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**Evidence for an involvement of supraspinal delta and spinal mu opioid
receptors in the antihyperalgesic effect of chronically administered
clomipramine in mononeuropathic rats**

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Abbreviations : CTOP: D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-ThrNH₂; NTI: naltrindole;
Nor-BNI: nor-binaltorphimine; ADs: antidepressants; TCAs: tricyclic antidepressants;
CMI: clomipramine; i.t.: intrathecal; i.c.v.: intracerebroventricular.

Abstract

The mechanisms of involvement of the opioidergic system in the antinociceptive effect of antidepressants remain to be elucidated. The present study was designed to determine what type of opioid receptors may be involved at the spinal and supraspinal levels in the antihyperalgesic effect of clomipramine, a tricyclic antidepressant commonly prescribed in the treatment of neuropathic pain. Its antihyperalgesic effect on mechanical hyperalgesia (paw pressure test) in rats induced by chronic constriction injury of the sciatic nerve was assessed after repeated administrations (5 injections every half-life, a regimen close to clinical use). Naloxone administered at a dose of 1 mg/kg, i.v., which blocks all opioid receptors, or at a low dose of 1 µg/kg, i.v., which selectively blocks the mu opioid receptor, inhibited the antihyperalgesic effect of clomipramine and hence indicated that mu opioid receptor is involved. Depending on whether they are administered by the intracerebroventricular or intrathecal route, specific antagonists of the various opioid receptor sub-types (CTOP = mu; naltrindole = delta and Nor-binaltorphimine = kappa) differently modify the antihyperalgesic effect of chronically injected clomipramine. The effect was inhibited by intrathecal administration of CTOP and intracerebroventricular administration of naltrindole whereas norBNI was ineffective whatever the route of injection. These results demonstrate a differential involvement of opioid receptors according to the level of the central nervous system : delta receptors at the supraspinal level and mu receptors at the spinal level. Clomipramine could act via a neuronal pathway in which these two receptors are needed.

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Antidepressants (ADs) are effective in neuropathic pain treatment (Onghena and Van Houdenove, 1992; Sindrup et al., 1999). Their analgesic effect seems to be independent of their thymo-analeptic action and is greater with tricyclic drugs (TCAs; non specific monoamine reuptake inhibitors) than specific reuptake inhibitors (Onghena and Van Houdenove, 1992). Although ADs have long been used, the mechanism of their analgesic action remains unknown. It probably involves a complex interaction between several neurotransmitter systems.

The psychotropic action of ADs, the ineffectiveness of peripheral analgesics in neuropathic pain, and various clinical and experimental results (see review Eschalier et al., 1999) suggest a predominant central effect even though some papers have also suggested a peripheral site of action (Sawynock et al., 1999).

The most usual hypothesis of mechanism of action is an involvement of the central monoaminergic systems. Their blocking of monoamine reuptake seems to play a major role by activating descending inhibitory pathways. Thus, the antinociceptive effect of various ADs is inhibited by parachlorophenylalanine (an inhibitor of serotonin synthesis), α -methyl-p-tyrosine (an inhibitor of noradrenaline synthesis), and serotonin and noradrenergic receptor antagonists (Yokogawa et al., 2002; Valverde et al., 1994). Likewise, Ardid et al. (1995) reported that the antinociceptive effect of clomipramine was inhibited by lesions of the dorsolateralis funiculus which conveys monoaminergic bulbo-spinal neurons. However, other explanations have been suggested such as an interaction with NMDA receptors (Kiefer et al., 1999), with tachykinin receptors (Iwashita et al., 1992), or with different ion channels (Anthiewicz Michaluk et al., 1991).

One other possible mechanism of action for the analgesic effects of TCAs involves interaction with the opioid system. ADs are able to displace radiolabeled

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opioid receptor ligands from their binding sites and, after repeated administration, to modify the density of opioid receptors (Isenberg and Cicero, 1984; Hamon et al., 1987). Several studies have evidenced the inhibition of the antinociceptive effect of ADs by naloxone (Ardid and Guilbaud, 1992; Valverde et al., 1994; Gray et al., 1998; Shreiber et al., 2000), an opioid receptor antagonist, and its enhancement by enkephalinase inhibitor (Gray et al., 1998). However, few studies have been conducted to determine the involvement of the different opioid receptor subtypes in the analgesic effect of ADs. Schreiber et al. (2000) and Gray et al. (1998) demonstrated that the antinociceptive effect of different ADs was mainly influenced by different opioid receptor antagonists. However, these authors failed to determine the involvement of a specific opioid subtype receptor according to the ADs used. The precise point of the neuroaxis at which the opioid receptor is involved was not evaluated and the studies were performed with acute injections and in acute pain conditions, which does not reflect the clinical use of ADs. Eschalier et al. (1988) and Ardid and Guilbaud (1992) showed that repeated injection (every half-life) induced higher antinociceptive effect than acute administration in acute or chronic pain models, respectively.

The aim of the present study was to identify subtypes of opioid receptors involved in the antihyperalgesic effect of repeated administration of clomipramine (CMI) and the site, spinal and/or supraspinal, of their involvement in chronic constriction injury (CCI), a mononeuropathic pain model (Bennet and Xie, 1988).

Methods and Materials

Animals

Male Sprague-Dawley rats (CD1 Charles River, France) weighing 180-200 g were used. They were housed in standard laboratory conditions with free access to food and water one week before experiments. Since the experiments were a potential cause of suffering, they were monitored by a local ethical committee and the guidelines of the Committee for Research and Ethical Issues of the IASP (1983) were followed. Great care was taken, particularly with regard to housing conditions, to avoid or minimize discomfort to the animals.

Induction of mononeuropathy

Unilateral peripheral mononeuropathy was induced according to the method previously described by Bennett and Xie (1988). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and four chromic gut (5-0) ligatures were tied loosely (with about 1 mm spacing) around the right common sciatic nerve. The nerve was constricted to a barely discernible degree, so that circulation through the epineurial vasculature was not interrupted. Only animals with a decrease $\geq 15\%$ of the pre-surgery value of vocalization threshold were selected

Assessment of nociceptive thresholds

Rats were submitted to the paw pressure test as described by Randall and Selitto (1957). Nociceptive thresholds, expressed in grams, were measured with a Ugo Basil analgesimeter (Apelex type 003920, tip diameter of probe 1 mm, Bioseb, France) by applying increasing pressure to the right hind paw of unrestrained rats

until a squeak and/or a struggle (vocalization threshold) was obtained (a cut off level of 750 g was applied). Thresholds to paw pressure (the mean of two consecutive stable values that differed no more than 10 %) were determined before surgery and just before and after drug treatments.

Treatment protocol

The experiments were performed blind in a quiet room by a single experimenter using the method of equal blocks with randomization of treatments in order to avoid any uncontrollable environmental influence that might induce a modification in the behavioral response.

Testing took place 14 days after ligature of the sciatic nerve. Rats were randomly assigned to cages, with each animal receiving either drug or saline in the same volume (1 and 2 ml/kg of body weight for i.v. and s.c. injections, respectively). Each experiment was performed with different rats.

Preliminary study: Influence of low and high doses of naloxone on the antihyperalgesic effect of specific opioid agonists.

The aim of this study was to determine the dose of systemically administered naloxone that specifically blocks mu opioid receptors and the dose that simultaneously blocks the three opioid receptors (mu, delta and kappa receptors). There is no clear evidence or guidelines in the literature to help choose the systemic selective or unselective doses of naloxone for mu receptors. Accordingly, we determined these doses before testing their influence on the effect of CMI. The influence of a low (1 µg/kg, i.v.) and a high (1 mg/kg, i.v.) dose of naloxone, chosen after preliminary testing, was evaluated on the antihyperalgesic effect of three

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specific opioid receptor agonists: DAMGO, a mu agonist (2 mg/kg, i.v.: Desmeules et al., 1993); BUBUC, a delta agonist (3 mg/kg, i.v.: Catheline et al., 1996) and U-50,488H, a kappa agonist (10 mg/kg, i.v.: Leighton et al., 1988).

Vocalization thresholds were determined before and 15 and 30 min after i.v. injections of agonists. Naloxone was injected 10 minutes after the last vocalization threshold determination, and the vocalization thresholds were determined 10, 20, 30, 50 and 80 min after naloxone injection (i.e. 50, 60, 70, 90 and 120 min after agonist injection).

Six groups (n=6 for each group) were used for each agonist : a control group saline + saline; saline + naloxone (1 µg/kg); saline + naloxone, (1 mg/kg); agonist + saline; agonist + naloxone (1 µg/kg); agonist + naloxone (1 mg/kg).

Influence of low and high systemic doses of naloxone on the antihyperalgesic effect of repeated injections of clomipramine.

The aim of this experiment was to determine whether mu opioid receptors were involved in the antihyperalgesic effect of CMI and if so whether they were the only receptors involved.

Vocalization thresholds were determined before any injection and 80 min after the last of the five sub-cutaneous injections of CMI (5 mg/kg/injection), performed every half-life (2h35). This pattern of administration achieved a maximal antihyperalgesic effect of CMI, as previously observed by Ardid and Guilbaud (1992) in the same model. A delay of 80 min before testing was observed after the fifth injection to allow plasma concentration steady state to reach a mean level at testing time, as previously described by Eschalier et al. (1988). After this vocalization threshold was determined, naloxone (1mg/kg and 1µg/kg) was i.v. injected and

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vocalization thresholds were determined 15, 30, 45, 60, 75 and 105 min after its injection.

Four groups (n=8 for each group) were used for each dose of naloxone: saline + saline; saline + naloxone; CMI + saline; CMI + naloxone.

Influence of specific opioid receptor antagonists, i.t. administered, on the antihyperalgesic effect of repeated injections of clomipramine.

These experiments were performed to evaluate the involvement of spinal mu, delta and kappa opioid receptors in the antihyperalgesic effect of CMI.

Vocalization thresholds were determined before any injection and 80 min after the last of the five sub-cutaneous injections of CMI (5 mg/kg/injection) performed every half-life. Specific antagonists of each opioid receptor were then i.t. injected in a 10 µl saline solution: CTOP for mu, naltrindole (NTI) for delta, and Nor-binaltorphimine (Nor-BNI) for kappa opioid receptors. The doses used have been shown to inhibit the antinociceptive effect of specific opioid agonists: 10 µg/rat for CTOP (Tseng et al., 1995), 30 µg/rat for NTI (Hao et al., 1998) and 40 µg/rat for Nor-BNI (Wongchanapai et al., 1998). Vocalization thresholds were determined 15, 25, 35, 50, 80 and 110 min after injection of the antagonist.

Four groups (n=8 for each group) were used for each antagonist: saline + saline; saline + antagonist; CMI + saline; CMI + antagonist.

Influence of specific opioid receptor antagonists, i.c.v. administered, on the antihyperalgesic effect of repeated injections of clomipramine.

The purpose of this experiment was to assess the nature of opioid receptors involved in the antihyperalgesic effect of CMI at the supraspinal level.

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Vocalization thresholds were determined before any injection and 80 min after the last of the five sub-cutaneous injections of CMI (5 mg/kg/injection) performed every half-life. The specific antagonists were then injected in a 5 μ l saline solution through cannula implanted in the right lateral ventricle. The doses used have been shown to inhibit the antinociceptive effect of specific opioid agonists: 0.05 μ g/rat for CTOP (Suh et al., 1995), 1 μ g/rat for NTI (Calcagnetti et al., 1991) and 5 μ g/rat for Nor-BNI (Xu et al., 1992). Vocalization thresholds were determined 15, 25, 35, 50, 80 and 110 min after antagonist injection.

Four groups (n=8 for each group) were used for each antagonist: saline + saline; saline + antagonist; CMI + saline; CMI + antagonist.

Procedure for central injections

Intrathecal injections

Injections were performed by lumbar puncture as described by Mestre et al. (1994). Briefly, the rat, slightly anesthetized with isofluoran gas (Minerve, France), was held in one hand by the pelvic girdle, and a 25-gauge x1-inch needle connected to a 25 μ l Hamilton syringe was inserted into the sub-arachnoidal space between the spinous processes of L5 and L6, until a tail flick was elicited. The syringe was held in position for a few seconds after the injection of a volume of 10 μ l/rat for all drugs.

Intracerebroventricular injections

Seven days after the induction of neuropathy, the rats were deeply anesthetized with a mixture of acepromazine (10 mg/kg) and ketamine (35 mg/kg) administered by intraperitoneal route. The skull was exposed by a midline incision and a hole was drilled on the right side 2 mm posterior to the bregma and 1.5 mm lateral to the sagittal suture. The cannula was placed 3 mm below the brain surface.

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After placement, the cannula was held in position with dental cement (Aquacem®, Germany). The animals were kept warm during recovery from anesthesia. Following cannula insertion, they were singly housed for a recovery period of 5-7 days prior to i.c.v drug administration (5 µl/rat). Drugs was slowly administered. At the end of the study, the position of the cannula was verified by injection of 5 µl of methyl blue. Results from animals which did not exhibit a clear distribution of methyl blue in the ventricular system were excluded.

Drugs

BUBUC, ([D-] Enkephalin-Thr(OtBU), was a generous gift from Bernard Roques (INSERM U266-CNRS UMR 8600, Paris, France). DAMGO, [D-Ala², N-Me-Phe⁴, Gly⁵Cys(StBu)², leu⁵ ol]Enkephalin, naloxone, CTOP, (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-ThrNH₂), Naltrindole HCl (NTI) and Nor-binaltorphimine (Nor-BNI) were obtained from Sigma Chemical Co (S^t Quantin Fallavier, France). U-50,488H(trans(± 3,4-dichloro-N-methyl-N-[2-(1-pyrrolidiny)cyclohexil] benzene acetamide)) was obtained from Tocris (Illkirch, France). All drugs were dissolved in physiological saline (NaCl 0.9%).

Expression of results and statistical analysis

Results were expressed as mean ± SEM of raw data (grams) corresponding to the pressure applied when vocalization was emitted.

For the first experiment, results were expressed by the analgesia score determined 60 min after the injection of the agonist. Analgesia score was calculated by subtracting from the score of the agonist treated group the score of the corresponding control groups. For example, [(DAMGO + saline) – (saline + saline) treated groups or [(DAMGO + naloxone) – (saline + naloxone)] treated group. The

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analgesia score was expressed as mean \pm SEM in gram. In order to quantify the influence of opioid antagonists on the antihyperalgesic effect of specific opioid agonists or CMI, we calculated the percentage of reduction each time the difference raw scores of (drug + saline) and (drug + antagonist) treated groups were significantly different. We used the following formula : $[(\text{drug+saline}) - (\text{drug+antagonist corresponding value})] \times 100 / [(\text{drug+saline}) - (\text{saline+antagonist corresponding value})]$. The percentage of reduction was expressed as mean \pm SEM.

Statistical analysis was performed using a two way analysis of variance (ANOVA) followed by a Student-Neuwmans-Keuls test for statistical evaluation of time-course effect. The results presented by analgesia score were analyzed by a Student's *t* test. In all cases the significance level was 0.05.

Results

Preliminary study: Influence of low and high doses of naloxone on the antihyperalgesic effect of specific opioid agonists.

The two doses of naloxone, like saline, did not modify vocalization thresholds in our experimental conditions (fig. 1A). DAMGO induced a statistically significant increase in vocalization thresholds between 15 and 60 min (fig. 1A). The two other opioid receptor agonists induced, like DAMGO, a statistically significant increase in the analgesia score for the different groups treated with U-50,488H and BUBUC + saline (fig. 1B).

The high dose of naloxone (1 mg/kg, i.v.) significantly inhibited all these effects (fig. 1A&B). Inhibition was complete for DAMGO ($-117.1 \pm 21.9\%$) and U-50,488H ($-90.5 \pm 6.4\%$) and a little less marked for BUBUC ($-84.1 \pm 20.6\%$) at the 60th min. The low dose of naloxone (1 μ g/kg, i.v.) only significantly reduced the effect of DAMGO ($-83.5 \pm 8.8\%$).

These findings showed that it was possible to use the low dose of naloxone (1 μ g/kg, i.v.) as a systemic dose, specific to mu receptor, whereas the higher dose of 1 mg/kg inhibited mu, delta and kappa receptors.

Influence of low and high doses of naloxone on the antihyperalgesic effect of repeated injections of clomipramine.

Five successive injections of saline did not induce any change in the vocalization thresholds of neuropathic rats. Naloxone had no effect by itself (fig. 2 A&B).

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CMI induced a significant increase in the vocalization thresholds assessed from the fifth injection that remained significant after saline injection for at least 60 min. The antihyperalgesic effect of CMI was decreased by the two doses of naloxone. The effect of the high dose was significant at the 45th min ($112.3 \pm 6.5\%$ of reduction). At the low dose, its effect was significant between the 30th and 45th minute ($63.4 \pm 4.2\%$, $72.5 \pm 5.6\%$ of reduction, respectively).

Influence of opioid antagonists, i.t. administered, on the antihyperalgesic effect of repeated injections of clomipramine.

In neuropathic saline-pretreated rats, saline and the three antagonists injected alone did not induce any statistically significant change in the vocalization thresholds.

In all experiments, CMI induced a significant antihyperalgesic effect after the fifth injection. For the CMI+saline treated group, vocalization thresholds were statistically different from those of the saline+saline treated group for 120 minutes.

The effect of CMI was inhibited by CTOP, a mu opioid antagonist (fig. 3A), with a significant reduction at the 15th, 25th, 35th mins after CTOP injection. The greatest inhibition was $-70.6 \pm 16.6\%$ at the 25th min. Neither NTI (fig. 3B) nor Nor-BNI (fig. 3C) modified the antihyperalgesic effect of CMI.

Influence of opioid antagonists, i.c.v. administered, on the antihyperalgesic effect of repeated injections of clomipramine.

In neuropathic saline-treated rats, saline and the three antagonists injected alone did not induce any statistically significant change in the vocalization thresholds.

In all experiments, CMI induced a significant antihyperalgesic effect after the fifth

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injection. For the CMI+saline treated group, vocalization thresholds were statistically different from those of the saline+saline treated group for 90 minutes.

CTOP and Nor-BNI (fig. 4A&C) did not induce any change in the vocalization thresholds. In contrast, NTI (fig. 4B), a delta receptor antagonist, totally suppressed the antihyperalgesic effect of CMI, as illustrated by a significant decrease in vocalization thresholds between the 25th and the 90th minute. Inhibition was greatest at the 35th min ($-97.1 \pm 1.9\%$).

Discussion

The main finding of the present study is that the antihyperalgesic effect of repeatedly administered CMI, is mediated by the opioidergic system in mononeuropathic rats. For the first time, we demonstrate an inhibition of this antihyperalgesic effect by systemic naloxone in conditions as close as possible to clinical use. We also observed that the effect of CMI was antagonized by NTI at the supraspinal level and that of CTOP at the spinal level, which suggests a differential involvement of delta and mu opioid receptors according to the site of the central nervous system considered. In contrast, kappa opioid receptors did not seem to be involved in the antihyperalgesic effect of CMI.

CMI, repeatedly injected every half-life, induced a clear antihyperalgesic effect, as evidenced by an increase in the vocalization threshold to paw pressure. This is consistent with the results of Ardid and Guilbaud (1992), who demonstrated that repeated administrations of CMI (5 x 0.75 and 1.5 mg/kg, s.c.) induced a more potent and prolonged antihyperalgesic effect than acute administration in the same model. Both findings confirm that this pattern of administration, which take into account differences in the pharmacokinetic parameters of drugs between man and rat and is closely related to clinical use (see for review Eschalier et al., 1999), is effective when combined with the use of neuropathic pain models (Eschalier et al., 1988).

In our experimental conditions, naloxone, at 1mg/kg, i.v., which inhibited the antihyperalgesic effect of the three opioid receptor (mu, delta and kappa) agonists in the same model, also significantly suppressed the antihyperalgesic effect of CMI. Many authors have reported the inhibition of antinociceptive effect of ADs by naloxone but exclusively after acute (Michael Titus and Costentin, 1987; Ardid and

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Guilbaud, 1992; Valverde et al., 1994; Gray et al., 1998) or daily and twice daily administrations (Ansuategui et al., 1989) in different pain tests and models. The total inhibition of the effect demonstrates that CMI needs a functional opioid system to induce its antihyperalgesic effect in conditions of repeated treatment in a model of neuropathic pain. The fact that the low dose of naloxone (1µg/kg, i.v.) was specific to mu receptors is consistent with its preferential affinity for these receptors (Magnan et al., 1982). The low dose also reduced the antihyperalgesic effect of CMI. However, the partial inhibitory effect demonstrates that mu opioid receptors are markedly, but not exclusively, involved in the antihyperalgesic effect of CMI. To determine the nature of the other opioid receptors involved, specific antagonists were used, and to clarify the site (spinal or supraspinal) of their involvement they were administered either i.t. or i.c.v. The few works, which have studied the influence of specific opioid receptor antagonists on the antinociceptive effect of ADs, have exclusively used systemic administration of these ligands (Gray et al., 1998; Schreiber et al., 2000).

The selective mu opioid antagonist, CTOP, intrathecally administered, partially inhibited the antihyperalgesic effect of CMI while NTI, a selective delta opioid antagonist, given i.c.v., totally suppressed the effect. In similar conditions, nor-BNI failed to inhibit the effect of CMI, whether administered i.t. or i.c.v., which could exclude the involvement of kappa opioid receptors. These results confirm the opioidergic mechanism involved in the antihyperalgesic effect of repeatedly administered CMI in mononeuropathic rats. They also indicate a differential involvement of mu and delta opioid receptors. Hence, the degree of inhibition by NTI and CTOP suggests that the activation of both supraspinal delta and spinal mu opioid receptors is successively needed for the antihyperalgesic effect of CMI, thereby implying a sequential functioning. The supraspinal effect of CMI leading to the

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activation of delta opioid receptors is essential, as shown by both the total inhibition obtained by i.c.v. injected NTI and to a lesser degree by the high unspecific dose of systemic naloxone. It is in line with the inhibition by NTI of the effect of ADs (Gray et al., 1998), with both the presence of these receptors in brain structures involved in the regulation of nociceptive message (Ossipov et al., 1999). Thus, this site of action, consistent with the documented antinociceptive effect of i.c.v. administered CMI and of other ADs, seems to be the first target of a sequential mechanism which also involves spinal mu opioid receptors. The coexistence of these supraspinal and spinal effects suggests the involvement of the bulbospinal pathways. This hypothesis is consistent with various other findings. For instance, bulbospinal pathways are required in the antinociceptive effect of CMI (Ardid et al., 1995). The antinociceptive effect and the spinal Fos-like immunoreactivity suppressive activities of [D-ala², glu⁴] deltorphin, given into the medullary reticular formation, were abolished by bilateral lesions of dorsolateral funiculus (Kovelowski et al., 1999). Supraspinally administered opioid receptor agonists, including morphine, activate bulbospinal pathways (Ossipov et al., 1999). However, the mechanism by which CMI interacts with the opioidergic system needs to be clarified. Several animal studies have shown an increase in cerebral opioid peptide levels or immunoreactivity after ADs-treatment. Sacerdote et al. (1987) and Dziedzicka-Wasylewska and Rogoz (1995) found an increase in hypothalamic beta-endorphin and in nucleus accumbens endogenous enkephalin concentration after acute and chronic treatment with ADs, respectively. Met-enkephalin-like immunoreactivity was increased in the striatum, nucleus accumbens (De Felipe et al., 1985), spinal cord, hypothalamus and cerebral cortex (Hamon et al., 1987) of rats treated with CMI or other ADs. Gray et al. (1998) showed that all ADs tested for their antinociceptive activity against acetic acid-induced abdominal writhes

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were potentiated by a sub active dose of acetorphan, an enkephalinase inhibitor. Clinically, Hameroff et al. (1982) reported that doxepin raised plasma enkephalin levels in 14 chronic pain patients and also reduced pain scores. These changes could activate the delta opioid receptors, to which both endorphins and enkephalins bind. However, the mechanism by which ADs might enhance the release of opioid peptides has not been elucidated. A recent study (Dziedzicka-Wasylewska et al., 2002) showed that repeatedly administered tianeptine (a serotonin reuptake enhancer) and fluoxetine (a serotonin reuptake inhibitor) induced similar changes in the level of Met-enkephalin in various regions of rat brain, which suggests that changes in Met-enkephalin levels are not linked to the inhibition of serotonin reuptake. ADs seem to affect only opioid peptide release because the authors of the study observed no change in the levels of proenkephalin mRNA. The activation of the opioid system by CMI and other ADs could also be due to a direct effect on opioid receptors, as suggested by Michael Titus and Costantin (1987). However, the affinity of ADs for opioid receptors is low (10^{-5} M) (Isenberg and Cicero, 1984) and hence an agonist-like effect of ADs on opioid receptors seems unlikely.

All these results suggest that an increase in endogenous opioid levels by CMI could be the main mechanism of the interaction. At the supraspinal level, endogenous opioids could activate delta opioid receptors, which would lead, directly or indirectly (e.g., by an inhibition of gabaergic interneurons, Millan, 2002), to an increase in the activity of descending inhibitory pathways (Ossipov et al., 1999). Activation of descending monoaminergic pathways could stimulate spinal enkephalin containing interneurons (Millan, 2002) and secondly mu opioid receptor which predominate in the dorsal horn of the spinal cord. However, since complete inhibition

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is not achieved by i.t. CTOP and low dose of naloxone, another non-opioid mediated spinal mechanism cannot be ruled out.

In conclusion, this work suggests a central mechanism of action of CMI initiated at the supraspinal level by activation of delta opioid receptor. This hypothesis argues against a primary spinal effect due to the inhibition of monoamines reuptake at the endings of the bulbospinal pathways, the generally acknowledged mechanism of action of ADs (Eschalier et al., 1999). However, further work is needed to clarify the neurochemical mechanisms, including monoamines, involved in the suspected neuronal network and to verify if such an opioidergic mechanism is also found with other non TCAs.

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Legends

Figure 1 A : Influence of naloxone (1 mg and 1 µg/kg, i.v.) on the antinociceptive effect of DAMGO (2 mg/kg, i.v.), mu agonist, on the vocalisation threshold to paw pressure in mononeuropathic rat. Results were expressed by the time-course curve of mean (\pm SEM) in gram. n=8 in each group.

*p<0.05 versus corresponding groups treated by saline + saline or saline + naloxone.

○ p<0.05 versus agonist + saline group (Student-Neuwmans-Keuls test).

1 B : Effect of naloxone (1 mg and 1 µg/kg, i.v.) on the analgesia score of DAMGO (2 mg/kg, i.v.), mu agonist, BUBUC (3 mg/kg, i.v.) delta agonist, and U-50,488H (10 mg/kg, i.v.) kappa agonist. Results of the vocalisation thresholds were expressed by analgesia score calculated by the difference of the values of the vocalisation thresholds between agonist groups and the corresponding control group in gram (\pm SEM). n=8 in each group.

*p<0.05 versus agonist + saline group (Student's *t* test).

Figure 2 : Influence of naloxone (1 mg (A) and 1 µg/kg (B), i.v.) on the antinociceptive effect of five successive injections every half life (2h35) of clomipramine (5 mg/kg, s.c.) on the vocalisation threshold to paw pressure in mononeuropathic rat. Results were expressed by the time-course curve of mean (\pm SEM) in gram. n=8 in each group.

* p<0.05 versus corresponding groups treated by saline + saline or saline + naloxone.

○ p<0.05 versus clomipramine + saline group (Student-Neuwmans-Keuls test).

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Figure 3 : Influence of CTOP, mu antagonist (A), of naltrindole, delta antagonist (B), or nor-binaltorphimine, kappa antagonist (C), administered by intrathecal route on the antinociceptive effect of five successive injections every half life (2h35) of clomipramine (5 mg/kg, s.c.) on the vocalisation threshold to paw pressure in mononeuropathic rat. Results were expressed by the time-course curve of mean (\pm SEM) in gram. n=8 in each group.

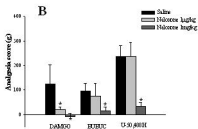
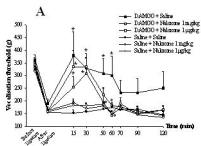
* $p < 0.05$ versus corresponding groups treated by saline + saline or saline + antagonist.

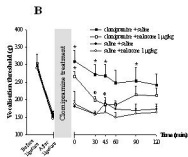
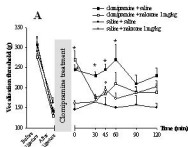
o $p < 0.05$ versus clomipramine + saline group (Student-Neuwmans-Keuls test).

Figure 4 : Influence of CTOP, mu antagonist (A), of naltrindole, delta antagonist (B), or nor-binaltorphimine, kappa antagonist (C), administered by intracerebroventricular route on the antinociceptive effect of five successive injections every half life (2h35) of clomipramine on the vocalisation threshold to paw pressure in mononeuropathic rat. Results were expressed by the time-course curve of mean (\pm SEM) in gram. n=8 in each group.

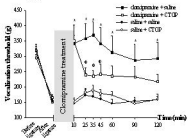
* $p < 0.05$ versus corresponding groups treated by saline + saline or saline + antagonist.

o $p < 0.05$ versus clomipramine + saline group (Student-Neuwmans-Keuls test).

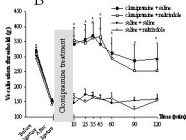




A



B



C

