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EFFECT OF α_1-ACIDGLYCOPROTEIN ON MYOCARDIAL UPTAKE AND PHARMACODYNAMICS OF QUINIDINE IN PERFUSED RAT HEART

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Abstract—The myocardial uptake and pharmacodynamics of quinidine were examined in the isolated perfused rat heart preparation under conditions of varying concentrations of bovine α_1-acidglycoprotein (AAG) in the perfusate. Three hearts were perfused for five consecutive 35 min phases with buffer containing quinidine and AAG in concentrations of 0, 0.1, 0.5, 1.5 and 0 g/L, in that order, with a 55 min washout period between each phase. The equilibration rate constant for the quinidine output concentration increased with increasing AAG concentration, but not as much as predicted by the conventional pharmacokinetic uptake model, which assumes constant capillary permeability among the phases. Estimates of the permeability surface product for the two zero AAG phases (17.7 ± 1.91 and 19.1 ± 0.82 mL/min/g) were significantly greater than those for the three AAG phases (8.94 ± 0.99, 8.70 ± 0.26, 9.01 ± 0.26 mL/min/g; $P < 0.05$). This effect of AAG is the same as that observed previously by us with bovine serum albumin in this same experimental preparation. This suggests that the mechanism of reduced capillary permeability is the same for both proteins, i.e. the formation of a steric barrier to paracellular transport rather than an electrostatic barrier. There was a direct, linear relationship between lengthening of the *QT* interval of the electrocardiogram and total and unbound quinidine concentrations, but the relationship for unbound concentration was independent of quinidine unbound fraction. Therefore, the electrocardiogram effect of quinidine was directly related to the circulating unbound rather than total drug concentration.

The rate of myocardial drug uptake depends on the permeability of the drug, the blood flow rate through the coronary vasculature (Q†) and the unbound fraction of drug in the blood (f_u) [1–3]. During uptake the main barriers encountered are the capillary endothelium and the myocardial cell membrane. Drug transport across these lipoid membranes is generally thought to involve partitioning of the drug into the membrane and transport by passive diffusion, i.e. transcellular transport [4]. Plasma protein binding of drug restricts drug uptake so that the drug must first dissociate from the protein before being taken up in the unbound form [5].

We have found recently in the isolated perfused rat heart preparation that the effect of albumin on the uptake of quinidine was not in accordance with this widely accepted mechanism [6]. Addition of albumin to the perfusion medium caused a halving of the myocardial permeability of quinidine. It was postulated that this was due to obstruction of capillary transport of quinidine ions by binding of albumin within the introit of intercellular junctions which form the paracellular pathway for the passive transport of water and small molecules [7–9]. Quinidine binds strongly to α_1-acidglycoprotein (AAG) [10] and there is evidence that AAG may also modify capillary permeability [11–13]. Therefore, it was of interest to study the effect of AAG on the myocardial permeability of quinidine.

The pharmacodynamic effect of a drug is usually assumed to be directly related to the unbound rather than the total concentration of the drug in the plasma [5]. However, in the presence of added AAG the activity of several α_1-adrenergic antagonists in the rabbit aortic strip preparation and also in the rabbit *in vivo* was less than predicted by the unbound drug concentration [14, 15]. Activity was consistent with unbound drug concentration in the presence of added albumin. This effect of AAG was not seen with the α-agonist, phenylephrine, but whether this phenomenon involves any other drugs is not known. Therefore, the aim of our study was to examine the influence of AAG on the myocardial uptake and pharmacodynamics of quinidine in the isolated perfused rat heart preparation.

MATERIALS AND METHODS

Materials

Quinidine sulphate and bovine AAG (purified from Cohn Fraction VI, 99% pure) were obtained

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† Abbreviations: f_u, equilibrium unbound fraction; ECG, electrocardiogram; C_{out}, perfusate drug output concentration; C_{in}, perfusate drug input concentration; a, fraction of perfusate shunted; k, rate constant for equilibration of C_{out}; A, zero time intercept of C_{out} vs time curve; C_{cor}, calculated coronary output concentration; V_u, volume of distribution referenced to unbound drug; Q, perfusate flow rate; PS, capillary permeability surface product; ΔQT, change in QT interval of ECG; ΔQT_{max}, maximum ΔQT; C_T, total drug concentration; C_u, unbound drug concentration; AAG, α_1-acidglycoprotein; k_e, rate constant for equilibration of effect.

from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Dextran T-70 was obtained from Pharmacia LKB (Uppsala, Sweden).

Experimental preparation

Male Sprague–Dawley rats weighing 250–300 g were used in this study. The rats were lightly anaesthetized with ether and given heparin (500 IU/kg i.v.). 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 then subjected to non-recirculating retrograde perfusion at 2.5 mL/min with a modified Krebs–Henseleit buffer via the aorta at 37°. The perfusion buffer consisted of (in mM): $CaCl_2$, 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; KCl 4.7; glucose 11.1; NaCl 121 and $NaHCO_3$ 25, and was saturated with oxygen–carbon dioxide (95/5). The pH of the perfusate was 7.46 ± 0.03. 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, U.S.A.) were used for delivery of the perfusate via two separate circuits, one for perfusion buffer (with or without AAG) only, and the other for perfusion buffer containing quinidine (with or without AAG). The design of the apparatus allowed rapid switching between the two channels as described previously [16].

Measurements

The heart was paced at 200 bpm via a platinum electrode placed in the region of the atrioventricular node with an isolated stimulator and a period generator (JRAK Biosignals, Melbourne, Victoria, Australia). Platinum electrodes for electrocardiogram (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, U.S.A.) via an amplifier (JRAK Biosignals) and an analogue–digital interface (MacADIOS 8 ain, GW Instruments). At the end of the experiment selected ECGs were retrieved and the *QT* interval measured on screen using the cursor of the 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. At the end of the experiment, the heart was blotted to remove excess perfusate and weighed.

Experimental design

In vitro *binding experiments.* Protein binding of quinidine in perfusate solutions of different drug and AAG concentrations was determined by equilibrium dialysis. Perfusate (2 mL) containing drug and AAG was dialysed against an equal volume of blank perfusate in Perspex chambers separated by cellulose dialysis membrane (Type 20, molecular cut-off 14,000, Union Carbide Corp., New York, NY, U.S.A.). The blank perfusate contained Dextran T-70 to adjust the osmolarity to that of the AAG compartment [17]. The dialysis cells were flushed with oxygen–carbon dioxide (95/5) and shaken at 37° for 20 hr. At equilibrium the blank perfusate pH was checked and the blank perfusate was checked for protein contamination. Drug f_u was calculated as the ratio of drug concentration in blank perfusate compartment/AAG compartment at equilibrium. Quinidine protein binding was measured at concentrations of AAG of 0.1, 0.5 and 1.5 g/L and the quinidine concentration was varied from 0.1 to 50 μM at each AAG concentration. The relationship between unbound and total quinidine for each AAG concentration was fitted by a polynomial function (Sigmaplot, Jandel Scientific, San Rafael, CA, U.S.A.).

Effect of AAG on quinidine uptake. Three hearts were used in these experiments and each heart was perfused for five consecutive phases. After the initial 30 min period of perfusion with drug-free perfusate to allow the ECG to stabilize, the heart was perfused in phase 1 with AAG-free perfusate containing quinidine (20 μM) for 35 min. This was followed by perfusion with quinidine-free perfusate for 40 min to wash out quinidine from the heart followed by perfusion for 15 min with quinidine-free perfusate containing AAG (0.1 g/L) and quinidine (21.6 μM), and this was followed by a washout period as before. Phases 3, 4 and 5 consisted of perfusion with 0.5 g/L AAG and 29.3 μM quinidine, 1.5 g/L AAG and 48.6 μM quinidine, and zero AAG and 20 μM quinidine, respectively. There was a 55 min washout period between each phase, as before, the AAG concentration used in the last 15 min of the washout period being the same as that used in the ensuing phase. The quinidine concentration was increased with increasing AAG concentration to maintain the unbound quinidine concentration at 20 μM. The perfusate flow rate during each phase was measured volumetrically. Quinidine concentration in output perfusate (0.5 mL) and *QT* interval were measured at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, 20, 25 and 35 min after commencement of quinidine administration in each phase.

Drug analysis

Quinidine concentration was assayed by HPLC. Perfusate (20 μL) was injected directly into the chromatography system, which consisted of a 200 μL loop injector (Rheodyne Inc., Cotati, CA, U.S.A.), a reverse phase C_{18} precolumn (New Guard, Applied Biosystems, Foster City, CA, U.S.A.), a large pore size (300 Å) reverse phase C_{18} analytical column (Selectosil 10 μ, 250 × 4.6 mm, Phenomenex, Torrance, CA, U.S.A.), a UV detector operated at 250 nM (model 510 Waters, Milford, MA, U.S.A.) and an Omniscribe chart recorder (Houston Instrument, Austin, TX, U.S.A.). The mobile phase was water, acetonitrile, acetic acid, triethylamine, orthophosphoric acid in the ratio 340:56:4:2.6:1.2 (pH 3.5) pumped at 1.0 mL/min. Under these conditions the retention time of quinidine was 8 min. The intra-assay coefficient of variation for the assay at quinidine concentrations of 0.2, 1 and 100 μM was 2.01, 0.74 and 1.30%, respectively.

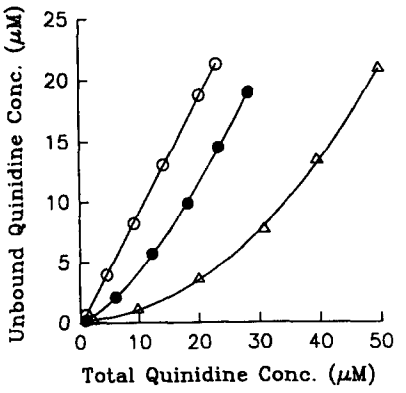

Fig. 1. Relationship between unbound and total quinidine concentration in perfusate containing 0.1 (○), 0.5 (●) and 1.5 (△) g/L AAG. Each point represents a single experiment.

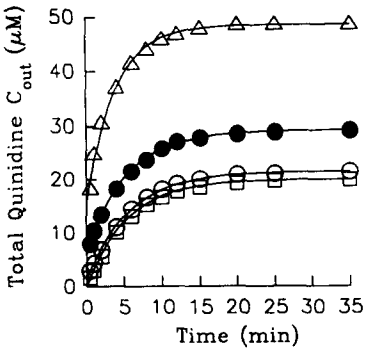

Fig. 2. Effect of perfusate AAG concentration [0 (□) (first phase only), 0.1 (○), 0.5 (●) and 1.5 (△) g/L] on total quinidine output concentration in a single experiment.

Data analysis and statistics

In all experiments, the perfusate drug output concentration (C_{out}) versus time data were fitted with the following equation [18] by non-linear least squares regression (Sigmaplot, Jandel Scientific):

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

where C_{out} is the 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 $a = A/C_{in}$.

The coronary output drug concentration (C_{cor}) was then calculated according to Eqn 2 [18].

$$C_{cor} = C_{out} - aC_{in}\,e^{-kt}. \quad (2)$$

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 modified by Morgan and Huang [3]:

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

In our experiments, Q was the coronary artery perfusion flow rate, calculated as the total measured flow rate of output perfusate × $(1 - a)$. Estimates of PS and V_u for quinidine were obtained by fitting (Sigmaplot) the total C_{out} versus time data from each phase with Eqn 3 substituted into Eqn 1. As f_u varied with total C_{out} in some cases, the f_u value for each individual total C_{out} value was used in the fitting procedure. Likewise, the measured value of Q at each sampling time was used in the fitting procedure.

The time course of the pharmacologic effect of quinidine (ΔQT) during the uptake experiments is given by:

$$\Delta QT = \Delta QT_{max}(1 - e^{-k_e t}) \quad (4)$$

where ΔQT_{max} is the maximum effect of the drug, observed at equilibrium, and k_e is the time constant for attainment of ΔQT_{max}. During each phase of the experiments, the pharmacological effect, ΔQT, was also plotted against C_{corr}, calculated from Eqn 2.

Differences among groups were examined by two-way analysis of variance and the Bonferroni multiple comparison t-test [19]. Correlations between variables were examined by linear regression. A probability less than 0.05 was considered significant.

RESULTS

Protein binding

In perfusate containing 0.1 g/L AAG there was a linear relationship between unbound and total concentration (C_u and C_T, respectively) of quinidine, i.e. f_u was constant. However, at 0.5 and 1.5 g/L AAG there was a non-linear relationship between C_u and C_T of quinidine (Fig. 1). The polynomial fit for each of these relationships was as follows:

0.1 g/L AAG $C_u = -0.278 + 0.941\ C_T$

0.5 g/L AAG $C_u = -0.041 + 0.237\ C_T + 0.0214\ (C_T)^2 - (2.08 \times 10^{-4}) \times (C_T)^3$

1.5 g/L AAG $C_u = 0.145 + 0.0268\ C_T + 0.00680(C_T)^2 + (2.18 \times 10^{-5}) \times (C_T)^3$

These equations were used to determine quinidine C_u for any AAG concentration and quinidine C_T used in the perfusion studies over the C_T range used (0.1–50 μM).

Pharmacokinetics of quinidine myocardial uptake

The time courses of total quinidine C_{out} at the different perfusate AAG concentrations are shown for one experiment in Fig. 2. As quinidine f_u varied with AAG concentration, higher total quinidine concentrations were used with the higher perfusate AAG concentrations (Fig. 2) so as to maintain the unbound quinidine concentration at 20 μM in all phases. Equation 3 substituted into Eqn 1 produced

Table 1. Effect of perfusate AAG on quinidine uptake

Phase	AAG (g/L)	f_u	k (min^{-1})	k_e (min^{-1})	Shunt fraction (%)
1	0	1.0	0.172 ± 0.008	0.141 ± 0.039	0.55 ± 0.97
2	0.1	0.928	0.194 ± 0.031	0.149 ± 0.020	10.8 ± 8.8
3	0.5	0.682	0.245 ± 0.029*	0.208 ± 0.019	21.6 ± 0.5†
4	1.5	0.412	0.394 ± 0.030*	0.293 ± 0.109†	34.0 ± 1.6*
5	0	1.0	0.153 ± 0.006	0.151 ± 0.051	6.88 ± 4.40

* Significantly different from all other groups ($P < 0.05$).
† Significantly different from zero AAG group ($P < 0.05$).

Table 2. Effect of perfusate AAG on myocardial disposition of quinidine

Phase	AAG (g/L)	PS (mL/min/g)	V_u (mL/g)
1	0	17.7 ± 1.91	17.3 ± 0.9
2	0.1	8.94 ± 0.99*	15.8 ± 2.4
3	0.5	8.70 ± 0.26*	17.9 ± 2.0
4	1.5	9.01 ± 0.26*	18.4 ± 1.3
5	0	19.1 ± 0.82	19.5 ± 0.8

* Significantly less than zero AAG group ($P < 0.05$).

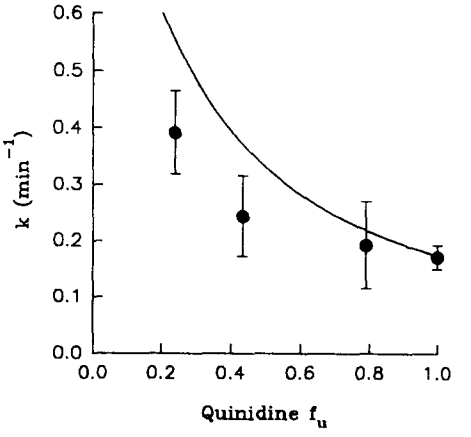

Fig. 3. Relationship between k and f_u for quinidine in the presence of AAG. Predicted using mean PS and V_u estimates (N = 3) from zero AAG perfusate in Eqn 3 (—). Mean observed values (±95% confidence interval; N = 3) (●).

an excellent fit to the data and the fitted values for k and a, the shunt fraction, are shown in Table 1. With 0.5 and 1.5 g/L AAG, equilibration of total C_{out} was significantly faster than that with zero and 0.1 g/L AAG (Table 1). The equilibration rate constant, k, increased from 0.172 to 0.394 min^{-1} when f_u in the perfusion medium was decreased from 1 to 0.412 (Table 1). There was no significant difference in k with zero AAG perfusate between phases 1 and 5, which attests to the stability of the experimental preparation. The fraction of perfusate shunted around the coronary circulation increased with increasing perfusate AAG concentration but returned to near the phase 1 value in the fifth phase (Table 1).

Fitting Eqn 3 substituted into Eqn 1 also provided estimates of PS and V_u (Table 2). In each of phases 2, 3 and 4, in which AAG was present in the perfusate, PS was reduced significantly by about half compared to those values of PS obtained in phases 1 and 5, in which AAG was absent. This effect of AAG was maximal at the lowest AAG concentration used (0.1 g/L). The presence of AAG had no significant effect on V_u (Table 2). The mean PS and V_u values for the zero AAG perfusate were used in Eqn 3 to predict the relationship between k and f_u assuming that AAG had no effect on PS. The resulting simulation is shown in Fig. 3. The mean value of k (±95% confidence interval) observed at each of the three values of f_u (corresponding to 0.1, 0.5 and 1.5 g/L AAG) is also shown in Fig. 3. This shows that in the presence of 0.5 and 1.5 g/L AAG, k was significantly less than that predicted assuming constant PS.

Pharmacodynamics of quinidine

During the drug-free stabilization period before the beginning of each phase the mean baseline QT interval increased slightly from the first to the fifth phase, but the increase was not significant (Table 3). The time course of increase in QT interval (ΔQT) in each of the five phases of one of the experiments is shown in Fig. 4. The rate constant, k_e, for equilibration of the effect, according to Eqn 4, is shown in Table 1. The mean maximum ΔQT interval did increase slightly from the first to the fifth phase, but the increase was not significant (Table 3). At each perfusate AAG concentration there was a linear relationship between ΔQT and the total quinidine C_{cor} calculated from Eqn 2, except for the highest AAG concentration where some curvature was evident (Fig. 5A). The slope of this relationship decreased significantly with increasing perfusate AAG concentration (Table 3). The slope of the relationship between ΔQT and unbound quinidine C_{cor} was linear for all AAG concentrations (Fig. 5B), but this did not change with perfusate AAG concentration (Table 3). In each of the phases with

Table 3. Effect of perfusate AAG on quinidine pharmacodynamics

Phase	AAG (g/L)	Baseline QT (msec)	Maximum ΔQT (msec)	Slope: ΔQT vs total C_{cor}	Slope: ΔQT vs unbound C_{cor}
1	0	81.7 ± 11.0	51.7 ± 4.4	2.57 ± 0.27	2.57 ± 0.27
2	0.1	86.0 ± 12.8	49.8 ± 6.7	2.34 ± 0.49	2.51 ± 0.34
3	0.5	86.7 ± 10.5	59.5 ± 6.1	1.96 ± 0.19*	2.64 ± 0.25
4	1.5	90.5 ± 9.6	62.0 ± 4.7	1.22 ± 0.14†	2.66 ± 0.32
5	0	95.8 ± 16.4	60.2 ± 16.5	3.18 ± 0.50	3.18 ± 0.50

* Significantly different from zero AAG phase 5 ($P < 0.05$).
† Significantly different from zero and 0.1 g/L AAG phases ($P < 0.05$).

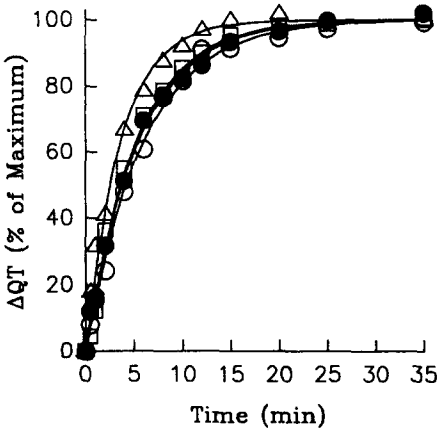

Fig. 4. Time course of effect of quinidine on ΔQT interval with different perfusate AAG concentrations (symbols as in Fig. 2) in a single experiment.

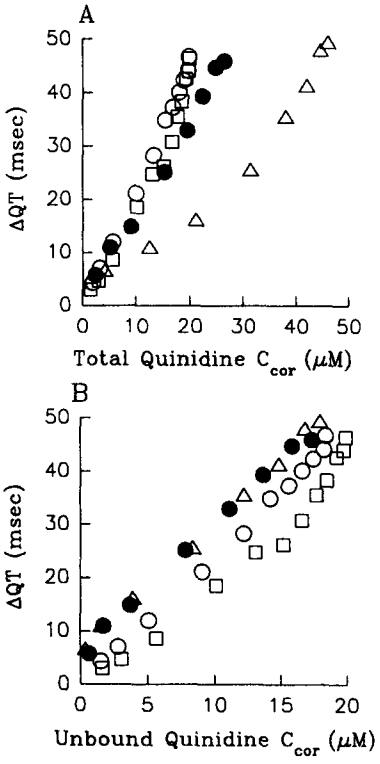

Fig. 5. Effect of perfusate AAG concentration (symbols as in Fig. 2) on relationship between ΔQT interval and (A) total quinidine C_{cor}, (B) unbound quinidine C_{cor} in a single experiment.

0.5 and 1.5 g/L AAG the intercept on the ΔQT axis was significantly greater than zero (Fig. 5B).

DISCUSSION

In the single-pass isolated perfused rat heart preparation, the rate of equilibration of quinidine C_{out} increased with increasing binding of quinidine to AAG added to the perfusate (Fig. 2). However, the increase in k with quinidine binding was not as great as would be predicted if PS and V_u had remained constant (Fig. 3). Analysis of the data showed that k did not change as predicted because AAG in the perfusate reduced the PS for quinidine by half (Table 2).

In a previous study in our laboratory with the same experimental model, we examined the effect of bovine serum albumin in the perfusate on the myocardial uptake of quinidine [6]. Using the same experimental design as in the present study, we found that albumin in the perfusate at concentrations of 0.1, 1 and 6% reduced the PS for quinidine by half. Thus, albumin and AAG had the same effect on quinidine PS in this preparation. We also examined the effect of albumin on antipyrine PS in our previous study [6]. In contrast to quinidine, albumin had no effect on antipyrine PS. Using a similar experimental approach, albeit with only one phase per experiment rather than the five phases used by us, Gillis and Kates [16] examined the effect of AAG on myocardial propafenone uptake and Machard and Chaumet-Riffaud [20] examined the effect of albumin on myocardial isradipine uptake. Kinetic analysis of these data indicated that the relationship between k and f_s for both drugs was in

accordance with Eqn 3, i.e. *PS* was not affected by the presence of protein [3]. Therefore, of the four drugs studied the effect of protein on myocardial drug permeability was confined to quinidine.

Plasma albumin has an important function in regulating the paracellular transport of water and small hydrophilic solutes across the capillary endothelium [7–9]. According to the fibre matrix model of capillary permeability, albumin binds to the luminal glycocalyx of continuous endothelium [21, 22] and its binding within the introit of intercellular junctions apparently forms a molecular filter that can electrostatically and sterically restrict paracellular transport [11, 23, 24]. We suggested that the effect of albumin in reducing myocardial quinidine permeability was consistent with reduction in paracellular capillary transport of quinidine ions according to the fibre matrix model [6]. It is known that AAG also plays a role in regulating capillary transport but evidence to date suggests that this effect is limited to the transport of polyanionic macromolecules such as albumin and α-lactalbumin [11, 12], whereas transport of water and small hydrophilic solutes is not affected [11, 12]. AAG binds to the endothelial glycocalyx and it has been hypothesized that the increase in density of negative wall charges within or at the entrance of endothelial transport pathways may create a charge-selective barrier [11–13]. The lack of effect of AAG on transport of small molecules led to the suggestion that AAG may not bind appreciably within the paracellular pathway but may bind selectively within the macromolecule transport pathways such as the plasmalemmal vesicular system [13]. However, in the present study, AAG caused an unambiguous reduction in quinidine myocardial permeability (Table 2) which closely resembled the reduction in permeability caused by albumin [6]. This is contrary to the conclusion from the previous investigations that AAG did not affect transport of relatively small solutes [11, 12]. The present findings with quinidine also suggest that the effect of AAG is probably not due to a charge-selective barrier because the increased negative charge of the glycocalyx due to AAG would be expected, if anything, to promote the paracellular transport of the oppositely charged quinidine ions as observed previously with the cationic macromolecule ribonuclease [12]. Therefore, these findings suggest that the mechanism of regulation of capillary permeability may be the same for both albumin and AAG, i.e. the formation of a steric barrier rather than an electrostatic barrier.

The experimental design used in the present study enabled us to study the effect of different perfusate concentrations of AAG on quinidine myocardial pharmacodynamics within the same preparation. Removal of the atria and pacing at 200 bpm facilitated the precise measurement of the effect of quinidine on the QT interval of the electrocardiogram. The baseline QT interval and maximum ΔQT induced by quinidine tended to increase during the five phases (Table 3), but the equilibration rate constant for the effect, k_e, was the same in the first and last phases ($P > 0.05$, Table 1). The increase in k_e with AAG concentration paralleled the concomitant increase in k (Table 1). There was a marked increase in the calculated shunt fraction with increasing AAG concentration which returned toward the initial value in the final phase (Table 1). In our previous study with varying perfusate albumin concentration there was also an increase in shunt fraction with protein concentration, reaching a maximum of 24% shunted at 60 g/L albumin [6]. This was probably due to increased vascular resistance caused by the viscosity of the albumin solution, but it is surprising that only 0.5 g/L AAG in the present study caused a similar degree of shunting (Table 1) to that caused by 60 g/L albumin in the previous study.

To account for the shunting C_{cor} was calculated from C_{out} using Eqn 2. There was a linear relationship between ΔQT and total quinidine C_{cor} with the 0, 0.1 and 0.5 g/L AAG perfusates (Fig. 5A), as found previously [6], but there was some curvature in this relationship with the 1.5 g/L AAG perfusate. Linear regression applied to each of the plots in Fig. 5A showed that the slope of this relationship decreased significantly with increasing AAG concentration (Table 3). However, there was no significant difference in the slope of the ΔQT versus unbound quinidine C_{cor} among the different AAG concentrations (Table 3). Moreover, the curvature that was evident in the ΔQT versus total quinidine C_{cor} with the highest AAG concentration disappeared when unbound rather than total drug concentration was plotted (Fig. 5B). The small intercept for the two highest AAG concentrations which was apparent in the ΔQT versus unbound C_{cor} in each of the experiments (Fig. 5B) may have arisen from using the f_u value corresponding to the quinidine output concentration. As f_u varies with quinidine concentration at the two highest AAG concentrations (Fig. 1), f_u would be expected to decrease with decreasing drug concentration along the length of the coronary circulation with these perfusates. In this case the f_u for the output concentration would be lower than at other points in the coronary circulation, which would have the effect of shifting the plots in Fig. 5B to the left and producing an intercept. The plots in Fig. 5 indicate that the electrocardiographic effect of quinidine is directly related to the unbound drug concentration in the circulation. This is in contrast to previous *in vitro* and *in vivo* findings with several α_1-adrenergic antagonists which showed that in the presence of AAG the drug response was lower than predicted by the unbound drug concentration in the circulation [14, 15].

The findings from these perfused rat heart experiments have potential clinical relevance. There is great variability in the electrophysiological response versus total quinidine concentration relationship among human subjects [25]. AAG is the main binding protein for quinidine in plasma [26] and there is great inter-subject variability in the plasma AAG concentration [27]. The data in Fig. 5 suggest that variability in plasma concentration of AAG may contribute to the great inter-subject variability in the response–total plasma concentration relationship. Moreover, in acute myocardial infarction, in which the plasma AAG concentration increases [28], the data in Fig. 5 would predict a shift to the right in the response–total plasma

quinidine concentration relationship. The total plasma quinidine concentrations defining the therapeutic range would rise accordingly.

In conclusion, this study shows that, like albumin, AAG reduces the myocardial capillary permeability for uptake of unbound quinidine by the perfused heart preparation. The electrocardiographic effect of quinidine was directly related to the circulating unbound drug concentration, as is usually assumed but rarely proven.

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