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BINDING OF QUINIDINE IN SERUM AND HEART FROM NORMAL AND ANURIC RATS, AND THE SIGNIFICANCE FOR DISTRIBUTION

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Abstract—Quinidine is extensively bound to serum proteins and has a large apparent volume of distribution in rats. Quinidine concentration ratio heart/serum is higher and about 12 in normal rats and decreases to about 5 in anuric rats with simultaneous increase in serum binding. To evaluate whether the observed distribution pattern of quinidine can be explained by binding only, the quinidine binding was determined in heart homogenate and serum in normal and anuric rats. The results show that the extent of quinidine binding evaluated by both binding capacities and association constants, is similar in heart homogenate from anuric and normal rats but greater than in serum. The anuric state induced an increased binding of quinidine in serum. The results show that the *in vivo* distribution of quinidine to hearts of normal and anuric rats can be explained by pH-dependent distribution of unbound quinidine between tissue and plasma and the observed *in vitro* capacities and association constant of binding to heart homogenates and serum.

Many basic drugs are characterized by a large volume of distribution influencing the disposition kinetics [1]. Such drugs are also often extensively bound to plasma protein and distribution of such drugs between plasma and tissue has to be explained by pH-dependent distribution, and by tissue and plasma protein binding [2]. Quinidine has a large volume of distribution in rats [3] and in humans [4]. It has been shown that the volume of distribution decreases in anuric rats [3] with increase in plasma binding of quinidine [5]. The aim of the present study is to investigate whether quinidine is bound in a tissue like the heart and if binding in heart and serum can explain observed distribution of this drug and the decrease in distribution observed in anuric rats [3].

MATERIALS AND METHODS

Chemicals. Quinidine hydrochloride was supplied by the Norwegian Drug Monopoly, and ^{3}H-labelled quinidine with specific activity of about 500 mCi·mmole^{-1} was obtained from Buchler & Co., Braunschweig, W. Germany. Quinidine hydrochloride and tritiated quinidine was established to 96.7 and 98.8% pure in three t.l.c. systems (ethanol–methanol–chloroform 2:2:6 by vol.; cyclohexan–chloroform–diethylamin 5:4:1 by vol. and methanol-concentrated ammonia 100:1.5 v/v).

Animals and experimental procedure. Male rats, 250–350 g of the Wistar albino strain, were maintained for at least 5 days in a 12 hr light/12 hr dark cycle, and at constant temperature and humidity before use. They received laboratory rat chow and water *ad lib.* Acute anuria was induced as described by ligating the ureteres in aether narcosis [3].

Blood or heart tissue was sampled in aether narcosis 25–30 hr after the operation. Blood samples were placed for 30 min at 20° in sealed tubes and serum was then obtained after centrifugation at 1000 *g* for 30 min. Sera from 4 rats were pooled for binding experiments. Great vessels and the atria were removed and the remaining heart ventricles were rinsed in ice-cold saline, blotted on filter paper and kept at −20° until analysis.

The frozen hearts were pulverized in a stainless steel percussion mortar cooled in liquid nitrogen. Frozen tissue powder was homogenized in a glass homogenizer in 0.15 moles·l^{-1} potassium phosphate buffer of pH 7.0 (1:9 w/v). Homogenates from 3–4 hearts were pooled for each binding experiment. Supernatant of homogenate obtained after a light centrifugation (600 *g* for 2.5 min) was used for binding experiments.

Determination of protein binding. Equilibrium dialysis was performed in duplicate for each concentration of quinidine as described by Nilsen *et al.* [5]. Serum was dialyzed against an equal volume (0.5 ml) of Krebs–Ringer bicarbonate buffer of pH 7.35 in an atmosphere of 5.2% (v/v) carbon dioxide in air, and the homogenate supernatant was dialyzed against 0.15 moles·l^{-1} potassium phosphate buffer of pH 7.0. The serum binding of quinidine was not affected by varying pH between 7.35 and 7.45 at 20–23°. The equilibrium dialysis chambers were shaken mechanically at room temperature (20–23°). When quinidine in varying concentrations and ^{3}H-labelled quinidine were added to the protein side, equilibrium was reached within 4 hr. Serum was dialyzed for 18 hr and heart homogenate for 6 hr. Association constants and number of binding sites were determined from Scatchard plots as described by Rosenthal [6] using a computer method to obtain the best fit. The lines of the best fit are given with the Scatchard plots.

To reduce bacterial growth all glasswares were heat sterilized, the equilibrium dialysis cells were soaked in 70% ethanol before a final rinse in distilled water. Distilled water and buffer solutions were run through filters with pore size 0.22 μm.

The relative concentrations of quinidine in buffer and protein solution after dialysis were determined by

liquid scintillation, using duplicate samples of 50 μl from the buffer and protein side and 10 ml scintillation fluid: 4 g of 2.5-bis(5-*p*-butylbenzolyl)2′-thiothiphene (BBOT) and 80 g of naphthalene dissolved in 600 ml toluene and 400 ml ethylene glycol–monoethylether, (Koch-Light Laboratories Ltd., England). Counting efficiency was 26% and the relative counting efficiency of quinidine in homogenate supernatant was 98.2 ± 0.3 S.D. per cent compared to the efficiency in buffer (n = 16). No corrections for this quenching was made.

The concentration of protein was determined before and after equilibrium dialysis, in serum by the biuret method [7] and in homogenate by the method of Lowry *et al.* [8], using bovine serum albumin as standard. The dilution factor of proteins by dialysis was 1.23 and 1.20 for serum from normal and anuric rats respectively. Dilution factor for both types of heart homogenate supernatants was 1.24.

*p*H *in blood.* The rats were kept in aether narcosis during the sampling and kept warm with an infrared lamp, and the tail was submerged in lukewarm water, before blood was sampled from a cut in the tail. pH was measured at 37° by a pH meter 27 from Radiometer according to the technique described by Siggard-Andersen *et al.* [9].

RESULTS

Serum binding. The binding in serum samples from normal and anuric rats was determined at different concentrations of quinidine. The results are given in Fig. 1 for different concentrations of unbound quinidine. The results show that the binding is dependent on the concentration of unbound quinidine and is higher in serum from anuric than normal rats. The serum binding results are also given as Scatchard plots (Figs. 2 and 3). The lines of best fit were determined and the derived binding capacity and association constants are given in Table 1. Binding expressed by the product of association constant and binding capacity is higher in serum from anuric than normal rats, due to an increased binding capacity of the primary binding sites and association constant.

Binding to heart homogenates. Binding were determined at different concentrations of quinidine in supernatant of heart homogenates from normal and anuric rats. Binding expressed as amount of quinidine bound per g of protein at different concentrations of unbound quinidine as given in Fig. 1, is somewhat higher at heart homogenates from anuric rats than from normal rats. However, the binding capacity of heart homogenates is higher in normal than in anuric rats (Table 1). Binding results were also plotted according to Scatchard [10] (Fig. 4), and derived binding capacities, association constants, and the products of these are given in Table 1. Binding of quinidine to heart homogenates expressed by the product of association constant and binding capacity is similar in the two groups of rats.

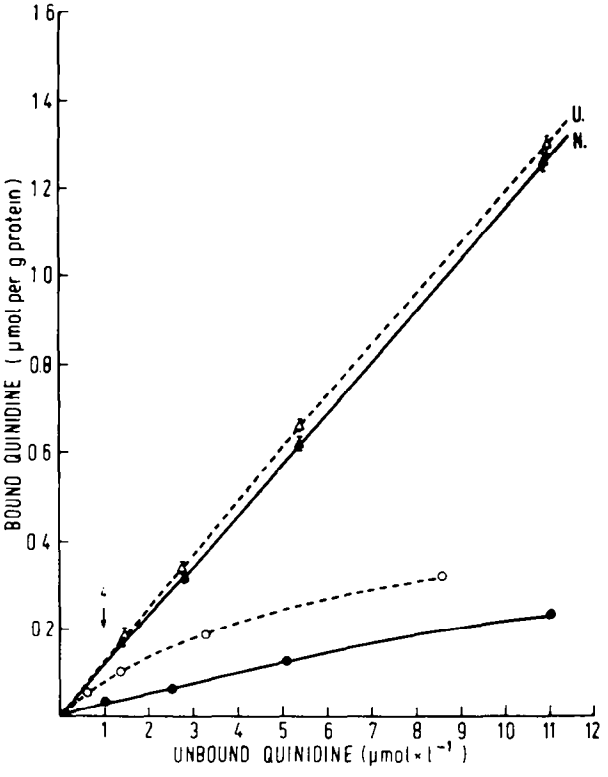

Fig. 1. Binding of quinidine in serum (○ and ●) and in heart homogenates (△ and ▲) at different concentrations of unbound quinidine. Closed signs and open signs represent samples from normal rats and anuric rats respectively. Each point represents mean (± S.E.M.) of duplicate determinations on one pool of serum or 2–3 pools of homogenates. Each pool was prepared from 4 rats.

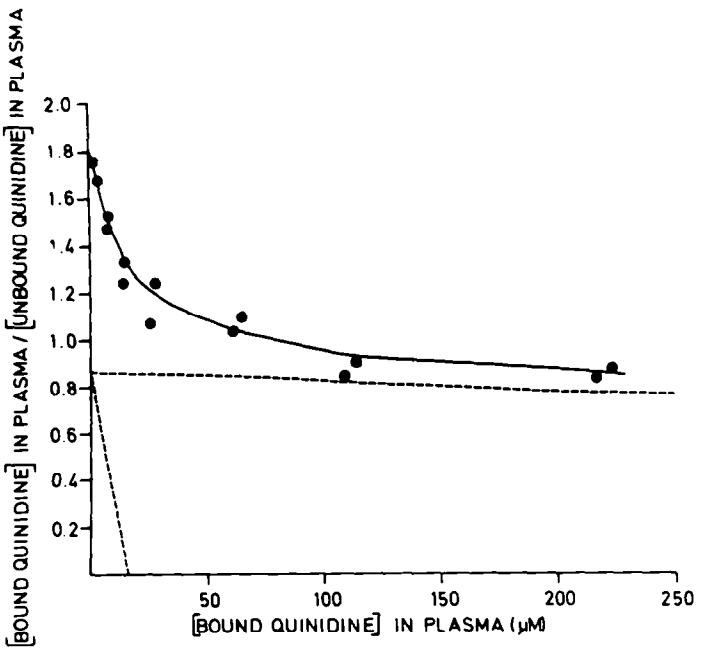

Fig. 2. Binding of quinidine to serum from normal rats at different concentrations given as a Scatchard plot and with the 2 best-fit lines for this curve.

Calculated and observed distribution heart/serum. Binding of quinidine to serum and heart was calculated from the mass-law derived equation.

$$[\text{bound}] = \frac{\text{binding capacity} \times [\text{unbound}]}{\text{dissociation constant} + [\text{unbound}]}$$

using observed binding values (Table 1) and a given unbound serum concentration of quinidine

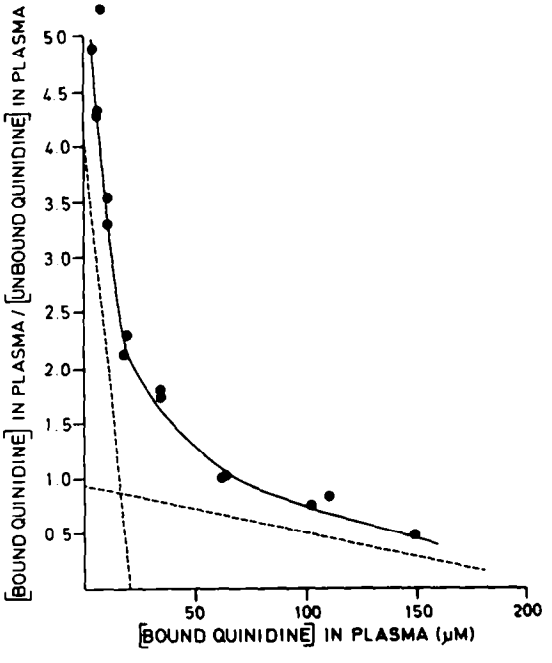

Fig. 3. Binding of quinidine to serum from anuric rats given as a Scatchard plot and with the 2 best-fit lines for this curve.

($1\ \mu\text{mole} \cdot l^{-1}$) observed after intravenous administration to normal and anuric rats. Calculations were performed both from an even distribution of unbound quinidine and from a pH-dependent distribution of unbound quinidine across plasma membrane. The pH-dependent distribution of unbound quinidine were derived from Henderson–Hasselbalch equation using a pK_a of 8.57 for quinidine [11], an intracellular pH of 7.0 [12], and the observed plasma pH value of 7.46 and 7.31 in normal and anuric rats, respectively. Calculated binding and distribution ratios, and observed concentrations and distribution of quinidine between heart and serum obtained from similar experiments after intravenous administration of quinidine [3] are given in Table 2. Assuming a pH-dependent distribution, unbound cellular concentrations of quinidine are 2.7 and 2.0 times higher than in serum of normal and anuric rats, respectively.

The results show that at the same unbound concentration of quinidine in heart and serum, serum binding is higher in anuric rats, but similar in hearts from normal and anuric rats. However, a pH-dependent distribution of unbound quinidine to heart provoke an increase of unbound and bound concentrations of the drug in heart and more in normal than anuric rats. The quinidine concentration ratios calculated from binding results (Table 2) are higher in normal than in anuric rats, and higher and more consistent with the observed ratios when assuming a pH-dependent distribution of unbound quinidine. The lower ratios in anuric rats are caused by an increase in serum binding, and a decrease of unbound heart concentration of quinidine.

DISCUSSION

In the present work *in vitro* methods have been used to evaluate the contribution of binding in the heart and

Table 1. Binding of quinidine to serum and heart homogenates

	Protein concentration $g \cdot l^{-1}$ or kg^{-1}	Binding capacity (NP) * moles $\cdot l^{-1}$, moles $\cdot kg^{-1}$	Association constant (K) $moles^{-1} \cdot l$	$K \cdot NP$ +
Normal rats (12)‡				
Heart homogenates §	166.3 ± 4.3	$8.72 \cdot 10^{-3}$	$2.16 \cdot 10^{3}$	18.8
Serum	75.5 ± 6.4	1¶ $1.97 \cdot 10^{-5}$	$5.06 \cdot 10^{4}$	0.997
		2¶ $2.36 \cdot 10^{-3}$	$4.52 \cdot 10^{2}$	1.067
Anuric rats (7)‡				
Heart homogenates §	139.7 ± 7.8	$5.74 \cdot 10^{-3}$	$2.98 \cdot 10^{3}$	17.1
Serum	71.3 ± 8.6	1¶ $2.48 \cdot 10^{-5}$	$2.02 \cdot 10^{5}$	5.01
		2¶ $2.60 \cdot 10^{-4}$	$4.27 \cdot 10^{3}$	1.110

* Number of binding sites times protein concentration.

+ Product of association constant and binding capacity, which from the mass-law equation gives an expression of extent of drug–protein interaction for comparison at unbound concentration lower than the dissociation constant.

‡ Number of animals.

§ Corrected for dilution of homogenates (1:9 w/v).

¶ 1 and 2 represent first and second class of binding sites.

serum and extra-intracellular pH gradient to the observed distribution of quinidine between serum and heart in normal and anuric rats [3]. Gillette [16] proposed that *in vivo* tissue binding of drugs could be estimated adequately from binding obtained in homogenate. By the homogenization procedure used, the cellular structures and possibly the subcellular organelles are broken, and this may imply denaturation and/or uncovering of binding sites as well as release of enzymes which can change the tissue macromolecules [13, 14]. Furthermore the structure in an aqueous phase in a homogenate may be different compared to the intact cell [15] and may influence binding properties. These effects of tissue homogenization can influence the reported results and explain the higher calculated than observed concentration ratios.

In serum from normal and anuric rats quinidine is bound to both albumin and lipoproteins [5] and apparently to α_1 acid glycoprotein [17, 18]. Binding of quinidine in heart homogenate has been demonstrated in microsomes, mitochondria and nuclei [19, 20]. Bickel *et al.* [22] have reported two classes of binding sites for chlorpromazine and imipramine to liver microsomes and mitochondria. Thus, both serum and homogenate of heart contain a complex mixture of macromolecules and structures capable of binding quinidine. The Scat-

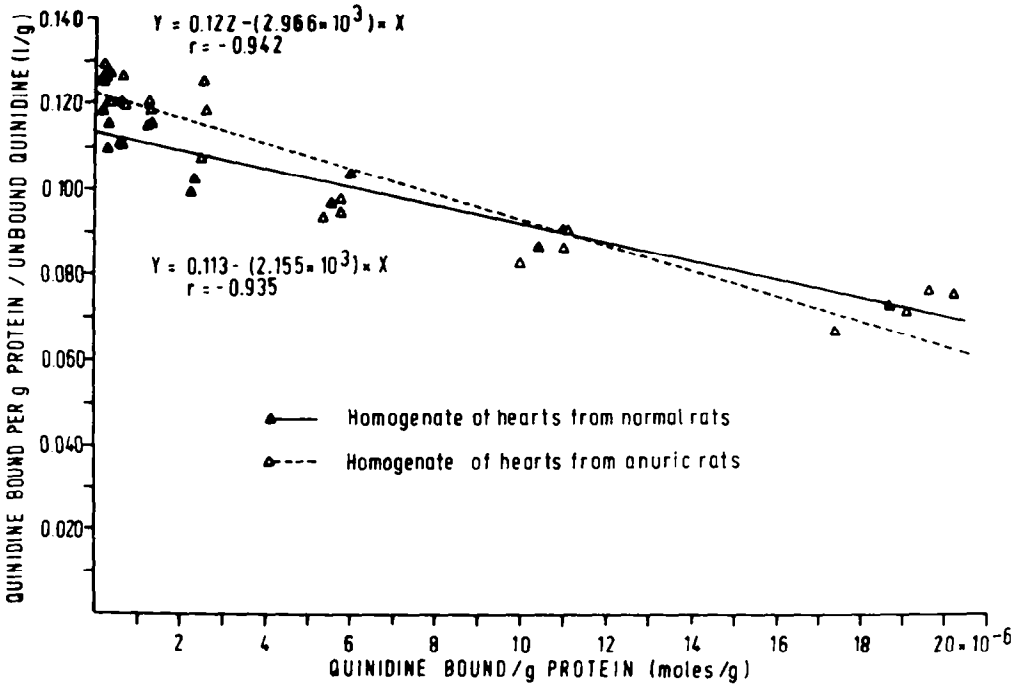

Fig. 4. Binding of quinidine in homogenates (1:9 w/v) of hearts obtained from normal (▲) and anuric (△) rats, is given as a Scatchard plot with the regression lines.

Table 2. Calculated and observed distribution of quinidine to heart and serum

	Calculated*				Observed†		
	Unbound quinidine μmoles·l^{-1}	Bound quinidine μmoles·kg^{-1} or l^{-1}	Total concentration quinidine μmoles·kg^{-1} or l^{-1}	[Heart]/[Serum] ratio	Unbound quinidine μmoles·l^{-1}	Total concentration μmoles·l^{-1} or kg^{-1}	[Heart]/[Serum] ratio
Normal rats							
Heart‡	2.7 (1.0)	50.6 (18.9)	53.3 (19.9)	16.2 (6.1)	1.0	38.5	12.5
Serum	1.0	2.3	3.3			3.2	
Anuric rats							
Heart‡	2.0 (1.0)	33.9 (17.0)	35.9 (18.0)	5.5 (2.8)	1.0	27.3	4.6
Serum	1.0	5.5	6.5			6.0	

* Based in the mass-law derived equation [bound] = binding capacity + [unbound]/dissociation constant + [unbound] and a given unbound serum concentration 1 μmoles·l^{-1} observed after *in vivo* administration of quinidine. Unbound concentration was derived both from the Henderson–Hasselbalch equation for pH-dependent partitition of quinidine (base pK_a 8.57) and from an even and pH independent distribution of quinidine across cell membranes. Binding parameters were taken from Table 1.

† Observed values after intravenous administration under equivalent experimental conditions [3].

‡ Values based on pH-dependent distribution of unbound quinidine are given and with values based on an even and pH-independent distribution of unbound quinidine in parenthesis.

chard plots are most compatible with 2 separate binding sites in serum and one binding site in heart homogenates. Binding characteristics do not have the same stringent meaning in a complex mixture of macromolecules, as when one single type of binding exists which is equal within classes and generally independent [21]. Accordingly, the quinidine binding in serum and heart was referred to association constants and the binding capacity.

The product of association constant and binding capacity is an adequate expression for the extent of drug–protein interaction for comparison of binding to different protein mixtures, when unbound concentration is lower than the association constant [23]. Somewhat greater binding of quinidine per g of protein (Table 1) was found in heart homogenate from anuric than from normal rats. This may be caused by changes in tissue structures [13], or in the interstitial fluid, reflecting the increased binding in serum. However, this difference in tissue binding disappears because of lower protein concentration in heart from anuric than from normal rats. Judged from the product of association constant and binding capacity, the extent of binding was 9 and 2.8 times higher in heart than in serum of normal and anuric rats, respectively. This difference in the two groups of rats is explained by an increase in serum binding of quinidine in anuric rats. The observed increase in serum binding in anuric rats confirms the previous observations [3, 5] and is probably explained mainly by an increase in serum concentration of α_1 acid glycoprotein [17, 18].

The distribution of the unbound fraction of drugs across biological membranes is presumably pH-dependent and demonstrated for several drugs [24, 25]. The unbound concentration of quinidine was calculated from the pK_a = 8.57, pH values determined in plasma and on intracellular pH accepted to be 6.9–7.0 [12]. In anuric rats the intracellular pH may be lower, but observations indicate a cellular resistance against acidosis [26], and the intracellular pH is supposed to be similar in normal and anuric rats.

A pH-dependent distribution of unbound quinidine between the extracellular and intracellular space produces an unbound intracellular concentration of 2.7 and 2.0 times that in serum of normal and anuric rats, respectively.

When quinidine concentration ratios heart tissue/plasma (Table 2) were calculated from binding values, those obtained when using unbound concentrations based on a pH-dependent partition across cell membranes are more consistent with the ratios observed after intravenous administration of quinidine. However, the calculated were somewhat higher than the observed ratios. This may be explained by an overestimation either of binding in heart by transfering results from tissue homogenates or of the pH gradient.

Qualitatively, the present *in vitro* results seem to demonstrate that the extent of quinidine binding is higher in heart tissue than in serum as reflected by the observed high tissue/serum concentration ratio. The observed decrease in this ratio in anuric rats can be explained by the accompanying increased serum binding of quinidine. Quantitatively, the observed concentration ratio from quinidine in normal and anuric rat can apparently only be accounted for the by the binding properties in heart homogenates and serum samples, and by a pH-dependent distribution of unbound quinidine between intra- and extracellular space.

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