Effect of Perfusate pH on Reduction of Quinidine Capillary Permeability by Albumin in Isolated Perfused Rat Heart

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It has been suggested that albumin reduces quinidine capillary permeability (PS) in the single-pass perfused heart preparation by reducing paracellular transport of quinidine ions. Using this preparation, we examined the effect of albumin (0.1 per cent) on quinidine PS at perfusate pH's of 7.1 and 7.9 during uptake of quinidine (19 μM) and also during washout of the drug using a randomized design. Quinidine PS was approximately 16 ml/min/g heart at pH 7.9 and was not altered by the presence of albumin in perfusate. At pH 7.1, in the absence of albumin, quinidine PS was also 16 ml/min/g, but in the presence of albumin (0.1 per cent) PS was reduced significantly to approximately 5 ml/min/g ($P < 0.001$). In the absence of albumin PS was the same at pH 7.1 and 7.9 in spite of a greater degree of ionisation of quinidine at pH 7.1. This suggests that there is significant uptake of ionised quinidine at pH 7.1. The greater effect of albumin on PS at pH 7.1 supports the hypothesis that albumin reduces paracellular transport of quinidine ions.

KEY WORDS: isolated perfused heart; quinidine; capillary permeability; perfusate pH; perfusate albumin concentration.

INTRODUCTION

It is well known that albumin and other plasma proteins reduce capillary permeability to water and hydrophilic solutes, such as Na^+, EDTA and cyanocobalamin, by about half (1–4). In the absence of protein, capillary permeability of the latter three solutes correlated with their aqueous free diffusion coefficients, suggesting paracellular transport through aqueous channels (3). This effect of albumin is apparently due to its presence within the introit of intercellular junctions which form the paracellular pathway for the passive transport of water and small molecules. Albumin is thought to restrict transport either by forming a molecular filter within the channels, by adsorbing to the walls of the channels or by modifying the properties of the glycocalyx which lies over and within the channels (5–7).

We have found recently in the isolated perfused rat heart preparation that the permeability-surface product (PS) for the uptake of unbound quinidine is reduced by about half by addition of either bovine serum albumin or bovine α_1-acidglycoprotein to the perfusate (8,9). The threshold albumin concentration for this effect is less than 0.1 per cent, which is consistent with the threshold observed for the effect of albumin on capillary hydraulic conductivity (2,10). We also found that myocardial PS for quinidine was not influenced by perfusion pH over the range 7.0 to 7.9 and concluded that quinidine ions and non-ions are taken up the same rate (11). Efficient uptake of ionised quinidine, which is hydrophilic, would be much more likely via the aqueous paracellular pathway than via the lipid transcellular pathway which is the conventional pathway for unionised drug. We postulated that the reduction of PS by albumin and α_1-acidglycoprotein may be due to an action by these proteins on the paracellular pathway (8,9). If this hypothesis is correct, the effect of albumin in reducing quinidine PS in the perfused heart preparation should increase with decreasing perfusate pH because of increasing quinidine ionised fraction. To test this hypothesis, we have examined in the single-pass perfused heart preparation the combined effects of perfusate pH and albumin on quinidine PS.

MATERIALS AND METHODS

Experimental Preparation

Male Sprague Dawley rats weighing 250–300g were used in this study. The rats were lightly anesthetised with ether and given heparin (500 IU/kg iv). The heart was rapidly removed and immersed in ice-cold perfusion buffer. Both atria were excised along with excess fat and tissue. The hearts were perfused at 2.5 ml/min with a pH 7.4 modified Krebs-Henseleit buffer via the aorta at 37°C for an initial 15 min stabilization period. During the experiments perfusion buffers of pH 7.1 and 7.9 were used. The composition of the buffers was as follows (in mM): $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; KCl 5; Glucose 11.1; NaCl 121 (pH 7.4), 136 (pH7.1), or 86 (pH7.9) and $NaHCO_3$ 25 (pH 7.4), 10 (pH 7.1), or 60 (pH 7.9) and was saturated with oxygen-carbon dioxide (95/5). Oxygenation and pH were maintained throughout the perfusion period by a silastic membrane oxygenator in the perfusion circuit. Two peristaltic pumps (Masterflex, Cole Parmer, Chicago, IL, USA) were used for delivery of the perfusate via two separate circuits, one for drug-free perfusion buffer (with or without albumin), and the other for perfusion buffer containing quinidine (with or without albumin). The design of the apparatus allowed rapid switching between the two channels.

Measurements

The heart was paced at 200 beat/min via a platinum electrode placed in the region of the AV node with an isolated stimulator and a period generator (JRAK Biosignals, Melbourne, Victoria, Australia). Platinum electrodes for ECG monitoring were positioned on the epicardial surface of the ventricle, one on the left ventricle free wall and the other near the pulmonary artery opening. The ECG was displayed and stored on a Macintosh LC microcomputer using the Superscope program (GW Instruments, Somerville, MA, USA) via an amplifier (JRAK Biosignals) and an analogue-digital interface (MacADIOS 8 ain, GW Instruments). At the end of the experiment selected ECG's were retrieved and the QT interval was measured on screen using the cursor of the

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Superscope program. At each sampling time, the QT interval was taken as the mean of five consecutive readings. Outflow perfusate was also collected at various times and assayed for quinidine immediately by HPLC (12). At the end of the experiment, the heart was blotted to remove excess perfusate and weighed.

Experimental Design

Fourteen hearts were studied and each heart was perfused for three consecutive phases. In the first set of experiments (n = 6) all perfusates contained 0.1 per cent (w/v) bovine serum albumin (prepared with charcoal treatment and extensive dialysis to remove low molecular weight substances, Sigma Chemical Co, St Louis MO, USA). Perfusate pH in phases 1, 2 and 3 was 7.1, 7.9 and 7.1, respectively, in three experiments and 7.9, 7.1 and 7.9, respectively, in the other three experiments. After the initial 15 min stabilization period (pH 7.4), the heart was perfused in phase 1 with drug-free perfusate at the selected pH for 20 min followed by perfusion with this same perfusate containing quinidine (19 μM) for a further 35 min (pH 7.1) or 45 min (pH 7.9). This was followed by a washout period with drug-free perfusate for 35 min (pH 7.1) or 45 min (pH 7.9). After finishing phase 1, a 30 minute stabilization period was employed using drug-free perfusate and the alternative pH to that used in phase 1. Phase 2 was then commenced and the procedure was identical to that used in phase 1 except for the perfusate pH. Following another 30 min stabilization period at the end of phase 2, phase 3 was commenced using the same procedure and pH as in phase 1.

The perfusate flow rate was measured volumetrically during each phase. Quinidine concentration in perfusate was measured at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, 20, 25 and 35 min (and 45 min for pH 7.9) both during the uptake and washout periods of each phase. Baseline QT interval was measured prior to the start of each phase. Both input and output perfusate pH's were monitored regularly.

In the second set of experiments (n = 4) the perfusate pH was maintained at 7.1 throughout the three phases. Perfusate albumin concentration in phases 1, 2 and 3 was 0, 0.1 per cent and 0, respectively, in two experiments and 0.1 per cent, 0 and 0.1 per cent in the other two experiments. The procedure for phases 1, 2 and 3 was identical to that used in the first set of experiments, except perfusate albumin concentration was changed instead of perfusate pH.

The third set of experiments (n = 4) was identical to the second set except that the perfusate pH was maintained at 7.9 throughout.

Protein Binding

Protein binding of quinidine (19 μM) in perfusate containing albumin (0.5 per cent) was determined before perfusion at pH 7.0 and 7.9, as described previously (8).

Data Analysis

In all experiments, the perfusate drug output concentration (C_{out}) versus time data for uptake of quinidine were fitted with the following equation (13) by nonlinear least squares regression (Sigmaplot, Jandel Scientific, San Rafael, CA, USA).

$$C_{out} = (1\text{-}a)C_{in}(1\text{-}e^{-kt}) + aC_{in} \quad (1)$$

where C_{out} is perfusate drug output concentration, C_{in} is the input concentration, a is the shunted fraction of the perfusate around the coronary circulation, and k is the drug equilibration rate constant assuming a one compartment model. This equation was necessary to take into account a zero-time intercept (A) on the C_{out} versus time profile in some experiments, where $a = A/C_{in}$.

During washout of quinidine from the heart, C_{out} versus time data were fitted with:

$$C_{out} = (1\text{-}a)C_{eq}e^{-kt} \quad (2)$$

where C_{eq} is the equilibrium C_{out} quinidine concentration, prior to washout. This equation is derived in the Appendix.

The volume of distribution referenced to unbound drug (V_u), f_u, flow rate (Q) and permeability surface product (PS) can be related to k via the classical Kety-Renkin-Crone relationship for drug uptake as derived by Johnson and Wilson (14) and modified to incorporate protein binding effects (15):

$$k = \frac{Q}{f_u V_u}(1 - e^{-f_u PS/Q}) \quad (3)$$

In our experiments, Q is the coronary artery perfusion flow rate, calculated as the total measured flow rate of output perfusate x (1-a). Estimates of PS and V_u for quinidine were obtained by fitting (Sigmaplot) the total C_{out} versus time data from each phase with Eq. (3) substituted into Eq. (1)(uptake) or Eq. (2)(washout).

Differences between groups of data were analysed by paired t-test, accepting $P < 0.05$ as statistically significant.

RESULTS

Protein Binding

Protein binding of quinidine in perfusate containing 0.5 per cent albumin was very low. There was no significant difference in f_u at pH 7.0 and 7.9 ($f_u = 0.964 \pm 0.04$ and 0.956 ± 0.02, respectively, n = 6, $P > 0.05$).

Quinidine Pharmacokinetics

In the first set of experiments, in which albumin was present in perfusate throughout, there was no significant difference in baseline QT interval in phases 1 and 2 between pH 7.1 and pH 7.9 perfusates (Table 1). At constant pH there was also no significant difference in baseline QT interval in phases 1 and 2 between zero albumin and 0.1 per cent albumin perfusates (Tables 2 and 3) when the combined data were analysed. When all 14 experiments were examined, there was a significant 10 per cent difference in mean baseline QT interval between the first and third phases (83.9 ± 9.1 msec and 92.4 ± 11.0 msec, respectively, $P = 0.013$).

The time-course of the quinidine C_{out} during uptake and washout of drug during one experimental phase is shown for a typical experiment in Fig. 1. The fits of Eqs. (1) and (2) were excellent, as illustrated in Fig. 1 and the fitted k values are shown in Tables 1–3. Analysis of the combined data from

Table I. Effect of Albumin on Myocardial Quinidine Pharmacokinetics at High and Low Perfusate pH (n = 6)

	Perfusate		k	PS	V_u	Baseline QT
	pH	Albumin	(min^{-1})	(ml/min/g)	(ml/g)	interval (ms)
Uptake	7.1	0.1%	0.266 ± 0.033[a]	5.25 ± 0.59[a]	9.80 ± 0.65[a]	88.6 ± 8.7
	7.9	0.1%	0.0928 ± 0.013	16.5 ± 0.55	33.4 ± 2.9	82.0 ± 9.7
Washout	7.1	0.1%	0.222 ± 0.021[a]	4.97 ± 0.44[a]	10.3 ± 0.33[a]	
	7.9	0.1%	0.0967 ± 0.006	16.4 ± 0.48	30.0 ± 1.1	

[a] Significantly different from pH 7.9 value.

experiments conducted a pH 7.1 (Tables 1 and 2) showed that k from uptake data was significantly greater than k from washout data (grand mean 0.257 ± 0.03 and 0.214 ± 0.018 min^{-1}, respectively, n = 21, $P < 0.001$). At pH 7.9, k from uptake data was significantly less than k from washout data (grand mean 0.0922 ± 0.0098 and 0.0963 ± 0.0069 min^{-1}, respectively, n = 21, $P < 0.05$). When Eq. (3), substituted into Eq. (1) or (2), was fitted to the C_{out} versus time data, very good fits were obtained and the fitted values of PS or V_u are shown in Tables 1–3. There was no significant difference in either PS or V_u between uptake and washout experiments, nor was there any difference for either parameter measured in phases 1 and 3.

Tables 1 and 3 show that with perfusate pH 7.9 PS was approximately 16 ml/min/g and not influenced by the presence of albumin ($P > 0.05$). With perfusate pH 7.1, PS was also approximately 16 ml/min/g if albumin was not present (Table 2), but was reduced significantly to approximately 5 ml/min/g if albumin was present in perfusate ($P < 0.001$, Tables 1 and 2). The presence of albumin did not influence V_u (Tables 2 and 3), but V_u was significantly greater at pH 7.9 than pH 7.1 ($P < 0.001$, Tables 1–3).

DISCUSSION

In the absence of albumin in perfusate, k at pH 7.9 was significantly less than that at pH 7.1 (Tables 2, 3). This has been shown previously to be due to the larger V_u of quinidine at the higher pH, which results from increased partitioning of unionised quinidine, a weak base ($pk_a = 8.3$), into myocardial tissue (11). In the absence of albumin quinidine PS was not affected by pH (Tables 2, 3) despite the difference in degree of ionisation. This behaviour has been observed previously and led to the conclusion that both ionised and unionised quinidine moieties readily enter the myocardium (11).

At pH 7.1, albumin (0.1 per cent) in perfusate reduced quinidine PS by about two-thirds (Table 2). In previous experiments with this preparation conducted at pH 7.4 albumin (0.1 per cent) caused a similar reduction in quinidine PS (14.4 to 5.3 ml/min/g) (8). It was suggested that this may have been due to a physical obstruction by albumin of paracellular transport of quinidine ions into the myocardium in analogous fashion to that seen with other hydrophilic moieties such as Na^+, EDTA and cyanocobalamin by Mann (3). At pH 7.9 in the present experiments albumin did not affect quinidine PS (Table 3). The greater effect of albumin on PS at pH 7.1 compared with pH 7.9 is consistent with a greater importance of paracellular transport at pH 7.1 due to the greater degree of ionisation of quinidine at this pH. However, at pH 7.9 more than half of the quinidine molecules are still in the ionised state and therefore albumin might have been expected to exert some influence on PS at this pH. Moreover, at pH 7.1, with 94 per cent of quinidine in the ionised form, quinidine PS was still one-third of that observed in the absence of albumin (Table 2). While this latter observation is consistent with previous work with, for example, Na^+, which showed that albumin only partly blocks the paracellular pathway (3), it would appear that the pH-dependent effect of albumin cannot be explained solely in terms of the proportion of ionised quinidine present.

The possibility that the pH-dependent effect of albumin on quinidine PS could have been due to an effect of pH on capillary permeability and was independent of quinidine ionisation should also be considered. Previous work by Gamble with the perfused rat mesentery preparation showed that with perfusate containing either albumin or Ficoll (a high molecular weight polymer) capillary permeability increased when the perfusate pH was reduced below 7.05 (16). However, capillary permeability, as measured by the capillary filtration coefficient, was unchanged between pH 7.05 and 8.2. Increased free Ca^{2+} or Mg^{2+} can potentially decrease

Table II. Effect of Albumin on Myocardial Quinidine Pharmacokinetics at Low Perfusate pH (n = 4)

	Perfusate		k	PS	V_u	Baseline QT
	pH	Albumin	(min^{-1})	(ml/min/g)	(ml/g)	Interval (ms)
Uptake	7.1	0%	0.249 ± 0.03	16.5 ± 0.04[a]	12.0 ± 1.3	91.8 ± 0.9
	7.1	0.1%	0.250 ± 0.027	5.08 ± 0.5	10.0 ± 0.9	92.0 ± 4.4
Washout	7.1	0%	0.210 ± 0.02	16.4 ± 0.5[a]	12.3 ± 0.9	
	7.1	0.1%	0.208 ± 0.02	4.76 ± 0.4	10.6 ± 0.4	

[a] Significantly different from 0.1% albumin value.

Table III. Effect of Albumin on Myocardial Quinidine Pharmacokinetics at High Perfusate pH (n = 4)

	Perfusate		k	PS	V_u	Baseline QT
	pH	Albumin	(min^{-1})	(ml/min/g)	(ml/g)	Interval (ms)
Uptake	7.9	0%	0.0913 ± 0.009	16.4 ± 0.3	32.4 ± 3.4	79.7 ± 6
	7.9	0.1%	0.0921 ± 0.007	16.4 ± 0.4	33.9 ± 1.7	85.5 ± 6
Washout	7.9	0%	0.0954 ± 0.007	16.7 ± 0.3	29.2 ± 1.2	
	7.9	0.1%	0.0967 ± 0.009	16.6 ± 0.3	30.6 ± 1.6	

vascular permeability (17) and Gamble did observe a slightly increased free Ca^{2+} at pH 7.05 compared with pH 8.2 resulting from lower albumin binding at pH 7.05, but this increased Ca^{2+} did not affect permeability (16). These findings suggest that the effect of albumin on quinidine PS at pH 7.1 in the present study was not due to direct effects of either pH or Ca^{2+} or Mg^{2+} on the microvasculature.

Consideration should also be given to the possibility that the pH-dependent effect of albumin may be due to changes in albumin rather than to quinidine. For example, the association of albumin with the pore matrix may have increased at low pH blocking more of the openings, or albumin may have triggered an increase in quinidine binding sites within or around the entrance of the pores. Although such effects of albumin may be less likely in light of the absence of pH effects on the capillary filtration coefficient (16), they do merit consideration.

In the present experiments, neither albumin nor perfusate pH influenced baseline QT interval (Table 1–3). There was a 9 per cent increase in baseline QT interval between the first and third phases indicating a slight change in the preparation with time. This would not have had any influence on the findings because the order of the treatments in the three phases was randomized. Moreover, both PS and V_u returned in phase 3 of each experiment to the values observed in phase 1. At pH 7.1 k for uptake was about 17 per cent higher than k for washout whereas at pH 7.9 the difference was relatively minor (<5 per cent). The reason for this is not known, but a much more profound difference between uptake and washout rate of isradipine has been observed in the isolated perfused rabbit heart. The authors suggested this may be due to a change in sarcolemmal binding parameters after initial rapid uptake (18). In the present study the small difference in k between uptake and washout did not influence the determination of PS or V_u which were not significantly different between uptake and washout in the same phase. It is also worth noting that with pH 7.1, while albumin appeared to have no effect on k (Table 2), the small degree of binding was sufficient to produce significantly different PS values which were highly reproducible within and between experiments.

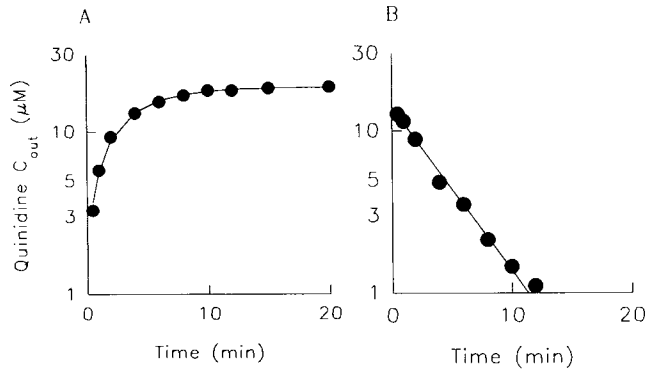

Fig.1 Time course of quinidine C_{out} during uptake (A) and washout (B) in a typical experiment.

In conclusion, this study shows that the inhibition by albumin of transport of quinidine into the myocardium increases as the degree of ionisation of quinidine in the circulation increases. While this provides further support to the hypothesis that albumin reduces paracellular transport of quinidine ions across the myocardial capillary endothelium, the precise mechanism of the pH-dependent effect of albumin warrants further investigation.

APPENDIX

If there is shunting of perfusate past the coronary circulation, C_{out} will be given by:

$$C_{out} = (1\text{-}a)C_{cor} + aC_{shunt} \quad \text{(A1)}$$

where C_{cor} is the perfusate drug concentration emerging from the coronary circulation and C_{shunt} is the output drug concentration of perfusate that bypassed the coronary circulation. During washout of drug from the myocardium, the time-course of C_{cor} is given by:

$$C_{cor} = C_{eq}e^{-kt} \quad \text{(A2)}$$

If there is no transport of drug from the myocardium into shunted perfusate,

$$C_{shunt} = 0 \quad \text{(A3)}$$

Substituting Eqs (A2) and (A3) into Eq (A1):

$$C_{out} = (1\text{-}a)C_{eq}e^{-kt} \quad \text{(A4)}$$

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