Br. J. Pharmac. (1981), **72**, 471–475

ENKEPHALIN RECEPTORS IN THE EMETIC CHEMORECEPTOR TRIGGER ZONE OF THE DOG

K.P. BHARGAVA, K.S. DIXIT & Y.K. GUPTA

Neuropharmacology Unit (C.S.I.R.), Department of Pharmacology & Therapeutics
King George's Medical College, Lucknow-226003 India

1 The emetic action of Met-enkephalin, morphine and naloxone was studied following their administration into the cerebral ventricles of dogs through chronically implanted cannulae and the effect on the responses of ablating the chemoreceptor trigger zone (CTZ) was investigated. The opiate antagonist, naloxone, was used to determine the role of enkephalin receptors in emetic responses.

2 Administration of Met-enkephalin (1.0 μg/kg) into the IVth ventricle regularly evoked emesis with an average latency of 35 s. A dose of morphine (2.5 μg/kg) which was five times larger was required for a consistent emetic response when introduced into the lateral cerebral ventricle (i.c.v.) as compared to the dose required by the IVth ventricular route. The latency of emetic responses by the latter route of injection of morphine was shorter. This is in accord with an action of morphine on the emetic CTZ.

3 After bilateral ablation of the CTZ, intraventricular injections of Met-enkephalin and morphine failed to produce emesis even when given in doses that were 5 to 10 times the dose which regularly elicited emesis in animals with intact CTZ. The emesis produced in dogs by intraventricular Met-enkephalin and morphine is thus fully accounted for by an action on the CTZ.

4 Naloxone (i.c.v.) in doses up to 10.0 μg/kg did not cause emesis. However, higher doses of naloxone elicited dose-dependent emesis in dogs. The 100% emetic dose of naloxone was found to be 160 μg/kg and the latency of emesis was 180 s. Unlike Met-enkephalin and morphine, naloxone continued to elicit emesis in CTZ-ablated animals.

5 Pretreatment with intraventricular naloxone (1 to 8 μg/kg) blocked the emetic responses induced by intraventricular Met-enkephalin and morphine but not that to apomorphine. The selective protective action of the opiate antagonist against Met-enkephalin and morphine supports the presence of enkephalin receptors in the emetic CTZ.

Introduction

The presence of enkephalins in the CNS suggests a neurotransmitter role for these substances and they seem to serve as ligands for the enkephalin-opiate receptors (Hughes, 1975; Simantov & Snyder, 1976; Johansson, Hokfelt, Elde, Schultzberg & Terenius, 1978). Morphine is the prototype opiate and is chemically allied to the strongest emetic agent apomorphine. In man, vomiting is a common side effect of narcotic agents. Emesis to morphine has been reported in dogs (Wang & Galviano, 1954) and cats (Borison, Fishburn, Bhide & McCarthy, 1962) and the emetic responses to morphine as well as apomorphine are successfully blocked by ablation of the emetic chemoreceptor trigger zone (CTZ) which is topographically distinct from the vomiting centre. Since the narcotic antagonist, naloxone, specifically antagonized the emetic response to morphine but not that to apomorphine (Costello & Borison, 1977), and haloperidol had opposite effects in this respect (Gylys, Doran & Szaraz, 1974), the receptors for the emetic action of morphine and apomorphine in the CTZ appear to be different.

The present study was carried out to investigate the emetic effect of intraventricular administration of Met-enkephalin, morphine and naloxone in dogs and to study the influence of CTZ ablation on the responses. Furthermore, the protective effect of naloxone was studied against the emetic response to Met-enkephalin, morphine and apomorphine to determine the role of enkephalin receptors in the emesis induced by morphine and apomorphine.

Methods

A total of 51 dogs of either sex weighing between 8

0007-1188/81/030471-05 $01.00

and 12 kg were used in the present study. Chronic implantation of a metal cannula into either a lateral cerebral ventricle or into the IVth ventricle was performed aseptically under pentobarbitone sodium anaesthesia, according to the method described by Bhargava & Dixit (1968). The correct placement of cannula was ascertained by withdrawal of clear cerebrospinal fluid from the cannula, by a postive emetic response to an injection of apomorphine into the cannulated lateral (2 μg) or IVth ventricle (0.5 μg) 3 to 4 days after the implantation and, finally, on autopsy.

Bilateral ablation of the chemoreceptor trigger zone (CTZ) was carried out by gentle thermal cauterization of a small discrete area in the lateral border of the area postrema of the floor of the IVth ventricle in the medulla oblongata, under aseptic conditions. Streptomycin (0.5 g) and penicillin (2 miu) were injected post operatively for 5 days. No neurological deficit was observed when the animals recovered. Functional elimination of the CTZ was determined by failure of the animals to vomit in response to intravenous apomorphine (200 μg/kg, 8 times the ED_{100}, Borison & Wang 1951). In some animals the extent of the lesion was determined histologically. The intactness of the vomiting centre was established by a positive emetic response to copper sulphate (300 mg in 25 ml water) administered orally, through an intragastric tube on an empty stomach.

For study of the emetic response, the dogs were fed porridge and milk 10 to 15 min before administration of the emetic agent and observed until they vomited, or for a period of 2 h. Actual expulsion of the gastric contents through the mouth was taken as the criterion of the emetic response. On each trial only a single dose of a test agent was used. An interval of at least 5 days was allowed between two successive tests. No animal was used for more than five emetic tests. The drug solutions were prepared in sterile 0.9% w/v NaCl solution (saline). All drugs injected into the ventricle were administered in a volume not exceeding 0.2 ml and were followed by 0.2 ml saline.

The drugs used in the study were apomorphine hydrochloride (Mallinckrodt, Chemical Works, New York), naloxone hydrochloride (by courtesy of Endo Laboratories Inc., New York), methionine enkephalin (by courtesy of Prof. J.C. Schwartz, Paris), copper sulphate and morphine sulphate (Government Opium and Alkaloid Works, Ghazipur, India).

Results

Emetic response to Met-enkaphalin, morphine and naloxone

The results of emetic studies with Met-enkephalin, morphine and naloxone when administered through the lateral and/or the IVth ventricles are summarized in Table 1. Met-enkephalin, when injected into the IVth ventricle, elicited a consistent emetic response in

Table 1 Emetic effect of intraventricularly administered Met-enkephalin, morphine and naloxone in dogs

Emetic agent	*Route*	*Dose* (μg/kg)	*No. of dogs vomited/ tested*	*Latency of vomiting range and average* (s)	*% emetic response*
Met-enkephalin	IVth ventricle	0.1	0/2		0
		0.5	4/8	35–50 (40)	50
		1.0	8/8	30–50 (35)	100
		2.5	11/11	30–50 (40)	100
Morphine	Lateral ventricle	0.5	1/3	213 (213)	33
		1.0	2/3	170–172 (171)	66
		2.5	11/11	161–183 (172)	100
		5.0	13/13	153–177 (165)	100
	IVth ventricle	0.1	0/2	—	0
		0.25	1/2	105 (105)	50
		0.5	5/5	70–80 (75)	100
		1.0	2/2	60–75 (70)	100
Naloxone	Lateral ventricle	10.0	0/3	—	—
		20.0	2/9	258–268 (263)	22
		40.0	1/4	253 (253)	25
		80.0	5/9	179–202 (192)	55
		160.0	7/7	170–205 (182)	100

a dose of 1.0 μg/kg with an average latency of 35 s. Following administration into the lateral and IVth ventricle, morphine (2.5 and 0.5 μg/kg respectively) elicited a consistent emetic response. The average latent periods were 172 s and 75 s respectively. Naloxone elicited 100% emetic response in a dose of 160 μg/kg given i.c.v., although doses of naloxone up to 10 μg/kg produced no emetic response.

The animals became sedated and less responsive to external stimuli following morphine or Met-enkephalin. Other effects included respiratory depression, shivering and panting. Naloxone in the doses tested did not induce any significant behavioural changes.

Effect of bilateral ablation of the chemoreceptor trigger zone

A dose of Met-enkephalin that normally was 100% effective in producing emesis when given into the IVth ventricle was without effect in 10 CTZ-ablated dogs; five times this dose also failed to produce emesis in 5 other CTZ-ablated dogs (Table 2). Similarly, morphine when injected into the lateral ventricle of CTZ-ablated dogs failed to induce emesis even when twice the 100% effective emetic dose was given. Morphine in doses up to 10 times the 100% effective emetic dose failed to induce emesis in CTZ-ablated dogs when injected into the IVth ventricle. On the other hand, naloxone (160 μg/kg) was able to elicit emesis in all 5 CTZ-ablated dogs.

Effect of naloxone pretreatment on the Met-enkephalin-, morphine- and apomorphine-induced emetic response

In order to test the receptor specificity of the emetic action of Met-enkephalin, morphine and apomor-

Table 2 Effect of chemoreceptor trigger zone ablation on the emetic action of Met-enkephalin, morphine and naloxone

Emetic agent	*Route*	*Dose* (μg/kg)	*No. of dogs tested*	*No. of dogs vomited*	*% response*
Met-enkephalin	IVth ventricle	1.0	10	0	0
		2.5	5	0	0
		5.0	5	0	0
Morphine	Lateral ventricle	2.5	3	0	0
		5.0	7	0	0
	IVth ventricle	0.5	3	0	0
		1.0	3	0	0
		5.0	3	0	0
Naloxone	Lateral ventricle	160.0	5	5	100

Table 3 Blockade of emesis by intraventricular naloxone in dogs

Naloxone pretreatment Route & dose (μg/kg)	*Emetic agent*	*Route*	*Dose* (μg/kg)	*No. of dogs vomited/tested*	*% response*	*% protection*
IVth ventricle 1.0	Met-enkephalin	IVth ventricle	1.0	0/5	0	100
IVth ventricle 1.0		IVth ventricle	5.0	1/3	33	67
Lateral ventricle 2.0	Morphine	Lateral ventricle	2.5	3/6	50	50
Lateral ventricle 4.0		Lateral ventricle	2.5	2/7	28	72
Lateral ventricle 8.0		Lateral ventricle	2.5	0/6	0	100
Lateral ventricle 8.0		Lateral ventricle	5.0	0/4	0	100
Lateral ventricle 1.0	Apomorphine*	Lateral ventricle	0.2	4/4	100	0
Lateral ventricle 2.0		Lateral ventricle	0.2	3/3	100	0
Lateral ventricle 4.0		Lateral ventricle	0.2	5/5	100	0
Lateral ventricle 8.0		Lateral ventricle	0.2	4/4	100	0

*Only those dogs were included in the study which showed at least two consecutive positive emetic responses to 0.2 μg apomorphine administered i.c.v. before pretreatment with naloxone.

phine, the antagonistic effect of the opioid antagonist, naloxone, was studied. Central naloxone pretreatment with small non-emetic doses, given 15 min before the emetic agents protected the animals against the emetic action of both Met-enkephalin and morphine. Even twice and five times the 100% emetic doses of Met-enkephalin and morphine respectively failed to elicit the emetic response after naloxone pretreatment. However, naloxone failed to protect against apomorphine-induced emesis (Table 3).

Discussion

From the results of the present study it is clear that administration of Met-enkephalin into the fourth ventricle of the dog induced emesis with a very short latency of 35 to 40 s. The latency of emesis with morphine by the same route was much longer. The 100% emetic dose of Met-enkephalin required for this route was found to be 1.0 μg/kg. Furthermore, in dogs with their CTZ ablated, five times this 100% emetic dose of Met-enkephalin failed to evoke emesis. These results demonstrate the central action of Met-enkephalin on the emetic CTZ. Since small doses of naloxone (8.0 μg/kg) successfully prevented the emesis evoked by exogenous Met-enkephalin injected into the IVth ventricle, it appears that there are specific opiate (enkephalin) receptors in the emetic CTZ. Furthermore, Atweh & Kuhar (1977), employing receptor autoradiographic techniques, have suggested the presence of opiate receptors in the area postrema, which is the anatomical site of the chemoreceptor trigger zone.

Enkephalins have been located in brain neurones and there is a dense population of these neurones in the brain stem region (Johansson *et al.*, 1978). Enkephalins have also been detected in the cerebrospinal fluid (Terenius & Wahlstrom, 1975) and are thus readily accessible to the superficially located CTZ in the area postrema on the floor of the IVth ventricle.

Catlin, George & Li (1978) observed a typical emetic response to low doses of intravenous human β-endorphin which was blocked by prior administration of naloxone. The CTZ is a periventricular structure which is readily accessible from the cerebrospinal fluid as well as the blood stream. It is our contention that endorphin-induced emesis arises from stimulation of enkephalin receptors in the CTZ.

Hatcher & Weiss (1923) demonstrated emesis by direct application of morphine to the floor of the IVth ventricle. Wang & Glaviano (1954) found that parenteral or oral morphine was totally ineffective in evoking emesis in CTZ-ablated dogs. Bhargava, Gupta & Chandra (1961) reported that extremely low doses of apomorphine elicited emesis when injected into the lateral ventricle and CTZ ablation rendered the dogs refractory. Similarly, ablation of the CTZ eliminated the emetic response to i.c.v. apomorphine and morphine in cats (Costello & Borison, 1977). These workers found that the anti-emetic dose requirement of morphine was less than that required to evoke emesis. Moreover, morphine continued to exert its anti-emetic effects in CTZ ablated animals. They contend that while the emetic action of morphine is at the CTZ, the anti-emetic effect is through inhibition of a synaptic mechanism at the deeper lying vomiting centre in the reticular formation.

In the present study, the 100% emetic dose of morphine injected into the lateral ventricle was 2.5 μg/kg. The latency of the response was 2 to 3 min. However, only 0.5 μg/kg of morphine was required to elicit a consistent emetic response when injected into the IVth ventricle and the latency of the response was reduced to approx. 1 min. The emetic responses to intraventricular morphine were completely blocked by prior ablation of CTZ. Ten times the 100% emetic dose of morphine injected into the IVth ventricle failed to evoke emesis in CTZ ablated dogs. Pretreatment of the animals with i.c.v. naloxone (8.0 μg/kg) also afforded full protection against the emetic effects of i.c.v. morphine. However, naloxone failed to afford any protection against i.c.v. apomorphine-induced emesis. These results suggest that although the CTZ is the site of emetic action of morphine and apomorphine, the receptors for the two drugs are different. Morphine and morphine-like drugs are believed to act on μ-type opiate receptors which are blocked selectively by naloxone (Kosterlitz & Leslie, 1978). In the present study the emetic effects of Met-enkephalin and morphine (unlike apomorphine) were also selectively blocked by naloxone. We believe that morphine induces emesis by an action on the enkephalin receptors in the emetic trigger zone and apomorphine may be acting on dopamine receptors in the CTZ. There is already evidence in support of histamine receptors in the chemoreceptor trigger zone (Bhargava & Dixit, 1968; Bhargava, Dixit & Palit, 1976). Thus, there may be a variety of receptors in the CTZ.

Naloxone, as discussed above, prevented the emesis induced by Met-enkephalin and morphine, in a dose of 1 to 8 μg/kg (see Table 3). Higher doses of naloxone, on the other hand, elicited an emetic response and the 100% emetic dose was found to be 160 μg/kg. The latency of the emetic response was about 3 min. Similar results have been obtained by Costello & Borison (1977) in the cat. Since ablation of the CTZ did not afford any protection against naloxone-induced emesis, an agonist action of naloxone (high doses) on the receptors in the CTZ can be excluded as the site of emetic action in dogs. The site of action of naloxone-induced emesis remains unexplored.

The significance of enkephalin-receptors in the emetic CTZ lies not only in the emetic response to opiates; they may be playing a key-role in emesis as-

sociated with various states where the levels of enkephalin in the CNS may be elevated. It would be interesting to estimate the levels of enkephalin in the CSF obtained from patients who exhibit vomiting as an important symptom, arising, for example, from intracranial space-occupying lesions.

References

ATWEH, S. & KUHAR, M.J. (1977). Autoradiographic localization of opiate receptors in rat brain II. The brain stem. *Brain Res.*, **129**, 1–12.

BHARGAVA, K.P. & DIXIT, K.S. (1968). Role of the chemoreceptor trigger zone in histamine-induced emesis. *Br. J. Pharmac. Chemother.*, **34**, 508–513.

BHARGAVA, K.P., DIXIT, K.S. & PALIT, G. (1976). Nature of histamine receptors in the emetic chemoreceptor trigger zone. *Br. J. Pharmac.*, **57**, 211–213.

BHARGAVA K.P., GUPTA, P.C. & CHANDRA, O. (1961). Effect of ablation of the chemoreceptor trigger zone (CT-Zone) on the emetic response to intraventricular injection of apormorphine and emetine in the dog. *J. Pharmac. exp. Ther.*, **134**, 329–331.

BORISON, H.L., FISHBURN, B.R., BHIDE, N.K. & MCCARTHY, L.E. (1962). Morphine induced hyperglycaemia in the cat. *J. Pharmac. exp. Ther.* **138**, 229–235.

BORISON, H.L. & WANG, S.C. (1951). Locus of the central emetic action of cardiac glycosides. *Proc. Soc. exp. Biol. Med.*, **76**, 335–338.

CATLIN, D.H., GEORGE, R. & LI, C.H. (1978). Endorphines: Pharmacologic and behavioral activity in cats after low intravenous doses. *Life Sci.*, **23**, 2147–2154.

COSTELLO, D.J. & BORISON, H.L. (1977). Naloxone antagonizes narcotic self blockade emesis in the cat. *J. Pharmac. exp. Ther.*, **203**, 222–230.

GYLYS, J.A., DORAN, K. & SZARAZ, M. (1974). Antagonism of morphine and apomorphine in dogs and rats. *Fed. Proc.*, **33**, 515.

HATCHER, R.A. & WEISS, S. (1923). Studies on vomiting. *J. Pharmac. exp. Ther.*, **22**, 139–193.

HUGHES, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88**, 295–308.

JOHANSSON, O., HOKFELT, T., ELDE, R.P., SCHULTZBERG, M. & TERENIUS, L. (1978). Immunohistochemical distribution of enkephalin neurons. pp. 51–70. In *Advances in Biochemical Psychopharmacology* Vol. 18, ed. Costa E. Trabucchi, M. New York: Raven Press.

KOSTERLITZ, H.W. & LESLIE, F.M. (1976). Comparison of the receptor binding characteristics of opiate agonists interacting with μ- or κ-receptors. *Br. J. Pharmac.*, **64**, 607–614.

SIMANTOV, R. & SNYDER, S.H. (1976). Morphine like peptides in mammalian brain. Isolation, structure, elucidation and interaction with the opiate receptor. *Proc. natn. Acad. Sci.* U.S.A. **73**, 2515–2519.

TERENIUS, L. & WAHLSTROM, A. (1975). Morphine like ligand for opiate receptor in human CSF. *Life Sci.*, **16**, 1759–1764.

WANG, S.G. & GALVIANO, V.V. (1954). Locus of emetic action of morphine and hydergine in dogs. *J. Pharmac. exp. Ther.*, **111**, 329–334.

(Received June 4, 1980. Revised July 30, 1980.)