Acknowledgements—We wish to express our gratitude to Mrs G. Reynes, Mrs E. Fonta and Ms M. H. Seguelas for technical assistance and Mrs J. Jousseaume for typing.

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Biochemical Pharmacology, Vol. 35, No. 12, pp. 2044–2046, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Protein binding of nomifensine and its three main metabolites

(*Received* 4 *September* 1985; *accepted* 6 *December* 1985)

The binding of drugs to plasma proteins can have a profound effect on their pharmacokinetics [1, 2]. A large inter-individual variation in plasma protein concentrations may cause different clinical responses when highly bound agents are used [3–5]. In recent years it has been recognized that in addition to albumin, α_1-acid glycoprotein (α_1-AG) also plays a significant role in the binding of basic drugs.

Nomifensine is a psychotropic agent having antidepressive properties. It has three main metabolites, 4-hydroxynomifensine (M_1), 4-hydroxy-3-methoxynomifensine (M_2) and 3-hydroxy-4-methoxynomifensine (M_3) [6] (Fig. 1). Nomifensine is slightly basic, so binding to α_1-AG could be assumed. Plasma protein binding of nomifensine has been reported to be about 60% [7]. The binding of nomifensine to different components of plasma, as albumin or α_1-acid glycoprotein, has not been published. There is no information of the binding of nomifensine metabolites to plasma proteins.

In the present study the binding of nomifensine and its main metabolites to whole plasma protein, albumin and α_1-acid glycoprotein was studied. Protein binding was evaluated over a wide concentration range. The effect of protein concentrations was also evaluated.

Materials and methods

Materials. Nomifensine maleate as a pure substance and in gelatine capsules (Nomival®) were obtained from Leiras Pharmaceuticals (Turku, Finland). 4-hydroxy-, 4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxynomifensine were gifts from Hoechst AG (Frankfurt am Main, F.R.G.). Human serum albumin (Fatty acid free) and α_1-acid glycoprotein (orosomucoid, human) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were analytical grade. Fresh human plasma of a healthy subject was obtained from Finnish Red Cross.

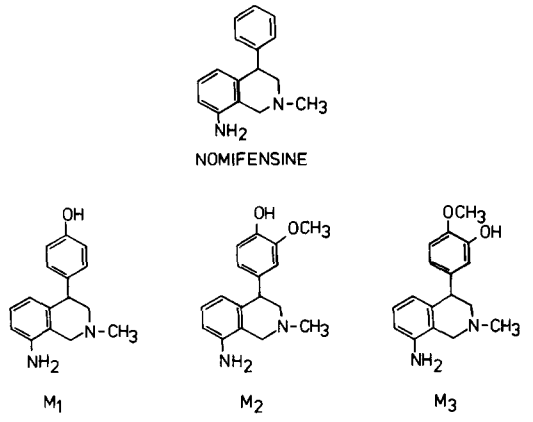

Fig. 1. Structure of nomifensine and its three principal metabolites M_1, M_2 and M_3.

Equilibrium dialysis. The binding experiments were performed by equilibrium dialysis using plexiglass cells with two chambers (2.5 ml) separated by a dialysis membrane (Visking membrane, 8-32/32", Medicell International Ltd., London, U.K.). The membrane was washed with distilled water containing a small amount of EDTA. Two ml of the protein solution or plasma was dialyzed against 2.5 ml of 0.05 M phosphate buffer (pH 7.4, containing 0.9% NaCl and 0.1% NaN_3) usually for 17 hr at 37° under gentle rotatory shaking. Albumin and α_1-AG solutions were made in 0.05 M phosphate buffer, pH 7.4. The pH value was adjusted in all experiments and checked after dialysis. Binding assays were made in duplicate at all protein concentrations.

Drug assays. After dialysis nomifensine and metabolite concentrations on both sides of the dialysis membrane were analyzed with HPLC [8, 9]. The percentage of binding was calculated according to the equation:

$$\% \text{ Bound} = \left(1 - \frac{\text{nomifensine or metabolite in buffer}}{\text{nomifensine or metabolite in protein solution}}\right) \times 100$$

Binding parameters were calculated according to Scatchard [10].

Results and discussion

The equilibrium of the binding of nomifensine to proteins was studied by incubation of the samples in dialysis chambers for 4, 6, 8, 10, 17 and 24 hours. Equilibrium was reached quite soon, already after 6–8 hr of incubation at 37°. There were no differences in the binding at equilibrium whether the drug was added to the buffer or protein solutions. Furthermore, the binding remained constant even after 24 hr of incubation, indicating the stability of the plasma proteins that contribute to the binding.

Table 1 shows the binding of nomifensine and its main metabolites to human plasma, human serum albumin (HSA) and α_1-acid glycoprotein. The concentration of nomifensine and its metabolites used was 0.5 μmol/l. The concentrations of albumin and α_1-acid glycoprotein were 50 g/l and 0.75 g/l, respectively. Both nomifensine and its principal metabolites bound extensively to plasma and albumin, while less than 20% were bound to α_1-acid glycoprotein. The reduced binding ability might be due to unfavourable space conformations of nomifensine and its metabolites. The difference in percent binding in plasma and the sum of percent binding to albumin and α_1-AG might be due to some other proteins like lipoproteins in plasma. In the present study the binding of nomifensine and its metabolites to lipoproteins was not studied because the pure protein was not available.

Table 2 shows the effect of nomifensine and metabolite concentrations on binding to different fractions of plasma proteins. Physiological concentrations of HSA (50 g/l) and α_1-acid glycoprotein (0.75 g/l) were used. The percent binding of nomifensine and metabolites to plasma and albumin was relatively constant over the concentration range 0.25–

Table 2. Effect of nomifensine and its metabolite concentrations on their binding to plasma, HSA and α_1-acid glycoprotein*

Nomifensine conc. (μmol/l)	% Nomifensine/metabolites bound to Plasma	HSA (50 g/l)	α_1AG (0.75 g/l)
0.25	73.2	54.9	15.6
0.50	78.2	55.0	14.5
0.75	73.1	53.6	19.8
1.00	75.3	56.0	17.1
5.00	70.9	60.3	14.3
10.00	74.1	63.8	15.6
M_1 conc. (μmol/l)			
0.25	45.2	37.1	8.3
0.50	57.0	32.5	5.9
0.75	69.0	37.7	6.3
1.00	64.7	31.4	7.7
5.00	69.3	41.5	—
10.00	68.4	30.3	—
M_2 conc. (μmol/l)			
0.25	68.8	44.7	11.1
0.50	67.0	38.2	8.5
0.75	73.6	33.6	6.5
1.00	68.7	40.5	8.6
5.00	70.3	42.6	—
10.00	67.5	40.1	—
M_3 conc. (μmol/l)			
0.25	70.5	44.6	10.0
0.50	71.6	39.3	7.4
0.75	74.3	44.8	6.6
1.00	71.1	44.0	5.3
5.00	68.3	52.6	—
10.00	69.9	30.6	—

* Determinations are made in duplicate.

Table 1. Binding of nomifensine and its metabolites to human plasma, HSA and α_1-acid glycoprotein*

	Nomifensine	% Bound M_1	M_2	M_3
Plasma	78.2	57.0	67.0	71.6
Albumin (50 g/l)	55.0	32.5	38.2	39.3
α_1-Acid glycoprotein (0.75 g/l)	14.5	5.9	8.5	7.4

* Determinations are made in duplicate.

10.00 μmol/l. The binding of nomifensine to α_1-acid glycoprotein was also constant. The metabolites bound little to α_1-acid glycoprotein and at the concentrations over 5.0 μmol/l the binding was negligible. At very high concentrations (1000–2500 μmol/l) the percent binding of nomifensine to plasma, HSA and α_1-acid glycoprotein was slightly reduced (Table 3). Nomifensine and its metabolites are poorly soluble in water, therefore it was impossible to confirm the decrease in binding at higher levels of the compounds. In any case, the concentrations of nomifensine and metabolites reached during maintenance treatment with nomifensine [11] or even after intravenous administration of the drug [12] are much lower.

Table 3. Effect of high nomifensine concentrations on its binding to plasma, HSA and α_1-acid glycoprotein*

Nomifensine conc. (μmol/l)	% Nomifensine bound to Plasma	HSA (50 g/l)	α_1-AG (0.75 g/l)
10	74.1	63.8	15.6
100	67.4	67.2	10.2
500	68.7	60.3	8.8
1000	59.4	51.6	8.8
2500	38.8	36.8	6.8

* Determinations are made in duplicate.

The binding of nomifensine to albumin was low affinity (the association constant $K_a = 2.2 \cdot 10^3\ M^{-1}$) and low capacity (a number of binding sites, N = 1). The glycoprotein bound nomifensine to two sets of sites with rather high and low affinities (K_a-values were $1 \cdot 10^4$ and $2.4 \cdot 10^3\ M^{-1}$, respectively). The capacity of the first site was lower (N = 1) than the second site (N = 20). The binding parameters for nomifensine metabolites were not possible to calculate reliably in the narrow concentration range used here.

The binding of nomifensine and its metabolites to plasma obtained from ten healthy volunteers after a single oral 100 mg dose of nomifensine maleate was determined. The bound fractions of nomifensine, M_1, M_2 and M_3 were 81.3 ± 4.2, 54.8 ± 6.6, 48.9 ± 9.9 and 52.3 ± 8.7%, respectively. The binding of nomifensine to plasma in nine depressed patients during nomifensine therapy was 85.8 ± 4.5% (±S.D.) which did not differ significantly from that of healthy volunteers (Student's *t*-test was used for the statistical calculations).

The binding of nomifensine to plasma proteins was about 80%. Heptner *et al.* [7] found the value of 60%. They used another method by Scholtan [13]. Because the details of that study were not published, it is difficult to clarify the difference between present results and those of Heptner. The three main metabolites were less bound than the parent drug. The hydroxyl and methyl groups might cause more steric hindrance between protein and metabolite molecules and make in that way the complexes difficult to form. The binding of nomifensine metabolites to proteins has not been published earlier.

It has generally been assumed that the binding of drugs to α_1-acid glycoprotein may have clinical significance. The concentration of α_1-acid glycoprotein can vary more than 3-fold in normal males. Furthermore, α_1-AG concentrations in plasma are markedly elevated in inflammatory diseases, stress and in the last trimester of pregnancy [14]. The binding of nomifensine and metabolites was constant in the concentration range, from 0.25 to 1.00 g/l of α_1-AG. Because the binding of nomifensine and its metabolites to α_1-acid glycoprotein is relatively low and independent of the protein concentration it is of hardly any pharmacological or clinical importance.

The effect of albumin concentration on the binding of nomifensine and its metabolites was also studied. In the concentration range, from 30 to 70 g/l, there were no changes in the percent binding of nomifensine and its metabolites. This suggests that protein concentrations in patients with nomifensine therapy do not cause any variation in amounts of free, biologically active forms of nomifensine or its metabolites.

In conclusion, nomifensine and its three principal metabolites are mainly bound to albumin in human plasma. Because the binding at therapeutic drug levels is not affected by changes of protein concentrations within the physiological range protein binding is not expected to result in any interindividual variation in psychiatric patients during customary nomifensine therapy. Anyhow, we cannot exclude other factors, like the inhibitory effect of some endogenous compounds on protein binding, which might cause interindividual variation during nomifensine treatment.

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Acknowledgements—Nomifensine maleate was kindly donated by Leiras Pharmaceuticals and nomifensine metabolites were a gift from Hoechst AG.

I am also indebted to Mr Jarmo S. Salonen, MSc, for the valuable discussions on mechanisms of protein binding of drugs and for his help when constructing the manuscript. I also thank Mrs. Ulla Heikonen for the excellent technical assistance.

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