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The Effect of α_1-Acid Glycoprotein on the Pharmacological Activity of α_1-Adrenergic Antagonists in Rabbit Aortic Strips

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Abstract—The pharmacological activity of three α_1-adrenergic antagonists, prazosin, tiodazosin and WB4101 has been studied in the presence and absence of 20 μM α_1-acid glycoprotein (AAG) in rabbit aortic strips, and measured as the ability to increase the EC50 value of the α_1-adrenergic agonist phenylephrine. For all three drugs, the presence of AAG diminished the pharmacological activity when compared with equivalent unbound concentrations in the absence of AAG. In the presence of AAG the EC50 value of phenylephrine at 5·69 nM unbound prazosin was on average 47% lower than in the absence of AAG ($P < 0.002$), at 122 nM unbound tiodazosin, 39% lower ($P < 0.01$), and at 25·6 nM unbound WB4101, 68% lower ($P < 0.002$). Albumin showed no ability to modify the α_1-adrenergic blocking activity of prazosin ($P > 0.7$). The EC50 value for phenylephrine in the absence of antagonists was not affected by AAG. The effect of AAG on the antagonistic activity of prazosin was concentration-dependent with a maximum suppression of prazosin activity of 79% and with a half-maximum concentration of 1·1 μM AAG. AAG significantly decreased prazosin's ability to reduce α_1-adrenergic stimulation of calcium influx ($P < 0.05$), while it had no effect on prazosin's ability to decrease α_1-adrenergic-stimulated formation of inositol phosphate. These results suggest that the effect of AAG on adrenoceptors appears to act selectively via α_{1a}-receptors. Consistent with these observations was the observation that WB4101, a selective α_{1a}-antagonist was more affected by AAG than was prazosin or tiodazosin.

In recent in-vivo studies we have found that α_1-acid glycoprotein (AAG), an acute phase reactant, could decrease the pharmacological activity of prazosin, measured as percent reduction in systolic blood pressure as a function of the unbound concentration in plasma (Chiang & Øie 1990). The result for prazosin is at variance with data reported for other drugs such as the β-blocker propranolol (Yasuhara et al 1985; Belpaire et al 1986) and the Na channel blockers disopyramide (Huang & Øie 1982), lignocaine (De Rick et al 1987) and propafenone (Gillis & Kates 1986) for which no alterations in pharmacological activities in the presence of AAG have been observed. Because prazosin is a specific α_1-adrenergic antagonist (Cavero & Roach 1980), the question was raised as to whether the ability of AAG to decrease activity of drugs was a general phenomenon for α_1-adrenergic antagonists. A study was therefore undertaken to determine if α_1-adrenergic antagonists other than prazosin could be affected by AAG. In addition to prazosin, tiodazosin, a selective α_1-adrenergic blocker of similar structure to prazosin (Buyniski et al 1980; Cohen et al 1980), and WB4101, a selective α_1-adrenergic blocker structurally unrelated to prazosin (Giardina et al 1985) were investigated.

Because AAG is a strong binder of prazosin (Vincent et al 1985), the possibility that the effect of AAG on prazosin may be related to a simple binding phenomenon was also tested. This was achieved by determining the effect of albumin on the pharmacological activity of prazosin at concentrations of albumin producing similar unbound fraction values as in the AAG studies.

These studies also allowed for testing of whether the observed activity of AAG in-vivo may have been related to a kinetic phenomenon. AAG is known to decrease the clearance of unbound prazosin without altering the half-life (Øie et al 1987), suggesting that at a given steady state unbound concentration, a lower infusion rate of prazosin is needed in the presence of AAG. The lower infusion rate will result in a lower formation rate of active metabolites which may alter the unbound concentration-effect relationship of prazosin. Because aortic strips from the rabbit have a low ability to metabolize prazosin, these studies could be carried out without the additional complication of formation of active metabolites. To further isolate the mechanism by which AAG affects α_1-adrenergic antagonists, the degree by which AAG modifies the interaction of prazosin with α_{1a}- and α_{1b}-adrenoceptors was tested in aortic tissue.

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Materials and Methods

Experimental design

Aortic strips from male New Zealand White rabbits, 2·5 to 3 kg were used throughout the studies. The animals were killed by decapitation under light ketamine anaesthesia, the chest opened and the thoracic aorta quickly excised. Three helical strips, each approximately 4 mm wide and 30 mm long, were prepared from each aorta using the method of Furchgott & Bhadrakom (1953). The three strips were mounted in jacketed glass organ baths (Harvard Apparatus, South Natick, MA) with one end attached to a glass holder and the other to an isometric transducer (FT03, Grass, Quincy, MA) connected to a polygraph (model 7, Grass). The organ bath contained 25 mL Krebs-Henseleit buffer maintained at 37°C and gassed with 95% O_2–5% CO_2. The resting tension of the aortic strips was adjusted to 1·0 g. After 1 h equilibration, 1 μM phenylephrine was added to the organ baths for 10 min and the preparations subsequently washed four times with Krebs-Henseleit buffer, and the preparations re-equilibrated for a further 1 h.

Cumulative concentration-response curves of phenylephrine were obtained in each of the organ baths by step-wise increase of the concentration of phenylephrine. For each step the phenylephrine concentration was increased 3-fold. When the maximum effect was attained, the preparations were washed with Krebs-Henseleit buffer 4 times and allowed a further 60 min equilibration. Various concentrations of prazosin, tiodazosin and WB4101 in the absence and presence of AAG were then added and 30 min later, a second cumulative concentration-response curve of phenylephrine determined. In the prazosin study, concentrations of 5 and 10 nM prazosin in the absence, and 20 nM prazosin in the presence of 30 μM AAG were investigated. Preliminary studies suggested that a mixture of 20 nM prazosin and 20 μM AAG would yield approximately 5 nM unbound prazosin. Similarly, concentrations of 100 nM and 300 nM tiodazosin, and 400 nM tiodazosin with 20 μM AAG (yielding approximately 100 nM unbound tiodazosin) and 25 nM, 50 nM, and 720 nM WB4101 with 20 μM AAG (yielding approximately 25 nM unbound WB4101) were investigated. In order to normalize the data between the individual preparations, the response to phenylephrine is reported as a percent increase in the tension, setting the maximum increase in the tension by phenylephrine to 100%.

In a separate study, the effect of 120 μM albumin on the pharmacological response of prazosin was investigated. Twenty nM prazosin at this albumin concentration yields approximately 5 nM unbound prazosin. The experimental protocol was otherwise as described above.

Two control studies were carried out. In one study the ability of 30 μM AAG to alter the cumulative concentration-response of phenylephrine was determined in the absence of α_1-adrenergic antagonists. The experiment was otherwise carried out as described above. In a separate study the ability of AAG alone to cause contraction of rabbit aortae was determined by sequentially adding AAG to aortic strip preparations to yield concentrations of 10, 20, 30 and 40 μM in the absence of phenylephrine and measuring the changes in the baseline tension.

In studies of the concentration dependence of the effect of AAG, unbound concentrations of approximately 10 nM prazosin were used in the absence and presence of 0·1–20 μM AAG. The total concentration of prazosin in these experiments in the presence of AAG ranged from 10 to 75 nM and the unbound concentration ranged from 8·0 to 12·1 nM as measured at the end of the experiment.

For Schild regression the dose ratio effect of various concentrations of prazosin using phenylephrine as the agonist was measured in the absence and presence of 20 μM AAG. Prazosin concentrations of 3, 5, 35 and 250 nM were used in the absence of AAG and total concentrations of 12–833 nM were used in the presence of AAG with unbound concentrations of 2·0–302 nM measured at the end of the experiment.

Measurement of phosphatidylinositol turnover

Aortae were cut into 4–5 mm wide rings and equilibrated for 30 min in 30 mL Krebs-Henseleit buffer (KHB) (pH 7·4, 37°C) and gassed with 95% O_2–5% CO_2. The rings were subsequently incubated for 1 h in 4 mL KHB containing 0·3 μM [^{3}H]inositol. After a 5 × 5 mL KHB rinse the rings were transferred to tubes containing 10 mM LiCl and various concentrations of prazosin in the presence and absence of 20 μM AAG. After 10 min incubation (37°C), 10 μM noradrenaline or 100 μM phenylephrine was added and the mixture incubated for 1 h. The reaction was terminated by adding 0·94 mL of a mixture of chloroform and methanol (1:2), and [^{3}H]inositol-1-monophosphate was isolated and measured as described by Chiu et al (1987).

Calcium influx studies

Aortae were cut into 3–5 mm wide rings and incubated in physiological saline solution (PSS) (containing (mM), NaCl 140, $MgCl_2$ 1·0, $CaCl_2$ 1·5, KCl 4·6, dextrose 10, Hepes 5 (pH 7·4)) at 37°C for 3 h under constant oxygenation. The rings were separated into 4 groups, control rings (Group 1), rings incubated with 10^{-5} M noradrenaline (Group 2), rings incubated with 10^{-5} M noradrenaline and 10^{-7} M prazosin (Group 3), and rings incubated with 10^{-5} M noradrenaline, 3×10^{-7} M prazosin and 20 μM AAG (Group 4). The procedure followed the general outline as described by Meisheri et al (1981): each group was incubated in 10 mL PSS for 15 min without agonist. Noradrenaline was added and the mixture incubated for 4 min. The rings were removed and placed in 5 mL PSS solution containing ^{45}Ca (5 μCi mL^{-1}), AAG, prazosin and noradrenaline as described above and incubated for 1·5 min. At the end of the incubation the aortic rings were removed and placed in ice-cold Ca^{2+}-free PSS with 2 mM EGTA to remove extracellular Ca^{2+} (Meisheri et al 1980; Cauvin & Malik 1984). The tissues were blotted, weighed and incubated overnight in 3 mL 5 mM EDTA. The solution was added to 17 mL scintillation fluid containing Triton X-100 and the concentration of $^{45}Ca^{2+}$ determined by scintillation counting. Twenty μL of the solution before incubation with EGTA was incubated with 5 mM EDTA overnight and used as the background value.

Protein binding

Protein binding of prazosin, tiodazosin, and WB4101 was measured by equilibrium dialysis. Six hundred μL of the organ bath solution was dialyzed against 600 μL KHB at 37°C for either 7 (prazosin and tiodazosin) or 9 h (WB4101) using a dialysis membrane (Spectra/por II, Spectrum, Los Angeles, CA) with a mol. wt cut-off of 12 000. In the binding studies of prazosin and WB4101, 300 nM [^{3}H]prazosin or 1·5 μM [^{3}H]WB4101 was added to the buffer side, respectively. The dialysis was carried out under a 95% O_2–5% CO_2 atmosphere in a Dubnoff shaking water bath to maintain the pH at approximately 7·4. After dialysis, the concentrations of radiolabelled prazosin or WB4101 on both sides of the cell membrane were determined by scintillation counting. The concentrations of unlabelled tiodazosin after dialysis were determined by HPLC (mobile phase: acetonitrile: 5M NaOH: 85% H_3PO_4: water (45:0·1:0·1:55); column: Alltech C-8 column (250 × 4·6 mm); flow rate: 1·5 mL min^{-1}. Tiodazosin was measured using a spectrophotofluorometer (FS970, Schoeffel, Westwood, NJ) with an excitation wavelength set at 250 nm and an emission cut-off filter of 389 nm.

Determination of metabolite formation

Ten nM [^{3}H]prazosin was incubated in organ baths containing 20 mL KHB with a mounted aortic strip for 10 h. Fifty μL

portions of the incubation solutions were then applied to TLC plates (0·2 mm silica gel 60 F-254; Alltech, Deerfield, IL) and prazosin separated from its metabolites using a mobile phase of ethyl acetate:methanol:diethylamine (75:20:5) (Taylor et al 1977). The plates were divided into 13 mm zones and the radioactivity in each zone determined by scintillation counting. The difference in radioactivity between the incubated solution and pure [^{3}H]prazosin at R_f values for the various prazosin metabolites was used to assess the ability of rabbit aorta to metabolize prazosin.

Data analysis

The increase of the strip tension by phenylephrine was related to the cumulative concentration of phenylephrine by the equation:

$$E/E_{max} = \frac{c}{EC50 + c} 100\% \quad (1)$$

where E/E_{max} is the tension compared with the maximum tension, c is the cumulative concentration of phenylephrine and EC50 is the concentration of phenylephrine producing 50% of the maximum effect. The EC50 values were obtained by fitting the above equation to the obtained data using the SYSTAT NONLIN program (Systat Inc, Evanston, IL).

The concentration-dependent effect of AAG on prazosin was evaluated using a modification of equation 1:

$$I = 100 - \frac{I_{max} c}{IC50 + c} \quad (2)$$

I_{max} is maximum % reduction of the ratio of the EC50 values of phenylephrine with and without prazosin, by AAG. I is the observed percent reduction caused by AAG of the EC50 ratio of phenylephrine in the presence and absence of prazosin, IC50 is the concentration of AAG causing a half-maximum reduction in the activity of prazosin, and c the concentration of AAG. The value 100 is the normalized baseline value for prazosin's activity in the absence of AAG.

The predicted EC50 values of the various α_1-adrenergic antagonist solutions containing AAG or albumin were calculated by linear regression of the EC50 values from zero, low, and high concentrations of the respective α_1-adrenergic antagonists in the absence of binding proteins.

For Schild regression, the linear regression of the relationship

$$\ln(DR - 1) = -\ln(k_I) + n\ln(c) \quad (3)$$

was determined (Kenakin 1987). DR is the ratio of the agonist concentrations eliciting 50% of the maximum effect in the presence vs absence of antagonist. k_I is the apparent inhibition constant of the antagonist, c is the concentration of antagonist and n is the slope of the regression line.

Statistical analysis

Test for significance was evaluated by a paired *t*-test. $P < 0{\cdot}05$ was considered significant.

Materials

Prazosin and tiodazosin were obtained from Pfizer (New York, NY). Human AAG and L-phenylephrine HCl were purchased from Sigma (St Louis, MO). Human albumin fraction V was obtained from Sigma and purified as described by Biserte et al (1960) to remove traces of AAG. [^{3}H]Prazosin was purchased from New England Nuclear (Boston, MA) and WB4101 and [^{3}H]WB4101 from Amersham (Arlington Heights, IL). The composition of KHB is (mM): NaCl 117, KCl 4·8, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 2·5, $CaCl_2$ 2·5, and glucose 11·1.

Results

The effect of AAG on the concentration-response curves of phenylephrine in the presence of three α_1-adrenergic antagonists

Concentration-response curves for phenylephrine in the presence and absence of prazosin, tiodazosin, and WB4101 with and without AAG for representative experiments are shown in Fig. 1. The presence of AAG decreased the apparent activity of all three α_1-adrenergic antagonists. The observed EC50 values of phenylephrine in the presence of α_1-adrenergic antagonists with AAG present were statistically significantly lower than the predicted EC50 values (Tables 1–3). The average unbound fraction values, f_u, obtained for prazosin, tiodazosin, and WB4101 in the 20 μM AAG experiments were $0{\cdot}29 \pm 0{\cdot}01$, $0{\cdot}30 \pm 0{\cdot}02$ and $0{\cdot}038 \pm 0{\cdot}004$, respectively.

Control studies

AAG had no effect on the concentration-response for phenylephrine; almost identical curves were observed in the presence and absence of AAG and no statistically significant changes in the EC50 values of phenylephrine were seen (Table 4). AAG alone had no effect on smooth muscle contractions of 10–40 μM in the aortic strip preparation. The baseline tension remained at $1{\cdot}01 \pm 0{\cdot}01$ g at all concentrations of AAG.

The effect of albumin on the concentration-response curve of phenylephrine in the presence of prazosin

Concentration-response curves for phenylephrine in the presence and absence of prazosin with and without 120 μM albumin are shown in Fig. 2. The presence of albumin did not alter the antagonistic activity of prazosin (Table 5). In separate studies, it was found that albumin had no effect on the EC50 value of phenylephrine alone and the binding of phenylephrine was negligible (< 10%). The unbound fraction of prazosin in the presence of 120 μM albumin was $0{\cdot}33 \pm 0{\cdot}02$, similar to the binding in the AAG experiment.

Determination of metabolite formation

The percentage increase in the radioactivity of the prazosin metabolite R_f values over 10 h corresponds to < 5% of the total radioactivity, indicating that the formation of prazosin metabolites during the study period (3 h) was negligible.

Concentration dependent effect of AAG on the inhibitory activity of prazosin

The effect of AAG on the ability of prazosin to inhibit the activity of phenylephrine was detectable even at very low concentrations of AAG (Fig. 3). An IC50 value of $1{\cdot}09 \pm 0{\cdot}24$ μM and a maximum inhibitory effect (I_{max}) of $79 \pm 5\%$ was determined at unbound concentrations of approximately 10 nM prazosin.

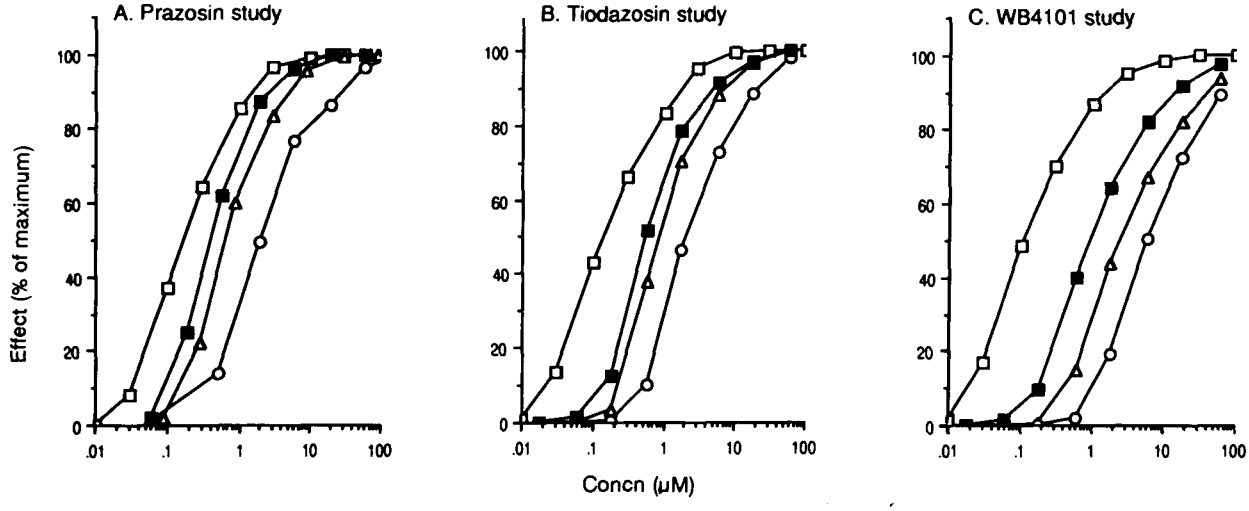

FIG. 1 Concentration-response curves of phenylephrine in the presence of three α_1-adrenergic antagonists in individual preparations. A: representative concentration-response curves of phenylephrine in control (□), in the presence of 5 nM prazosin (△), 10 nM prazosin (○), and 5·92 nM unbound prazosin + 20 μM AAG (■). B: representative concentration-response curves of phenylephrine in control (□), in the presence of 100 nM tiodazosin (△), 300 nM (○), and 132 nM unbound tiodazosin + 20 μM AAG (■). C: representative concentration-response curves of phenylephrine in control (□), in the presence of 25 nM WB4101 (△), 50 nM WB4101 (○), and 26·1 nM unbound WB4101 + 20 μM AAG (■).

Table 1. The average EC50 values (μM) of phenylephrine in controls, in the presence of 5 and 10 nM prazosin, and the observed and predicted EC50 values in the presence of prazosin plus 20 μM AAG.

	Number of experiments	Control	Prazosin 5 nM	Prazosin 10 nM	Unbound prazosin 5·69 ± 0·11 nM with AAG	
					Observed[a]	Predicted[b]
Mean	6	0·22	1·24	2·03	0·70	1·29[c]
s.e.		0·01	0·21	0·20	0·18	0·14

[a]The average observed EC50 values obtained from averaging each individual datum at similar levels of unbound concentration of prazosin. [b]Linear regression of the EC50 values in the presence of 0, 5, and 10 nM prazosin to interpolate the theoretical EC50 at the observed unbound concentration. [c]Significantly different from the observed EC50 of phenylephrine in the presence of prazosin and AAG ($P < 0·002$).

Table 2. The average EC50 values (μM) of phenylephrine in controls, in the presence of 100 and 300 nM tiodazosin, and the observed and predicted EC50 values in the presence of tiodazosin plus 20 μM AAG.

	Number of experiments	Control	Tiodazosin 100 nM	Tiodazosin 300 nM	Unbound tiodazosin 122 ± 10 nM with AAG	
					Observed[a]	Predicted[b]
Mean	6	0·26	1·11	3·00	0·81	1·29[c]
s.e.		0·03	0·19	0·54	0·19	0·16

[a]The average observed EC50 values obtained from averaging each individual datum at similar levels of unbound concentration of tiodazosin. [b]Linear regression of the EC50 values in the presence of 0, 100, and 300 nM tiodazosin to interpolate the theoretical EC50 at the observed unbound concentration. [c]Significantly different from the observed EC50 of phenylephrine in the presence of tiodazosin and AAG ($P < 0·01$).

Schild regression

The ratio of the EC50 values of phenylephrine in the presence and absence of prazosin was significantly affected by AAG. A Schild regression (Fig. 4) indicates a shift in the slope of the regression in the presence of AAG (from 1·12 ± 0·07 to 1·44 ± 0·13; $P < 0·05$) with the largest differences at low concentrations.

Phosphatidylinositol turnover in the presence of AAG

Prazosin significantly decreased the noradrenaline-stimulated formation of inositol phosphate from phosphatidylinositol (from 100 to 28·6 ± 4·5% [mean ± s.e.], $P < 0·001$) (Fig. 5). AAG had no effect on inositol phosphate formation either in the presence of prazosin (26·7 ± 5·4% with AAG vs 28·6 ± 4·5% without AAG, $P > 0·5$) or in the absence of

Table 3. The average EC50 values (μM) of phenylephrine in controls, in the presence of 25 and 50 nM WB4101, and the observed and predicted EC50 values in the presence of WB4101 plus 20 μM AAG.

	Number of experiments	Control	WB4101 25 nM	WB4101 50 nM	Unbound WB4101 27·6±2·5 nM with AAG	
					Observed[a]	Predicted[b]
Mean	6	0·20	3·60	7·51	1·19	3·87[c]
s.e.		0·02	0·30	1·48	0·16	0·45

[a] The average observed EC50 values obtained from averaging each individual date at similar levels of unbound concentration of WB4101. [b] Linear regression of the EC50 values in the presence of 0, 25, and 50 nM WB4101 to interpolate the theoretical EC50 at the observed unbound concentration. [c] Significantly different from the observed EC50 of phenylephrine in the presence of WB4101 and AAG ($P < 0·002$).

Table 4. The average EC50 values (μM) of phenylephrine in the absence and presence of 20 μM AAG.

	Number of experiments	Without AAG	With AAG
Mean	4	0·24	0·27[a]
s.e.		0·04	0·05

[a] Not significantly different from the EC50 of phenylephrine alone ($P > 0·1$).

prazosin (104·6±6·5% vs 100%, $P > 0·5$) (Fig. 5). Using the more specific α_1-adrenergic agonist epinephrine gave similar results. AAG did not alter the formation of inositol phosphate by adrenaline and had no ability to alter the inhibitory effect of prazosin on this reaction (data not shown). Because activation of α_{1b}-adrenoceptors induces alterations in the phosphatidylinositol turnover (Minneman 1988), these results suggest that AAG does not interact directly or indirectly with α_{1b}- adrenoceptors.

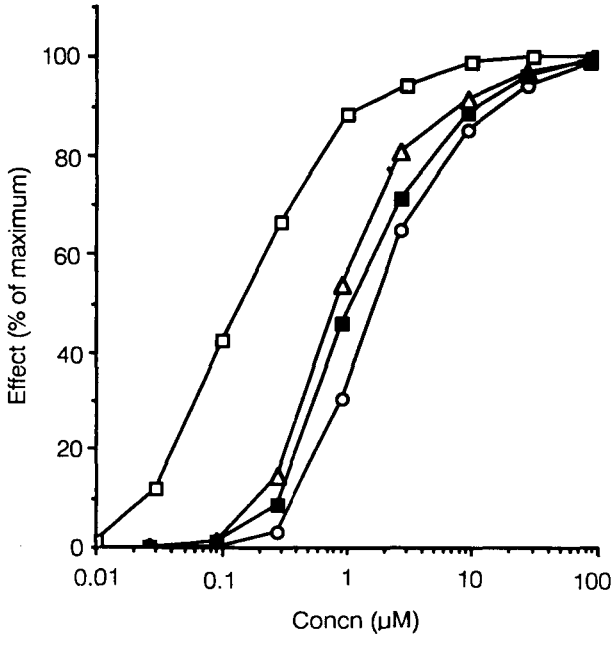

FIG. 2 Representative concentration-response curves of phenylephrine in control (□), in the presence of 5 nM prazosin (△), 10 nM prazosin (○), and 6·6 nM unbound prazosin with 120 μM albumin (■).

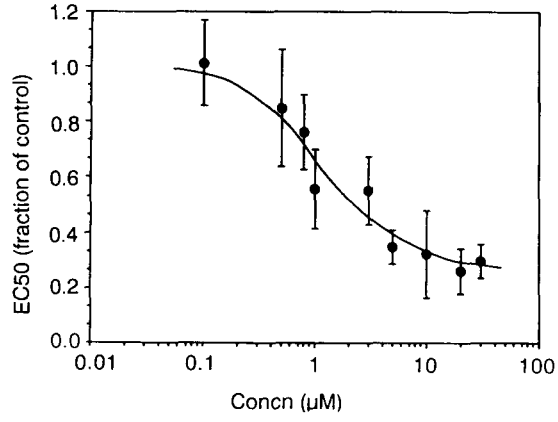

FIG. 3. Concentration dependent effect of AAG on the activity of prazosin in aortic strip preparations. The ratio of the EC50 value of phenylephrine in the presence and absence of 10 nM unbound concentrations of prazosin in the absence of AAG is set to 100%. Each data point represents the average value of 3 to 13 measurements. The vertical bars represent the standard error around each point. The solid line represents a fit of equation 3 to the data points.

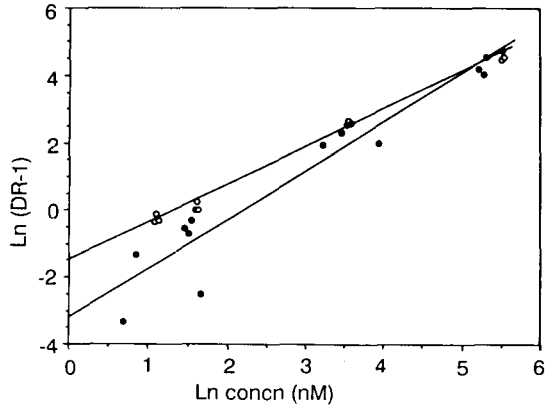

FIG. 4. Schild regression of DR-1 for phenylephrine vs prazosin concentration in the absence (○) and presence (●) of 20 μM AAG. In the absence of AAG the curve is described by ln (DR-1) = ln (0·213) + 1·12 × ln (prazosin concentration in nM) and in the presence of AAG: ln (DR-1) = ln (0·039) + 1·44 × ln (prazosin concentration in nM).

Calcium influx studies

The results were normalized to 20 mg tissues. The calcium influx in the control group (Group 1) was set to 100% and the calcium influx in the other groups expressed as a percent of

Table 5. The average EC50 values (μM) of phenylephrine in controls, in the presence of 5 nM and prazosin, and the observed and predicted EC50 values in the presence of prazosin plus 120 μM albumin.

	Number of experiments	Control	Prazosin 5 nM	Prazosin 10 nM	Unbound prazosin 6·13±0·15 nM with albumin	
					Observed[a]	Predicted[b]
Mean	6	0·22	1·31	2·14	1·40	1·43[c]
s.e.		0·02	0·19	0·19	0·13	0·10

[a] The average observed EC50 values obtained from averaging each individual datum at similar levels of unbound concentration of prazosin. [b] Linear regression of the EC50 values in the presence of 0, 5, and 10 nM prazosin to interpolate the theoretical EC50 at the observed unbound concentration. [c] Not significantly different from the observed EC50 of phenylephrine in the presence of prazosin and albumin ($P > 0.7$).

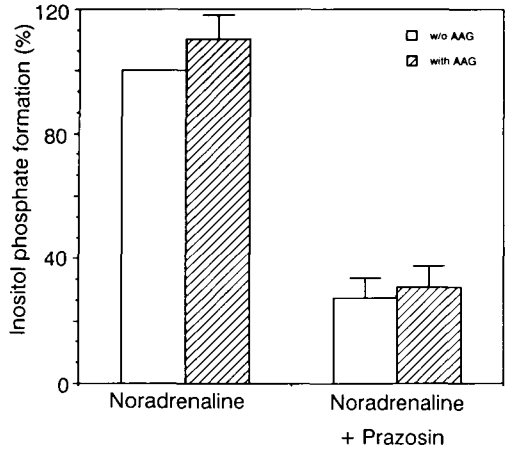

FIG. 5. Inositol phosphate accumulation (mean±s.e.) in the presence of 10 μM noradrenaline with or without 20 μM AAG and 10 μM noradrenaline plus approximately 0·1 μM unbound prazosin with or without AAG. The formation of inositol phosphate by 10 μM noradrenaline in the absence of prazosin and AAG is set to 100%. Average values of 6 experiments.

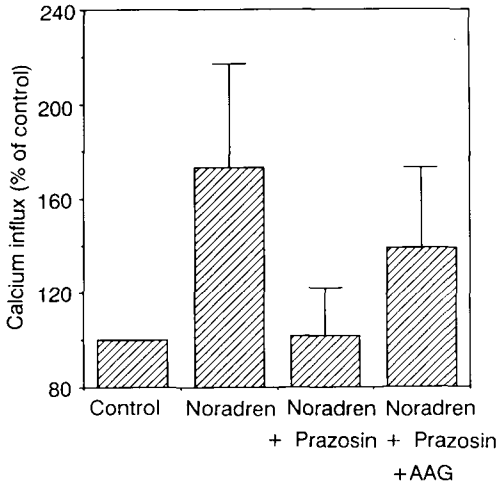

FIG. 6. Effect of prazosin and the combination of prazosin and AAG on noradrenaline induced calcium influx in the rabbit aorta. Influx of Ca^{2+} in rabbit aorta without α_1-adrenergic agonists and antagonists was set to 100% (left-most bar). The presence of 10^{-5} M noradrenaline (second bar from left), increased the influx, 10^{-7} M prazosin decreased the influx elicited by 10^{-5} M noradrenaline (second bar from right) and 20 μM AAG diminished the ability of $1{\cdot}1 \times 10^{-7}$ M unbound prazosin to suppress the calcium-induced influx by 10^{-5} M noradrenaline (right bar).

these values. The results are presented in Fig. 6. As can be seen, noradrenaline enhanced the calcium influx significantly (173±44%, [mean±s.d.] n=6, $P < 0.02$). Prazosin (10^{-7} M) significantly decreased the noradrenaline-stimulated calcium influx (102±20%, $P < 0.02$). Addition of 20 mM AAG to prazosin preparations (3×10^{-7} M total, $1{\cdot}1 \times 10^{-7}$ M unbound) increased the calcium influx in comparison with the preparations in the absence of AAG (139±34%, $P < 0.05$). Because activation of α_{1a}-adrenoceptors is associated with influx of calcium via nifedipine-sensitive calcium channels (Minneman 1988), these results indicate that AAG directly or indirectly influences α_1-adrenergic antagonists via the α_{1a}-adrenoceptors.

Discussion

AAG is an avid binder of many organic bases (Müller et al 1986) including many of the α_1-adrenergic antagonists. If one accepts the notion that the pharmacological effect is related to unbound rather than total drug concentrations, one would expect a significant shift in the effect-total concentration relationship but no shift in the effect-unbound concentration relationship of α_1-adrenergic antagonists when adding AAG to the system. From the obtained data one sees that the α_1-adrenergic antagonist activity at equal unbound concentrations of drugs is much lower in the presence of AAG than in the absence of AAG. AAG, therefore, appears to counteract α_1-adrenergic antagonists. This effect was seen both with prazosin and tiodazosin, two compounds having a similar chemical structure, as well as with WB4101, an α_1-adrenergic antagonist of a different structural class. The effect of AAG, therefore, does not seem to relate to specific chemical structures and we postulate that the effect may be extrapolated to other α_1-adrenergic antagonists. Interestingly, AAG has neither an apparent effect on α_1-adrenergic agonists nor any adrenergic activity by itself. The effect of AAG, therefore, does not appear to relate to a traditional agonist/antagonist activity of AAG.

Because insignificant amounts of prazosin metabolites were generated during the incubation period in these experiments, the observed effect of AAG is not related to a differential formation of active metabolites in the absence and presence of AAG. The alteration in the activity of α_1-adrenergic antagonists, therefore, is likely to be a direct effect of AAG itself.

It is also clear that this ability of AAG to reduce the activity of α_1-adrenergic antagonists does not extend to all

proteins capable of binding α_1-adrenergic antagonists. For example, albumin at concentrations that bind prazosin to the same degree as in the AAG experiments did not cause any diminution of the effect of prazosin. Further, this lack of effect of albumin indicates that the observed effect of AAG is not due to methodological artefacts in the protein binding technique employed.

It has been suggested (Minneman 1988; Minneman et al 1988) that α_1-adrenergic receptors can be subdivided into two classes, α_{1a}- and α_{1b}-adrenergic receptors. Both subtypes apparently act via G-proteins. α_{1a}-Adrenergic receptors act mainly by stimulating nifedipine-sensitive calcium channels causing extracellular calcium influx while α_{1b}-adrenergic receptors act via phosphatidylinositol turnover and intracellular mobilization of calcium. The ability of AAG to influence prazosin's inhibition of α_{1a}-adrenergic stimulated extracellular calcium influx together with its virtual lack of effect on phosphatidylinositol turnover, would suggest that AAG elicits its effect via α_{1a}-adrenergic receptors and will have little or no effect on α_{1b}-adrenoceptors. This hypothesis would predict that α_1-adrenergic antagonists working via the α_{1a}-receptors would be more susceptible to the effect of AAG than antagonists mainly working via α_{1b}-adrenoceptors. The fact that WB4101 has been characterized as a relatively selective α_{1a}-adrenergic antagonist while prazosin is an equipotent α_{1a}- and α_{1b}-adrenergic antagonist (Minneman 1988) and that AAG had a more pronounced effect on WB4101 than prazosin in the aortic strip studies supports the hypothesis that AAG mainly acts on α_1-adrenergic antagonists.

The Schild regression data show a slope of greater than one in the presence of AAG. Slopes greater than one can be encountered if an equilibrium has not been reached (Kenakin 1987). We do not believe this explains our results as: (1) AAG did not itself affect the effect-concentration curves for phenylephrine (see Results); (2) in the presence of prazosin, with and without AAG, a higher dose of phenylephrine was added only after a constant pharmacological activity had been observed for about two min. In preliminary studies, extending the time of constant response to ten min before the next dose did not affect the outcome. An alternative explanation for having a slope greater than one would be the ability of prazosin at high concentrations, due to a direct or indirect interaction, to decrease the activity of AAG.

It is not clear why the effect is only directed toward antagonists and not agonists. One may speculate that α_1-adrenergic antagonist and agonists do not bind to the same binding sites although they may partly overlap as has been indicated for β-adrenergic receptors (Strader et al 1989a, b), and that AAG may alter the region of the receptor which binds the antagonists thereby reducing the efficacy of the antagonists to inhibit the activity of α_1-agonists. The finding by Cheresh et al (1984) that AAG does not alter the binding of concanavalin A and sIg to lymphocytes but prevents the capping of the receptors associated with the binding suggest that AAG may have subtle effects on receptors. This suggestion would indicate that the α_{1a}- and α_{1b}-adrenergic receptors would have sufficiently different structures for AAG to interact only with the α_{1a}-adrenergic receptor. Confirmation or rejection of this suggestion is not available from the current literature.

The effect of AAG on prazosin in the aortic strip system is seen even at very low concentrations of AAG (Fig. 4). At clinical concentrations of AAG (5–15 μM) the effect approaches its maximum value. This would suggest that alterations of plasma concentrations of AAG may not have any clinical effect on the pharmacological activity of α_1-adrenergic antagonists. However, in in-vivo studies in rats where human AAG was administered to raise the AAG plasma level, the effect of prazosin was significantly decreased after the AAG administration (Chiang & Øie 1990). In those animals the plasma AAG concentration was approximately 10 μM at the beginning of the study. Administration of human AAG increased the total AAG concentration to 20 μM. Whether those results indicate that the concentration-dependent effect of AAG on prazosin is different in-vivo compared with aortic strip preparations or whether rat AAG has a different effect (lower absolute effect and/or higher IC50 value) compared with human AAG is not known and further investigations are needed.

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