J. Pharm. Pharmacol. 1998, 50: 197–203
Received August 4, 1997
Accepted October 28, 1997

Analgesic and Thermic Effects, and Cerebrospinal Fluid and Plasma Pharmacokinetics, of Intracerebroventricularly Administered Morphine in Normal and Sensitized Rats

HEMENDRA N. BHARGAVA, VINCENT M. VILLAR*, JULIO CORTIJO* AND ESTEBAN J. MORCILLO*

*Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, The University of Illinois at Chicago, Chicago, Illinois, U.S.A. and *Department of Pharmacology, Faculty of Medicine and Odontology, University of Valencia, Valencia, Spain*

Abstract

The relationship between asthma and opioids has barely been investigated. This study examines whether active sensitization of rats changes the analgesic and thermic effects of intracerebroventricular morphine or the pharmacokinetics of the drug.

Morphine (5, 10 and 20 μg) was given intracerebroventricularly to sensitized (active immunization to ovalbumin and $Al(OH)_3$ then airway challenge with ovalbumin after 12 days) and normal (i.e. non-sensitized) male Sprague-Dawley rats. The tail-flick latencies and changes in colon temperature were determined before morphine injection and at 30 min intervals for a period of 300 min afterwards. Results were expressed as the area under the time–response curve. The analgesic and hyperthermic response to morphine for sensitized rats was less than that obtained for normal rats. Cerebrospinal fluid and blood samples were collected periodically for a period of 240 min and morphine levels were determined by a highly sensitive radioimmunoassay. The pharmacokinetic parameters half-life, terminal elimination rate constant and the mean residence time were determined in both cerebrospinal fluid and plasma by non-compartmental analysis. The area under the cerebrospinal fluid concentration–time curve from time zero to infinity was higher for sensitized rats than for normal rats for all three doses of morphine but these differences did not correspond with similar changes in pharmacological responses.

In conclusion, the attenuated analgesic and thermic responses to intracerebroventricular morphine in the sensitized rats might be a result of pharmacodynamic alterations rather than to pharmacokinetic changes.

The existence of a possible relationship between asthma and opioids has barely been studied (Copolov & Helme 1983). Asthmatic episodes can occur in the absence of detectable external factors other than psychological stress (McFadden 1995) and endogenous opioids are known to be involved in the physiological reaction to stress (Sibinga & Goldstein 1988). On the other hand, exogenously administered opioids can precipitate attacks of asthma (Popa 1994) and alternatives to narcotic therapy have been studied to provide analgesia in asthmatics (Jahangir et al 1993). To the best of our knowledge there is no literature report of any possible influence of asthma on the pharmacodynamics and pharmacokinetics of opiates.

In this study the effects of intracerebroventricular administration of morphine on thermally induced pain and changes in colon temperature were determined for normal (non-sensitized) rats and for rats actively sensitized to ovalbumin, an established model of experimental asthma (Blythe et al 1986). The relationship between the magnitude of the analgesic and thermic responses to morphine and the cerebrospinal fluid (CSF) and plasma pharmacokinetic parameters has also been studied. An estimate of the amount of morphine reaching the central nervous system (CNS) after three intravenous doses of morphine was made in a previous study (Bhargava et al 1991); the same amounts were administered intracerebroventricularly in this study.

Correspondence: E. J. Morcillo, Department of Pharmacology, Faculty of Medicine and Odontology, Av. Blasco Ibanez 15, E-46010 Valencia, Spain

Materials and Methods

Materials

Adult male specific-pathogen-free Sprague-Dawley rats (Sasco King, Madison, WI), 225–250 g, were housed in a room with controlled temperature ($23 \pm 1°C$) and humidity ($50 \pm 10\%$) and a 12-h dark-light cycle (lights on 0600 to 1800 h) for at least 4 days before being used. Food and water were freely available.

Morphine sulphate was obtained from the National Institute on Drug Abuse (Rockville, MD), courtesy of Mr Robert L. Walsh. The drug was dissolved in physiological saline and injected intracerebroventricularly through a 27-gauge needle in a volume of 10 μL. The RIA kit 'Coat-a-Count Serum Morphine', was obtained from the Diagnostic Products Corporation (Los Angeles, CA). Other chemicals were from standard commercial sources.

Sensitization protocol

Rats were actively sensitized to ovalbumin, as described elsewhere (Sorkness et al 1990), by administration of a single subcutaneous injection of a suspension containing 1 mg ovalbumin (Sigma, St Louis, MO) and 200 mg $Al(OH)_3$, and an intraperitoneal injection of 0·5 mL killed *Bordetella pertussis* (Difco, Detroit, MI). This protocol produces anti-ovalbumin IgE antibody at a level sufficient to elicit an immediate allergic response on challenge with ovalbumin (Sorkness et al 1990). Twelve days after sensitization to ovalbumin rats were challenged with ovalbumin as previously outlined (Blythe et al 1986). In brief, a solution of ovalbumin in saline (1 mg mL^{-1}; 100 μL) was insufflated into the trachea by means of an 18-gauge plastic catheter. After intra-tracheal antigen provocation, sensitized rats developed audible wheezing that lasted for 15 to 30 min. The control group of normal (i.e. non-sensitized) rats received saline (100 μL) intratracheally. For both the pharmacodynamic and pharmacokinetic studies the airway challenge was performed 45 min before intracerebroventricular injection of morphine.

Preparation of animals

Rats were anaesthetized lightly with diethyl ether. Polyethylene catheters (PE 60, Silastic Brand Medical Grade Tubing, Dow Corning, Midland, MI; 0·51 mm i.d. × 0·94 mm o.d.) were surgically implanted into the left jugular vein for pharmacodynamic and pharmacokinetic studies. A chronic cannula was implanted in the lateral ventricle by the method of Noble et al (1967). A midsagittal incision was made from the level of the eyes to the level of the ears exposing the bregma. A hole large enough to accommodate a 27-gauge needle was drilled through the skull 1·5 to 2·0 mm lateral and caudal to the intersection of the coronal and sagittal sutures. A 27-gauge needle with 4·0 mm exposed beyond a stop was inserted into the lateral ventricle while being held in a vertical position, and then fixed with dental cement. This technique was used both for injection of morphine and for collection of cerebrospinal fluid.

Pharmacodynamic studies with normal and sensitized rats

An appropriate dose (5, 10 or 20 μg) of morphine sulphate was administered in the lateral ventricle through the in-dwelling cannula. The analgesic effect and colon temperature of each rat were then determined.

Measurement of analgesic effect. The analgesic effect of morphine was determined by the tail-flick method described elsewhere (Bhargava et al 1991). The tail-flick latencies to thermal stimulation were determined before intracerebroventricular injection of morphine and at various times for 300 min afterwards. The basal tail-flick latency was approximately 2 s. A cut-off time of 10 s was used to prevent damage to the tail. The basal latency was subtracted from that induced by morphine. The analgesic response of each rat was converted into the area under the curve from 0 to 300 min ($AUC_{0-300\ min}$; Bhargava et al 1991), expressed as the mean ± s.e.m. Eight rats were used for each dose of morphine sulphate. Differences between analgesic responses to different doses of morphine were compared by use of Student's *t*-test. A value of $P < 0·05$ was regarded as indicative of significance.

Measurement of thermic effect. The change of colon temperature in response to morphine administered intracerebroventricularly to the rats was determined with the same rats used to examine the analgesic effect (Bhargava et al 1991). The temperature of each rat was recorded before injection of morphine and at various times for 300 min afterwards, by means of a telethermometer. The change in temperature from the basal value was plotted with time and the $AUC_{0-300\ min}$ was calculated. Statistical evaluations were performed as described for the analgesic response.

Pharmacokinetic studies

Twenty-four hours after surgery an appropriate dose of morphine sulphate was injected intracerebroventricularly through the in-dwelling cannulas. After the injection of morphine the intracere-

broventricular cannula was washed with 5 μL saline to avoid contamination of CSF samples drawn afterwards, as described previously (De Balbian Verster et al 1971). Blood samples (0·15 mL) were drawn from the jugular vein at various times for a period of 240 min after intracerebroventricular injection of morphine sulphate. The dose of morphine sulphate was converted into the corresponding amount of morphine free base before analysis of the pharmacokinetic data (1·33 mg sulphate salt ≡ 1 mg base). Blood samples were centrifuged at 3000 rev min^{-1} for 10 min at − 4°C. Plasma and CSF samples were stored at − 80°C. The concentration of morphine in plasma and in CSF was determined by radioimmunoassay (RIA) using duplicate 25-μL samples. This procedure could detect morphine concentrations as low as 0·8 ng mL^{-1}. The antiserum had only 0·2% cross-reactivity with morphine glucuronides. CSF and plasma concentrations of morphine were expressed as morphine free base. Non-compartmental analysis (Gibaldi & Perrier 1982) was used to obtain values of mean residence time (MRT). Details of the procedure used and the recovery of morphine from extracted and unextracted samples have been described elsewhere (Bhargava et al 1991). The values of all the pharmacokinetic parameters were compared by analysis of variance then Scheffe's test. A value of $P < 0.05$ was regarded as indicative of statistical significance.

Results

The analgesic effect of intracerebroventricular morphine in normal and sensitized rats

The effects of 5-, 10- and 20-μg doses of morphine on the tail-flick latency at various times after drug injection for sensitized and normal rats are shown in Figure 1 (upper panels). For both groups of rats 5 μg intracerebroventricular morphine increased the tail-flick latency; this returned to the pre-drug level after approximately 90 min. With the other two doses (10 and 20 μg) the values returned to pre-drug values between 240 and 300 min. The tail-flick latency data were transformed into $AUC_{0-300\ min}$ for the three doses of morphine in both groups of rats and individual data were plotted

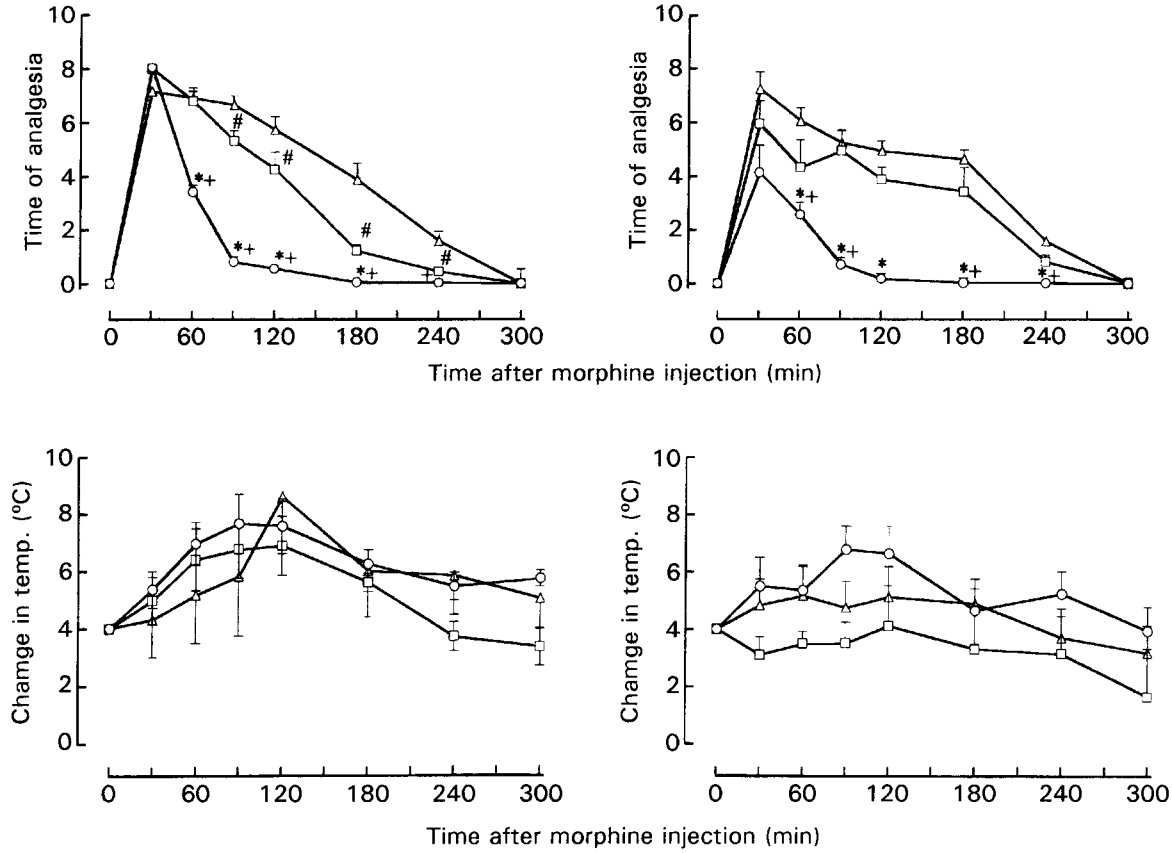

Figure 1. Analgesic (tail-flick latencies; upper panels) and thermic (changes in colon temperature; lower panels) effects of intracerebroventricularly administered morphine (5 (○), 10 (□) and 20 (△) μg) in normal (left panels) and sensitized (right panels) rats. Data are means ± s.e.m. of results from 8–10 animals per group; *$P < 0.05$, significant difference between results for 5 and 10 μg; +$P < 0.05$, significant difference between results for 5 and 20 μg; #$P < 0.05$, significant difference between results for 10 and 20 μg.

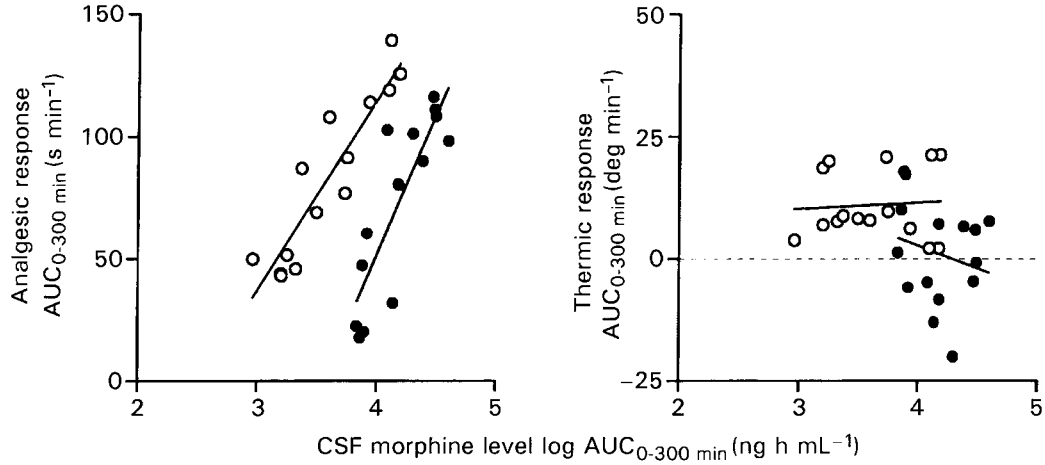

Figure 2. Plot of individual data for the level of morphine in the cerebrospinal fluid (CSF) for assessment of correlation with the analgesic and thermic responses of normal (○) and sensitized (●) rats. CSF morphine levels after dosing of intracerebroventricular morphine (5, 10 and 20 μg; 5 animals per dose in each group) is expressed as $\log_{10}$ of the $AUC_{0-300\ min}$ values and the pharmacological responses are expressed as $AUC_{0-300\ min}$ values. Linear regression analysis showed the analgesic response to be dose-dependent (correlation coefficients were 0·854 for the normal group and 0·711 for the sensitized group; slopes were significantly different from zero, $P < 0.05$) but not the thermic response (slopes not significantly different from zero). The differences between the values for the analgesic and thermic responses in the normal and sensitized groups were statistically significant ($P < 0.05$).

against the corresponding dose of morphine expressed as $AUC_{0-300\ min}$ (CSF). Morphine induced a dose-dependent analgesic response in both groups but for similar CSF morphine levels analgesia was significantly reduced for sensitized rats (Figure 2).

Thermic effect of intracerebroventricular morphine for normal and sensitized rats

The effects of 5-, 10- and 20-μg doses of intracerebroventricular morphine on colon temperature at various times after drug injection in sensitized and normal rats are shown in Figure 1 (lower panels). The time-course of changes in colon temperature were transformed into $AUC_{0-300\ min}$ for the three doses of morphine in both groups of rats and individual data were plotted against the corresponding dose of morphine expressed as $AUC_{0-300\ min}$ (CSF). In normal rats morphine consistently increased colon temperature but the increment was not dose-related (Figure 2). In sensitized rats, the colon temperature either increased or decreased in response to morphine but the $AUC_{0-300\ min}$ (CSF) values for the thermic response were lower ($P < 0.05$) for these animals than for normal rats (Figure 2).

Pharmacokinetics of intracerebroventricular morphine in the CSF and plasma of normal and sensitized rats

Semilogarithmic plots of mean CSF and plasma concentrations as a function of time after intracerebroventricular injection of morphine (5, 10 and 20 μg) are shown in Figure 3. The pharmacokinetic

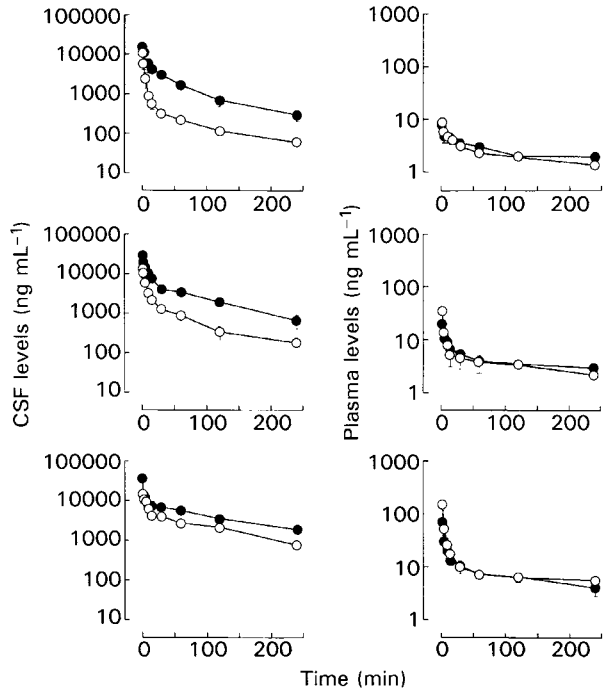

Figure 3. Time-course of morphine levels in CSF (left panels) and plasma (right panels) after intracerebroventricular administration of 5 μg (upper panels), 10 μg (middle panels) or 20 μg (lower panels) morphine to normal (○) and sensitized (●) rats. Data are means ± s.e.m. of results from five animals in each group.

parameters derived on the basis of non-compartmental analysis (Gibaldi & Perrier 1982) of CSF, and plasma morphine concentration–time data for the three doses of morphine for both groups of rats are presented in Table 1. The curves representing the decay of CSF morphine in sensitized rats con-

Table 1. Cerebrospinal and plasma pharmacokinetic parameters of morphine after intracerebroventricular administration of 5, 10 or 20 μg to normal and sensitized rats.

Kinetic parameters	Dose (μg)	Cerebrospinal fluid pharmacokinetics		Plasma pharmacokinetics	
		Normal rats	Sensitized rats	Normal rats	Sensitized rats
Area under the concentration-time curve from time 0 to ∞ (μg h mL^{-1} for normal rats and ng h mL^{-1} for sensitized rats)	5	1·61 ± 0·22	7·55 ± 0·27*	15·15 ± 0·82	12·4 ± 1·92
	10	4·11 ± 0·57†	15·23 ± 1·45†*	54·33 ± 2·12†	27·1 ± 1·36†*
	20	12·9 ± 1·35†‡	31·0 ± 2·78†‡ *	118·7 ± 17·9†‡	44·0 ± 3·06†‡ *
Elimination half-life (h)	5	0·72 ± 0·19	0·75 ± 0·16	2·93 ± 0·19	2·24 ± 0·35
	10	1·05 ± 0·13	2·03 ± 0·72	1·59 ± 0·27	2·29 ± 0·29
	20	1·39 ± 0·11‡	4·45 ± 1·66*	25·4 ± 6·84	1·49 ± 0·17
Terminal elimination rate constant (h^{-1})	5	1·43 ± 0·25	1·69 ± 0·42	0·35 ± 0·02	0·49 ± 0·08
	10	1·02 ± 0·13	0·97 ± 0·34	0·69 ± 0·09	0·46 ± 0·05
	20	0·82 ± 0·10	0·44 ± 0·16	0·06 ± 0·02	0·74 ± 0·12*
Mean residence time (h)	5	0·76 ± 0·12	1·08 ± 0·23	4·23 ± 0·27	3·23 ± 0·50
	10	1·51 ± 0·19	2·93 ± 1·03	2·29 ± 0·39	3·31 ± 0·41
	20	1·87 ± 0·22	6·41 ± 2·40	36·7 ± 10 ± 10·	2·15 ± 0·24*

Data are means ± s.e.m. of results from five experiments. †$P < 0·05$, significantly different from result for preceding dose in the same group; ‡ $P < 0·05$, significantly different from result for 5 μg in the same group; *$P < 0·05$, significantly different from the corresponding value in the normal group.

sistently lay above those representing decay in normal rats, and the values of $AUC_{0-\infty}$ (CSF) were significantly greater for sensitized rats than for normal rats for all three doses of morphine. The $AUC_{0-\infty}$ (plasma) values for sensitized rats after 10- and 20-μg doses were significantly smaller than values for normal rats. A dose-dependent non-linear increase of $AUC0_{0-\infty}$ values (CSF and plasma) was observed for normal rats whereas for the sensitized animals the increase was linear. Other pharmacokinetic parameters studied were not significantly different between groups, except for the 20-μg dose, although the difference found for half-life (plasma) is statistically uncertain because of the large variability observed for the normal group (Table 1).

Discussion

Intractable pain in patients has been treated successfully by intracerebroventricular administration of morphine (Obbens et al 1987). We have shown that intracerebroventricular morphine in normal rats induces dose-related analgesia which lasted for 5 h with the highest dose tested (20 μg). These results confirm results from previous studies using this route of administration and others in which morphine was given subcutaneously or intracerebroventricularly (Stain et al 1995).

In this study the analgesic response to similar CSF morphine levels (measured as AUC) was significantly attenuated in sensitized rats examined during the early allergic response. The analgesic response to intracerebroventricular morphine for normal rats was accompanied by a modest but significant hyperthermic response that was not dose-dependent. These results confirm previous studies in which low doses of morphine administered intravenously or subcutaneously also produce a hyperthermic response (Bhargava et al 1991). As for the analgesic response, the hyperthermic response to intracerebroventricular morphine (measured as AUC) was reduced in sensitized rats (this study). Taken together these results suggest that although differences between analgesic and thermic responses to intracerebroventricular morphine for normal and sensitized rats are not a prominent phenomenon, sensitized animals showed a generally reduced response to intracerebroventricular morphine. The possible causes of this attenuated response to morphine might have a pharmacodynamic or pharmacokinetic basis.

Actively sensitized rats subjected to airway challenge with the antigen is a stressful condition in which endogenous opioids are surely involved (Sibinga & Goldstein 1988). Thus, enkephalins might be released from the adrenal medulla (Viveros et al 1979) and β-endorphins from ante-

rior pituitary (Sibinga & Goldstein 1988). It is commonly accepted that receptor activity is regulated by the concentration of endogenous ligands; hence, down-regulation of μ-receptors might eventually occur in response to a local increase in the endogenous ligands of such receptors. In fact, we have recently shown that active sensitization resulted in down-regulation of μ-receptors in rat pulmonary tissues (Bhargava et al 1997). If this occurred in the CNS, a reduced analgesic response would be a consequence.

On the other hand, sensitized rats were actively immunized against an antigen administered systemically and any possible influence of the immunization process on the endogenous opioid system was ignored. Cells of the immune system have specific receptors to opioids (Carr 1991) and changes in peripheral opioid receptors have been reported in neurogenic and allergic inflammation processes which are also known to be involved in experimentally-induced asthma (Stein 1995). Because we have not performed binding studies of opioid ligands in CNS tissues obtained from normal and sensitized rats, the relevance of these mechanisms to differences in the effects of morphine observed in the current study remain to be confirmed in further studies.

Alternatively, alteration of the pharmacokinetic process of intracerebroventricular morphine could occur in sensitized rats but not in normal animals. We have studied CSF and plasma pharmacokinetics after intracerebroventricular morphine at the same doses used in the analgesic and thermic experiments. The pharmacokinetic data derived from a non-compartmental model (Gibaldi & Perrier 1982) are consistent with data from other studies which examine the decay of brain extracellular fluid after subcutaneous morphine (Stain et al 1995).

The curves representing the decay of CSF morphine levels in sensitized rats consistently lay above those for normal animals. This finding suggests that morphine levels in the CNS at a given time are higher for sensitized than for normal rats. The AUC (CSF) values were also higher for sensitized than for normal animals and, therefore, these higher levels of CSF morphine are not consistent with the attenuated analgesic and thermic responses to morphine found in this study. Because in the rat (−)-morphine is metabolized only to morphine-3-glucuronide (Coughtrie et al 1989), a metabolite lacking any analgesic effect (Labella et al 1979; Smith et al 1990), and not to the potent analgesic morphine-6-glucuronide (Shimomura et al 1971), the possibility that a higher level of CSF morphine reflects a lesser extent of biotransformation in the brain to metabolites endowed with potent analgesic properties seems unlikely as an explanation of the attenuated responses observed with sensitized animals.

On the other hand, differences in plasma levels with time and in AUC (plasma) values for sensitized compared with normal rats appear of little relevance to differences observed in the functional studies. Other pharmacokinetic alterations found for sensitized animals, for example the linear increase in CSF and plasma AUC values compared with a non-linear increase for normal rats, are difficult to interpret but do not seem relevant to the differences observed in pharmacological responses.

In conclusion, the analgesic and thermic responses to intracerebroventricular morphine are attenuated for sensitized rats compared with those for normal animals. For sensitized rats the area under the CSF concentration–time curve from time zero to infinity was higher than the corresponding values for normal rats. Therefore, the differences found in the pharmacological responses to intracerebroventricular morphine of the sensitized animals might be because of pharmacodynamic rather than pharmacokinetic changes.

Acknowledgements

This study was supported by a Research Scientist Development Award K02-DA-00130 from the National Institute of Drug Abuse. Dr V. Villar is the recipient of a re-incorporation contract from the Ministry of Education and the University of Valencia (Spain) under research projects SAF96-0200 and SAF97-0047 from CICYT (Comision Interministerial Investigación Científica y Técnica, Madrid, Spain).

References

Bhargava, H. N., Villar, V. M., Gulati, A., Chari, G. (1991) Analgesic and hyperthermic effects of intravenously administered morphine in the rat are related to its serum levels. J. Pharmacol. Exp. Ther. 258: 511–516

Bhargava, H. N., Villar, V. M., Cortijo, J., Morcillo, E. J. (1997) Binding of [^{3}H][D-Ala^2, MePhe^4, Gly^5-ol]enkephalin, [^{3}H][D-Pen^2, D-Pen^5]enkephalin, and [^{3}H]U-69,593 to airway and pulmonary tissues of normal and sensitized rats. Peptides (in press)

Blythe, S., England, B., Esser, B., Junk, P., Lemanske Jr, R. F. (1986) IgE antibody-mediated inflammation of rat lung: histologic and bronchoalveolar lavage assessment. Am. Rev. Respir. Dis. 134: 1246–1251

Carr, D. J. J. (1991) The role of endogenous opioids and their receptors in the immune system. Proc. Soc. Exp. Biol. Med. 198: 710–720

Copolov, D. L., Helme, R. D. (1983) Enkephalins and endorphins. Clinical, pharmacological and therapeutic implications. Drugs 26: 503–519

Coughtrie, M. W. H., Ask, B., Rane, A., Burchell, B., Hume, R. (1989) The enantioselective glucuronidation of morphine in rats and humans. Evidence for the involvement of more

than one UDP-glucuronosyltransferase isoenzyme. Biochem. Pharmacol. 38: 3273–3280

De Balbian Verster, F., Robinson, C. A., Hengeveld, C. A., Bush, E. S. (1971) Freehand cerebroventricular injection technique for unanaesthetized rats. Life Sci. 10: 1395–1402

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn, Marcel Dekker, New York

Jahangir, S. M., Islam, F., Aziz, L. (1993) Ketamine infusion for postoperative analgesia in asthmatics: a comparison with intermittent meperidine. Anesth. Analg. 76: 45–49

Labella, F. S., Pinsky, C., Havilicek, V. (1979) Morphine derivatives with diminished opiate receptor potency show enhanced central excitatory activity. Brain Res. 174: 263–271

McFadden, E. R. (1995) Asthma. In: Isselbacher, K. J., Braunwald, E., Wilson, J. D., Martin, J. B., Fanci, A. S., Kasper, D. L. (eds) Harrison's Principles of Internal Medicine. 13th Edn, McGraw-Hill, New York, pp 1341–1348

Noble, E. S., Wortman, R. J., Axelrod, J. A. (1967) A simple and rapid method for injecting norepinephrine into the lateral ventricles of the rat brain. Life Sci. 6: 281–291

Obbens, E. A. M. T., Hill, C. S., Leavens, M. E., Ruthenbeck, S. S., Otis, F. (1987) Intraventricular morphine administration for control of chronic cancer pain. Pain 28: 61–68

Popa, V. (1994) Codeine-induced bronchoconstriction and putative bronchial opiate receptors in asthmatic subjects. Pulm. Pharmacol. 7: 333–341

Shimomura, K., Kamata, O., Ueki, S., Ida, S., Oguri, K., Yoshimura, H., Tsukamoto, H. (1971) Analgesic effect of morphine glucuronides. Tohoku J. Exp. Med. 105: 45–52

Sibinga, N. E. S., Goldstein, A. (1988) Opioid peptides and opioid receptors in cells of the immune system. Annu. Rev. Immunol. 6: 219–249

Smith, M. T., Watt, J. A., Cramond, T. (1990) Morphine-3-glucuronide—a potent antagonist of morphine analgesia. Life Sci. 47: 579–585

Sorkness, R., Johns, K., Castleman, W. L., Lemanske Jr, R. F. (1990) Late pulmonary allergic responses in actively but not passively IgE-sensitized rats. J. Appl. Physiol. 69: 1012–1021

Stain, F., Barjavel, M. J., Sandouk, P., Plotkine, M., Sherrmann, J. M., Bhargava, H. N. (1995) Analgesic response and plasma and brain extracellular fluid pharmacokinetics of morphine and morphine-6-β-D-glucuronide in the rat. J. Pharmacol. Exp. Ther. 274: 852–857

Stein, C. (1995) The control of pain in peripheral tissue by opioids. N. Engl. J. Med. 332: 1685–1690

Viveros, O. H., Diliberto Jr, E. H., Hazum, E., Chang, K. J. (1979) Opiate-like materials in the adrenal medulla: evidence for storage and secretion with catecholamines. Mol. Pharmacol. 16: 1101–1108