v., F(ab')2 25 mg kg^{-1}i.v., PY 108-068 50 μg kg^{-1} sublingually.

The controls (n=3) which had not received antibodies developed ventricular extrasystoles on average 10 min after the third injection of digoxin, and 15 min later went into ventricular tachycardia, followed within less than 1 h by fatal ventricular fibrillation. Initially the mean arterial pressure was unchanged, while mesenteric blood flow fell continuously and the resistance increased. Before terminal ventricular fibrillation supervened mesenteric blood flow was less than 50% of its initial value and the resistance had risen by more than 80%.

The animals (n = 5) treated with F(ab')2 after the third injection of digoxin had isolated ventricular extrasystoles for a brief period, but did not develop

persistent arrhythmias. They remained in sinus rhythm until the end of the experiment. Heart rate and mean arterial pressure 90 min after treatment with antibodies were within the range of their initial values. Mesenteric blood flow did not fall any further after injection of F(ab')2, and at the end of the experiment rose slightly, but did not reach the initial value. After antibody treatment the resistance elevated by digoxin fell slightly but remained 35% higher than the initial value 90 min after antibody injection.

Sublingual administration of the calcium antagonist PY 108-068 to four animals 100 min after the last injection of digoxin, led to a pronounced increase in mesenteric blood flow back to pretreatment values and decrease in resistance below its initial values within a few min. There was, however, a 25% decrease in mean arterial pressure, associated with a 5% increase in heart rate.

DISCUSSION

Our experiments in cats confirm the findings of Shanbour et al (1971), Bynum et al (1973), Pawlik & Jacobson (1974) and Mikkelsen et al (1979) that digoxin causes vasoconstriction in the mesenteric circulation and in-vitro contraction of strips of isolated mesenteric artery lasting for a number of hours. In-vivo digoxin caused a significant decrease in blood flow and an increase in resistance in the mesenteric circulation. The 'prophylactic' addition of antibodies prevented this effect of the glycoside in-vitro. A similar protective effect against digitalis-induced arrhythmias has been observed in-vivo in cats (Hess et al 1978). This effect is probably attributable to rapid binding and inactivation of the free digoxin by an excess of antibodies. Despite prophylactic use of these antibodies not being a practical proposition in man, specific antibodies may be of great value for the treatment of toxic effects of glycosides, e.g. in life-threatening arrhythmias (Smith et al 1976; Hess 1981; Smith et al 1982).

The in-vivo experiments confirmed earlier results (Hess et al 1978), in as much as the animals treated with antibody immediately after the digoxin injections remained in sinus rhythm until the end of the experiment, whereas all the control animals succumbed to toxic arrhythmias.

The delay occurring before any effect is discernible in response to cardiac glycoside antibodies is well known from earlier studies. For example, the $t\frac{1}{2}$ for abolition of the inotropic effect of ouabain by ouabain-specific antibodies was 124 ± 6 min (Gold & Smith 1974), while the $t\frac{1}{2}$ for abolition of the effects of digoxin on trabecular fibres of calf hearts by digoxin-specific antibodies was 70 min (Hess & Müller 1982). In the ouabain experiments a 1·1–1·5-fold molar antibody excess sufficed, whereas in the digoxin experiments a 2·5 molar excess of antibody was needed. Similarly, in experiments on dogs and cats with digitalis-induced toxic arrhythmias and in the clinical use of digoxin-specific antibodies, it was frequently a matter of hours before all signs of intoxication had completely regressed after administration of the antibodies (Smith et al 1977; Hess 1981). The time lag between administration of antibodies and a recognizable response in these situations is probably due to time for distribution of the antibody, binding of the glycoside in the extracellular space, dissociation of the glycoside from the receptor and binding of the glycoside to the antibodies as well as the time needed by the cells to recover from the abnormalities induced by the glycoside. In other words the latency is mainly governed by the glycoside-receptor dissociation constant, the effective antibody concentration and the specificity of the antibody or the glycoside-antibody association constant. In our experiments we were not able to determine all these variables. However, it may be assumed that if the antibodies had had a still higher affinity or had been used in greater concentration, the onset of action might have been more rapid.

Calcium antagonists merit consideration as treatment to bridge this latent period occurring before the onset of action of antibody therapy in impaired mesenteric blood flow. In our experiments mesenteric vasoconstriction and impaired blood flow were restored to normal within a few min by treatment with a new dihydropyridine calcium antagonist PY 108-068 (Hof et al 1981, 1982), which was administered 90 min after treatment with antibodies. However, there was an accompanying fall in mean arterial pressure reflecting general vasodilatation. The use of a calcium antagonist (verapamil) to abolish glycoside-induced mesenteric vasoconstriction has already been tested in animal experiments, and its efficacy confirmed (Brobmann et al 1976). Glycoside-induced impairment of mesenteric blood flow has also been reversed with glucagon (Danford 1971; Schwaiger et al 1979), and with perhexiline and histamine (Schwaiger et al 1979). However, increased mesenteric blood flow in response to histamine is to the muscle layers, at the expense of the mucosa and submucosa, while with perhexiline and glucagon normal blood flow is reinstated in all parts of the intestinal wall (Schwaiger et al 1979). Accordingly, histamine is probably unsuitable for

treatment of incipient intestinal necrosis due to digitalis.

In our view calcium antagonists should be the treatment of first choice for impaired mesenteric blood flow occurring in patients treated with cardiac glycosides, since calcium antagonists abolish the glycoside-induced mesenteric vasoconstriction immediately. Treatment with specific cardiac glycoside antibodies may also be expected to have a favourable effect, especially where intoxication is associated with arrhythmias. However, the response is not apparent for some time, so that calcium antagonists are probably useful as a means of bridging the gap.

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