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Effect of quinidine on the tissue binding of digoxin in guinea-pigs

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The mechanism of quinidine-induced decrease in the tissue distribution of digoxin to heart, liver and skeletal muscle has been examined in guinea-pigs. Quinidine, in the presence of adenosine-5′-triphosphate (ATP), inhibited the specific binding of digoxin in homogenates of heart, liver and muscle, while in the absence of ATP the inhibition was observed only in heart. The decrease in the tissue-to-plasma unbound concentration ratios (Kpu) of heart and muscle determined from in-vitro binding studies was comparable to that in the Kpu values observed in-vivo, while in liver it was not sufficient to account for the fall in $Kpu_{in\text{-}vivo}$ values. It is concluded that quinidine-induced decrease in the tissue distribution of digoxin in heart and muscle is due to inhibition of tissue binding of this drug, while that in liver could be partially attributed to the decrease in the tissue binding.

The concomitant use of digoxin and quinidine is an accepted drug combination, but there are hazards in its use (Gold et al 1932). When quinidine is given to patients (Ejvinsson 1978; Doering 1979; Leahey et al 1978; Schenck-Gustafsson et al 1981), dogs (Gibson & Nelson 1980) or guinea-pigs (Kim et al 1981; Geiger et al 1982; Sato et al 1983) receiving digoxin, the serum digoxin concentration increases. A reduction in both the total body clearance and the volume of distribution of digoxin, which account for the elevated digoxin concentration, have been observed (Gibson & Nelson 1980; Schenck-Gustafsson et al 1981; Sato et al 1983).

We have reported that, in guinea-pigs, quinidine decreased the volume of distribution, the tissue to plasma partition coefficient (Kp) and the total body clearance of digoxin, but did not change the blood to plasma concentration ratio (R_b) and plasma protein binding (Sato et al 1983). It has been reported that quinidine decreases the binding of ouabain and digoxin to (Na^+, K^+) ATPase in the heart (Straub et al 1978; Ball et al 1981). One possible explanation for the decrease in the tissue distribution of digoxin may be inhibition of digoxin tissue binding by quinidine. As possible mechanisms for the tissue binding of digoxin, specific binding in the presence of ATP (ATP-dependent binding) and non-specific binding (ATP-independent binding) were considered. The present study examined the effect of quinidine on ATP-dependent and independent bindings of digoxin to heart, liver and muscle homogenates.

Methods

Male Hartley guinea-pigs, 200–250 g, were used. [^{3}H]Digoxin (12α-labelled, specific activity 10·1 Ci $mmol^{-1}$) was obtained from New England Nuclear Co., Boston, Mass. Digoxin, ouabain and Tris-ATP were obtained from Sigma Chemical Co., St. Louis, MO. Quinidine was purchased from Tokyo Kasei Co., Tokyo, Japan. All other chemicals were commercial products and reagent grade.

A tissue binding study was made to determine ATP-dependent specific binding of digoxin using the membrane filtration method. Under light ether anaesthesia, the portal vein was cannulated and liver perfused with cold saline (4 °C) for 1 min. Then heart, liver and skeletal muscle were excised. 6% homogenate was prepared in 0·32 M sucrose using a motor-driven Potter type homogenizer. The binding of digoxin was determined according to Gelbart & Goldman (1977), with a slight modification. A final concentration of 10 nM [^{3}H]digoxin and $MgCl_2$ 3, NaCl 100, EGTA 1 (ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid), Tris-ATP 3 and Tris-HCl buffer 25 mM (pH 7·4) in a total volume of 5 ml was pre-incubated at 37 °C for 5 min. The binding reaction was started by addition of 0·5 ml of homogenate to the incubation mixture. An amount, 0·5 ml, was removed from the incubation mixture 12 min after initiation of the incubation and was filtered through a Millipore filter (0·45 μm). This was washed twice with 5 ml of ice cold 25 mM Tris-HCl buffer (pH 7·4) and dissolved in Bray's counting solution (60 g naphthalene, 100 ml of methanol, 20 ml of ethylene glycol, 4 g of PPO and 0·2 g of POPOP $litre^{-1}$ of dioxene). Radioactivity was measured in a liquid scintillation counter (Packard model 3255, Packard Instruments Corp., Downers Grove, IL). ATP-dependent binding was defined as the difference between the total binding and binding in the absence of ATP. Similar studies were carried out in the presence of 4·4 μM quinidine (the unbound concentration of quinidine in this system corresponded to the in-vivo plasma unbound concentration).

Tissue binding studies with the equilibrium dialysis method were made to determine ATP-independent binding of digoxin. Heart homogenate 20%, liver homogenate 33%, and muscle homogenate 25%, were prepared in 50 mM Tris-HCl buffer (pH 7·4). To avoid metabolism during incubation with digoxin, liver homogenate was pre-dialysed against 50 mM Tris-HCl buffer (pH 7·4) at 4 °C for 24 h. Equilibrium dialysis was at 37 °C for 12 h against 50 mM Tris-HCl buffer (pH 7·4) using a spectrapore membrane and semimicrocells. The binding of digoxin to tissue homogenates was un-

* Correspondence.

changed during dialysis for 12 to 18 h at 37 °C. The initial concentration of digoxin was 10 nM and that of quinidine was 200 μM (the unbound concentration of quinidine in this system corresponds to the in-vivo plasma concentration). After equilibrium was attained, aliquots of the buffer side were transferred into scintillation vials containing toluene scintillation cocktail (0·1 g of POPOP, 4·0 g of PPO and 500 ml of Triton X-100 litre^{-1} of toluene) and the radioactivity measured. Metabolites and degradation products arising during the incubation were determined according to Harrison & Gibaldi (1976), and were negligible.

The tissue-to-plasma partition coefficient (Kp), which is defined as the ratio of the drug concentration in the tissue to that in the venous plasma, was determined as follows. Adult, male Hartley guinea-pigs, 280–300 g, were simultaneously given 250 μg kg^{-1} of digoxin (containing 100 μCi kg^{-1} of [^{3}H]digoxin) and 25 mg kg^{-1} quinidine sulphate through a jugular vein cannula. The animals were killed 6 h after digoxin administration and the tissues were quickly excised, rinsed and blotted. The separation of metabolites from digoxin was by the method of Harrison & Gibaldi (1976). According to the equations proposed by Chen & Gross (1979), the Kp value was corrected from the apparent Kp value, which was defined as the tissue concentration divided by the arterial plasma concentration.

The in-vivo tissue-to-plasma unbound concentration ratio ($Kpu_{in\text{-}vivo}$) was defined by the following equation:

$$Kpu_{in\text{-}vivo} = Cb/Cu = Kp/fu \quad (1)$$

where Cb, Cu and fu are the bound drug concentration, the unbound drug concentration in the tissue and the plasma unbound fraction, respectively.

The in-vitro tissue-to-plasma unbound concentration ratio ($Kpu_{in\text{-}vitro}$) was determined according to Lin et al (1982) and Harashima et al (1984). Since it is difficult to perform binding studies using homogenized tissue without dilution, tissue homogenates were prepared in the buffer. If the drug binding characteristics are independent of the protein concentration, the bound drug concentration (Cb) can be described by the following equation:

$$Cb = d \cdot Cb' \quad (2)$$

where Cb′ and d are the observed bound drug concentration and the dilution factor, respectively. Thus the $Kpu_{in\text{-}vitro}$ value is given by

$$Kpu_{in\text{-}vitro} = d_1 \cdot Cb_1/Cu_1 + d_2 \cdot Cb_2/Cu_2 + 1 \quad (3)$$

where Cb_1 and Cu_1 are the bound and unbound drug concentrations obtained from ATP-dependent binding studies, and Cb_2 and Cu_2 are those obtained from ATP-independent binding studies. Protein concentration was determined by the method of Lowry et al (1951).

Statistical significance was analysed according to a two-tailed Student's *t*-test.

Results

The Kp values of digoxin determined from in-vivo studies are shown in Fig 1. In the control animals the Kp values were 8–15, while in the quinidine-treated animals the values of all tissues studied significantly decreased. The decrease in the Kp values was largest in liver (to 15% of control). To examine the mechanism of quinidine-induced decrease in the tissue distribution, we first determined the tissue binding of digoxin in the presence of 3 mM ATP with the membrane filtration method. The binding ratios (Cb/Cu) of digoxin in heart, liver and muscle after correction using equation 2 are shown in Fig. 2. The ATP-dependent binding ratios of all tissues studied were significantly decreased in the presence of quinidine. The washing procedure, which is unavoidable for the filtration method, may affect weak binding such as ATP-independent binding in the absence of

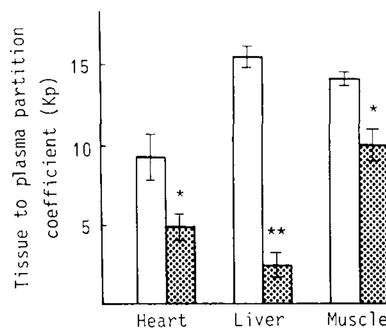

FIG. 1. Effect of quinidine on the in-vivo tissue-to-plasma partition coefficient at 6 h after bolus administration of 250 μg kg^{-1} digoxin. Open bars represent the control (without quinidine), while shaded bars represent the simultaneous administration of 25 mg kg^{-1} quinidine. * Significantly different from the control values ($P < 0·05$). ** Significantly different from the control values ($P < 0·01$).

Table 1. Relations between in-vivo and in-vitro Kpu values.[a]

	Heart		Liver		Muscle	
	in-vivo[b]	in-vitro[c]	in-vivo	in-vitro	in-vivo	in-vitro
Control	15·53 ± 2·43	9·24 ± 0·56	26·05 ± 1·05	3·88 ± 0·15	23·66 ± 0·65	8·70 ± 0·42
+ quinidine	8·28 ± 1·43	5·19 ± 0·49	4·10 ± 1·28	3·40 ± 1·58	16·64 ± 1·58	7·04 ± 0·47
% of control	53	56	16	88	70	81

[a] Results are given as the mean ± s.e. (n = 3–5).
[b] $Kpu_{in\text{-}vivo}$ was calculated by using equation 1.
[c] $Kpu_{in\text{-}vitro}$ was calculated by using equation 3.

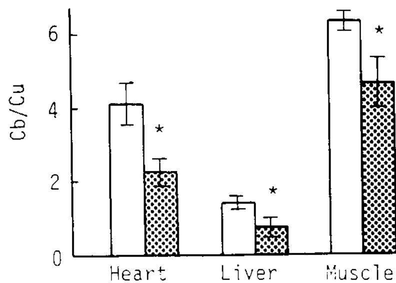

FIG. 2. Effect of quinidine on ATP-dependent binding. ATP-dependent binding was estimated with the membrane filtration method. Tissues were homogenized and incubated with 10^{-8} M digoxin at 37 °C for 12 h. Open bars represents the control (without quinidine), while shaded bars represent the presence of quinidine (the final concentration in the incubation mixture was 4·4 μM). * Significantly different from the control values ($P < 0.05$).

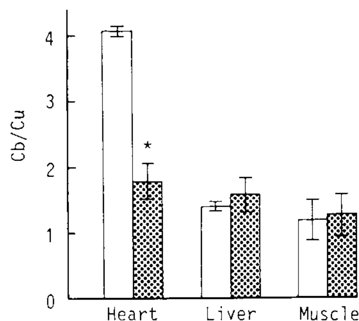

FIG. 3. Effect of quinidine on ATP-independent binding. ATP-independent binding was estimated with the equilibrium dialysis method. Tissues were homogenized and dialysed with 10^{-8} M digoxin at 37 °C for 12 h. Open bars represent the control, while shaded bars represent the presence of quinidine (the initial concentration in the incubation medium was 200 μM). * Significantly different from the control values ($P < 0.05$).

ATP, so we determined ATP-independent binding by the equilibrium dialysis method and the results are shown in Fig 3. The ATP-independent binding ratio of heart was decreased approximately 50% in the presence of quinidine, while quinidine did not alter ATP-independent binding in liver and muscle.

The comparison between the in-vivo and in-vitro Kpu values of heart, liver and muscle in the presence and absence of quinidine are summarized in Table 1. In heart and muscle the $Kpu_{in\text{-}vitro}$ values were one-half and one-third of those of the $Kpu_{in\text{-}vivo}$ values, respectively. Also decreases in the $Kpu_{in\text{-}vivo}$ and $Kpu_{in\text{-}vitro}$ values were observed in heart and muscle. A remarkable decrease in the $Kpu_{in\text{-}vivo}$ value was observed in liver in the presence of quinidine, while the $Kpu_{in\text{-}vitro}$ value showed no alteration.

Discussion

It has been reported that quinidine reduces the binding of cardiac glycosides to the supposed glycoside receptor, i.e. (Na^+, K^+) ATPase. Straub et al (1978) showed that quinidine reduced the number of digitalis binding sites as determined by in-vitro binding studies using (Na^+, K^+) ATPase from beef heart membrane. Ball et al (1981) reported that quinidine was capable of decreasing the affinity for digoxin of cardiac glycoside receptor sites on purified (Na^+, K^+) ATPase in guinea-pigs and on intact human erythrocyte membranes. In those studies, however, the concentration of quinidine was higher than the therapeutic range. Within the therapeutic range, Doering et al (1979) reported that quinidine failed to change the binding of ouabain to lamb ventricular sarcolemma. In the present study we have provided evidence that quinidine, in therapeutic concentration, inhibited ATP-dependent binding of digoxin to all tissues studied, and ATP-independent binding to heart homogenates (Fig. 2). This discrepancy might be due to the interspecies difference in the tissue binding (Fig. 2).

Kuschinsky et al (1968) demonstrated that the total tissue content of digoxin represented mainly the binding to non-specific sites. Our present results also suggested that ATP-independent binding might be one of the determinant factors for the tissue distribution of digoxin, though in the present study quinidine had no effect on ATP-independent binding to tissue homogenates except for heart (Fig. 3). ATP-independent binding might mainly represent the non-specific binding, but that the inhibitory effect of quinidine was observed only in heart indicated the existence of different characteristic binding in heart tissue. This is suggested by the fact that ATP-independent binding of digoxin to heart in the absence of quinidine was much greater than that to heart in the presence of quinidine and to other tissues in the presence or absence of quinidine (Fig. 3).

In heart and muscle, discrepancies were shown between the $Kpu_{in\text{-}vitro}$ and $Kpu_{in\text{-}vivo}$ values (Table 1). These discrepancies may be due to the inapplicability of extrapolation of the tissue binding data using diluted homogenates to those for 100% homogenates by using equation 2. But the inhibitory percent of $Kpu_{in\text{-}vitro}$ values in the presence of quinidine to that in the absence of quinidine was in good agreement with that of the $Kpu_{in\text{-}vivo}$ values. Furthermore, in liver, the discrepancy was shown between $Kpu_{in\text{-}vitro}$ and $Kpu_{in\text{-}vivo}$ values and between in-vivo and in-vitro inhibitory percentages of these parameters in the presence of quinidine. This finding revealed that in liver there may be other mechanism(s) for digoxin distribution than tissue binding. It has been reported that another cinchona alkaloid, quinine, decreased the initial uptake rate of ouabain by rat isolated hepatocytes (Eaton & Klaassen 1978). Similarly digoxin uptake by guinea-pig liver might be inhibited by quinidine.

In conclusion, the inhibitory effects of quinidine on the distribution of digoxin in the guinea-pig were due not only to the decrease in ATP-dependent binding in heart, muscle and liver but also to the inhibition of

ATP-independent binding in heart. Furthermore, in heart and muscle we could explain the decrease in the $Kpu_{in\text{-}vivo}$ values by in-vitro binding studies, while in liver this was not so.

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Mechanisms of the relaxations induced by 5-hydroxytryptamine on the rat isolated caecum

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The mechanism by which 5-HT produces relaxations of the rat caecum has been examined. Propranolol and cocaine markedly attenuated the relaxations whereas tetrodotoxin had no effect. Higher doses of propranolol and cocaine ($>10^{-6}$ M), as well as reserpinization, converted the relaxations into contractions. There was a residual relaxation resistant to propranolol, cocaine and reserpine treatment. High doses of 5-HT ($>10^{-5}$ M) were thus thought to relax the rat caecum indirectly, through the release of noradrenaline from the tissue by a tyramine-like action. The relaxations do not seem to be due only to the release of noradrenaline.

The resting tone of the rat caecum is such that the preparation responds to application of a drug with contractions or relaxations depending on the drug used. Sympathomimetic drugs cause relaxation and cholinomimetics (bradykinin and prostaglandins E_1, E_2 and F) contraction. Low doses of 5-hydroxytryptamine ($<10^{-6}$ M) produce a contraction which is converted to relaxation in the presence of atropine (Tayo & Acholem 1981). Higher doses of 5-HT ($>10^{-6}$ M) produce relaxation of the caecum in the absence of any antagonist (Uguru 1983). The possibility that this relaxation might be due to the release of noradrenaline from the tissue has been investigated. Adrenergic axons in other tissues are known to be capable of taking up 5-HT and releasing noradrenaline (Innes 1962; Owman 1964; Pluchino 1972).

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Materials and methods

Albino rats, 200–350 g, were killed and exsanguinated. The intestine was exposed and the caecum cut at the ileo-caecal junction. The appendix was removed and the caecal contents flushed out with Tyrode solution of the composition (mM); NaCl 137, KCl 2·7, $MgCl_2$ 0·5, $CaCl_2$ 1·36, NaH_2PO_4 0·3, $NaHCO_3$ 11·9, glucose, 11·1. The tissue, mounted in a 50 ml organ bath containing Tyrode solution between 35–37 °C and bubbled with air, was left for 1 h to equilibrate, the Tyrode solution being replaced every 15 min. Responses were recorded on an Ugo Basile Gemini recorder (CAT 7070) by means of an Ugo Basile isotonic transducer (CAT 7006). The tension on the tissue was between 0·8–1 g.

5-HT was added non-cumulatively to the bath and was left for 1 min before washing. 2–3 min were allowed between applications of 5-HT. Increasing concentrations of propranolol, cocaine or tetrodotoxin were added cumulatively to the Tyrode solution in the reservoir and tissues were exposed to each concentration for at least 30 min before addition of 5-HT. Other rats were treated with reserpine 4 mg kg^{-1}, 18–20 h before the addition of 5-HT.