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Does α_1-Acid Glycoprotein Act as a Non-functional Receptor for α_1-Adrenergic Antagonists?

MINGSHENG QIN AND SVEIN ØIE

Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143-0446, USA

Abstract—The ability of a variety of α_1-acid glycoproteins (AAG) to affect the intrinsic activity of the α_1-adrenergic antagonist prazosin was studied in rabbit aortic strip preparations. From these studies, the activity of AAG appears to be linked to their ability to bind the antagonist. However, a capability to bind prazosin was not the only requirement for this effect. The removal of sialic acid and partial removal of the galactose and mannose residues by periodate oxidation of human AAG all but eliminated the ability of AAG to affect the intrinsic pharmacologic activity of prazosin, although the binding of prazosin was not significantly affected. The presence of bovine AAG, a protein that has a low ability to bind prazosin, reduced the effect of human AAG on prazosin activity. Based upon these results, we propose that AAG is able to bind in the vicinity of the α_1-adrenoceptors, therefore extending the binding region for antagonists in such a way as to decrease the ability of the antagonist to interact with the receptor. The carbohydrate side-chains are important for the binding of AAG in the region of the adrenoceptor.

The pharmacologic response to α_1-adrenergic antagonists has been found to be decreased in the presence of human α_1-acid glycoprotein (AAG), an acute phase-response protein, both in-vivo and in-vitro (Chiang & Øie 1990; Chiang et al 1991). Because the pharmacological effect is usually only related to the unbound drug in plasma, the effect of AAG can in part be explained by the ability of human AAG to bind α_1-adrenergic antagonists. However, because the effect at a given unbound concentration is also reduced in the presence of human AAG, a significant component of the observed decrease in activity is due to an alteration in the intrinsic antagonistic activity (Chiang & Øie 1990; Chiang et al 1991). This effect of human AAG on the intrinsic antagonistic activity of α_1-adrenergic blockers appears to be relatively specific as described in studies of sodium-channel blockers (Huang & Øie 1982; Gillis & Kates 1986; Belpaire et al 1986; de Rick et al 1987) and β-blockers (Yasuhara et al 1985), where human AAG was shown to alter the total concentration-effect relationship but not the intrinsic antagonistic activity.

This effect is also unique in that other binding proteins of the α_1-adrenergic antagonists do not appear to possess this ability of human AAG. For example, albumin binds the α_1-adrenergic antagonist prazosin to a significant degree but does not alter the unbound concentration-effect relationship as does human AAG (Chiang et al 1991). In contrast to human AAG, rat AAG does not affect the unbound concentration-effect relationship of prazosin. In unpublished studies where the rat AAG was elevated in-vivo by occlusion of the infrarenal aorta, we found no change in the unbound concentration-effect relationship. The most distinct difference between rat and human AAG is that rat AAG binds prazosin much less ($< 10\%$ at $20\,\mu$M) than human AAG ($\sim 75\%$ at $20\,\mu$M). These results would suggest that the ability of human AAG to bind prazosin is important for the observed decrease of the intrinsic activity, but also that the binding is not the only characteristic necessary for the effect.

However, because the occlusion of the infrarenal aorta causes a hind leg ischaemia and activates the acute-phase response (Griffeth et al 1983), the lack of response to an elevated rat AAG concentration may be the result of a combination of factors that change during the acute-phase response.

The purpose of this study was to investigate whether the ability of AAG to decrease the unbound concentration-effect relationship of α_1-adrenergic antagonists is related to the ability of AAG to bind these compounds and to determine whether other potential factors may be involved.

Correspondence: S. Øie, Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143-0446, USA.

Materials and Methods

Materials

Prazosin, L-phenylephrine hydrochloride, human AAG, bovine AAG, and canine AAG were obtained from Sigma (St Louis, MO). [^{3}H]Prazosin was purchased from New England Nuclear (Boston, MA).

Asialo-AAG was prepared from human AAG both by a chemical method (Eylar & Jeanloz 1962) and by an enzymatic method (Casey et al 1987). After treatment the AAG samples were dialysed against distilled water and freeze dried. No detectable residual AAG was found in the different preparations using crossed affino-immunoelectrophoresis (Bøg-Hanssen 1983). The residual fractions of sialic acid in the chemically and enzymatically treated preparations were 0·5% and 3·2%, respectively, using the thiobarbituric acid method (Warren 1959).

Oxidized AAG or oxidized asialo-AAG was prepared by dissolving 28 mg human AAG or asialo-AAG (prepared by the enzymatic method) in 10 mL acetate buffer (0·65 M, pH 4·5), mixing with 1·5 mL 0·1 M $NaIO_4$ and reacting for 1·5 h at 4°C. The reaction was terminated by the addition of 0·2 mL glycerol. The solution was subsequently dialysed

against 2 L double-distilled water at 4°C for 20 h. SDS-PAGE indicated a diffuse band around 38 kDa and the product did not separate on crossed affino-immunoelectrophoresis from concanavalin A, suggesting that removal of galactose and mannose, as described by Eylar & Jeanloz (1962) was achieved.

^{125}I-AAG was prepared from human AAG according to the Enzymobead method (Bio-Rad, Richmond, CA). The reaction mixture was subsequently passed through a Sephadex G-50 column, eluted with 0·2 M phosphate buffer (pH 7·2) and the fractions containing the ^{125}I-AAG collected.

Separation of human AAG into concanavalin A-non-reactive (AAG-A) and concanavalin A-reactive (combination of highly reactive and intermediate reactive, AAG-B) was carried out as described by Bierhuizen et al (1988). AAG-A contains only oligosaccharide side-chains of tri- and tetra-antennary nature, while AAG-B contains one or more biantennary structures.

Deglycosylated human AAG was obtained by trifluoromethanesulphonic acid hydrolysis (Sojar & Bahl 1987) or by enzymatic hydrolysis using *N*-glycopeptidase F (Tarentino et al 1985).

Methods

Measurements of the pharmacologic activity of α_1-adrenergic antagonists. Aortic strips from male New Zealand White rabbits, 2–3 kg, were used throughout the studies. The animals were anaesthetized with 50 mg kg^{-1} ketamine, and subsequently killed by decapitation. The chest was opened and the thoracic aorta excised. Four strips each approximately 4 mm wide and 25 mm long were prepared from each aorta using the method of Furchgott & Bhadrakom (1953). The four strips were mounted in jacketed glass organ baths (Harvard, MA) with one end attached to a glass holder and the other to an isometric transducer connected to a polygraph (Model 7, Grass, Quincy, MA). The organ bath contained 25 mL Krebs–Henseleit buffer (pH 7·4) maintained at 37°C and gassed with 95% O_2–5% CO_2 at a rate of 0·1 L min^{-1}. The resting tensions of the aortic strips were adjusted to 1·0 g. After 1-h equilibration, phenylephrine was added (final concentration 1 mM) to ensure the contracting ability of the tissue in the organ baths. The preparations were washed four times with Krebs–Henseleit buffer, and re-equilibrated for an additional hour.

Cumulative concentration-response curves of phenylephrine were obtained in each of the organ baths by stepwise increase of the phenylephrine concentration. In each step the phenylephrine concentration was increased 3-fold. When the maximum effect was attained, the preparations were washed with Krebs–Henseleit buffer four times, and a 1-h equilibration time was allowed. Various concentrations of prazosin in the absence and presence of human AAG (20 μM), bovine AAG (20 μM), canine AAG (20 μM), asialo-AAG (20 μM), oxidized asialo-AAG (5 μM), oxidized AAG (10 μM), AAG-A (5 μM), AAG-B (5 μM) or non-glycosylated AAG (5 and 20 μM) were then added and, 30 min later, a second cumulative concentration-response curve of phenylephrine was determined as described above. In competition studies, 20 μM bovine and 5 μM human AAG solutions were used. Concentrations of 5 nM and 10 nM prazosin were used in the absence of AAG to generate a reference curve for the effect of unbound prazosin. In the presence of human, bovine and canine AAG, asialo-AAG, AAG-A, AAG-B, and non-glycosylated AAG, the concentrations of total prazosin were chosen to generate unbound concentrations between 5 and 10 nM in each case based upon preliminary binding studies.

To normalize the data between the individual preparations, the response to phenylephrine is reported as a percentage of the maximum tension achieved.

Protein binding. Protein binding of prazosin to the different AAGs was measured by equilibrium dialysis as described previously (Øie & Fiori 1985). Briefly, 800 μL of the organ bath solution was dialysed against an equal volume of 1·5 nM [^{3}H]prazosin in Krebs–Henseleit buffer solution (pH 7·4) at 37°C in a shaking water bath for 7 h. A Spectra/Por 2 (Spectrum, Houston, TX) membrane with a molecular cut-off of 12–14 kDa was used. The membrane had been washed eight times in distilled water, soaked in methanol for 15 min and rewashed four times in distilled water and subsequently soaked in dialysis buffer solution overnight. After dialysis, the concentration of radiolabelled prazosin was determined in the buffer and organ bath solution by scintillation counting.

Binding of human AAG to aorta rings. New Zealand White rabbits, 2–3 kg, were anaesthetized with 50 mg kg^{-1} ketamine, and subsequently killed by decapitation. The chest was opened and the thoracic aorta excised. After removing the attached fat, the aorta was cut into rings approximately 2 mm wide. The rings were incubated in 30 mL Krebs-Henseleit buffer (pH 7·4) for 60 min under an atmosphere of 95% O_2–5% CO_2. The rings were separated into six groups and transferred to tubes containing ^{125}I-AAG and [^{3}H]inulin with or without various concentrations of WB4101. [^{3}H]Inulin was used as an indicator of the water associated with the aortic surface. The preparations were incubated for another hour, gassed with 95% O_2–5% CO_2. The rings were subsequently centrifuged, weighed and dissolved in 0·2 mL 37% perchloric acid : H_2O_2 (1 : 1) at 75°C. ^{125}I and ^{3}H were determined using gamma-counting and scintillation spectroscopy, respectively.

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

$$E/E_{max} = \frac{C}{EC50 + C} \times 100\,\% \qquad (1)$$

Table 1. Binding of prazosin to different AAGs[a].

Fraction bound	Human AAG	Asialo-AAG	Canine AAG	Bovine AAG
Mean values	0·66	0·59	0·25	0·04
s.d.	0·04	0·04	0·01	0·02
n	8	8	8	8

[a] The AAG concentration used was 20 μM; the prazosin concentrations varied between 8 and 20 nM.

Table 2. Observed and predicted[a] DR-1 values for phenylephrine in the presence of prazosin and AAG (mean ± s.d.).

	Observed	Predicted	Ratio observed/predicted	Prazosin concn		n
				Unbound (nM)	Total (nM)	
Human AAG	4·37 ± 1·12*	8·02 ± 1·83	0·54 ± 0·06	6·94 ± 0·88	20	6
Asialo-human AAG	4·09 ± 1·01*	7·25 ± 2·52	0·58 ± 0·12	6·83 ± 1·93	16	6
Canine AAG	5·40 ± 1·78*	7·45 ± 0·86	0·72 ± 0·21	7·50 ± 0·17	10	6
Bovine AAG	11·09 ± 3·53	12·07 ± 3·12	0·91 ± 0·11	7·67 ± 0·07	8	6

[a] The predicted values were estimated from the observed inhibitory activity of prazosin on phenylephrine at identical unbound concentrations in protein free media. * $P < 0.05$ compared with predicted value.

Where E/E_{max} is the increase in tension over baseline expressed as a percentage of the maximum increase (E_{max}), C is the concentration of phenylephrine, and EC50 is the concentration of phenylephrine producing 50% of the maximum effect. The EC50 and E_{max} were obtained by fitting the above equation to the data from the experiments using the MINIM 1·8 program.

The dose ratio (DR) of phenylephrine in the presence of the individual α_1-adrenergic antagonists was determined at the EC50 value from:

$$DR = \frac{\text{EC50 in the presence of antagonist}}{\text{EC50 of the control}} \quad (2)$$

Linear regression of the logarithms of DR-1 and the unbound α_1-adrenergic antagonist concentration was carried out (Kenakin 1987) using the least squares method. The results were used to predict the values of DR-1 at the observed unbound α_1-adrenergic antagonist concentrations in the presence of AAG.

Statistical analysis. Differences between the observed and predicted DR-1 values in the presence of AAG were evaluated by unpaired Student's *t*-test.

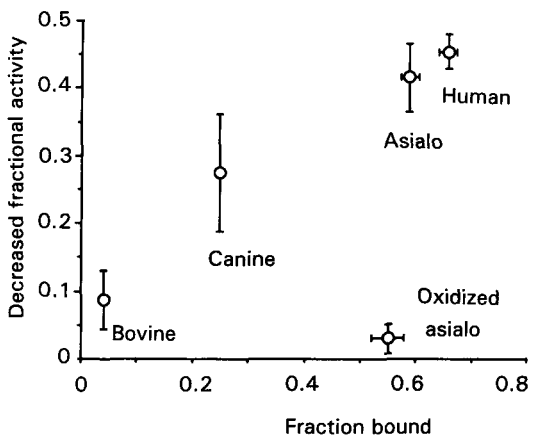

FIG. 1. Relationship between the decreased fractional activity of prazosin in the presence of various AAGs and the binding of prazosin to the AAGs. The decreased activity of prazosin was measured as the fractional lower antagonistic activity against phenylephrine in aortic strip preparations in the presence of the various AAGs of that expected from the same measured unbound concentrations in protein free preparations (values are mean ± s.e.m.).

Results

The fractions of bound prazosin in the presence of human, bovine, canine and asialo-AAG at concentrations of 20 μM are given in Table 1. The corresponding effects of the individual AAGs on the antagonistic activity of prazosin in aortic strips are given in Table 2, together with the predicted values calculated from the observed unbound concentrations. It is clear that human AAG, with the highest ability to bind prazosin, has the highest ability to decrease the intrinsic activity of prazosin. Canine AAG with an intermediate binding ability also has an intermediate effect on the intrinsic activity of prazosin. Bovine AAG does not bind prazosin to any significant degree and is almost devoid of an ability to affect the intrinsic activity of prazosin. This lack of both an ability to bind prazosin and to affect its pharmacologic intrinsic activity is similar to the effect seen in-vivo (unpublished) with rat AAG, a protein that neither binds prazosin nor affects its pharmacologic activity. Removing the terminal sialic acids from the oligosaccharide side-chains of human AAG did not significantly alter the binding of prazosin or the effect of the protein on the intrinsic activity of prazosin (Tables 1, 2, Fig. 1).

Differences in the branching of the oligosaccharide side-chains appear to play only a minor role in the activity of AAG. The decrease in the intrinsic activity of prazosin is greater with AAGs containing biantennary side-chains (AAG-B) than without (AAG-A) (Table 3). However, the difference between the two types of human AAG was small and did not reach statistical significance.

When human AAG was oxidized by sodium periodate, the protein binding remained relatively unaltered in comparison to intact AAG at concentrations of 10 μM (0·43 ± 0·05 vs 0·43 ± 0·04, respectively). Similar results were

Table 3. Observed and predicted DR-1 values for phenylephrine in the presence of prazosin and AAG-A (no biantennary oligosaccharide side-chains) and AAG-B (one or more biantennary side-chains) (mean ± s.d.).

	Observed	Predicted	Ratio observed/predicted	n
AAG-A	3·91 ± 1·67	5·30 ± 1·68	0·75 ± 0·16	4
AAG-B	3·14 ± 0·85	5·38 ± 2·10	0·62 ± 0·16*	4

* $P < 0.10$.

obtained using 5 μM oxidized asialo-AAG and intact asialo-AAG ($0{\cdot}76 \pm 0{\cdot}03$ vs $0{\cdot}79 \pm 0{\cdot}04$, respectively). However, for both compounds the ability to reduce the intrinsic activity of prazosin was essentially lost when the protein was oxidized. A ratio of 0·97 for the observed agonist to the predicted value for the antagonistic activity of prazosin for both the oxidized AAG and oxidized asialo-AAG was observed. These values contrast with the significant reduction in the intrinsic activity of prazosin observed using intact AAG and asialo-AAG (Table 2). This observation indicates that the binding of the α_1-adrenergic antagonists is not the only criterion for activity of AAG and that a relatively intact glycan side-chain is also necessary for the activity. The aglycone (20 μM) generated by trifluoromethanesulphonic acid essentially lost all ability to affect the pharmacological activity of prazosin (not statistically significantly different from control). However, this aglycone also showed a diminished ability to bind prazosin in comparison with intact AAG (bound fraction of $0{\cdot}02 \pm 0{\cdot}07$ vs $0{\cdot}66 \pm 0{\cdot}04$, respectively, at concentrations of 20 μM). Further, the generated aglycone showed only a weak interaction with AAG antibodies and had a significantly broadened UV absorption band, suggesting that at least a partial denaturation had taken place. Treatment with *N*-glycopeptidase F which is less likely to denature the protein, only partially cleaved the oligosaccharide side-chains as found by SDS-PAGE and studies with this product were, therefore, inconclusive.

Human AAG at 5 μM has a significant ability to reduce the response to prazosin (fractional activity $= 0{\cdot}55 \pm 0{\cdot}10$, $P < 0{\cdot}05$). Bovine AAG at 20 μM has little or no effect on the activity of prazosin. Addition of bovine AAG (20 μM) to 5 μM human AAG significantly decreased the ability of human AAG to affect the antagonistic activity of prazosin (fraction activity $= 1{\cdot}14 \pm 0{\cdot}33$).

The binding of ^{125}I-AAG to aortic rings was relatively weak with no apparent saturable component (Fig. 2). The individual values were determined with a relatively high degree of uncertainty (the coefficient of variation was on the average 34% with a range of 6 to 60%) as 70% of the uncorrected binding of human AAG was associated with the residual water attached to the aortic rings. Addition of the α_1-adrenergic antagonist, WB4101, which binds strongly to human and bovine AAG, did not significantly change the binding of prazosin to the aortic rings.

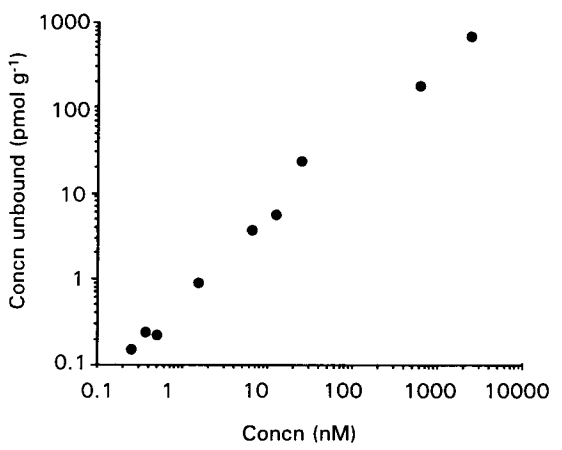

FIG. 2. Binding of human AAG to aortic rings as a function of concentration. Each point is the average of 2–6 measurements.

Discussion

From the data presented, there appears to be a correlation between the ability of various AAGs to bind prazosin and their ability to decrease its intrinsic pharmacologic activity. This indicates that the strength of binding is important for the ability of AAG to affect the antagonists. However, the ability to bind antagonists is not the only necessary characteristic. For example, it is possible, by oxidation, to decrease the effect of AAG or asialo-AAG on the intrinsic activity of prazosin without reducing the ability to bind the antagonist. Similarly, Chiang et al (1991) have found that albumin can bind prazosin but that this protein has no effect on the pharmacologic activity of prazosin. These observations suggest that the effect of AAG is not merely related to the binding of α_1-adrenergic antagonists but that it must also relate to other characteristics of AAG. From the decrease in the activity of human AAG (strong binder of prazosin) in the presence of bovine AAG (weak binder of prazosin) it appears that this nonbinding AAG acts as a competitive inhibitor of an active AAG. We therefore speculate that the activity of human AAG must also involve an ability to associate with the α_1-adrenoceptors. Because the effect is not one of direct competition between AAG and α_1-adrenergic antagonists (Chiang & Øie 1990), the following model may explain the observations presented. The different AAGs bind in the vicinity of the α_1-adrenoceptor in a competitive manner; AAGs that can also bind α_1-adrenergic antagonists, in effect extend the binding region for antagonists; such a delocalized binding region will decrease the probability of the antagonist to block the agonist binding site and, thereby, inhibit the interaction of the agonist with receptor; removal of the

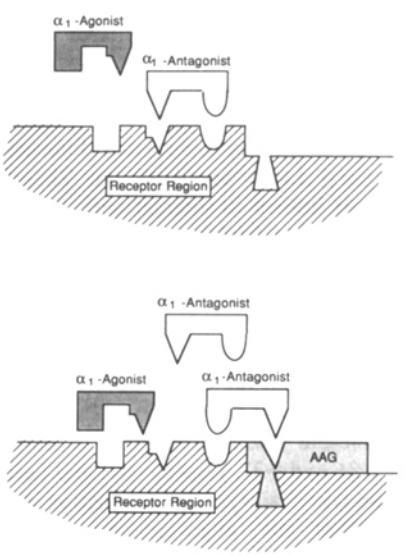

FIG. 3. Suggested model for AAG's interaction with α_1-adrenoceptors. When AAG binds in the vicinity of the receptor, the binding region of the α_1-adrenergic antagonists is extended. This extension decreases the probability of the α_1-adrenergic antagonists to inhibit association of the α_1-adrenergic agonists with the adrenoceptor.

carbohydrate side-chains reduces the binding of AAG to the adrenoceptor. A cartoon of the model is presented in Fig. 3.

The model suggests that AAG should bind to the aortic ring. Binding of human AAG to the aortic rings was detectable, although weak, and showed no indication of being saturable. Two possible reasons for the inability to detect saturable binding can be put forward.

Binding of human AAG in the vicinity of the α_1-adrenoceptors may be small in comparison with the non-specific binding and because 70% of the total apparent associated AAG was related to residual water attached to the ring, relatively high standard deviations were obtained preventing detection of any minor saturable binding.

If the α_1-adrenergic antagonists in the presence of AAG straddle the adrenoceptor and AAG, the binding of AAG to the receptor should theoretically increase in the presence of an antagonist by anchoring AAG more strongly to the α_1-adrenoceptors. We did not observe any change in the binding of AAG to aortic rings in the presence of WB4101, an antagonist that is strongly bound to AAG (>90% bound, data not shown). However, if the binding of AAG is largely non-specific, any change in the binding of AAG at the α_1-adrenoceptors due to the presence of WB4101 may be too small to be readily detectable.

Both the oligosaccharide side-chains and the aglycone of AAG appear to be important for the activity seen. For example, the oxidation of AAG and asialo-AAG removes the majority of the galactose, fucose and mannose sugars from the oligosaccharide side-chains. This does not alter the binding of prazosin significantly, but it causes a significant decrease in the intrinsic pharmacologic activity of prazosin. In addition, AAG containing biantennary glycan chains (AAG-B) also had the highest activity (although the differences did not reach statistical significance at the 5% level). Differences in the branching of the sugar side-chains have been demonstrated to have profound effects on other aspects of the activity of AAG. For example, AAGs containing highly branched glycan chains have a higher immunosuppressive activity than AAGs with biantennary structures (Bories et al 1990). The mechanism for the effect of the oligosaccharide side-chains is not clear. Highly branched sugar side-chains, in contrast to biantennary glycans, are frequently focusylated (Fournet et al 1978) creating a sialyl Lewis configuration. The sialyl Lewis configuration is known to interact with specific receptors on the cell surface, e.g. ELAM 1 receptors (Walz et al 1990). On the other hand, biantennary branching is associated with interaction with lectins and lectin-like compounds. Both of these observations indicate that the oligosaccharide side-chains are likely to be involved in cell-surface interactions. Because we found that the biantennary forms had a somewhat higher activity but showed no difference in binding of prazosin (unbound fractions of AAG-A and AAG-B at 20 μM were 0·36 and 0·37, respectively) and because oxidizing the glycan side-chains caused a reduction in activity, it is conceivable that the oligosaccharide side-chains are involved in the postulated association of AAG with the α_1-adrenoceptors.

The binding of drugs to AAG is usually assumed to be both hydrophobic in nature and associated with its protein component (Müller 1989). This assumption is consistent with the data for the oxidized AAG where the unbound fraction did not change. On removing the oligosaccharide side-chains with trifluoromethanesulphonic acid, not only was the pharmacological activity lost, but the binding of prazosin was also significantly diminished. This would suggest that the oligosaccharide side-chains play some role in the protein binding of prazosin. However, the decrease in binding may also be related to a partial denaturing of the aglycone. Although Sojar & Bahl (1987) have indicated that trifluoromethanesulphonic acid treatment does not denature the protein under the preparative conditions used, our studies revealed that trifluoromethanesulphonic acid generated an AAG aglycone which had a significantly reduced ability to interact with AAG antibodies and which showed a dramatic broadening of the UV absorptive region. Both of these observations indicate that denaturation may have, at least partially, taken place.

These data are consistent with the hypothesis that AAG interacts with α_1-adrenoceptors and thereby extends the binding region of α_1-adrenergic antagonists. However, verification of this hypothesis requires further experimental work.

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References

Belpaire, F. M., Bogaert, M. G., Mugabo, P., Rosseel, M. T. (1986) Binding to serum α_1-acid glycoprotein and effect of β-adrenoceptor antagonists in rats with inflammation. Br. J. Pharmacol. 88: 697–705

Bierhuizen, M. F. A., De Wit, M., Govers, G., Ferwerda, W., Koeleman, C., Pos, O., Van Dijk, W. (1988) Glycosylation of three molecular forms of human α_1-acid glycoprotein having different interactions with concanavalin A. Eur. J. Biochem. 175: 387–394

Bøg-Hanssen, T. C. (1983) Affinity electrophoresis of glycoproteins In: Scouten, W. (ed.) Solid Phase Biochemistry, Analytical and Synthetic Aspects. J.Wiley and Sons, New York, NY, pp 223–251

Bories, P. N., Feger, J., Benbernou, N., Rouzeau, J. D., Agneray, J., Durand, G. (1990) Prevalence of tri- and tetraantennary glycans of human α_1-acid glycoprotein in release of macrophage inhibitor of interleukin-1 activity. Inflammation 14: 315–323

Casey, C., Kragskow, S. L., Sorrell, M. F., Tuma, D. J. (1987) Chronic ethanol administration impairs the binding and endocytosis of asialo-orosomucoid in isolated hepatocytes. J. Biol. Chem. 262: 2704–2710

Chiang, J., Øie, S. (1990) Pharmacologic activity of prazosin is decreased by alpha-1-acid glycoprotein in vivo. J. Pharmacol. Exp. Ther. 254: 324–329

Chiang, J., Hermodsson, G., Øie, S. (1991) The effect of α_1-acid glycoprotein on the pharmacological activity of α_1-adrenergic antagonists in rabbit aortic strips. J. Pharm. Pharmacol. 43: 540–547

de Rick, A. F., Belpair, F. M., Dello, C., Bogaert, M. G. (1987) Influence of enhanced alpha-1-acid glycoprotein concentration on protein binding, pharmacokinetics and antiarrhythmic effect of lidocaine in the dog. J. Pharmacol. Exp. Ther. 241: 289–293

Eylar, E. H., Jeanloz, R. W. (1962) Periodate oxidation of α_1 acid glycoprotein of human plasma. J. Biol. Chem. 237: 1021–1025

Fournet, F., Montreuil, J., Strecker, G., Dorland, L., Haverkamp, J., Vliegenthart, J. F. G., Binette, J. P., Schmid, K. (1978) Determination of the primary structures of 16 asialo-carbohydrate units derived from human plasma α_1-acid glycoprotein by 360-MHz 1H NMR spectroscopy and permethylation analysis. Biochemistry 17: 5206–5214

Furchgott, R. F., Bhadrakom, S. (1953) Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther. 142: 39–58

Gillis, A.M., Kates, R.W. (1986) Influence of protein binding on the myocardial uptake and pharmacodynamics of propafenone. J. Cardiovasc. Pharmacol. 8: 1163–1167

Griffeth, L.K., Rosen, G.M., Tschanz, C., Rauckman, E.J. (1983) Effects of model traumatic injury on hepatic drug metabolism in the rat. I In vivo antipyrine metabolism. Drug Met. Dispos. 11: 517–525

Huang, J.D., Øie, S. (1982) Effect of altered disopyramide binding on its pharmacologic response in rabbits. J. Pharmacol. Exp. Ther. 223: 469–471

Kenakin, T.P. (1987) Pharmacologic Analysis of Drug Receptor Interaction Chapter 9. Raven Press, New York, pp 205–244

Øie, S., Fiori, F. (1985) Effect of albumin and alpha-1-acid glycoprotein on elimination of prazosin and antipyrine in the isolated perfused rat liver. J. Pharmacol. Exp. Ther. 234: 636–640

Müller, W.E. (1989) Drug binding sites on human alpha-1-acid glycoprotein. Prog. Clin. Biol. Res. 300: 363–378

Sojar, H.T., Bahl, O.P. (1987) A chemical method for the deglycosylation of protein. Arch. Biochem. Biophys. 259: 52–57

Tarentino, A.L., Gomez, C.M., Plummer, T.H. (1985) Deglycosylation of asparagine-linked glycans by peptide: *N*-glycosidase F. Biochemistry 24: 4665–4671

Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M., Seed, B. (1990) Recognition by ELAM-1 of the sialyl-Le^x determinant on myeloid and tumor cells. Science 250: 1132–1135

Warren, L. (1959) The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234: 1971–1975

Yasuhara, M., Fujiwara, J., Kitade, S., Katayama, H., Okumura, K., Hori, R. (1985) Effect of altered plasma protein binding on pharmacokinetics and pharmacodynamics of propranolol in rats after surgery: role of alpha-1-acid glycoprotein. J. Pharmacol. Exp. Ther. 235: 513–520