JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2010, 61, 3, 355-362
www.jpp.krakow.pl

H. JAVELOT[2], M. MESSAOUDI[2], S. GARNIER[2], C. ROUGEOT[1]

HUMAN OPIORPHIN IS A NATURALLY OCCURRING ANTIDEPRESSANT ACTING SELECTIVELY ON ENKEPHALIN-DEPENDENT δ-OPIOID PATHWAYS

[1]Institut Pasteur - Unite de Biochimie Structurale et Cellulaire/URA2185 - CNRS, Paris, France;
[2]ETAP-Ethologie Appliquee - Technopôle de Nancy-Brabois - 13, Vandoeuvre-les-Nancy, France

Human opiorphin protects enkephalins from degradation by human neutral endopeptidase and aminopeptidase-N and inhibits pain perception in various behavioral rodent models of pain *via* endogenous enkephalin-related activation of opioidergic pathways. In addition to pain control, endogenous opioid pathways are also implicated in the modulation of emotion-related behaviors. Thus, we explored the dose-dependent motivational responses induced by opiorphin using the forced swim test, the standard rat model of depression. In addition, to further understand the endogenous events triggered by opiorphin, we investigated the specific involvement of μ- or δ-opioid receptor-dependent pathways. In parallel, the locomotor activity test was used to detect possible sedation or hyperactivity. Here, we report for the first time that at 1-2 mg/kg i.v. doses, opiorphin elicited antidepressant-like effects by activating endogenous δ-opioidergic pathways, since that activation was reversed by the selective δ-opioid antagonist naldrindole (10 mg/kg i.p.). The antidepressive behavioral responses exerted by opiorphin are specific at systemically active doses. Treated-rats did not develop either hypo- or hyper-active responses in a locomotor test or amnesic behavioral response in the passive avoidance rat model. In addition, opiorphin did not induce either anxiolytic-, or anxiogenic-like responses in the conditioned defensive burying test. Taking the data together, we conclude that opiorphin is able to elicit antidepressant-like effects, mediated *via* δ-opioid receptor-dependent pathways, by modulating the concentrations of endogenous enkephalin released in response to specific physical and/or psychological stimuli. Thus, opiorphin or optimized derivatives is a promising single candidate to treat disorders that include both pain and mood disorders, particularly depression.

Key words: *enkephalin-inactivating neutral endopeptidase inhibitor, aminopeptidase-N inhibitor, opioid pathway, depression, learning-memory, locomotion*

INTRODUCTION

Human opiorphin QRFSR-peptide is a physiological dual inhibitor of both Zn-ectopeptidases, neutral endopeptidase (NEP EC3.4.21.11) and aminopeptidase N (AP-N EC3.4.11.2), which are implicated in the rapid inactivation of endogenous circulating opioid agonists, enkephalins, *in vivo*. The discovery of an endogenous inhibitor in humans followed the initial characterization of its functional homolog in rat, sialorphin (1, 2). Enkephalins are released according to the intensity and the nature of painful stimulus in nervous pathways specifically involved in the control of nociception. By increasing the half-life of circulating enkephalins opiophin, at systemically or centrally active doses (1-2 mg/kg, i.v. or 5-10 μg/kg i.c.v.), produces analgesia in rodent models of pain. These models included supraspinally controlled, mechanically-induced acute nociception in a rat model (1), spinally controlled, thermally-induced acute nociception in both rat and mouse models and peripheral chemically-induced acute and inflammatory tonic nociception in a rat model (3) (Rougeot *at al*., unpublished data). Moreover, the analgesic response induced by opiorphin, which requires activation of endogenous μ-opioid pathways, is comparable to that induced by the morphine μ-opioid agonist, in terms of effective doses as well as of analgesic response, delay and intensity. In addition, in contrast to exogenous μ-opioid agonists such as morphine, opiorphin did not develop significant abuse liability or antinociceptive drug tolerance when chronically administered at equi-effective analgesic doses (Rougeot *at al*., unpublished data).

This discovery of an endogenous peptide enhancer of opioid pathways in humans is of major interest from physiopathological and therapeutic points of view. Endogenous human opiorphin appears to intervene in the process of adaptation mediated by enkephalins, which is associated with the regulation of pain transmission and also of emotional homeostatic equilibrium (4-7). This led us to predict that the potentiation by opiorphin of the enkephalinergic pathway might also influence emotional states inducing antidepressive-like behavior.

The endogenous opioid peptides, enkephalins, have a high intrinsic efficacy and a high agonist potency towards both the mu (μ)- and delta (δ)-opioid membrane-bound receptors (8). The development of specific agonists and antagonists for each opioid receptor subtype, as well as of animal models lacking preproenkephalin or each of receptor genes, provided substantial

evidence for the role of endogenous opioid systems, particularly enkephalin-dependent δ-opioid, in regulatory processes of emotion inducing antidepressive-like behavior (9-11). The systemic delivery of a wide variety of δ-opioid selective agonists, *i.e*. (+)BW373U86 and SNC80 (nonpeptidic agonists) or DPDPE and deltorphin II (peptidic agonists), consistently showed an antidepressant-like action profile in the behavioral model of depression, the rat forced swim test (FST), which is mediated by endogenous δ-opioid receptors (11-13). Likewise, the lack of δ-receptors favors the establishment of a depressive-like behavior in mice deficient for this receptor subtype (14). Taken together, these data strongly suggest that the δ-opioid system could provide an important therapeutic target for mood disorders, in particular major depression.

In the present study, we explored the dose-dependent motivational responses induced by opiorphin using the FST paradigm, which has proven its usefulness and efficacy as a rodent model to assess the antidepressant activity of known medications used to treat human depression (15). The tests were performed with reference to imipramine, a serotonin-norepinephrin reuptake inhibitor which is a potent antidepressant used clinically, and to 8-OH-DPAT, a 5-HT1a-receptor agonist. In addition, we investigated the specific involvement of μ- or δ- opioid receptor-dependent pathways in relation to the endogenous events triggered by opiorphin. In parallel, the locomotor activity test was carried out to confirm that opiorphin did not induce side effects, such as sedation or hyperactivity, which could lead to a misinterpretation of the results obtained with the FST model. In the same way, the possible amnesic effect of opiorphin was also evaluated, using the passive avoidance test (16). Finally, we explored the pharmacological responses induced by opiorphin, using the conditioned defensive burying test, to determine its potency on anxiety behavior (17, 18).

MATERIALS AND METHODS

Animals

Male Wistar rats (Harlan, France) weighing 250 to 280 g at the beginning of the experiment were used in this study. After a 7-day acclimatization period, they were weighed and randomly housed according to the treatment groups in a room with a 12 hours alternating light/dark cycle (light on: 9:00 p.m./9:00 a.m.) and controlled temperature (21±1°C) and hygrometry (50±5%). Food and water were available *ad libitum.* Animals were acclimatized to the corresponding experimental apparatus for 2 consecutive days. They were experimentally tested only once.

Behavioral tests, care and euthanasia of study animals were in accordance with guidelines of the European Communities Directive 86/609/EEC and the ASAB Ethical Committee for the use of laboratory animals in behavioral research (Animal Behaviour, 2006; 71: 245-253).

Chemicals

Opiorphin (Genosphere Biotechnologies, France) was dissolved in vehicle solution (55% of PBS 100 mM - 45% of acetic acid 0.01N) and systemically injected *via* tail vein at doses ranging from 0.5 to 2 mg/kg body weight. Imipramine and 8-OH-DPTA (Sigma Chemical, France) was dissolved in physiological saline solution (0.9% sodium chloride) and injected *via* the i.p. route in a dose of 20 mg/kg and in a dose of 0.5 mg/kg, respectively. Naltrindole (δ-opioid antagonist) and CTAP (μ-opioid antagonist) were purchased from Sigma Chemical (France), dissolved in physiological saline solution and administered at 10 mg/kg i.p., 5 min and 1 mg/kg i.v., 10 min before the administration of opiorphin, respectively. All drugs were administered in a volume of 1 ml/kg body weight.

Forced swim test, FST

In the present study, rats were subjected to two sessions spaced 24-h apart during which they were placed individually into a glass cylinder (20 cm in diameter and 50 cm height) filled to 30 cm height with 25°C water and from which they could not escape. At the end of the first 15 min-session (conditioning test on day 1), the rats were withdrawn from water, dried delicately and then treated with the different products according to their treatment group. Then, they were placed in their home cage. The following day the rats were treated 5 hours and 15-30 min before exposure to the second 5 min-test session (swim test), according to their treatment group. For conditioning swim pretest and swim test sessions of the FST, the rat behaviors were video-recorded during the first 5-min using a CCD-TV camera in a poorly lit test room. Results were expressed as means of immobility-time scores ±SEM for n=8 rats, calculated as percentage of the pre-treatment immobility time obtained for each corresponding naive rat during the conditioning pretest session.

The locomotor test

A rectangular experimental cage (25x45x30 cm) with two compartments was used to evaluate the locomotor activity of rats. Experiments were performed during the first phase of the dark period, when the rats were most active (9:00 a.m. to 13:00 p.m). The locomotor activity was evaluated in parallel with FST, 24 hours after the conditioning session and 15-30 min after the second treatment. In the locomotor activity test, the number of rearings (vertical locomotor activity) and the number of passages (horizontal locomotor activity) between the two compartments were video-recorded during a 3 min-test period. The total locomotor activity was defined as the sum of the 2 parameters. Results were expressed as means of locomotor activity score ±SEM for n=8 rats.

The passive avoidance test

The behavioral passive avoidance test was performed according to the method described by Messaoudi *et al*. (18). The avoidance apparatus consisted of two compartments of identical size, one illuminated and one darkened, connected to each other with a vertically sliding door. The floor of the darkened box was equipped with a grid floor connected to a shock-source generator. On the first day (training pretest) the rat was placed in the illuminated box facing from the entrance to the dark box. After entry inside the dark compartment, the door was closed and a 1 mA electric foot shock was applied for 1 sec, then the door was immediately opened allowing the rat to escape from the aversive compartment. Rats were treated with opiorphin or vehicle immediately after the training pretest. On the second day (retention test) the rat was placed according to the pretest conditions except no shock was delivered and the session was videotaped for 3 min. Results are expressed as means of latencies before entering the dark box ±SEM for n=8 rats.

The conditioned defensive burying test

Burying, a natural defensive reaction elicited by aversive stimulation, is part of the rat's natural behavioral repertoire. On the first day, the rat was placed in the test chamber with 5 cm of

fresh bedding on the opposite side of the electric probe, inserted 2 cm above the bedding material and mounted on one end wall of the chamber. The first time the rat touched the probe with its forepaw, one single mild but aversive 2 mA shock was delivered and its behavior was recorded for 3 min (pretest session). On the following day, rats were given opiorphin or vehicle, 15 min before being placed according to the pretest conditions except that no shock was delivered. The amount of time the rats display burying responses (*i.e.*, pushing and spraying the bedding material with snout and forepaws towards and over the shock probe) and the number of approaches towards the probe, retreats away from the probe and head stretching towards the probe, were recorded for 5 min (test session). Global score of anxiety was calculated as the sum of scores for all signs and expressed as mean ±SEM for 8 rats.

Statistical evaluation

The results were expressed as mean ±SEM. The significance of differences between groups was evaluated using the Kruskal-Wallis one-way analysis of variance (KWT) for comparison between several independent variables across the experimental conditions. When a significant difference among the treatments was obtained, the Mann-Whitney *post hoc* test (MWT) was applied to compare each treated group to the control group. The non-parametric Wilcoxon matched pairs test (WT) was used to compare repeated measures in each treatment group. For all statistical evaluations, the level of significance was set at $p<0.05$. All statistical analyses were carried out using the software StatView®5 statistical package (SAS, Institute, Inc., USA).

Abbreviations

hNEP - human neutral endopeptidase; hAP-N - human aminopeptidase-N; FST - forced swim test; KWT - Kruskal-Wallis test; MTW - Mann-Whitney test; WT - Wilcoxon test.

RESULTS

Human opiorphin induces an antidepressant-like effect in the forced swim rat model by activating δ-opioid receptors

The forced swimming test (FST) is one of the most widely used behavioral rodent models for screening, with good reliability and predictive validity, the antidepressant-like activity of drugs (15). During the conditioning swim session the rats experience "helplessness" in the environmental conditions. Twenty-four hours later, during the swim test session after an initial period of swimming and climbing activity, rats adopt a characteristic immobile posture only making movements necessary to keep head above water (floating) reflecting that it has learned that escape is impossible and have given up hope. The duration of floating immobility represents "behavioral despair" and can be reduced by a wide range of clinically active antidepressants.

Using this behavioral rat model, the anti-depressive potency and the mechanism of opiorphin action was investigated. The tests were performed with reference to imipramine (20 mg/kg i.p.) or 8-OH-DPAT (0.5 mg/kg i.p.). Opiorphin, references, or vehicle treatment consisted of three injections: the first immediately after the first FST exposure on day 1, the second and third injection 5 hours and 15 min (30 min for references) before the second FST exposure.

By comparing pre-treatment values obtained during the conditioning pretest session, no significant difference between the immobility duration of the five groups was observed ($p=0.77$ by Kruskal-Wallis (KWT), n=8 rats/group). In contrast, a significant treatment effect on the occurrences of immobility behavior among these groups was revealed during the FST test session ($p=0.0005$ by KWT). Data obtained for vehicle, 8-OH-DPAT and opiorphin-treated (0.5-2 mg/kg i.v.) groups (n=8 rats/group) are expressed as immobility-time scores (means ±SEM), calculated as percentage of the pre-treatment immobility times obtained for each corresponding naive rat during the conditioning pretest session. Thus, as shown in *Fig. 1*, the 100% values represent the preconditioning baseline score.

In this first experimental set, the data revealed that opiorphin decreases the occurrences of immobility during the FST test session in a dose dependent manner, reaching maximal statistical significance at doses of 1 mg/kg i.v. and 2 mg/kg i.v. (*Fig. 1*). In comparison to vehicle-administered controls, the Mann-Whitney test (MWT) indicated that opiorphin treatment resulted in a significant decrease in immobility scores at 1 mg/kg i.v. and 2 mg/kg i.v doses: from 218±42% for vehicle to 69±6% and 58±12%, respectively ($p<0.05$ *vs.* vehicle) whereas tended to produce a decrease in immobility at 0.5 mg/kg i.v. (104±21%,

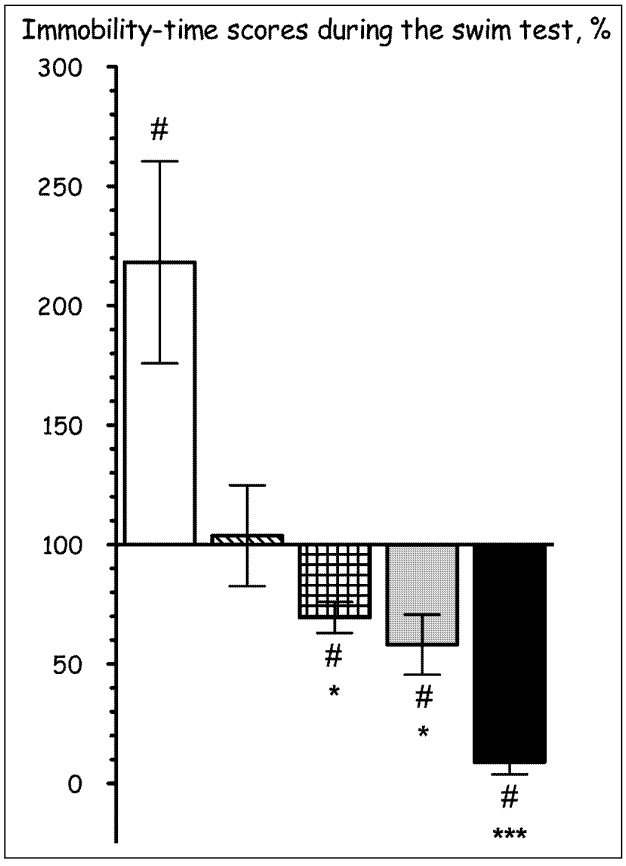

Fig. 1. Human opiorphin displays antidepressant-like effects in the FST rat model. Effects of opiorphin at 0.5 mg/kg i.v. (diagonal-striped bar), 1 mg/kg i.v. (crossed bar) and 2 mg/kg i.v. (grey bar) compared to vehicle (open bar) and 8-OH-DPAT reference (black bar), on the immobility scores during the swim test session. Results are expressed as means of immobility-time scores ±SEM for n=8 rats, calculated as a percentage of the pre-treatment immobility time obtained for each corresponding naive rat during the conditioning pretest session. The Y-axis 100% baseline values correspond to the mean of the immobility scores during the pretest session for 8X5 naive rats (100±11%). Asterisk indicates * $p<0.05$ and *** $p<0.001$ *vs*. vehicle by MWT and # $p<0.05$ *vs*. conditioning pretest by the WT.

p<0.1 *vs.* vehicle). In addition, a comparison between the conditioning pre-test and the test swim sessions revealed that immobility times of the control group are significantly increased in the test swim compared to the corresponding naive rats during the pretest session (from 100±18% to 218±42%, p=0.025 by Wilcoxon test, WT) demonstrating the well-established behavioral adaptation of rats that have already learned that escape is impossible. In contrast, opiorphin treatment induced a significant reduction in immobility scores at 1 mg/kg i.v. dose: from 100±21% to 69±6% (p=0.01 *vs.* conditioning pretest by WT) and at 2 mg/kg i.v. dose: from 100±26% to 58±12% (p=0.02). Thus, the data clearly indicate that opiorphin induces a powerful antidepressant-like effect at 1 mg/kg i.v. and 2 mg/kg i.v. in the rat FST. However, the magnitude of the effect induced by opiorphin is lower than that induced by the 5-HT1a serotonin receptor agonist, 8-OH-DPAT, used as a reference in this first FST experiment (from 100±24% to 9±5%, p=0.01 *vs.* conditioning pretest by WT, *Fig. 1*).

A second series of FST experiments was undertaken in order to define which specific endogenous opioidergic pathway contributes to the antidepressant-like effects induced by opiorphin at 1 mg/kg i.v. dose. As shown in *Fig. 2*, opiorphin again induced a clear and replicable antidepressant-like effect in this second set of assays. The systemic administration of opiorphin resulted in a 3.7-fold decrease in immobility scores relative to vehicle-treated controls during the swim FST session (p=0.0008 *vs*. vehicle by MWT, n=8 rats/group) and in a 1.8-fold decrease in immobility times relative to that of corresponding naive rats during the conditioning pretest session (p=0.017 *vs*. conditioning pretest by WT). Interestingly, the order of magnitude of the antidepressive-like behavioral response induced by opiorphin at 1 mg/kg i.v. was equivalent to that produced by the serotonin-norepinephrine reuptake inhibitor, imipramine (20 mg/kg, i.p.), used as reference in this second FST experiment (1.8-fold decrease on immobility time, p=0.018 *vs*. conditioning pretest by WT, n=7 rats and p=0.91 *vs*. opiorphin by MWT).

The administration of the δ-opioid selective antagonist, naltrindole in combination with opiorphin, totally reversed the antidepressant-like activity elicited by 1 mg/kg i.v. opiorphin to the level of the control group (p=0.0008 *vs*. opiorphin alone by MWT, n=8 rats/group). This strongly implicates the δ-opioid receptor in the observed opiorphin effect. Thus, similarly to vehicle-treated control rats, immobility scores of naltrindole plus opiorphin-treated rats significantly increased between conditioning pretest and swim test sessions (from 100±19% to 213±23% for vehicle, p=0.012 and from 100±12% to 174±25% for naltrindole plus opiorphin, p=0.012 by WT, n=8 rats/group). In contrast, there was no significant difference in immobility scores between the conditioning pretest and the swim test in the CTAP plus opiorphin-treated rats (from 100±12% to 109±39%, p=0.33 by WT, n=8 rats). Therefore, the μ-opioid selective antagonist, CTAP failed to block the antidepressive behavioral response of opiorphin when administered concurrently. Although CTAP plus opiorphin-treated rats displayed significantly lower immobility times compared to the control group (p=0.012 *vs*. vehicle by MWT) the apparent increase in immobility scores relative to the level of the opiorphin-treated group was not significant (p=0.25 *vs*. opiorphin alone by MWT, *Fig. 2*) again suggesting that the endogenous μ-opioid pathway is not preponderant for the activity of opiorphin on the FST. It is important to state that the dose of CTAP used in the present study blocks the analgesic effect of 1 mg/kg i.v. opiorphin (Rougeot *et al*., unpublished data).

In conclusion, the opiorphin-induced potentiation of endogenous enkephalin-dependent δ-opioidergic pathway consistently produces antidepressant-like effects similar to therapeutic antidepressant, such as imipramine, a modulator of endogenous serotoninergic and adrenergic pathways.

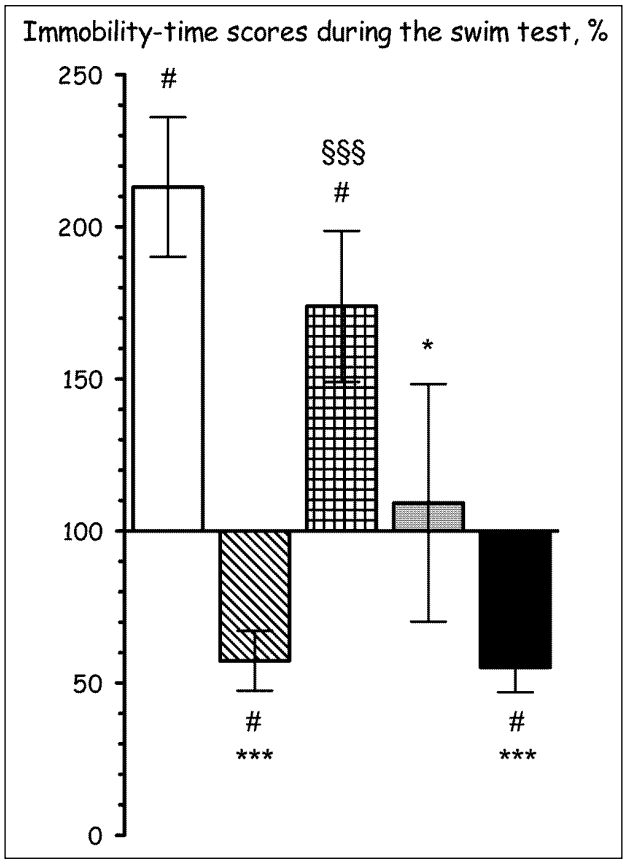

Fig. 2. Human opiorphin displays antidepressant-like effects in the FST model by activating the endogenous δ-opioid pathway. Effects of opiorphin at 1 mg/kg i.v., alone or in the presence of selective μ- or δ-opioid receptor antagonist on the immobility scores during the swim test session. Opiorphin 1 mg/kg i.v. (diagonal-striped bar), opiorphin plus δ-antagonist, naltrindole (crossed bar), opiorphin plus μ-antagonist, CTPA (grey bar) compared to vehicle (open bar) and imipramine reference at 20 mg/kg i.p. (black bar). Results are expressed as means of immobility-time scores ±SEM for n=8 rats, calculated as a percentage of the pre-treatment immobility time obtained for each corresponding naive rat during the conditioning pretest session. The Y-axis 100% baseline values correspond to the mean of the immobility scores during the pretest session for 8X5 naive rats (100±6%). Asterisk indicates * p<0.05 and *** p<0.001 *vs*. vehicle by MWT and $^{\#}$ p<0.05 *vs*. conditioning pretest by WT and §§§ p<0.001 *vs*. opiorphin by MWT.

Human opiorphin administration does not affect locomotion in the locomotor activity test

Certain drugs, notably psychomotor stimulants, which are not effective antidepressants, appear as false positives in the FST when administered systemically. In order to ascertain whether an increase in spontaneous locomotor activity contributes to the anti-immobility effect of opiorphin, we performed locomotion studies in parallel with the FST studies. The locomotor response is defined as the total number of movements measured by photocell beam breaks, *i.e*., rearings reflecting the index of exploratory behavior and horizontal crossing passages between

the two compartments. Studies were conducted in parallel with the FST, except at 24 hours after the first FST session and second treatment, when the rats were placed in automated activity chambers before being re-exposed to swim FST 5 hours later. The effect of opiorphin alone or concurrently with naltrindole or CTAP and vehicle on vertical and horizontal spontaneous locomotor activities (mean counts ±SEM) in rats is shown in *Fig. 3*. Imipramine was used as a reference.

The Kruskal-Wallis test, indicated a significant difference between the five treatment groups (p=0.004, n=8 rats/group). Indeed, the imipramine-treated rats displayed significant reduction in locomotor activity compared with vehicle-treated rats (19±2 *vs*. vehicle 30±3 counts, p=0.008 by MWT). Thus, the behavioral response to imipramine in the FST is not due to a generalized increase in locomotor activity, since imipramine administration, at a dose that was efficacious in the FST, has the opposite effect (decreased activity) on locomotor activity. In contrast, opiorphin, naltrindole plus opiorphin- or CTAP plus opiorphin-treated rats exhibited the same locomotor activity as control rats receiving vehicle (35±3, 28±2, 32±2 counts, p=0.25, p=0.49, p=0.67 *vs*. vehicle, respectively).

Thus, opiorphin does not alter spontaneous ambulatory activity in the locomotor test. Moreover, the results demonstrate that pretreatment with the selective δ-opioid antagonist, naltrindole, blocks the antidepressive effect of opiorphin due to a direct effect and is not linked in anyway to hypoactivity behavior (sedative effect). This contrasts to the undesirable sedative effects of imipramine.

Human opiorphin administration does not result in memory impairment in the passive avoidance test

We investigated the putative emergence of opiorphin-induced cognitive impairment of learning and memory using the passive avoidance test. The test determines the long-term memory potential in rats using an avoidance apparatus. Rats received 1 mg/kg i.v. administration of opiorphin or vehicle immediately after the training pretest and retention of the passive avoidance response was analyzed 24 hours later. The latencies to enter the dark box were recorded during the training pretest (conditioning aversive learning) and 24 hours later, during the retention test, and used as a direct measure of memory long-term retention (*Fig. 4*).

Like vehicle-treated control rats, latencies before entering the dark box of opiorphin-treated rats significantly increased between training pretest and retention test sessions (from 23±5 sec to 171±7 sec for vehicle, p=0.012 and from 25±7 sec to 159±21 sec for opiorphin, p=0.012 by WT, n=8 rats/group) indicating good learning acquisition for both treated groups. Moreover, opiorphin (1 mg/kg i.v.) administered after training pretest did not change the passive avoidance behavior tested 24 hours later, as latency to enter the dark compartment did not differ from vehicle control rats (p=0.99 *vs*. vehicle by MWT).

In conclusion, systemic administration of opiorphin (1 mg/kg) does not affect memory retention in the rat model of passive avoidance test.

Human opiorphin does not induce anxiogenic nor anxiolytic effects in the conditioned defensive burying test

It has been reported that the δ-opioid receptor system can regulate anxiety behaviors in the rat (12), therefore, we investigated the effects of opiorphin on anxiety-related behavior using the conditioned defensive burying test. This pharmacologically robust model detects therapeutic anxiolytic agents using electric shock-inducing anxiety. Only rats displaying a probe-burying activity of at least 25 sec duration during the pretest session were selected for the subsequent anxiety test performed 24 hours later. In the test session the rat did not receive an electric shock but was exposed to anxiety behavioral responses induced by simply viewing the electric probe. The duration of probe-burying *i.e*., pushing and spraying the bedding material with snout and forepaws toward and over the probe, and the number of responses displayed by rats towards the probe *i.e*., approaches, retreats away and head stretching, were recorded for 5 min. These different variables served as measure of a global score of anxiety for each rat receiving intravenous administration of opiorphin (1 mg/kg) or vehicle, 15 min before the test.

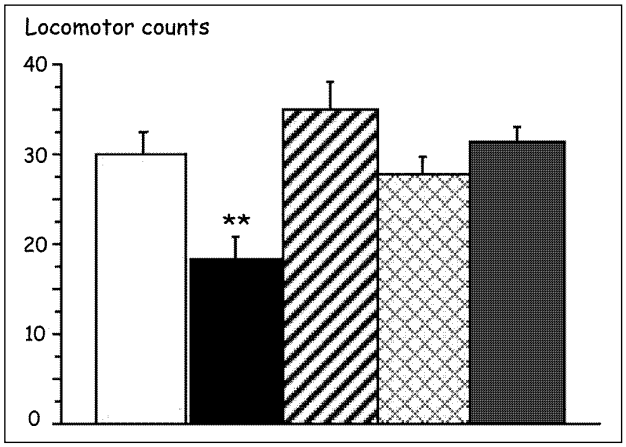

Fig. 3. Human opiorphin does not alter spontaneous ambulatory activity in the locomotor test. Effects of opiorphin at 1 mg/kg i.v., alone or in the presence of selective μ or δ-opioid receptor antagonist on the total number of vertical and horizontal movements over the 3 min period of the locomotor test. Opiorphin (diagonal-striped bar, 1 mg/kg i.v.), opiorphin plus δ-antagonist, naltrindole (crossed bar), opiorphin plus μ-antagonist, CTPA (grey bar) compared to vehicle (open bar) and imipramine reference (black bar, 20 mg/kg i.p.). results are expressed as mean ±SEM for n=8 rats of total locomotor counts. Asterisk indicates ** p<0.01 *vs*. vehicle by MWT.

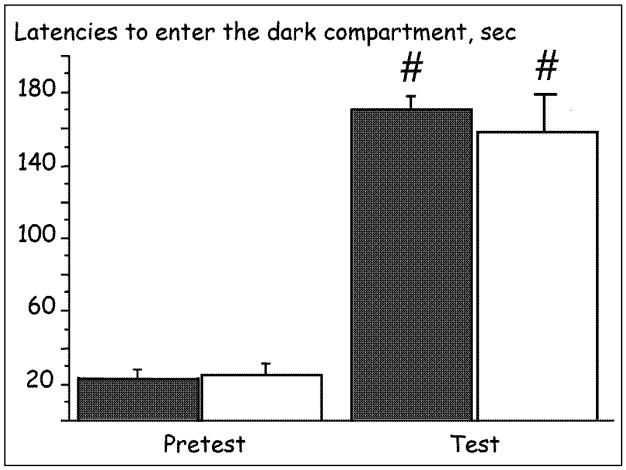

Fig. 4. Human opiorphin does not alter long-term memory retention in the passive avoidance test. Effects of i.v. administration of opiorphin at 1 mg/kg, compared to vehicle, on latency time to enter the black compartment during the test session. The Y-axis values represent the mean ±SEM of latency duration to enter the black compartment (sec) of 8 rats during both the pre-test and test sessions: open bar=vehicle, grey bar=opiorphin at 1 mg/kg i.v. Asterisk indicates # p<0.05 *vs*. pretest by WT.

Opiorphin at 1 mg/kg i.v. dose has no significant effect on defensive burying behaviors. Indeed, opiorphin-treated rats displayed equivalent probe burying responses to vehicle-treated rats: global score of anxiety 26±4 for opiorphin and 25±3 for vehicle, p=0.79 *vs*. vehicle by MWT, n=8 rats/group).

DISCUSSION

The aim of the present study was to investigate the pharmacological functional profile of opiorphin in pathways modulating emotionally motivated behaviors. Opiorphin was previously identified as a natural enkephalin-degrading peptidase inhibitor, the only natural enkephalinase peptide-inhibitor characterized in humans. Using the FST standard model of depression in rat, here we report that at systemic 1-2 mg/kg doses, opiorphin elicits a potent antidepressant-like effect. Collectively, the data also showed that the antidepressive behavioral response induced by opiorphin at 1 mg/kg i.v. dose in FST is abrogated by the δ-opioid receptor selective antagonist naltrindole, demonstrating that the expression of antidepressive-related behavior exhibited by opiorphin requires the endogenous δ-opioid pathways, whose activation is specifically dependent on enkephalins. The antidepressive-like behavioral responses exerted by opiorphin are specific as treated-rats did not develop either hypo- or hyper-active behavioral responses in a locomotor model. From these data we confirm that the anti-immobility effect of opiorphin at 1 mg/kg i.v. in the FST is associated with motivation-enhancing effects and not related to hyperactivity behavior (psychomotor stimulant effect). Furthermore, opiorphin-treated rats did not have impaired learning acquisition and memorization in the passive avoidance rat model. In addition, opiorphin did not induce either anxiolytic- or anxiogenic-like responses in the ethological rat model of conditioned defensive burying test.

These findings are in agreement with those demonstrating that enkephalins protected from rapid enkephalinase inactivation by synthetic dual NEP and APN inhibitors, such as RB-101, also produce antidepressant-like effects in the mouse FST. The effect is reversed by the selective δ-antagonist naltrindole, indicating that endogenous enkephalins produce antidepressant-like effects through specific interaction with the δ-opioid receptor subtype (19, 20). Strikingly, opiorphin showed 30-fold greater antidepressant potency, in terms of dose-effect, than the synthetic inhibitor RB101 (32 mg/kg i.v.) (19).

Interestingly, the antidepressive behavioral response induced by opiorphin at 1 mg/kg i.v. is similar, in terms of magnitude, to that produced by imipramine (20 mg/kg i.p.), a well-established antidepressant and a known serotonin-norepinephrine reuptake inhibitor, that acts by increasing the synaptic availability of these neurotransmitters. Thus, the decrease in resignation-related signs elicited by opiorphin-mediated modulation of enkephalin-dependent δ-opioidergic neurotransmission closely resembles that induced by imipramine-mediated modulation of monoaminergic neurotransmission. In addition, these data provide exciting evidence for the strong therapeutic potential of opiorphin, or functional optimized derivatives, for the treatment of depressive disorders, in particular because at effective doses, opiorphin does not have the undesirable sedative side-effect induced by imipramine. Our results also show that opiorphin does not induce amnesic responses while number of preclinical studies gives evidence for imipramine-induced impairment of memory retention. However, it is important to state that the impairment in psychomotor and memory performances associated with tricyclic antidepressants and/or with depression seems to be of questionable clinical relevance as there is a marked discrepancy between results obtained in preclinical and clinical studies (21-24).

Taking together the data presented here and previous findings (1, 3) (Rougeot *at al*., unpublished manuscript), we conclude that the natural enkephalin-degrading peptidase human inhibitor, opiorphin, by modulating the concentrations of endogenous enkephalins, released in response to specific physical and/or psychological stimuli is able to elicit both analgesic and antidepressive-like responses. Systemically active opiorphin produces analgesic and antidepressive-like effects by triggering the endogenous enkephalin-dependent μ- and/or δ-opioid pathways. Anatomical, neurochemical, and neurophysiological commonalities between chronic pain and chronic affective disorders lead to a striking overlap of pharmacopeia for pain and psychiatric disorders (25). For instance, a range of anti-depressant actions may contribute to the mechanisms by which pain suppression occurs, evidence for the therapeutic intersection of pain and affective disorders (26). Accordingly, the site of actions of serotonin-norepinephrine reuptake inhibitors as tricyclic antidepressants on the dorsal horn of the spinal cord, the center for opioid-mediated analgesia, contribute to their analgesic effects that is blocked by the opioid antagonist, naloxone (26, 27). Moreover, a synergistic antinociceptive interaction between opioid agonists and antidepressant drugs has been proved by number of authors. In particular, Wrzosek *et al.* demonstrated that co-administration of tramadol (μ-opioid analgesic) with doxepin (tricyclic antidepressant) in a rat model of neuropathic pain potently decreases the intensity of thermal hyperalgesia and that the nature of interaction between both drugs is synergistic (28). Conversely, some medications used for neuropathic pain induce strong psychotropic effects and, opioid- and cannabinoid-derived drugs are included in this group. In addition, several lines of evidence support the involvement of the endocannabinoid system in the modulation of nociception and emotional-like responses and the existence of cannabinoid-opioid cross-modulation of their effects (29-32).

There is extensive data supporting the notion that peripheral and spinal μ-opioid pathways are preferentially involved in the control of nociceptive transmission while central δ-opioid pathways are preferentially involved in the control of emotional behavior (7, 9, 33-36). Nevertheless, a functional cross-talk exists between the enkephalin-related μ- and δ-opioid receptors. Delta-opioid agonists have a facilitative role in μ-mediated antinociception while μ-opioid agonists modulate the anti-depressant potency produced by δ-opioid agonists.

In summary, opiorphin, is a systemically active key modulator of both pain perception and motivational adaptation responses to physical and psychological stimuli in humans. Its key integrative role is mediated through enkephalin-related activation of opioidergic pathways.

A striking and important feature of opiorphin is that the effective doses for the antidepressant-like and anti-pain potencies require the same titration, at least in rat models. Long-term pain is often associated with affective disturbances, and the converse is also true, as unrelenting affective disorders frequently amplify the pain sensation (37). When these conditions present simultaneously, treating both should be considered the best practice. In this regard, opiorphin is a very promising single drug agent to treat disorders that comprise both depression and pain.

Structural modifications of opiorphin, to increase its lipophilic character and metabolic stability in order to improve its bioavailability and potency compared to the native peptide, will provide new and exciting therapeutic treatments for mood disorders, in particular depression.

Acknowledgments: The research was funded by the "Agence Nationale de la Recherche" EMPB2005 program.

Conflict of interests: None declared.

REFERENCES

1. Wisner A, Dufour E, Messaoudi M, *et al*. Human opiorphin, a natural antinociceptive modulator of opioid-dependent pathways. *Proc Natl Acad Sci USA* 2006; 103(47): 17979-17984.
2. Rougeot C, Messaoudi M, Hermitte V, *et al*. Sialorphin, a natural inhibitor of rat membrane-bound neutral endopeptidase that displays analgesic activity. *Proc Natl Acad Sci USA* 2003; 100(14): 8549-8554.
3. Tian XZ, Chen J, Xiong W, He T, Chen Q. Effects and underlying mechanisms of human opiorphin on colonic motility and nociception in mice. *Peptides* 2009; 30(7): 1348-1354.
4. Konig M, Zimmer AM, Steiner H, *et al*. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. *Nature* 1996; 383: 535-538.
5. Ragnauth A, Schuller A, Morgan M, *et al*. Female preproenkephalin-knockout mice display altered emotional responses. *Proc Natl Acad Sci USA* 2001; 98(4): 1958-1963.
6. Belluzzi JD, Grant N, Garsky V, Sarantakis D, Wise CD, Stein L. Analgesia induced in vivo by central administration of enkephalin in rat. *Nature* 1976; 260: 625-626.
7. Noble F, Benturquia N, Bilkei-Gorzo A, Zimmer A, Roques BP. Use of preproenkephalin knockout mice and selective inhibitors of enkephalinases to investigate the role of enkephalins in various behaviours. *Psychopharmacology* (*Berl*) 2008; 196(2): 327-335.
8. Raynor K, Kong H, Chen Y, *et al*. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol Pharmacol* 1994; 45(2): 330-334.
9. Nieto MM, Guen SL, Kieffer BL, Roques BP, Noble F. Physiological control of emotion-related behaviors by endogenous enkephalins involves essentially the delta opioid receptors. *Neuroscience* 2005; 135(2): 305-313.
10. Skoubis PD, Lam HA, Shoblock J, Narayanan S, Maidment NT. Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice. *Eur J Neurosci* 2005; 21(5): 1379-1384.
11. Broom DC, Jutkiewicz EM, Folk JE, Traynor JR, Rice KC, Woods JH. Nonpeptidic delta-opioid receptor agonists reduce immobility in the forced swim assay in rats. *Neuropsychopharmacology* 2002; 26(6): 744-755.
12. Saitoh A, Kimura Y, Suzuki T, Kawai K, Nagase H, Kamei J. Potential anxiolytic and antidepressant-like activities of SNC80, a selective delta-opioid agonist, in behavioral models in rodents. *J Pharmacol Sci* 2004; 95(3): 374-380.
13. Torregrossa MM, Jutkiewicz EM, Mosberg HI, Balboni G, Watson SJ, Woods JH. Peptidic delta opioid receptor agonists produce antidepressant-like effects in the forced swim test and regulate BDNF mRNA expression in rats. *Brain Res* 2006; 1069(1): 172-181.
14. Filliol D, Ghozland S, Chluba J, *et al*. Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. *Nature Genetics* 2000; 25(2): 195-200.
15. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 1977; 266: 730-732.
16. Jacob W, Gravius A, Pietraszek M, *et al*. The anxiolytic and analgesic properties of fenobam, a potent mGlu5 receptor antagonist, in relation to the impairment of learning. *Neuropharmacology* 2009; 57(2): 97-108.
17. Craft RM, Howard JL, Pollard GT. Conditioned defensive burying as a model for identifying anxiolytics. *Pharmacol Biochem Behav* 1988; 30(3): 775-780.
18. Messaoudi M, Lalonde R, Schroeder H, Desor D. Anxiolytic-like effects and safety profile of a tryptic hydrolysate from bovine alpha s1-casein in rats. *Fundam Clin Pharmacol* 2009; 23(3): 323-330.
19. Jutkiewicz EM, Torregrossa MM, Sobczyk-Kojiro K, *et al*. Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. *Eur J Pharmacol* 2006; 531(1-3): 151-159.
20. Noble F, Roques BP. Protection of endogenous enkephalin catabolism as natural approach to novel analgesic and antidepressant drugs. *Expert Opin Ther Targets* 2007; 11(2): 145-159.
21. Zarrindast MR, Shamsi T, Azarmina P, Rostami P, Shafaghi B. GABAergic system and imipramine-induced impairment of memory retention in rats. *Eur Neuropsychopharmacol* 2004; 14(1): 9-64.
22. Song L, Che W, Min-Wei W, Murakami Y, Matsumoto K. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacol Biochem Behav* 2006; 83(2): 186-193.
23. Gorenstein C, de Carvalho SC, Artes R, Moreno RA, Marcourakis T. Cognitive performance in depressed patients after chronic use of antidepressants. *Psychopharmacology* (*Berl*) 2006; 185(1): 84-92.
24. Pokorski M, Siwiec P. Depression and memory: a comparative study of young and old women. *J Physiol Pharmacol* 2008; 59(Suppl 6): 573-578.
25. Moskowitz M, Fishman S. The neurobiological and therapeutic intersection of pain and affective disorders. *Focus* 2006; IV(4): 465-471.
26. Berrocoso E, Mico JA. Role of serotonin 5-HT1A receptors in the antidepressant-like effect and the antinociceptive effect of venlafaxine in mice. *Int J Neuropsychopharmacol* 2009; 12(1): 61-71.
27. Berrocoso E, Mico JA. Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *Int J Neuropsychopharmacol* 2009; 12(8): 1033-1044.
28. Wrzosek A, Obara I, Wordliczek J, Przewlocka B. Efficacy of tramadol in combination with doxepin or venlafaxine in inhibition of nociceptive process in the rat model of neuropathic pain: an isobolographic analysis. *J Physiol Pharmacol* 2009; 60(4): 71-78.
29. Berrocoso E, Sanchez-Blazquez P, Garzon J, Mico JA. Opiates as antidepressants. *Curr Pharm Des* 2009; 15(14): 1612-1622.
30. Maldonado R, Valverde O. Participation of the opioid system in cannabinoid-induced antinociception and emotional-like responses. *Eur Neuropsychopharmacol* 2003; 13(6): 401-410.
31. Cichewicz DL. Synergistic interactions between cannabinoid and opioid analgesics. *Life Sci* 2004; 74(11): 1317-1324.
32. Adamczyk P, Golda A, McCreary AC, Filip M, Przegalinski E. Activation of endocannabinoid transmission induces antidepressant-like effects in rats. *J Physiol Pharmacol* 2008; 59(2): 217-228.
33. Matthes HW, Maldonado R, Simonin F, *et al.* Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* 1996; 383(6603): 819-823.
34. Miranda HF, Pinardi G. Lack of effect of naltrindole on the spinal synergism of morphine and non-steroidal anti-inflammatory drugs (NSAIDS). *J Physiol Pharmacol* 2009; 60(2): 71-76.

35. Miranda HF, Silva E, Pinardi G. Synergy between the antinociceptive effects of morphine and NSAIDs. *Can J Physiol Pharmacol* 2004; 82(5): 331-338.
36. Goodchild CS, Nadeson R, Cohen E. Supraspinal and spinal cord opioid receptors are responsible for antinociception following intrathecal morphine injections. *Eur J Anaesthesiol* 2004; 21(3): 179-85.
37. Stahl SM. Does depression hurt? *J Clin Psychiatry* 2002; 63(4): 273-274.

Received: January 4, 2010
Accepted: May 25, 2010

Author's address: Dr. Catherine Rougeot; Institut Pasteur - Unite de Biochimie Structurale et Cellulaire/URA2185 – CNRS, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France; Phone: +33 (0)1 40 61 34 45; Fax: 33(0)1 45 68 83 99; E-mail: catherine.rougeot@pasteur.fr