animals. This model of IBD might thus be described as a sulphasalazine-resistant type.

The authors thank Dr T. G. Hodge for his help in preparation and analysis of the histological sections, Dr A. Onderdonk for valuable advice and generous donation of *Bacteroides vulgatus* used in these studies, and Dr A. J. Lewis for his encouragement and advice in this work.

References

Broughton-Smith, N. K., Whittle, B. J. R. (1985) Increased metabolism of arachidonic acid in an immune model of colitis in guinea pigs. Br. J. Pharmacol. 86: 439–446

Hoult, J. R. S. (1987) Sulphasalazine: mode of action and side-effects in rheumatoid arthritis and ulcerative colitis. In: Rainsford, K. D., Velo, G. P. (eds) Side Effects of Anti-inflammatory Drugs, vol. 2. Lancaster, MTP Press, pp 223–231

Kanerud, L., Hafstrom, I., Ringertz, B. (1990) Effect of sulphasalazine and sulphapyridine on neutrophil superoxide production: role of cytosolic free calcium. Ann. Rheum. Dis. 49: 296–300

Klotz, U., Maier, K., Fischer, C., Heinkel, K. (1980) Therapeutic efficacy of sulfasalazine and its metabolites in patients with ulcerative colitis and Crohn's disease. New Engl. J. Med. 303: 1499–1502

MacDermott, R. P., Kane, M. G., Steele, L. L., Stenson, W. F. (1986) Inhibition of cytotoxicity by sulfasalazine. I. Sulfasalazine inhibits spontaneous cell-mediated cytotoxicity by peripheral blood and intestinal mononuclear cells from control and inflammatory bowel disease patients. Immunopharmacology 11: 101–109

Miyachi, Y., Yoshioka, A., Imamura, S., Niwa, Y. (1987) Effect of sulphasalazine and its metabolites on the generation of reactive oxygen species. Gut 28: 190–195

Oestreicher, P., Nielsen, S. T., Rainsford, K. D. (1991) Inflammatory bowel disease induced by combined bacterial immunization and oral carrageenin in guinea pigs. Model development, histopathology, and effects of sulfasalazine. Dig. Dis. Sci. 36: 461–470

Onderdonk, A. B. (1985) Experimental models for ulcerative colitis. Ibid. 30: 40A–44S

Onderdonk, A. B., Cisneros, R. L., Bronson, R. T. (1983) Enhancement of experimental ulcerative colitis by immunization with *Bacteroides vulgatus*. Infect. Immun. 42: 783–788

Onderdonk, A. B., Steeves, R. M., Cisneros, R. L., Bronson, R. T. (1984) Adoptive transfer of immune enhancement of experimental ulcerative colitis. Ibid. 46: 64–67

Onderdonk, A. B., Bronson, R., Cisneros, R. (1987) Comparison of *Bacteroides vulgatus* strains in the enhancement of experimental ulcerative colitis. Ibid. 55: 835–836

Peppercorn, M. A. (1990) Advances in drug therapy for inflammatory bowel disease. Ann. Int. Med. 112: 50–60

Pfeiffer, C. J. (1986) Animal Models for Intestinal Disease. CRC Press, Boca Raton, FL

Remvig, L., Andersen, B. (1990) Salicylazosulfapyridine (Salazopyrin) effect on endotoxin-induced production of interleukin-1-like factor from human monocytes in vitro. Scand. J. Gastroent. 19: 11–16

Ruderman, W. B. (1990) New pharmacologic agents for the therapy of inflammatory bowel disease. Med. Clin. 74: 133–153

Sharon, P., Stenson, W. F. (1985) Metabolism of arachidonic acid in acetic acid colitis in rats. Similarity to human inflammatory bowel disease. Gastroenterology 88: 56–63

Taffet, S. L., Das, K. M. (1983) Sulfasalazine. Adverse effects and desensitization. Dig. Dis. Sci. 28: 833–842

J. Pharm. Pharmacol. 1992, 44: 531–533
Communicated July 27, 1991

Influence of changes in protein binding on the central activity of antidepressants

I. TORRES, E. GOMEZ, E. GARCIA, E. SUÁREZ, J. M. RODRIGUEZ-SASIAÍN, R. CALVO, *Department of Pharmacology, Faculty of Medicine, Basque Country, 48940 Leioa, Vizcaya, Spain*

Abstract—The central effect (expressed as analgesic response), protein binding and brain uptake of mianserin were measured in mice receiving drug intraperitoneally. A significant decrease of the central effect of mianserin (30 mg kg^{-1}) was seen in mice with experimental inflammation when compared with control animals (reaction time (s) = $12{\cdot}12 \pm 1{\cdot}22$ vs $25{\cdot}56 \pm 2{\cdot}92$; $P < 0{\cdot}001$) and the dose-analgesia response curve (10–60 mg kg^{-1}) was significantly shifted to the right in mice with inflammation. In serum of mice with inflammation, unbound concentration of mianserin was decreased from $19{\cdot}37 \pm 0{\cdot}73$ to $17{\cdot}83 \pm 0{\cdot}30\%$ ($P < 0{\cdot}05$) and seromucoid levels were significantly increased ($P < 0{\cdot}001$). Following the intraperitoneal administration of 30 mg kg^{-1} of mianserin, brain uptake decreased in diseased mice when compared with control animals ($P < 0{\cdot}02$), suggesting that the decrease in analgesia was secondary to a decrease in drug delivery to the brain because of increased protein binding.

Although the effects of binding on pharmacokinetics have been investigated (Dayton et al 1973; Gibaldi et al 1978; Wilkinson 1983), the effect of binding on pharmacodynamics is a relatively unexplored area; the relationship between free drug concentration and pharmacological effects is difficult to investigate, and is particularly complex for drugs that cross the blood-brain barrier to enter the central nervous system.

However, information is already available on the increased effect of central agents in several disease states, clearly identified as a cause of an increase of free fraction of acidic drugs in plasma (Greenblatt & Koch-Wesser 1974; Greenblatt & Allen 1978; Halliday et al 1985). In contrast, there is an apparent lack of clinical and experimental studies regarding the effect of augmented serum binding on the central effect of basic drugs (e.g. tricyclic antidepressants, neuroleptics, opiates). Basic drugs are avidly bound by α_1-acid glycoprotein (AAG) (Piafsky 1980; Abranson 1982; Tierlynck et al 1982). It is known that levels of AAG in serum show an important interindividual variability and increases in inflammatory conditions, such as rheumatoid arthritis or metastatic cancer (Piafsky 1980); consequently the drug response could be affected.

Thus we have set out to evaluate whether in mice with increased AAG plasma concentrations, there is an increase in binding of mianserin, thereby affecting its delivery to the brain. The central effect of mianserin was evaluated by its analgesic effect (Reichenberg et al 1985).

Correspondence: R. Calvo, Departamento de Farmacologia, Facultad de Medicina, Universidad del Pais Vasco, 48940 Leioa, Vizcaya, Spain.

Materials and methods

Drugs. [^{3}H]Mianserin chlorhydrate (sp. act. 80·0 Ci mmol^{-1}) was obtained from Itisa (Barcelona, Spain). Its radiochemical purity was over 97% by TLC. A mixture of appropriate amounts of radioactive mianserin and pure drug (Organon) was used to prepare a stock solution in 0·9% NaCl (saline) for injection.

Methods. In the first study, 180 male mice, 28–32 g, were used to determine the analgesic potency of mianserin. Inflammation was induced in 90 mice, 48 h before the experiment by subcutaneous injection of 0·5 mL of turpentine oil (Jamieson et al 1972). The rest of the mice were injected with 0·5 mL of saline and were used as controls. All animals (15 per dose) received an intraperitoneal injection of mianserin (0, 10, 20, 30, 40 or 60 mg kg^{-1}).

Mice were maintained at a constant temperature (24–26°C) and controlled humidity (approx. 50%) with a 12 h light cycle and had free access to food and water. Analgesic effect was determinated 60 min after injection of the antidepressant. The constant temperature hot plate test was used (Fialip et al 1989). The plate temperature was set to 54 ± 0·5°C throughout the experiment. The reaction time of each animal (licking of forepaws) was measured as the analgesic response. The latency to the first response was recorded. If no response was observed within 40 s, the test was terminated in order to avoid tissue damage.

In the second study, 24 male mice, 28–32 g, were used to determine the brain uptake index of mianserin. These animals were also divided into two groups, control (n = 10) and turpentine oil-treated (n = 14).

All mice received [^{3}H]mianserin as an intraperitoneal injection (30 mg kg^{-1}). Animals were decapitated 60 min after the mianserin administration and exsanguinated; 0·3 mL of blood was obtained from each. Blood samples (0·2 mL) were collected in heparinized tubes, immediately centrifuged (20°C, 2000 *g*) and plasma (50 μL) was separated for measurement of total mianserin concentration.

Brains were rapidly removed and cortex dissected according to Glowinski & Iversen (1966). Samples were accurately weighed and stored at 4°C until [^{3}H]mianserin assay.

Samples of mouse brain (ca 100 mg) and plasma samples (0·1 mL) were placed separately in scintillation counting vials, treated with 2 mL of Protosol, and heated at a temperature not exceeding 50°C for 48 h. When they were solubilized, 10 mL of Econofluor counting solution was added to each vial and radioactivity was measured by scintillation counting using a Packard model 300-Tri-Carb Spectrometer.

Binding of [^{3}H]mianserin (15 μg mL^{-1}) was determined in pooled (0·5 mL) plasma from control (n = 15) and turpentine-pretreated mice (n = 20) by ultrafiltration at 37°C using an Amicon MPS-1 micropartition system (March & Blanke 1985). This concentration (15 μg mL^{-1}) is equivalent to the concentration reached in mice receiving a therapeutic dose of 30 mg kg^{-1} intraperitoneally.

The concentration of unbound [^{3}H]mianserin was measured by scintillation spectrometry as described above.

Plasma samples from these animals were also used to evaluate AAG concentration measured as mucoproteins (Thaw & Albutt 1980).

Statistical analyses. All data are presented as the mean ± s.e.m. Analysis of variance was applied in the first study and Student's *t*-test in the second study. The criterion for significance was $P < 0·05$.

Table 1. Influence of experimental inflammation on the analgesic effect of mianserin (30 mg kg^{-1}). Analgesic effect was measured as the reaction time of each animal for licking of forepaws (mean ± s.e.m.).

	Control mice	Turpentine-treated mice
Reaction time (s)	25·56 ± 2·92	12·15 ± 1·22*

*$P < 0·001$.

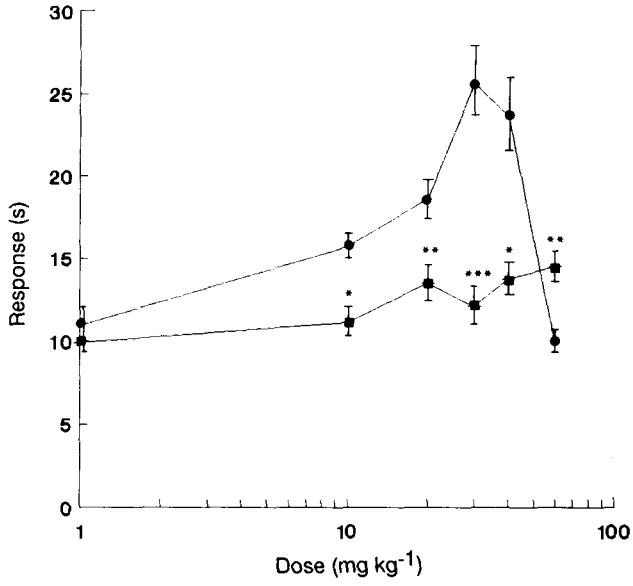

FIG. 1. Dose-analgesic response of mianserin in control mice (●) and mice with experimental inflammation (■) (n = 15 per dose). *$P < 0·01$, **$P < 0·02$, ***$P < 0·001$.

Results

Table 1 shows the influence of experimental inflammation on the analgesic effect of mianserin.

In mice with experimental inflammation, analgesic effects were significantly less ($P < 0·001$) after the same dose of mianserin. The effect of inflammation on the dose-response line for mianserin is shown in Fig. 1. The dose-response line is shifted to the right in turpentine oil-treated mice, when compared with

Table 2. Brain uptake index (brain/total plasma concentration ratio) of mianserin after intraperitoneal administration of 30 mg kg^{-1} in control and in mice with experimental inflammation (mean ± s.e.m.).

Mice	Brain uptake index
Control (n = 10)	1·20 ± 0·08
Turpentine-treated (n = 14)	0·87 ± 0·09*

*$P < 0·02$.

Table 3. Percentage of unbound mianserin and mucoprotein concentration in plasma from control mice and in mice with experimental inflammation (mean ± s.e.m.).

Mice	Unbound mianserin (%)	Mucoprotein (mg mL^{-1})
Control (n = 15)	19·37 ± 0·75	0·71 ± 0·03
Turpentine-treated (n = 20)	17·83 ± 0·30*	2·93 ± 0·07**

*$P < 0·05$; **$P < 0·001$.

the control group, suggesting a decrease in mianserin analgesic potency.

Table 2 shows the brain uptake index of mianserin expressed as the brain/total plasma concentration ratio after intraperitoneal administration of 30 mg kg^{-1} to control and turpentine-treated mice. The ratio was significantly less in the latter group (0.87 ± 0.09) when compared with the control group (1.20 ± 0.08, $P < 0.02$). Percentages of unbound mianserin in plasma from normal mice and mice with experimental inflammation are shown in Table 3. The percentage of unbound mianserin in control samples was 19.37 ± 0.75 vs $17.83 \pm 0.30\%$ ($P < 0.05$) in turpentine-treated mice indicating an increase in binding. Mucoprotein values are also increased in these mice ($P < 0.001$).

Discussion

It has been recognized that drug binding to plasma proteins limits passage of drug across the blood-brain barrier, and that only the unbound fraction is available for transport into the tissues of the central nervous system (CNS), but in general, when the unbound drug enters the CNS, there is more unbound drug available to diffuse into the tissues causing a re-equilibration. Therefore it could be argued that binding to plasma protein is not a restricting factor in the delivery of drugs to the brain. Pardridge et al (1983) observed that injecting propranolol (a basic agent which binds strongly to AAG), into the carotid artery of the rat, with serum from patients showing high AAG levels, exhibited reduced brain uptake compared with experiments with serum from normal volunteers. However, uptake of the propranolol into the central nervous system was greater than one would predict on the basis of the free drug hypothesis.

Similar findings have been reported for the brain uptake of benzodiazepines (Jones & Wilkinson 1986). However, despite these findings, the classic investigation of Ghoneim et al (1976) showed that in uraemic rats thiopentone binding is decreased and that the reduced binding led to accelerated distribution and increased drug concentrations in the brain and heart.

In other studies in patients, significant correlation has been found between the time required for induction of anaesthesia and serum albumin concentration for the intravenous anaesthetic midazolam, a highly protein bound benzodiazepine (Reves et al 1981).

In rabbits pretreated with valproate, which displaces midazolam from its albumin binding sites, a higher free fraction of midazolam produced a higher uptake of benzodiazepine in the brain with a consequent potentiation of response (Calvo et al 1988).

More recently a decrease of the central effect of penbutolol (a basic agent mainly bound to AAG) was reported in mice with inflammation. A parallel decrease in free fraction and brain uptake of penbutolol was observed in animals with high seromucoid levels (Martinez-Jordá et al 1990). Patients receiving bupivacaine by epidural injection and who developed CNS toxicity had lower AAG concentrations in serum than a group of patients not developing CNS side effects, in spite of similar total serum bupivacaine concentration in both groups (Denson et al 1984).

Our results show that after intraperitoneal administration of mianserin, its central effect is clearly decreased in mice with inflammation, and this change is related to reduced delivery to the brain and increased binding. This situation may also occur in other states involving AAG levels.

References

Abranson, F. P. (1982) Methadone plasma protein binding: alterations in cancer and displacement from alpha-1-acid glycoprotein. Clin. Pharmacol. Ther. 32: 652–658

Calvo, R., Suárez, E., Rodríguez-Sasiain, J. M., Aguilera, L. (1988) Effect of sodium valproate on midazolam distribution. J. Pharm. Pharmacol. 40: 150–152

Dayton, P. G., Israili, Z. H., Perel, J. M. (1973) Influence of binding on drug metabolism and distribution. Ann. NY Acad. Sci. 226: 172–174

Denson, D., Coyle, D., Thompson, G., Myers, J. (1984) Alpha-1-acid glycoprotein and albumin in human serum bupivacaine binding. Clin. Pharmacol. Ther. 35: 409–415

Fialip, J., Marty, H., Makambila, M., Civiale, M., Eschalier, A. (1989) Pharmacokinetic patterns of repeated administration of antidepressants in animals. II. Their relevance in a study of the influence of clomipramine on morphine analgesia in mice. J. Pharmacol. Exp. Ther. 248: 747–751

Ghoneim, M. M., Pandya, H., Kelley, S. E., Fischer, L. J., Corry, R. J. (1976) Binding of thiopental to plasma proteins: effects on distribution in the brain and heart. Anesthesiology 45: 635–639

Gibaldi, M., Levy, G., McNamara, P. J. (1978) Effect of plasma protein and tissue binding on the biological half-life of drugs. Clin. Pharmacol. Ther. 24: 1–4

Glowinski, J., Iversen, L. L. (1966) Regional studies of catecholamines in the rat brain. Part I. J. Neurochem. 13: 655–669

Greenblatt, D. J., Allen, M. D. (1978) Toxicity of nitrazepam in the elderly. A report from the Boston Collaborative Drug Surveillance Program. Br. J. Clin. Pharmacol. 5: 407–410

Greenblatt, D. J., Koch-Wesser, J. (1974) Clinical toxicity of chlordiazepoxide and diazepam in relation to serum albumin concentration. A report from the Boston Collaborative Drug Surveillance Program. Ibid. 7: 259–262

Halliday, M. J., Dundee, J. W., Collier, P. S., Loughren, P. G., Harper, K. W. (1985) Influence of plasma proteins on the onset of hypnotic action of intravenous midazolam. Anaesthesia 40: 763–766

Jamieson, J. C., Ashton, F. E., Friesen, A. D., Chou, B. (1972) Studies on acute phase proteins of rat serum. II. Determination of the contents of alpha-1-glycoprotein, alpha-2-macroglobulin and albumin in serum from rats suffering from induced inflammation. Can. J. Biochem. 50: 871–880

Jones, E. K., Wilkinson, G. R. (1986) Plasma binding and brain uptake of benzodiazepines. In: Tillement, J. P., Lindenlaub, E. (eds) Protein Binding and Drug Transport. Schattauer Verlag, Stuttgart-New York pp 311–331

March, C., Blanke, R. V. (1985) Determination of free valproic acid concentrations using the Amicon Micropartition MPS-1 ultrafiltration system. Ther. Drug Monit. 7: 115–120

Martinez-Jordá, R., Aguirre, C., Calvo, R., Rodríguez-Sasiain, J. M., Erill, S. (1990) Decrease in penbutolol central response as a cause of changes in its serum protein binding. J. Pharm. Pharmacol. 42: 164–166

Pardridge, W. M., Sakiyema, R., Fierer, G. (1983) Transport of propranolol and lidocaine through the rat blood-brain barrier. J. Clin. Invest. 71: 900–908

Piafsky, K. M. (1980) Disease-induced changes in the plasma binding of basic drugs. Clin. Pharmacokinet. 5: 246–262

Reichenberg, K., Gaillard-Plaza, G., Montastruc, J. L. (1985) Influence of naloxone on the antinociceptive effects of some antidepressant drugs. Arch. Int. Pharmacodyn. 275: 78–85

Reves, J. G., Newfield, P., Smith, L. R. (1981) Midazolam induction time: association with serum albumin. Anesthesiology 55: A 259

Tierlynck, O., Belpaire, F. M., Andreasen, I. (1982) Binding of aprindine and moxaprindine to human serum, alpha-1-acid-glycoprotein and serum of healthy and diseased humans. Eur. J. Clin. Pharmacol. 21: 427–431

Thaw, P. A., Albutt, E. C. (1980) A critical evaluation of a serum seromucoid assay and its replacement by a serum alpha-1-acid-glycoprotein assay. Ann. Clin. Biochem. 17: 140–143

Wilkinson, G. R. (1983) Plasma and tissue binding considerations in drug disposition. Drug Metab. Rev. 14: 427–465