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Attenuation of the morphine withdrawal syndrome by inhibition of catabolism of endogenous enkephalins in the periaqueductal gray matter

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Summary. We have investigated the effects of the local administration into the periaqueductal gray matter of thiorphan, a selective inhibitor of endopeptidase 24.11 "enkephalinase", kelatorphan, (R)-3-(N-hydroxy-carboxamido-2-benzylpropanoyl)-L-alanine, and RB 38 A, (R)-3-(N-hydroxycarboxamido-2-benzylpropanoyl)-L-phenylalanine, two almost complete inhibitors of enkephalin metabolism, on the naloxone-precipitated morphine withdrawal syndrome in rats.

Local administration of these inhibitors decreased the severity of the withdrawal syndrome. Jumping, chewing, diarrhea, piloerection, salivation and hypothermia were decreased by all drugs. Lacrimation and weight loss were reduced by kelatorphan and RB 38 A whereas teeth chattering, tremor, eye twitch and rhinorrhea were decreased only by RB 38 A. The rise in plasma corticosterone levels was only slightly reduced by the three inhibitors. Wet dog shakes and ptosis remained unchanged.

These results indicate that during the morphine withdrawal syndrome in rats there is a tonic or/and naloxone evoked release of opioid peptides, presumably enkephalins, into the periaqueductal gray matter and that inhibition of their degradation strongly decreases the severity of the withdrawal syndrome.

Key words: Morphine withdrawal – Periaqueductal gray matter – Enkephalin catabolism inhibitors – Kelatorphan – Thiorphan

Introduction

The periaqueductal gray matter (PAG) plays an important role in multiple responses mediated by the opioid systems including catatonia (Jacquet and Marks 1976), hyperreactivity (Jacquet et al. 1977), immunosuppression (Weber and Pert 1989), analgesia (Sharpe et al. 1974; Jacquet and Lajtha 1976; Yaksh et al. 1976) and physical dependence (Laschka et al. 1976). The implication of this area in the development of the opioid dependence has been demonstrated first by the precipitation of a strong withdrawal syndrome by administering opiate antagonists into the PAG (Laschka et al. 1976), and secondly, by the severe physical dependence observed after the chronic administration of enkephalin analogs (Wei 1981) and morphine (Bozart and Wise 1984) in this area. Autoradiographic studies have shown much higher densities of the μ-opioid receptor subtype than the δ-subtype in the PAG (Quirion et al. 1983; Waksman et al. 1986). A high concentration of opioid peptides (Hökfelt 1977) and endopeptidase 24.11 "enkephalinase" (NEP) (Waksman et al. 1986) has also been found in this area. The NEP and the aminopeptidase N (APN), an enzyme homogeneously distributed in the central nervous system (Gros et al. 1986), are the two main enzymes responsible of the in vivo degradation of the enkephalins (Roques 1991).

Several inhibitors of the enkephalin degrading enzymes, selective of one enzyme or mixed, have been designed (reviews in Roques and Fournié-Zaluski 1986; Thorsett and Wyvratt 1987; Fournié-Zaluski 1988). These compounds have been shown to protect in vitro and in vivo enkephalins from catabolism (Waksman et al. 1985; Bourgin et al. 1986) and to elicit pharmacological properties deriving from this protection (Roques et al. 1980; Fournié-Zaluski et al. 1984; Dickenson et al. 1987; Roques and Beaumont 1990; Schmidt et al. 1991). The administration into the lateral ventricle of specific NEP inhibitors, thiorphan and phosphoramidon (Dzoljic et al. 1986; Haffmans and Dzoljic 1987), or complete inhibitors of catabolism of enkephalin, kelatorphan ((R)-3-(N-hydroxycarboxamido-2-benzylpropanoyl)-L-alanine) and RB 38 A ((R)-3-(N-hydroxycarboxamido-2-benzylpropanoyl)-L-phenylalanine) (Maldonado et al. 1989), were shown to minimize the severity of the morphine withdrawal syndrome in rats. The intensity of this syndrome was also reduced by the peripheral administration of acetorphan, prodrug of thiorphan (Livingston et al.

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1988). These results show that an increase in opioid receptor occupation by endogenous opioid peptides, protected from peptidase-induced inactivation is able to modulate the severity of the opiate withdrawal syndrome.

The endogenous opioid peptides present in the PAG may play an important role in some physiological functions (Millan et al. 1987). Indeed, an antinociceptive response in thermal tests was observed after the injection of SCH 32615, selective inhibitor of the NEP, into the PAG suggesting that this thermal stimulus leads to a tonic release of enkephalins that may modulate nociceptive processing (Al-Rodhan et al. 1990).

In our study, the possible tonic release of enkephalins into the PAG during naloxone precipitated morphine withdrawal syndrome in rats has been investigated. The effect of the injection into the PAG of thiorphan, kelatorphan or RB 38 A on the naloxone-precipitated morphine withdrawal syndrome has been compared by measuring corticosterone levels in plasma, weight loss, colonic temperature and behavioral changes.

Materials and methods

Animals and surgery. One hundred and twenty male Sprague-Dawley rats (Depré), ranging in weight from 200 to 220 g at the beginning of the study, were used. Animals were anaesthetized with ketamine (100 mg/kg i.p.) and bilateral stainless-steel cannula guides (25 gauge) were stereotaxically implanted 1.5 mm above the PAG. The cannulae were secured to the skull with stainless-steel screws and dental cement. The cannula-guides were kept clear with wire stylets. The coordinates, according to Paxinos and Watson (1982) were: 8.1 posterior to the bregma; lateral: $\pm$ 0.5 mm; vertical: 5.7 mm from the skull. After surgery, the animals were housed in cages with water and food made available ad libitum.

Induction of physical dependence. One week after surgery the rats were divided into 8 groups ($n = 15$) corresponding to morphine treatment groups (4) and saline control groups (4). Saline and morphine hydrochloride were injected s.c. twice daily, in a volume of 2.5 ml/kg. The morphine dose was progressively increased from 8 mg/kg to 45 mg/kg over a period of 5 days. The first and second number inside the parenthesis represent the dose of morphine (mg/kg) injected at 9 a.m. and 6 p.m., respectively on consecutive days: 1st day (8, 15), 2nd day (20, 25), 3rd day (30, 35), 4th day (40, 45), 5th day (45; only at 9 a.m.).

Morphine withdrawal. Withdrawal syndrome was precipitated by injection of naloxone hydrochloride (5 mg/kg, s.c., in a volume of 2.5 ml/kg) two hours after the final morphine administration.

Immediately after naloxone injection, animals were placed individually into test chambers consisting of round boxes (30 cm diameter $\times$ 35 cm height and withdrawal) signs were evaluated during a 30 min period. Intensity of witdrawal was evaluated by measuring several classical signs of morphine abstinence (Bläsig et al. 1973; Neal and Sparber 1986).

Two classes of signs were distinguished: counted and checked signs. Jumping, wet dog shakes, teeth chattering and chewing were counted. Ptosis, diarrhea, tremor, eye twitch, rhinorrhea, lacrimation, piloerection and salivation were evaluated over 10 min periods with one point being given for the presence of each sign during each period (maximum score: 3).

Colonic temperature was determined by inserting a lubricated temperature probe 6 cm into the rectum of rats. The temperature was recorded 2 min later using a thermometer (Model TE 3, Ellab instruments, Carrieri, Paris, France.

Body weight and colonic temperature were determined two min before, and 30 and 60 min after naloxone injection.

65 min after naloxone injection rats were decapitated and blood was collected into test tubes for assay of corticosterone.

Corticosterone assay. Corticosterone was measured by radioimmunoassay using an antiserum raised in rabbits against corticosterone coupled to bovine serum albumin by the mixed anhydride method of Erlanger et al. (1957) and using {1,2,6,7-3H} corticosterone (Amersham, Les Ulis, France) as radiolabelled ligand.

Briefly, 100 µl of rat serum were added to 100 µl of radiolabelled corticosterone (5000 cpm) and 800 µl of assay buffer (0.05 M sodium phosphate pH 7.4 containing 0.1% gelatine). After extraction with 10 ml of cyclohexane/ethylacetate (50:50, v/v), the dried extracts were dissolved in 6 ml of assay buffer. 500 and 200 µl of extracts were directly assayed in duplicate by adding 100 µl of diluted antiserum (1/1800e), 100 µl of tritiated corticosterone (10000 cpm) and assay buffer to a final volume of 700 µl. The standard curve ranged from 15 to 2000 pg/tube of corticosterone. After 16 h incubation at 4°C, bound and free fractions were separated by adding 500 µl of a dextran-coated charcoal suspension followed by centrifugation. Bound fractions were added to 10 ml of scintillation liquid and counted in a LKB liquid scintillation counter. Corticosterone concentrations were calculated by interpolation from the standard curve and corrected for experimental losses during extraction. The method had a sensibility of 7 pg/tube and intra and inter assay c.v. of 4.7 and 6.8% respectively.

The antiserum used had a low cross-reactivity with most steroids, as controlled according to Abraham (1974): cortisol 0.005%; 11-deoxycortisol 0.005%; 21-deoxycortisol 0.18%; 11-deoxycorticosterone 5%; 21-deoxycorticosterone 0.16%; progesterone 0.002%; 17-hydroxyprogesterone, pregnenolone and 17-hydroxypregnenolone $< 0.001\%$.

Injection into the PAG. Bilateral administration into the PAG was performed with an injection apparatus consisting of 30.5 gauge stainless-steel needles attached to a 2 µl microsyringe (Hamilton, Reno, Nev., USA) by polyethylene tubing. All drugs were dissolved in saline (0.9% NaCl). Thiorphan (20 µg), kelatorphan (6.4 µg), RB 38 A (2.4 µg) and control solutions were administered into the PAG by an infusion pump (Precinorm, Bottmingen, FRG) in a constant volume of 1 µl (0.5 µl per side) at a rate of 0.0083 µl/s, 15 min before the naloxone injection. The needle was left in situ for 30 s to allow diffusion of the drug away from the cannula guide. Before each injection, the cannula guides were cleared of debris with a dental needle cut square to the exact length of the guide.

The doses used in this study were calculated from the analgesic ED_{50} of each compounds.

Drugs. The inhibitors of enkephalin-degrading-enzymes, thiorphan (Roques et al. 1980), kelatorphan (Bouboutou et al. 1984) and RB 38 A (Xie et al. 1989), were synthesized in our laboratory as described. Naloxone HCl was obtained from Sigma laboratories, St. Quentin, Fallavier, France.

Histology. Following completion of the experimental sequence, the animals were deeply anaesthetized and 0.1 µl of 1% Evans blue solution was injected into the microinjection site. After decapitation, the brain was removed and frozen. Coronal sections (100 µm thick) of the brain were cut on a cryostat at -17°C and stained with cresyl violet. Representative sections showing the deepest site were then compared to standard stereotaxic plates (Paxinos and Watson 1982; Fig. 1). At this time the site was assigned as being within or outside the PAG.

Statistical analysis. Changes in colonic temperature and weight loss were analyzed using repeated measures analysis of variance. The factors of variation were group and time. If a significant effect of treatment or interaction with treatment was observed, one way analysis of variance was used to determine the significance at each time point. Pairwise comparisons were made using the Newman-Keuls test. Plasma corticosterone levels were analyzed using single-factor ANOVA. Individual comparisons were made using the

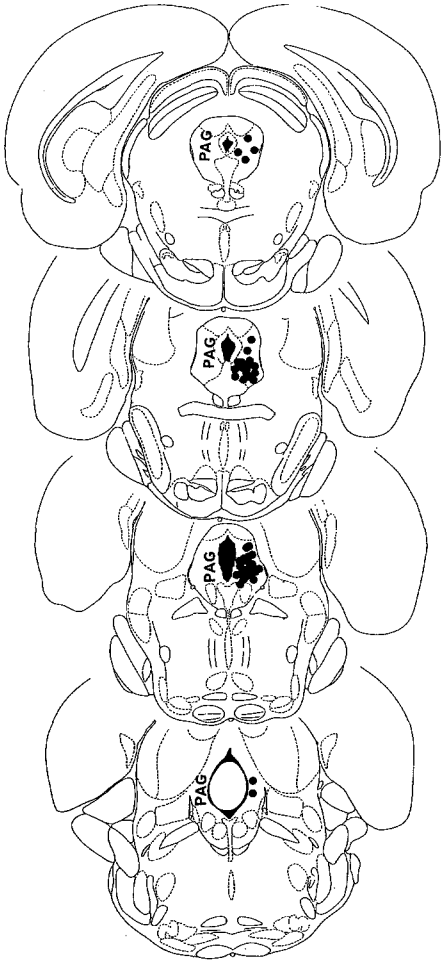

Fig. 1. Representative coronal section based on the atlas of Paxinos and Watson (1982): bregma A: −7.3; B: −7.8; C: −8.3; D: −8.8. Black circles correspond to the final site of injection into the PAG of rats

Newman-Keuls test. Withdrawal behavioral signs were statistically evaluated using the Kruskal-Wallis H test. Comparisons of treatment groups were made using the Mann-Whitney U test.

Results

Behavioral changes

Non-dependent animals. Control animals, which were chronically treated with saline and injected with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG, did not show any behavioral change characteristic of the opiate withdrawal after naloxone (5 mg/kg, s.c.) injection. The administration of the different inhibitors into the PAG induced an increase in locomotor activity in control animals during about 5 min.

Morphine dependent animals. In morphine dependent rats injected into the PAG with saline (1 µl), naloxone administration (5 mg/kg, s.c.) precipitated a withdrawal

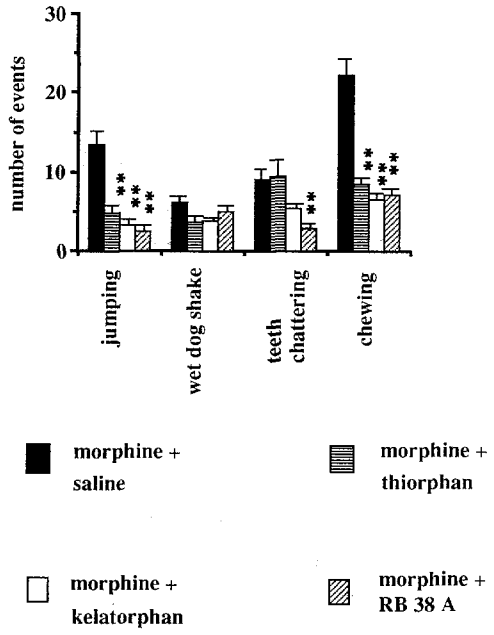

Fig. 2. Counted withdrawal signs after naloxone (5 mg/kg) in rats treated chronically with morphine: effect of pretreatment with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG, 15 min before naloxone. Values are mean ± SEM. Number of animals per group = 15. ** $P < 0.01$: vs morphine + saline group (Mann-Whitney U test)

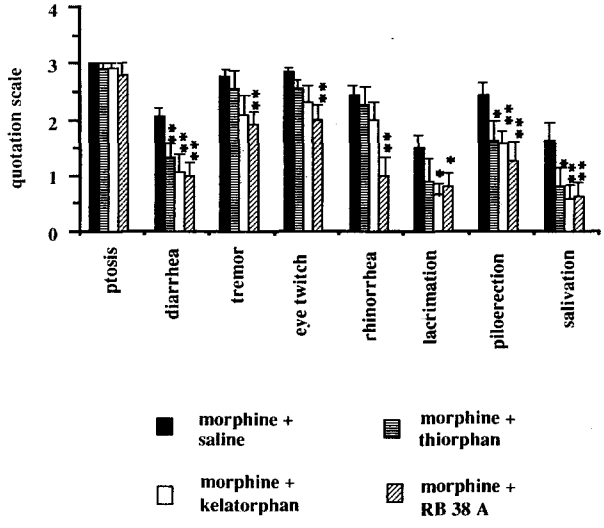

Fig. 3. Checked withdrawal signs after naloxone (5 mg/kg) in rats treated chonically with morphine: effect of pretreatment with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG, 15 min before naloxone. Values are mean ± SEM. Number of animals per group = 15. * $P < 0.05$, ** $P < 0.01$ vs morphine + saline group (Mann-Whitney U test)

syndrome characterized by numerous behavioral changes (Figs. 2 and 3).

Thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) injected into the PAG 15 min before the naloxone administration reduced significantly the severity of naloxone precipitated withdrawal symptoms. Thus jumping, chewing, diarrhea, piloerection and sali-

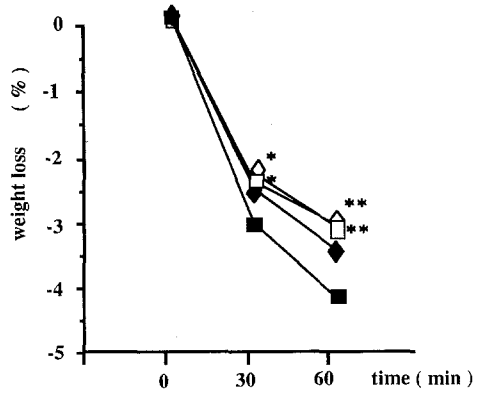

Fig. 4. Values of weight losses before and 30 and 60 min after naloxone (5 mg/kg) in morphine chronically treated rats following different pretreatments (saline 1 µl, thiorphan 20 µg/1 µl, kelatorphan 6.4 µg/1 µl or RB 38 A 2.4 µg/1 µl) into the PAG, 15 min before naloxone. Number of animals per group = 15. Values are means, * $P < 0.05$, ** $P < 0.01$ vs morphine + saline group at the same time interval (Newman-Keuls test). (■) morphine + saline; (◆) morphine + thiorphan; (□) morphine + kelatorphan; (◇) morphine + RB 38 A

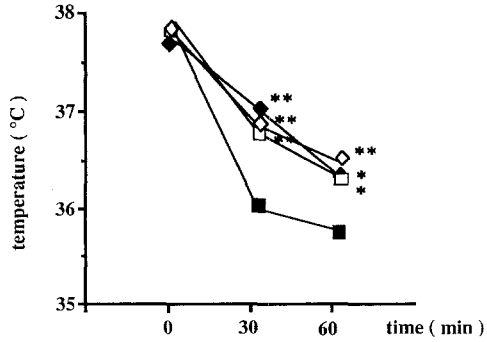

Fig. 5. Values of colonic temperature before and 30 and 60 min after naloxone (5 mg/kg) in morphine chronically treated rats following different pretreatments (saline 1 µl, thiorphan 20 µg/1 µl, kelatorphan 6.4 µg/1 µl or RB 38 A 2.4 µg/1 µl) into the PAG, 15 min before naloxone. Number of animals per group = 15. Values are means; * $P < 0.05$, ** $P < 0.01$ vs morphine + saline group at the same time interval (Newman-Keuls test). (■) morphine + saline; (◆) morphine + thiorphan; (□) morphine + kelatorphan; (◇) morphine + RB 38 A

vation were strongly reduced by the three drugs. Lacrimation was reduced by both kelatorphan and RB 38 A. Furthermore, teeth chattering, tremor, eye twitch and rhinorrhea were also decreased by RB 38 A. Kelatorphan and, with less intensity, thiorphan showed a tendency to reduce these last signs. Only wet dog shakes and ptosis were not modified by the three drugs used (Figs. 2 and 3).

Weight loss

Non-dependent animals. Small weight losses were observed in non-dependent animals after naloxone administration (less than 1% of body weight within 60 min). No significant modification was observed between the different groups injected with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG (data not shown).

Morphine dependent animals. After naloxone administration, morphine dependent rats treated with saline (1 µl) into the PAG lost 4 and 5% of their body weight within 30 and 60 min respectively. These weight losses were significantly reduced by the pretreatment with kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG (Fig. 4). Thiorphan did not induce any significant modification.

Colonic temperature

Non-dependent animals. After naloxone, no significant changes in colonic temperature were observed in non-dependent animals treated with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG (data not shown).

Morphine dependent animals. Dependent rats treated with saline (1 µl) into the PAG showed a hypothermia, measured 30 and 60 min after the naloxone administration. This hypothermia was significantly antagonized by the pretreatment with thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG (Fig. 5).

Table 1. Changes in plasma corticosterone levels (ng/ml) 65 min after naloxone (5 mg/kg) in saline or morphine chronically treated rats following different pretreatments (saline 1 µl, thiorphan 20 µg/1 µl, kelatorphan 6.4 µg/1 µl or RB 38 A 2.4 µg/1 µl) into the PAG, 15 min before naloxone. Values are mean ± SEM. Number of animals per group = 15. ** $P < 0.01$ vs saline chronic group (Newman-Keuls test)

	Chronic treatment	
	Saline	Morphine
Acute treatment		
Saline	119.2 ± 23.8	312.2 ± 12.6**
Thiorphan	117.6 ± 24.0	285.4 ± 12.1**
Kelatorphan	120.4 ± 33.6	276.7 ± 22.1**
RB 38 A	125.3 ± 25.1	280.7 ± 18.5**

Plasma corticosterone levels

Non-dependent animals. In non-dependent rats, the average plasma level of corticosterone 60 min after naloxone administration was similar after pretreatment with saline (1 µl), thiorphan (20 µg/1 µl), kelatorphan (6.4 µg/1 µl) or RB 38 A (2.4 µg/1 µl) into the PAG (Table 1).

Morphine dependent animals. A significant increase in plasma corticosterone levels appeared in morphine dependent animals 60 min after naloxone administration. This increase was not significantly modified by pretreatment with the various inhibitors, although a tendency to reduce the enhanced corticosterone levels was observed with the three drugs (Table 1).

Discussion

In the present study, the local administration of different inhibitors of the enkephalin catabolism into the PAG was shown to minimize the severity of the naloxone-induced morphine withdrawal syndrome in the rat. All the behavioral signs tested (with the exception of wet dog shakes and ptosis) as well as weight loss and hypothermia were significantly reduced. The increase in plasma corticosterone levels observed during the withdrawal syndrome was only slightly reduced. Kelatorphan and particulary RB 38 A were more effective in reducing withdrawal syndrome than thiorphan, in agreement with the biochemical and pharmacological potencies of these compounds (Fournié-Zaluski 1988). Some symptoms as teeth chattering, tremor, eye twitch, rhinorrhea, lacrimation and weight loss were unaffected by thiorphan but significantly decreased by kelatorphan and/or RB 38 A.

Previously, we reported that the administration of the enkephalin catabolism inhibitors into the lateral ventricle decreased the presence of some behavioral signs, hypothermia and increase in plasma corticosterone levels observed during morphine withdrawal syndrome in rats (Maldonado et al. 1989). The reduction observed in the present study after the administration into the PAG was more intense and widespread than the one observed after i.c.v. injection. However, the increase in plasma corticosterone levels and wet dog shake behavior, reduced after i.c.v. injection, were not significantly affected after the local administration into the PAG. Wet dog shake behavior is a complex sign implicating several neurotransmitter systems (Handley and Singh 1986). When the opiate antagonist methylnaloxonium was administered into the PAG in morphine-dependent rats, a very low frequency of wet dog shakes was observed; however, the frequency of this sign was much higher after the injection into the lateral ventricle suggesting that the PAG does not play an important role in the expression of this withdrawal sign (Maldonado et al. 1990 b). Haffmans and Dzoljic (1987) reported a decrease in the frequency of some behavioral signs of morphine withdrawal, including wet dog shakes, after administration of thiorphan into the PAG. The use of a different methodology for inducing morphine dependence and precipitating morphine withdrawal could explain the differences obtained in this study.

It has been reported that increases in plasma corticosterone levels are a sensitive indicator of the severity of withdrawal (Eisenberg and Sparber 1979; Neal and Sparber 1986). In morphine-dependent rats this increase is due to the secretion of hypothalamic peptides inducing an increase in the release of ACTH from the pituitary (Zimmerman et al. 1975). The local administration of enkephalin catabolism inhibitors into the PAG presumably does not exert any influence on this secretion. Moreover, the systemic administration of clonidine, a drug capable of attenuating the withdrawal syndrome, was neither found to have any effect on naloxone-induced rise in plasma corticosterone (Eisenberg 1983).

Histological verifications indicate that the final sites of injection are close to the aqueduct in several animals. However, the possibility that injected compounds are diffusing into the ventricular system and exerting effects outside the PAG seems unlikely. In order to minimize diffusion the injection volume was limited to 0.5 µl per side. This volume has been reported to result in a localized effect (as defined by onset and loss of activity changes in the position of the microinjection of less than 1 mm) after microinjections into the PAG (Yaksh et al. 1976). Moreover, in our study there was not difference between the results obtained in animals in which the injection was performed close or far (1 mm approximately) to the aqueduct. In contrast, the administration of the same enkephalin-catabolism inhibitors into the ventricular system induced a less intense effect on morphine withdrawal syndrome (Maldonado et al. 1989) than the one observed in our study.

Changes observed after the administration of the inhibitors into the PAG must be interpreted as a decrease in the severity of the morphine withdrawal syndrome. Indeed, all the inhibitors tested induced a strong decrease in the number of jumps, a sign which has been designated as a "dominant" behavioral response directly related to the severity of the abstinence (Marshall and Weinstock 1971; Bläsig et al. 1973; Williams and Thorn 1984). This behavior constantly increases when dependence becomes higher or the doses of precipitating antagonists are increased (Bläsig et al. 1973). Other signs well-correlated with the severity of withdrawal as teeth chattering, weight loss and hypothermia (Bläsig et al. 1973; Williams and Thorn 1984; Maldonado et al. 1990a) were also found significantly reduced. Ptosis and wet dog shakes, which were unaffected by the inhibitors, are considered in earlier studies as "recessive" signs (Bläsig et al. 1973) with poor relevance with the severity of the withdrawal syndrome (Bucket 1964; Williams and Thorn 1984).

The decrease in the severity of morphine abstinence syndrome was presumably induced by the increase of the opioid occupation by endogenous peptides released into the PAG and protected from their inactivation by the peptidase inhibitors. These endogenous peptides might inhibit the binding to opioid receptors of the competitive antagonist naloxone, that was administered to precipitate the morphine withdrawal syndrome. Due to the selective involvement of NEP and APN in the inactivation of Met^5- and Leu^5-enkephalins and of Met^5-enkephalin-Arg-Phe (Turner et al. 1985), it appears likely that these peptides released in the PAG are responsible of the reduction in naloxone evoked withdrawal syndrome. Accordingly, these inhibitors were shown to reduce in vivo binding of [^{3}H]diprenorphine (Meucci et al. 1989) and to increase the Met^5-enkephalin content of mouse striatum (Zhang et al. 1982) and the amounts of endogenous Met^5-enkephalin extracellularly released from the rat spinal cord in vitro and in vivo (Bourgoin et al. 1986). Several non-opioid peptides, as substance P, cholecystokinin and neurotensin, are also degraded by NEP and/or APN in vitro (review in Roques et al. 1991), and are present in both terminals and cell bodies in the PAG (Malick and Goldstein 1978; Jurna and Zetler 1981; Behbehani and Pert 1984; Rosén and Brodin 1989). It cannot be excluded that some of these non-opioid peptides participate in the

anti-withdrawal effects observed after the injection of the peptidase inhibitors into the PAG. However, several findings suggest that the participation of these peptides is not important. Thus the level of substance P in cerebrospinal fluid did not change 30 min after selective NEP administration whereas level of enkephalin was 9-fold increased (Yaksh et al. 1991). Likewise, brain microdialysis studies report no modification of extracellular concentration of cholecystokinin in the rat striatum after administration of bestatin and phosphoramidon (Butcher et al. 1989). Moreover, the effects of these peptides usually are opposite to opioid peptides (Faris et al. 1982; Kalivas et al. 1984; Vaught 1988).

The higher efficacy of the mixed inhibitors in reducing withdrawal indicate that the peptides released in the PAG are metabolized by several peptidases. Mixed inhibitors have also been observed to yield stronger analgesic (Fournié-Zaluski et al. 1984; Dickenson et al. 1987) and behavioral effects (Maldonado et al. 1989) after their i.c.v. administration, than selective NEP or APN inhibitors. This was also reported in electrophysiological studies after their local injection into the locus coeruleus (Williams et al. 1987). The greater efficiency of mixed inhibitors is correlated with studies showing that RB 38 A and kelatorphan are much more potent than thiorphan in protecting in vivo and in vitro enkephalins from degrading enzymes (Waksman et al. 1985; Bourgoin et al. 1986).

Our results indicate that during the morphine withdrawal syndrome there is a tonic or/and naloxone evoked release of some peptides, presumably enkephalins, into the periaqueductal gray matter of rats. Interestingly, electrophysiological studies reported that tonic release of opioid peptides is not observed in the locus coeruleus (Williams et al. 1987), another brain structure classically related with the expression of the physical morphine withdrawal (Aghajanian 1978). The presence of this release of opioid peptides in only some structures implicated in the manifestation of the morphine abstinence may explain the mild physical dependence that is observed after the chronic infusion of high doses of enkephalin catabolism inhibitors; this physical dependence was significantly less severe than the one observed after chronic infusion of opioid agonists (Maldonado et al. 1990a; Roques 1991). In fact, it can be supposed that chronic administration of opioid agonists stimulates opioid receptors in all brain structures where these receptors are present; while with enkephalin catabolism inhibitors this stimulation could occur only in the structures where a tonic or synaptically evoked release of enkephalins exist, such as in the PAG.

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