Biochemical Pharmacology, Vol. 34, No. 9, pp. 1591–1594, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

PRELIMINARY COMMUNICATIONS

MODIFICATION OF PROPRANOLOL BINDING TO ALPHA-1-ACID GLYCOPROTEIN BY SERUM ALBUMIN

L. Chauvelot-Moachon, O. Marquis et J-P. Giroud

Département de Pharmacologie, CHU Cochin Port-Royal, 27 rue du Fbg St Jacques
Paris, 75674, FRANCE

(Received: 6 December 1984; accepted: 6 February 1985)

Orosomucoid or alpha-1-acid glycoprotein (AAG) is the preferential serum binding protein for propranolol. This implies that variations in serum propranolol binding depend on AAG concentrations and not on human serum albumin (HSA). Actually, Piafsky et al. (1) and other authors (2-5) have shown that bound/free ratio of propranolol is linearly correlated with AAG concentrations which are several times increased in inflammatory disease (6). Therefore, binding studies with isolated, purified AAG should agree with binding results observed with serum. This is not the case : we have preliminary calculated from previous publications that propranolol binding to isolated AAG seems to be lower than necessary to account for variations of propranolol binding to whole serum (7,8) whatever the pathological states. Once the discrepancy between binding to isolated AAG and to serum is confirmed, two main hypotheses come to mind : 1) propranolol is bound to another acute phase protein ; 2) isolated AAG does not behave like AAG in serum. The first hypothesis was ruled out from the start because propranolol was slightly bound to other important acute phase proteins (9) and to gamma-globulins (7). The second hypothesis was confirmed by our results. A clear interaction was found between AAG and serum albumin for propranolol binding. The characteristics of the interaction highly suggest that HSA potentiates propranolol binding to AAG. Moreover, the extent of the potentiation appears to depend on lipids associated with those two proteins.

MATERIAL AND METHODS

Material. All proteins used were purchased from Sigma Chemical Co. Except otherwise specified, HSA, essentially fatty acid free (A 1887), was used ; a 40 g/l solution contains less than 0.1 g/l of AAG. (D-L)^{3}H-propranolol hydrochloride (84mCi/mg) was purchased from Amersham International. Radiochemical purity was superior to 98 % as assessed by thin-layer chromatography. AAG concentrations were determined by radial immunodiffusion (Nor-Partigen plates, Behringwerke).

Serum. Serum was obtained from 16 patients including 3 cases of arthritis, 4 of disseminated lupus, 4 of cancer and 3 of bacterial infection. Two patients had no organic disease. Patients did not take any beta-blocker. Blood was collected into glass tubes and serum samples were kept at - 20° before experimentation.

Binding experiments. Propranolol binding was measured in vitro by equilibrium dialysis according to Glasson et al. (7) except that experiments could be performed in 4 hours due to the low volume of half-cells (100 µl each). A constant concentration of ^{3}H-propranolol (2.5 ng/ml) was chosen in order not to reach saturation level of AAG at 0.66 g/l. This was determined by preliminary experiments with increasing concentrations of propranolol. Our results agreed with other authors' (7). Propranolol binding to HSA is not saturable from 1 to 10^{4} ng/ml of the drug. No significant degradation of AAG was observed during dialysis with AAG alone or associated with HSA. Results are expressed as mean $\pm$ SD of at least 3 experiments performed on different days.

Delipidation of AAG. Native AAG (Sigma, 99 % pure) was delipidated according to Ganguly et al (10).

Application of the law of mass action. In non-saturable conditions, drug binding to a mixture of two proteins (1 and 2) can be characterized by the simplified equation :

$$\frac{B}{F} = n1 \times K1 \times R1 + n2 \times K2 \times R2$$

B is the molar concentration of bound drug, F the molar concentration of free drug, n the number of binding sites per mole of protein, K the association constant (M^{-1}) and R the molar concentration of the protein. The application of this equation allows to compare bound/free ratio observed (in presence of both proteins) with the expected value represented by the sum of n1 x K1 x R1 plus n2 x K2 x R2 (determined for each protein alone).

RESULTS AND DISCUSSION

The bound/free ratio of propranolol to AAG at varying concentrations, alone or associated with a constant concentration of fatty acid free HSA (40 g/l) is illustrated in figure 1. The values of n1 x K1 for AAG alone (0.137 $\pm$ 0.029 μM^{-1}) are independent of AAG concentrations from 0.2 to 4.0 g/l. The linear regression curve in presence of HSA is not parallel to the previous one. The slope corresponds to a new value of n x K for AAG (n'1 x K'1 = 0.414 $\pm$ 0.072 μM^{-1}).

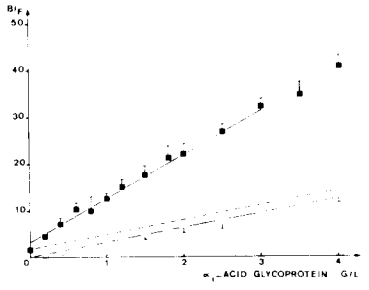

Figure 1. Mean ($\pm$ SD) bound/free ratio of propranolol as a function of AAG concentrations alone (□) and associated with 40 g/l of fatty acid free HSA (■). Dashed lines represent the expected values of B/F in presence of both proteins. The equation for AAG alone was y = 3.11 x AAG (g/l) - 0.11. Then, n1 x K1 for AAG alone = 0.137 $\pm$ 0.029 μM^{-1}. The equation for AAG associated with HSA was y = 9.179 x AAG (g/l) + 3.29. New value of n1 x K1 in presence of HSA = 0.414 $\pm$ 0.072 μM^{-1} (calculated by linear regression).

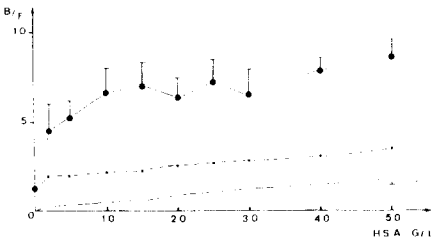

Figure 2. Mean ($\pm$ SD) bound/free ratio of propranolol as a function of HSA concentrations alone (○) and associated with 0.66 g/l of AAG (●). Dashed lines represent the expected values of B/F in presence of both proteins. New values of n1 x K1 for AAG in presence of HSA were calculated as (B/F ratio in presence of both proteins) - (B/F ratio in presence of HSA) for each concentration of HSA. New values of n1 x K1 in presence of HSA from 10 to 50 g/l = 0.405 $\pm$ 0.033 μM^{-1}

Studies of bound/free ratio with HSA at varying concentrations alone or associated with a constant concentration of AAG (0.66 g/l) (figure 2) showed again that the observed bound/free ratio in presence of both proteins was higher than the expected value. Since binding capacities (n2 x K2) for HSA alone were dependent on HSA concentrations (7), new values of n1 x K1 for AAG (n'1 x K'1) were calculated as (bound/free observed) - (n2 x K2 x R2) for each concentration of HSA. Mean n'1 x K'1 = 0.405 $\pm$ 0.033 μM^{-1} in presence of HSA from 10 to 50 g/l. In other words, the potentiation of AAG binding capacity by HSA was much the same either with varying concentration levels of AAG or HSA at physiological concentrations. Additional studies were performed with AAG at 0.66 g/l and HSA at very low concentrations. No potentiation was observed with 0.1 g/l of HSA. Then, the potentiation progressively increased with HSA concentrations from 0.2 to 10 g/l (results not shown).

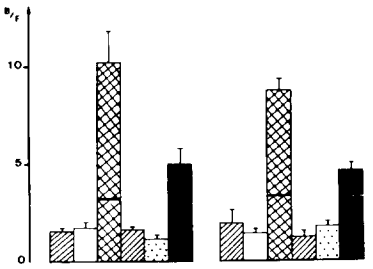

Figure 3. Mean (± SD) bound/free ratio of propranolol 1) with AAG alone at 0.66 g/l (▨), 2) with serum albumin fatty acid free (□) or fatty acid rich (▦), at 40 g/l and 3) with AAG associated with serum albumin fatty acid free (▩) or fatty acid rich (■).
Panel A. Human serum albumin, fatty acid free (A.1887) and fatty acid rich (A.1653).
Panel B. Bovine serum albumin, fatty acid free (A.7511) and fatty acid rich (A.4378).
Horizontal line represents the expected value of B/F ratio in presence of both proteins.

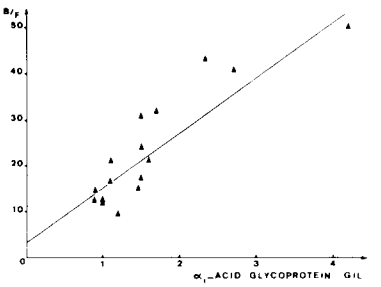

Figure 4. Correlation between bound/free ratio of propranolol and AAG concentrations in serum samples. Each value is the mean of a duplicate determination. The equation was $y = 12.78 \times AAG\ (g/l) + 3.09$. $r = 0.88$ $_1p < 0.001$. Then mean n1 x K1 = $0.56\ \mu M^{-1}$.

In studies with different kinds of albumin (figure 3), potentiation of AAG binding capacities were less marked with fatty acid rich serum albumin than with fatty acid free serum albumin (t test, $p < 0.001$). The origin of albumin, human or bovine, did not influence potentiation.

The interaction could be specific to serum albumin since no differences were found between the bound/free values expected and those observed when AAG, at varying concentrations, was mixed with gamma-globulins at 22.5 g/l (results not shown). The interaction between AAG and HSA could occur with other basic drugs, such as some beta blocking, antiarrhythmic or analgesic agents ... (3, 8, 11).The results of propranolol binding to patient sera (figure 4) agree with literature data (1-5). In this figure, the most striking point is that the linear curve and those obtained with the mixture of AAG plus HSA described in figure 1 are almost parallel. The slope determined from serum samples is only 1.3 time those obtained with the mixture of AAG plus HSA, whereas that slope is 4.1 times that obtained with AAG alone (figure 1).

Ganguly et al. (10) have established a 3.5 times increase in binding capacities of progesterone for AAG after its delipidation. The differences between albumin fatty acid rich and fatty acid free led us to examine the effect of AAG delipidation upon its potentiation by HSA. Experiments were performed as in figure 1. Results are illustrated in figure 5. Mean value of n1 x K1 for delipidated AAG used alone was near those calculated for non-delipidated AAG potentiated by HSA. Nevertheless, n1 x K1 values depended on AAG concentrations for an undetermined reason (propranolol binding to delipidated AAG at 1 g/l was not saturable in our conditions) (results not shown). Potentiation of delipidated AAG by HSA was clearly less marked than for native AAG : at 1.5 g/l of AAG, the presence of HSA allowed a 1.8 time increase of n1 x K1 for AAG and almost no potentiation occured at 2.5 g/l of AAG.

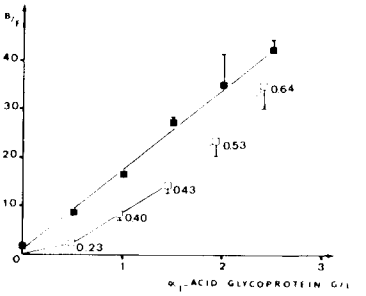

Figure 5. Mean (± SD) bound/free ratio of propranolol in presence of delipidated AAG alone (□) and associated with 40 g/l of fatty acid free HSA (■). Numbers under open symbols represent the values of n1 x K1 (μM^{-1}) for delipidated AAG alone. The equation in presence of both proteins was y = 16.73 x AAG $(g/l)_1$ + 1.059. Then, new value of n1 x K1 for delipidated AAG in presence of HSA = 0.736 ± 0.082 μM^{-1}.

These last experiments suggest that some inhibitory lipids associated with "native" AAG are picked up by HSA allowing a higher binding capacity of AAG for propranolol. This mechanism could account for the less marked potentiation observed with fatty acid rich albumin. The kind and role of lipids removed during AAG delipidation are under investigation ; the lipid-HSA interaction could involve fatty acids known to be bound to serum albumin. Whatever the underlying mechanism, large use of isolated and purified AAG for binding studies led us to consider this interaction. Our results indicate that binding studies with propranolol and probably with other basic drugs should rather be carried out with a mixture of AAG plus HSA. Competitive binding between those drugs could also be influenced by this phenomenon.

REFERENCES

1. K.M Piafsky, O. Borga, I. Odar-Cederlof, C. Johansson and F. Sjoqvist. N.Engl.J.Med.299,1435(1978)

2. U.K. Walle, T. Walle, S.A. Bai and L.S. Olanoff. Clin.Pharmacol.Ther.32,659(1983)

3. E. Pike, B. Skuterud, P. Kierulf, D. Fremstad, S.M. Abdel Sayed and P.K.M. Lunde. Clin.Pharmacokin.6,367(1981)

4. G. Alvan, K. Bergstrom, O. Borga, L. Iselius and N. Pedersen. Eur.J.Clin.Pharmacol.25,437(1983)

5. F.P. Abramson, J. Jenkinsand and Y. Ostchega. Clin.Pharmacol.Ther.32,659(1982)

6. J.W. Pawton. Meth.Find.Exp.Clin.Pharmacol.5,635(1983)

7. S. Glasson, R. Zini, P. d'Athis, J.P. Tillement and J.R. Boissier. Mol.Pharmacol.17,187(1980)

8. F.M. Belpaire, M.G. Bogaert and M. Rosseneu. Eur.J.Clin.Pharmacol.22,253(1982)

9. B.J. Scott, A.R. Braduell, R.E. Schneider and H. Bishop. Lancet,avril 28,930(1979)

10. M. Ganguly, R.H. Carnighan and U. Westphal. Biochemistry.6,2803(1967)

11. S.M. Owens, M. Mayersohnand and J.R. Woodworth. J.Pharmacol.Exp.Ther.226,656(1983)