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INTRACELLULAR LOCALIZATION OF QUINIDINE

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Abstract—The distribution of quinidine in the heart is relatively higher in the ventricles than in the auricles. The intracellular localization of the drug in the left ventricular tissue reveals that the nuclear fraction binds the maximum, the supernatant fraction being the next while the sarcosomal fraction shows insignificant affinity. Liver tissue, in contrast, shows that the supernatant fraction retains the highest concentration, whereas the nuclear and mitochondrial fractions share about equal amounts. The separation of the microsomal fraction has not been accomplished. Physico-chemical studies demonstrate that the binding of quinidine to the nuclear fraction is rather a "physical" process. Quinidine in the supernatant fractions of both heart and liver has been shown to be associated with the cytoplasmic proteins.

BINDING of quinidine with membrane lipoprotein has been shown to result in decreased membrane permeability to passive ion movements. This permeability change is held responsible for the quinidine-induced alterations in sodium and potassium exchange in cardiac muscle.[1–4] Uyeki *et al.*[5] suggested that the binding of quinidine with critical enzyme proteins may be responsible for the altered intermediary metabolism following administration of the drug. Also, quinidine has been shown indirectly to be bound to intracellular proteins[6] and, recently, to serum albumin in a rather specific manner.[7]

A possible specific binding of the drug at a definite intracellular site has not yet been investigated. If such a localization could be shown to exist, the mechanism of action of quinidine would be better understood. In an earlier communication from this laboratory the binding of quinidine with the myocardial cellular components was briefly reported.[8] In the present investigation the authors have studied in detail the distribution of quinidine in different chambers of the heart and the liver. Experiments designed to elucidate the mode of binding of quinidine with the various cellular components have also been included in this study.

METHODS

Healthy mongrel dogs of both sexes, weighing 7 to 14 kg were used in this investigation. Animals were fasted for 24 hr to deplete hepatic glycogen before performing the experiment. Quinidine sulfate (Merck), dissolved in distilled water with a few drops of 0·1 N H_2SO_4 and neutralized to pH 7·0, was administered i.v., for studies *in vivo*, in a dose of 15 mg/kg body weight. This dose was somewhat higher than the clinically recommended one, but was purposely chosen to induce significant concentration in the tissue. Fifteen min after injection, the chest of the animal was opened quickly under ether anesthesia and the heart excised and freed from blood. The tissue samples were cut from the heart and the liver, trimmed free from fibrous tags, and washed with cold distilled water to remove traces of blood; they were then squeezed and

wrapped in filter paper to absorb moisture. The tissue samples were homogenized for exactly 2 min in a chilled stainless steel–Monel metal Waring blender with cold isotonic sucrose solution. The heart tissue had been cut fine with scissors before transfer to the blender jar. The homogenates were passed through muslin to remove connective tissue and cell debris, and finally made to a volume to give a 10% (w/v) homogenate. The pH of the homogenates was checked and found to be about 7·0 in all cases.

Isolation of the particulate components of the cell was done according to the differential centrifugation technique of Schneider and Hogeboom;[9] we used the same gravitational field and period for the heart and the liver tissue. Ten ml of the 10% homogenate was subjected to centrifugation in an International refrigerated centrifuge, model PR-2, at 4° for 10 min at 800 *g* (International rotor No. 269) to sediment nuclei; this sediment was washed twice with 2·5 ml each time of isotonic sucrose solution and centrifuged for 10 min at the same force. The sediment resulting after the final washing was designated "nuclear fraction." It responded positively to staining with hematoxylin. The supernatant and the washings obtained from the nuclear fraction were combined and centrifuged for 10 min at 6,000 *g* on a multispeed head (International rotor No. 296). The sedimented residue was washed twice with 2·5 ml each time of isotonic sucrose solution and recentrifuged for 10 min at the same force. The final sediment was designated as "mitochondrial fraction" for the liver and "sarcosomal fraction" for the heart tissue. The two fractions stained with Janus green B. The supernatant and washings from the above fraction were combined and designed "supernatant fraction." The separation of microsomes from this fraction was, however, not attempted.

For studies *in vitro*, 10-ml samples of the 10% homogenates, obtained from the tissues of control animals which did not receive the drug, were first incubated with solution containing 20 μg quinidine at 37° for 15 min. This was followed by separation into nuclear, mitochondrial, and supernatant fractions.

Quinidine was estimated in the different fractions by the method of Kelsey and Geiling,[10] in a Klett-Summerson fluorimeter. Exogenously added quinidine gave 88 to 95% recovery in our hands. Protein estimation was by the biuret method of Gornall *et al.*[11] The concentration of quinidine was expressed in terms of micrograms quinidine per gram tissue or tissue equivalent, and the percentage distribution in each fraction was calculated on this basis.

RESULTS

I. Distribution of quinidine in different chambers of heart

The data (Table 1) demonstrated that the distribution of quinidine was not uniform in the four chambers of the heart, the concentration being relatively higher in ventricles as compared to auricles. Expressing the values as percentage to total, the distribution of quinidine was 34 in the left ventricle, 30 in the right ventricle, and 18 each in the left and the right auricles.

II. Intracellular localization of quinidine in the left ventricle and in the liver

In view of the fact that quinidine concentration reached the highest level in the left ventricle, all the intracellular studies on heart were confined to the left ventricular tissue.

TABLE 1. DISTRIBUTION OF QUINIDINE IN DIFFERENT CHAMBERS OF HEART*

	Quinidine	
	(μg/g tissue)	(%)
Left ventricle (10)	26·47 ± 1·29	34
Right ventricle (10)	23·10 ± 0·91	30
Left auricle (10)	14·33 ± 0·76	18
Right auricle (10)	14·06 ± 0·45	18

* For all tables, values are given ± standard error of the mean; numerals in parentheses represent number of experiments.

The findings, both *in vivo* and *in vitro*, presented in Table 2, demonstrated that the level of quinidine in cardiac muscle was significantly higher in the nuclear fraction than in the sarcosomal and supernatant fractions. The percentage distribution of quinidine in nuclear, sarcosomal, and supernatant fractions was respectively 52, 8, and 33 *in vivo*, and 49, 5, and 39 *in vitro*. The intracellular distributions of quinidine in the liver tissue, on the other hand, was of a different pattern, the highest concentration being found in the supernatant fraction. The percentage distribution in the nuclear, mitochondrial, and supernatant fractions was respectively 12, 11, and 72 *in vivo*, and 11, 13, and 67 *in vitro*. Expressing the values from different cellular fractions of the heart and the liver on the basis of the amount of quinidine per milligram protein followed a similar pattern, both *in vivo* and *in vitro*.

TABLE 2. INTRACELLULAR LOCALIZATION OF QUINIDINE IN LEFT VENTRICLE AND LIVER

		Homogenate	Separated fractions					
		(μg/g tissue)	Nuclear		Sarcosomal mitochon.		Supernatant	
			(μg/g tissue equiv.)	(unit)*	(μg/g tissue equiv.)	(unit)	(μg/g tissue equiv.)	(unit)
Left ventricle	*in vivo* (10)	24·91 ± 1·38	12·90 ± 0·93	66	1·87 ± 0·15	39	8·14 ± 0·26	41
	in vitro (6)	18·30 ± 0·29	9·00 ± 0·26	46	0·83 ± 0·11	17	7·15 ± 0·63	37
Liver	*in vivo* (8)	37·98 ± 5·08	4·39 ± 1·16	28	4·17 ± 0·66	31	27·32 ± 4·85	156
	in vitro (6)	18·65 ± 0·18	1·95 ± 0·11	13	2·46 ± 0·07	11	2·47 ± 0·79	71

* Unit = μg quinidine/mg protein × 10^2.

III. Physicochemical aspects of quinidine binding

Studies with isolated nuclear fraction. The nuclear fractions obtained from the tissues of normal animals, by the procedure already described, were suspended in 5 ml each of isotonic sucrose solution, unless mentioned otherwise. After incubation under the

conditions specified below, the suspensions were centrifuged at 800 *g* for 10 min, and quinidine was estimated in the sediment.

(*i*) Influence of quinidine concentrations. The nuclear suspensions from the heart and the liver were incubated for 15 min at 37° with quinidine in the range 10 to 100 μg contained in 1 ml solution. The results represented in Fig. 1 show that the nuclear fractions derived from the same weight of heart and liver tissue differ markedly in their binding capacity for quindine. With the heart fraction, the uptake of quinidine was progressive with the concentration of quinidine. At the highest level of the drug in the suspension medium, about 75% was recoverable in the isolated nuclear sediment. With the liver nuclei, the uptake was progressive only up to about 30 μg of the drug in the suspension medium. With further increase, there was only a gradual elevation in the uptake, and this tended to fall when the level of quinidine was raised to 50 μg, indicating that the saturation point was reached. The maximal uptake by the liver nuclei was less than 50% of the concentration prevailing in the medium.

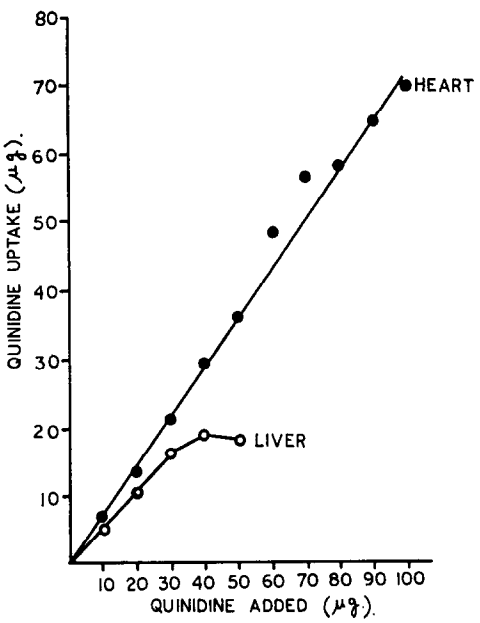

FIG. 1. Influence of quinidine concentration.

(*ii*) Influence of temperature. The nuclear suspensions from the heart and the liver were incubated with 20 μg quinidine in a volume of 1 ml for 15 min at 2°, 20°, 37°, and 60°. In a control experiment which was conducted at 37°, the nuclear preparation had been subjected to prior heat treatment in boiling water for 1 min. The results in Table 3 demonstrated that temperature had practically no effect on quinidine binding by nuclei, both in the heart and in the liver. The binding was also apparently unaffected by prior heat denaturation of proteins.

(*iii*) Effect of dialysis. The nuclear suspensions from the heart and the liver, after incubation with 20 μg quinidine in a volume of 1 ml solution for 15 min at 37°, were dialyzed against distilled water with occasional change for 24 hr at 5°; subsequently, quinidine was estimated in the whole suspension remaining inside the

TABLE 3. INFLUENCE OF TEMPERATURE ON QUINIDINE BINDING

Source of nuclear fraction	2°	20°	37°	60°	37°*
	(μg quinidine/g equivalent tissue)				
Left ventricle (4)	9·98 ±0·85	10·73 ± 0·65	9·78 ±0·55	10·20 ± 0·81	10·30 ± 0·76
Liver (4)	2·40 ±0·16	2·36 ± 0·14	2·44 ±0·09	2·39 ± 0·13	2·49 ± 0·15

* Nuclei heated at 100° for 1 min prior to incubation with drug.

bags without separation of the sedimentable fraction. The data (Table 4) showed that only 36% and 22% of the initial quinidine were left behind in the material derived from the heart and the liver respectively.

TABLE 4. EFFECT OF DIALYSIS ON UPTAKE OF QUINIDINE BY NUCLEI

Heart nuclear fraction (5)			Liver nuclear fraction (5)		
Before dialysis	After dialysis	Undialyzable quinidine	Before dialysis	After dialysis	Undialyzable quinidine
(μg quin./g equiv. tissue)		(%)	(μg quin./g equiv. tissue)		(%)
10·74 ± 0·98	3·84 ±0·27	36	2·41 ±0·12	0·52 ±0·09	22

(*iv*) Influence of pH. The nuclear sediments from the heart and the liver were directly suspended in 5 ml veronal buffer at pH 4, 5, 6, 7, 8, and 9 and incubated with 20 μg quinidine in a volume of 1 ml for 15 min at 37°. Analysis of the sedimentable fraction (Fig. 2) demonstrated that quinidine uptake, both by the heart and the liver nuclei, increased with increasing pH, reaching a maximum at pH 8·0. At all pH values the nuclear fraction derived from the heart tissue bound more quinidine then the corresponding fraction from the liver.

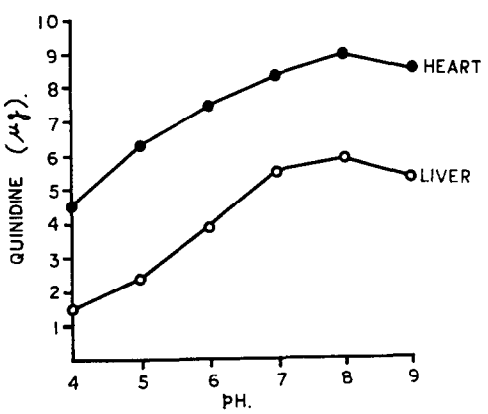

FIG. 2. Influence of pH.

Studies with the supernatant fraction. The supernatant fraction was obtained from homogenates of normal heart and liver tissue which had been incubated *in vitro* with 20 μg quinidine in a volume of 1 ml for 15 min at 37°.

(*i*) Protein binding of quinidine. The isolation of total protein was attempted by salting out with ammonium sulfate at full saturation. The protein fraction was allowed to flocculate overnight at 5°; it was collected by centrifugation and the quinidine estimated. The data, presented in Table 5, showed that 86% and 84% of the quinidine could be accounted for in the precipitated proteins from the heart and the liver respectively.

TABLE 5. PROTEIN BINDING OF QUINIDINE IN SUPERNATANT FRACTION

Heart supernatant fraction (5)			Liver supernatant fraction (5)		
Initial	Ammonium sulfate fraction	Recovery in protein fraction	Initial	Ammonium sulfate fraction	Recovery in protein fraction
(μg quin./g equiv. tissue)		(%)	(μg quin./g equiv. tissue)		(%)
6·27	5·37	86	9·88	8·30	84
±0·16	±0·19		±0·14	±0·11	

(*ii*) Effect of dialysis. The supernatant fractions from the heart and the liver were dialyzed against distilled water with occasional change for 24hr at 5°, and quinidine remaining inside the bags was estimated. The findings (Table 6) demonstrated that only 15% of the drug was left undialyzed in the supernatant from the heart, whereas over 50% remained undialyzed in the corresponding fraction from the liver.

TABLE 6. EFFECT OF DIALYSIS ON UPTAKE OF QUINIDINE IN SUPERNATANT FRACTION

Heart supernatant fraction (5)			Liver supernatant fraction (5)		
Before dialysis	After dialysis	Undialyzable quinidine	Before dialysis	After dialysis	Undialyzable quinidine
(μg quin./g equiv. tissue)		(%)	(μg quin./g equiv. tissue)		(%)
7·30	1·11	15	12·90	6·76	52
±0·22	±0·06		± 0·30	±0·56	

DISCUSSION

Once a drug has penetrated the boundaries of the various tissue cells, its degree of binding to the cell components becomes the significant factor in determining its intracellular binding, but very little information is available on this aspect. Precise conclusions may be drawn from experiments wherein the cell fractionation is accomplished subsequent to administration of drug to the intact animal, but valuable clues may be had also when the intracellular components are separated from homogenates of normal animal tissue which have been treated *in vitro* with the drug.

George *et al.*[12] studied the intracellular localization of digitoxin in heart and liver and found that on intravenous administration of the drug more than 85% was present in the supernatant fractions of both heart and liver, while mitichondria contained 3% and 0·1% respectively. Spratt and Okita,[13] using labelled digitoxin, demonstrated that over 90% of the unchanged drug was localized in the soluble fraction of the cells of rat heart, kidney, and liver. No marked quantitative binding could be observed with any of the particulate fractions. Studying the binding of several barbiturates on homogenates of various tissues, Goldbaum and Smith[14] suggested that the tissue binding and the plasma protein binding of these compounds may be governed by the same principle. On the other hand, almost limitless capacity of isolated liver cell nuclei to bind quinacrine suggested a type of binding different from various proposed types of plasma protein binding.[15, 16]

The findings of the present investigation demonstrate that 15 min after intravenous injection of quinidine the concentration of the drug in the ventricles is considerably higher than in the auricles. To the best of the authors' knowledge this preferential capacity of the ventricles to retain a drug has not hitherto been recognized.

Since quinidine is effective in cardiac arrhythmias, its intracellular localization in cardiac tissue, which is the locus of its action, and the nature of its binding to the various cell components assume special significance. A comparative picture in the liver would reveal any specificity of the cardiac tissue. A study of the intracellular distribution of quinidine in the left ventricular tissue reveals that its concentration in the nuclear fraction is considerably higher than in the sarcosomal and the supernatant fractions, although contamination of the nuclear fraction with myofibrils is inevitable at this speed. However, staining procedures demonstrate that it is insignificant. It contains mainly heart nuclei with sparse intact myofibrils. Moreover, this contamination has its experimental limitations. The intracellular distribution of the drug in the liver tissue, in contrast, is of a different pattern, the maximal concentration being in the supernatant fraction; the rest is distributed almost equally between the nuclear and the mitochondrial fractions. The differences observed are too great, under the conditions employed for homogenization, to be accounted for in terms of a possible rupture of liver nuclei and the retention of intact cells by the heart nuclear fraction. It is, in fact, possible that the presence of quinidine in the supernatant fraction of the heart may be due to leaching from the nuclear fraction, since the fractionation was accomplished in aqueous and not in nonaqueous medium. The findings of the studies, *in vitro* lend additional support regarding the distinctive pattern of intracellular distribution of quinidine in the heart and the liver. These conclusions may be contrasted with those of George *et al.*[12] and Spratt and Okita,[13] who reported that digitoxin is not bound at all to the nuclear fractions of the heart and the liver. Such a variation in the intracellular localization of quinidine and digitoxin may be associated with the difference in the mode of response by cardiac muscle to the two drugs.

Our studies on isolated nuclear preparation of the heart and the liver reveal certain characteristics in common, as shown by their response to temperature, dialysis, and pH. Temperature has practically no effect on capacity of the nuclear fractions for quinidine binding. It is interesting to note that prior denaturation of nuclear proteins by heat treatment also does not alter the binding property of the nuclear fractions. That quinidine is not attached firmly to the nuclear fractions is apparent from the

fact that about 70 to 80% of the drug is dialyzable from the liver and the heart nuclear fractions respectively. These observations suggest a "physical" quinidine binding to the nuclei. The results of experiments on incubation of isolated nuclear fractions with increasing concentration of the drug demonstrate that the fraction from the heart prossesses much higher capacity to bind the drug than does the fraction from the liver. The quantity of the drug taken up may be expected to depend to some extent also on the mass of the nuclei derived from the two tissues. Even after making allowances for a larger proportion of subcellular material in the heart fraction, the results would indicate a higher affinity for the drug by the heart than by the liver nuclear fraction.

The results with the 6,000 *g* supernatant fraction of the heart and the liver demonstrate that quinidine is attached to the cytoplasmic proteins, about 80% being recoverable from the total protein precipitated with ammonium sulfate. On dialysis, the heart supernatant retained only about 15% compared to 50% held back by the liver. It is, however, difficult at present to assign any differential property of the liver supernatant for binding quinidine on these data.

The separation of microsomes from the supernatant fractions and a study of their capacity for binding quinidine could not be accomplished in detail in the present study; yet, in a limited number of experiments it has been observed that the microsomes, both from the heart and the liver, have no appreciable affinity for quinidine, the per cent recovery of the drug being in the range of 4 to 5 in the experiments *in vivo*.

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