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Spinal effect of a neuropeptide FF analogue on hyperalgesia and morphine-induced analgesia in mononeuropathic and diabetic rats

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1 1DMe, a neuropeptide FF (NPFF) analogue, has been shown to produce antinociception and to enhance morphine analgesia in rats after intrathecal administration. To determine whether 1DMe could correct hyperalgesia and restore morphine efficacy in mononeuropathic (MN) and diabetic (D) rats we examined the spinal effect of 1DMe in MN and D rats without and after spinal blockade of μ- and δ-opioid receptors with CTOP and naltrindole, respectively. The influence of 1DMe on morphine-induced antinociception was assessed in the two models using isobolographic analysis.

2 Whereas 1DMe intrathecally injected (0.1, 1, 7.5 μg rat^{-1}) was ineffective in normal (N) rats, it suppressed mechanical hyperalgesia (decrease in paw pressure-induced vocalisation thresholds) in both MN and D rats. This effect was completely cancelled by CTOP (10 μg rat^{-1}) and naltrindole (1 μg rat^{-1}) suggesting that it requires the simultaneous availability of μ- and δ-opioid receptors.

3 The combinations of morphine : 1DMe (80.6 : 19.4% and 99.8 : 0.2%, in MN and D rats, respectively) followed by isobolographic analysis, showed a superadditive interaction, relative to the antinociceptive effect of single doses, in D rats only. In N rats, the combination of morphine : 1DMe (0.5 mg kg^{-1}, i.v.: 1 μg rat^{-1}, i.t., ineffective doses) resulted in a weak short-lasting antinociceptive effect.

4 These results show a different efficacy of 1DMe according to the pain model used, suggesting that the pro-opioid effects of the NPFF in neuropathic pain are only weak, which should contribute to hyperalgesia and to the impaired efficacy of morphine.

Keywords: Neuropeptide FF; neuropathy; chronic pain; morphine analgesia; μ- and δ-opioid; intrathecal; mononeuropathic rats; diabetic rats

Abbreviations: CCI, chronic constriction injury; CCK, cholecystokinin; D, diabetic; MN, mononeuropathic; N, normal; NPFF, neuropeptide FF

Introduction

Evidence has been accumulating that hyperalgesic states resulting from metabolic or chronic constriction nerve injury (CCI) are associated with modified efficacy of analgesic drugs (Raz *et al*., 1988; Attal *et al*., 1991; Yanamoto & Yaksh, 1992; Desmeules *et al*., 1993; Courteix *et al*., 1994; Lee *et al*., 1994; Kayser *et al*., 1995; Ossipov *et al*., 1995; Suh *et al*., 1996; Courteix *et al*., 1998). Pharmacological studies have indicated that, on a molar basis, morphine is half as potent in streptozocin-induced diabetic (D) rats as in healthy rats (Courteix *et al*., 1994). This may be partially attributed to altered morphine pharmacokinetics (Courteix *et al*., 1998). In mononeuropathy induced by CCI or spinal transection, morphine analgesia has been reported to be either reduced (Advokat & Gulati, 1991; Yanamoto & Yaksh 1992; Advokat & Rhein, 1995; Suh *et al*., 1996; Nichols *et al*., 1997; Kauppila *et al*., 1998) or increased (Attal *et al*., 1991; Desmeules *et al*., 1993; Lee *et al*., 1994; Kayser *et al*., 1995; Catheline *et al*., 1996).

Anti-opioid peptides have been shown to play an important role in modulating opioid sensitivity (Stanfa *et al*., 1994). It has been proposed that the cholecystokininergic (CCKergic) control of pain may influence the efficacy of opioids (Faris, 1985; Zhou *et al*., 1993). An induction of CCK precursor gene expression in the dorsal root ganglia and an increase in spinal release of CCK (Verge *et al*., 1993) have been described after peripheral axotomy in rats. The ability of CCK_B receptor antagonists to enhance morphine antinociception in CCI-induced mononeuropathic (MN) rats (Idänpään-Heikkilä *et al*., 1997) and to restore opioid analgesia in D and MN rats (Courteix *et al*., 1997), further suggests that CCK might modify opioid sensitivity in neuropathic pain states.

Interestingly, recent investigations have indicated that neuropeptide FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH2, NPFF or F8Famide), an octapeptide present in the rat central nervous system (Allard *et al*., 1991) and for which high concentrations of specific receptors are present in human spinal cord (Allard *et al*., 1994), exerts modulatory action on morphine-induced analgesia in healthy animals (Yang *et al*., 1985; Kavaliers, 1990; Gicquel *et al*., 1992; Million *et al*., 1993; Gouardères *et al*., 1993; Desprat & Zajac, 1994) and in unilateral CCI of spinal nerve in rats (Wei *et al*., 1998). Depending on the animal species, the route of administration (intracerebroventricular, i.c.v. or intrathecally, i.t.) and the dose, NPFF can function as an endogenous anti-opioid agent as well as a pro-opioid agent (Kavaliers *et al*., 1990; Gouardères *et al*., 1993a); 1996; Million *et al*., 1993; Roumy & Zajac, 1998) suggesting the existence of a balance between the anti-opioid and the pro-opioid effects of NPFF. NPFF analogues have been shown to enhance morphine antinocicep-

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tion in normal rats through an indirect activation of μ- and δ-opioid receptors (Gouardères *et al*., 1996a).

To clarify the role of this system in pathological conditions, we investigated the spinal effects of an NPFF analogue in two models of chronic neuropathy, (MN and D rats) which display hyperalgesia and modified morphine efficacy (Yamamoto & Yaksh 1992; Lee *et al*., 1994; Courteix *et al*., 1994). The enzyme resistant NPFF analogue [D-Tyr^1, (NMe)Phe^3]NPFF (1DMe), which binds with a high affinity to rat spinal cord (Gicquel *et al*., 1992), has been shown to modulate morphine activity similarly to NPFF (Gouardères *et al*., 1996a) and so constitutes a useful pharmacological tool to study the role of NPFF in spinal nociception.

Accordingly, we studied the effect of intrathecally (i.t.) administered 1DMe on mechanical hyperalgesia in normal (N), D and MN rats using the paw pressure procedure. The effect of μ- (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2) (CTOP) (Hawkins *et al*., 1989; Gulya *et al*., 1988) and δ-(naltrindole) (Calcagnetti & Holtzman, 1991; Drower *et al*., 1991) opioid antagonists on the spinal antinociception elicited by 1DMe was also examined.

Since the NPFF analogue and morphine alone exert effects on nociception in D and MN rats that differ from those observed in N rats, the interaction of 1DMe (i.t.) with morphine (i.v.) was investigated using isobolographic analysis in the two animal models of neuropathic pain. The association of an ineffective dose of morphine (i.v.) with an ineffective dose of 1DMe (i.t.) was also studied in N rats.

These results have been reported in part in the Proceedings Supplement of the September Meeting of the British Pharmacological Society (Courteix *et al*., 1997).

Methods

Animals

Male Sprague-Dawley rats (Charles River, Cléon, France) were used. They were housed three per cage under standard laboratory conditions, and given food and water *ad libitum*. Their weight at the beginning of the experiment (75–100, 150–175, 200–250 g) was chosen to obtain rats of similar weight (300 g) when the drugs were administered. The guidelines of the IASP Committee for Research and Ethical Issues concerning animal pain models (Zimmermann, 1983) were followed.

Induction of diabetes

The rats (200–250 g) were rendered diabetic with an intraperitoneal (i.p.) injection of streptozocin (STZ) (75 mg kg^{-1}) (Zanosar®, Upjohn, St-Quentin-en-Yvelines, France) dissolved in distilled water. Diabetes was confirmed 3 weeks later by measurement of tail vein blood glucose levels with Ames Dextrostix® and a reflectance colorimeter (Ames Division, Miles Laboratories, Puteaux, France). Only rats with a final blood glucose level of at least 14 mM were included in the study. This animal model of chronic pain with mechanical, thermal and chemical hyperalgesia has been described in detail elsewhere (Courteix *et al*., 1993a).

Control (normal) rats (75–100 g) were administered only distilled water (1 ml kg^{-1}, i.p.).

Induction of mononeuropathy

The rats (150–175 g) were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and four chronic gut (5-0) ligatures were tied loosely (with about 1 mm spacing) around the common sciatic nerve, according to the method described by Bennett & Xie (1988). The nerve was constricted to a barely discernible degree, to that circulation through the epineurial vasculature was not interrupted. This model, in which a chronic constrictive nerve injury produces allodynia and hyperalgesia, has been described as a model of neuropathic pain by Bennett & Xie (1988).

Nociceptive test

The rats were submitted to the paw pressure test previously described by Randall & Selitto (1957). Nociceptive thresholds, expressed in grams, were measured using a Ugo Basil analgesimeter (Apelex, tip diameter of probe 1 mm, weight 30 g) by applying an increasing pressure to the left hind paw until vocalisation was elicited (maximal pressure was 750 g).

Drugs and chemicals

Morphine hydrochloride (M.W. 321.81) was purchased from the Cooperation Pharmaceutique Française (Melun, France) and dissolved in saline (NaCl, 0.9%) on the day of the experiment.

1DMe ([D-Tyr^1, (NMe)Phe^3]NPFF) (M.W. 1171) was synthesized as described by Gicquel *et al*. (1992) and dissolved in saline on the day of the experiment.

CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2) (M.W. 1062.3) and naltrindole (M.W. 451) were purchased from RBI (Bioblock, Illkirsch, France) and dissolved in oxygen-free distilled water the day before the experiment and stored in appropriate aliquots at -20°C.

Injections

1DMe, CTOP, naltrindole or saline were intrathecally injected in a volume of 10 μl in the subarachnoid space between L5 and L6 using a 30 ½ Ga needle and a 25 μl Hamilton syringe as described by Mestre *et al*. (1994).

Morphine and saline were administered intravenously *via* a caudal vein in a volume of 1 ml kg^{-1}.

Experimental design

Tests took place 3 weeks after the induction of diabetes or the injection of distilled water, and 2 weeks after the sciatic nerve ligature. At that time, only D and MN rats in which the reduction in pain scores was more than 15% of the value obtained before the STZ injection on the nerve ligature, respectively, were included (i.e. 75% of D rats and 90% of MN rats). The animals were submitted to the paw pressure test before drug injection. Once two stable threshold values were obtained, drugs were injected as follows:

Experiment 1: 1DMe (0.1, 1 or 7.5 μg rat^{-1}, i.t.) or saline alone, in D, MN and N rats. A preliminary study had shown that the dose of 10 μg rat^{-1} induced an impairment of hindlimb function immediately after i.t. injection and convulsions 1–2 h later in 90% of the injected rats as reported by Gouardères *et al*. (1996a); this dose was therefore not included in this study.

Experiment 2: CTOP (10 μg rat^{-1}) or naltrindole (1 μg rat^{-1}) i.t., 15 min before an i.t. effective dose of 1DMe (1 μg rat^{-1} or 7.5 μg rat^{-1} in D or MN rats, respectively).

Experiment 3: morphine i.v. (0.1, 0.5, 2 or 4 mg kg^{-1} in D rats, and 0.005, 0.01, 0.05 or 0.1 mg kg^{-1} in MN rats) followed by i.t. 1DMe (0.06, 0.3, 1.2 or 2.4 μg rat^{-1} in D rats, and 0.36,

0.72, 3.6 or 7.2 μg rat^{-1} in MN rats). In MN rats, the ED_{50} of morphine was determined before the combination experiment. Morphine (0.5, 1 or 2 mg kg^{-1}, i.v.) exerted a dose-dependent antinociceptive effect with an ED_{50} of 0.83 mg kg^{-1}. The doses studied in combination were selected from the ratio $ED_{50\ (morphine)}:ED_{50\ (1DMe)}$. The doses of 1DMe expressed in μg rat^{-1}, were converted into mg kg^{-1} (mean body weight: 300 g) to perform the isobolographic analysis.

Experiment 4: morphine i.v. (0.5 mg kg^{-1}) or saline immediately followed by 1DMe (1 μg rat^{-1}) or saline in N rats. In these animals, the lack of 1DMe in the range of doses injected makes it impossible to calculate the ED_{50} and consequently to perform the isobolographic analysis which requires the ED_{50} of each drugs. Thus the doses administered were chosen according to their efficacy: an ineffective dose of morphine (0.5 mg kg^{-1}) with an intermediate dose of 1DMe (1 μg rat^{-1}).

The vocalization thresholds were measured every 10 or 15 min for 120 min after the injections. Each experiment was performed blind ($n=6-16$ according to the treatment) using different animals and in randomized blocks to avoid any chronobiological effects, and to assess the effect of different treatments in the same environmental conditions.

Data analysis

Results are expressed as mean$\pm$s.e.mean of raw data. Statistical significance was assessed using a two-way analysis of variance (ANOVA) followed, when the *F*-value was significant, by a Dunnett's test to analyse the time-course of the effect. The significant level was set at $P<0.05$.

The ED_{50} defined as the dose of a drug that produced 50% of the maximal effect i.e. 50% of the maximal pressure of 750 g) was calculated by computer assisted analysis of the graded dose-response curves.

Isobolographic analysis was performed according to Tallarida (1992). Firstly, the potency of the individual drugs was determined. The 1DMe ED_{50} was plotted on the ordinate and the morphine ED_{50} on the abscissa. A theoretical simple additive line for a combination of the two drugs was then generated by connecting the ED_{50} for 1DMe with that of morphine. Morphine and 1DMe were prepared in fixed ratios (proportions based on weights) and administered in various doses. For the combination morphine + 1DMe, the ED_{50} and CL defined as the confidence limit of the mixture were calculated by linear regression of the dose response curve and resolved into its component parts according to the dose ratio. The potency and 95% CL of the two drugs were compared with the theoretical additive value (ED_{50add}) obtained from the ED_{50} for morphine according to the formula $ED_{50add} = ED_{50(morphine)}/(p_1 + Rp_2)$ where R is the potency ratio of morphine to 1DMe ($R = ED_{50(morphine)}/ED_{50(1DMe)}$), p_1 is the proportion of morphine in the total dose and p_2 is the proportion of 1DMe in the total dose. Overlap of the 95% CLs suggests a simple additive effect of the two agents.

Results

Behavioural hyperalgesia

STZ injection and sciatic nerve ligature significantly reduced vocalization thresholds 3 (in 75% of animals) and 2 (in 90% of animals) weeks after the induction of diabetes and nerve surgery, respectively (before STZ: 317$\pm$11 g, week 3 of diabetes: 224$\pm$5 g; before nerve surgery: 251$\pm$6 g, week 2 after surgery: 103$\pm$3 g).

Effect of 1DMe alone in N, MN and D rats

Relative to N, MN or D rats, the general behaviour was unaffected by the i.t. injection of 1DMe (0.1, 1 or 7.5 μg rat^{-1}).

Intrathecal injection of saline did not affect the vocalization thresholds in N, MN or D rats (Figure 1a, b and c).

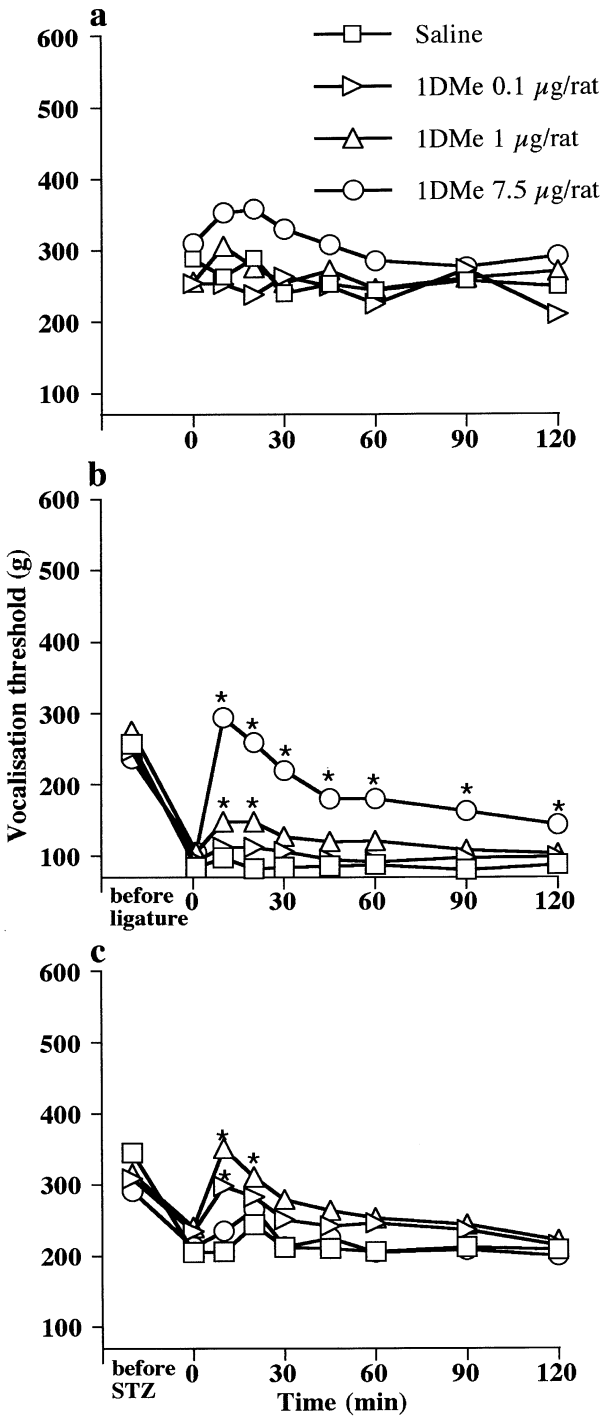

Figure 1 Time course of the effect of intrathecally administered saline or 1DMe on the paw pressure-induced vocalization thresholds in (a) normal, (b) mononeuropathic and (c) diabetic rats. Results are expressed as grams (g) (means$\pm$s.e.mean from 7–16 rats). The absence of an error bar indicates that the value of s.e.mean is smaller than the size of the symbol. *$P<0.05$, versus corresponding predrug values (time 0).

None of the three doses of 1DMe significantly affected vocalization thresholds in N rats (Figure 1a).

In MN rats, the dose of 0.1 μg rat^{-1} of 1DMe did not affect the vocalization thresholds, but the doses of 1 and 7.5 μg rat^{-1} significantly and dose-dependently increased the scores, resulting in a total reversal of hyperalgesia at the highest dose (Figure 1b). The maximal increase, obtained at 10 min, was $+42\pm10$ g ($+43\pm11\%$ of the predrug score) and $+189\pm18$ g ($+186\pm22\%$ of the predrug score) for 1 and 7.5 μg rat^{-1}, respectively. This antinociceptive effect lasted 20–120 min, depending on the dose.

In D rats, 1DMe induced a significant antinociceptive effect after doses of 0.1 and 1 μg rat^{-1}, resulting in a complete suppression of diabetes-induced hyperalgesia (Figure 1c). The antinociceptive effect corresponded to a maximal threshold elevation of $+63\pm34$ g (i.e. $+27\pm12\%$ of the predrug score) for 0.1 μg rat^{-1} and of $+111\pm38$ g (i.e. $+46\pm16\%$ of the predrug score) for 1 μg rat^{-1}. The effect of 1DMe in D rats was characterized by a short duration (10–20 min). The 1DMe dose of 7.5 μg rat^{-1} was inactive.

1DMe produced an antinociceptive effect in both MN and D rats with ED_{50} values of 60.3 μg rat^{-1} (i.e. 0.201 mg kg^{-1}) and 2.75 μg rat^{-1} (i.e. 0.0092 mg kg^{-1}), respectively.

Effect of CTOP and naltrindole on the antinociceptive effect of 1DMe in D and MN rats

The i.t. injection of the μ-opioid receptor antagonist, CTOP (10 μg rat^{-1}), 15 min before i.t. 1DMe totally suppressed the antinociceptive effect produced by 1DMe in both MN (Figure 2a) and D (Figure 2b) rats. Similarly, naltrindole (1 μg rat^{-1}), a δ-opioid receptor antagonist, suppressed the antinociceptive effect of 1DMe in MN (Figure 2a) and D (Figure 2b) rats.

Effect of the combined administration of morphine and 1DMe in MN rats

Since the peak effects of morphine (i.v.) and 1DMe (i.t.) occurred at 15 and 10 min, respectively, the injection of morphine was given 5 min before that of 1DMe. Morphine and 1DMe were co-administered at a fixed ratio determined as previously described, $ED_{50\ (morphine)}:ED_{50\ (1DMe)}=0.83$ mg kg^{-1} : 0.201 mg kg^{-1} (i.e. 4.13). The co-injection of morphine with 1DMe at the doses of 0.005 mg kg^{-1} morphine + 0.36 μg rat^{-1} 1DMe to 0.1 mg kg^{-1} morphine + 7.2 μg rat^{-1} 1DMe, did not produce any abnormal reaction. The combined doses of morphine: 1DMe 0.005 mg kg^{-1}: 0.36 μg rat^{-1} and 0.01 mg kg^{-1} : 0.72 μg rat^{-1} failed to modify the thresholds to paw-pressure. The combination of morphine 0.05 mg kg^{-1} with 3.6 μg rat^{-1} 1DMe significantly increased the vocalization thresholds from the 45th to the 90th min following injection (Figure 3). The highest doses of the combination (morphine 0.1 mg kg^{-1} and 1DMe 7.2 μg rat^{-1}) exerted a greater antinociceptive effect with a maximal score elevation of $+241\pm55$ g ($+198\pm68\%$ of the predrug score) at 30 min, which was significantly higher than that of morphine 0.5 mg kg^{-1} or 1DMe 7.5 μg rat^{-1} alone. In order (i) to avoid the adverse effects (motor impairment) of 1DMe at doses higher than 7.5 μg rat^{-1}, and (ii) to respect the ED_{50} ratios as described by Tallarida (1992), higher doses of morphine were not tested.

The total ED_{50} for the combination morphine: 1DMe is 0.228 mg kg^{-1}, representing 0.184 mg kg^{-1} of morphine and 0.044 mg kg^{-1} (13.2 μg rat^{-1}) of 1DMe (Figure 4). The theoretical additive ED_{50} for the combination 1DMe + morphine is: $[0.83/(0.806+(4.13\times0.194)]=0.516$ mg kg^{-1}, plotted at (0.416, 0.100). The graphic illustration on the isobologram (Figure 4) shows that the CLs do overlap. The *t*-test applied to the potency ratio between the total ED_{50} and the ED_{50} for the theoretical additive point reveals no significant difference; thus this combination does not present a superadditive interaction.

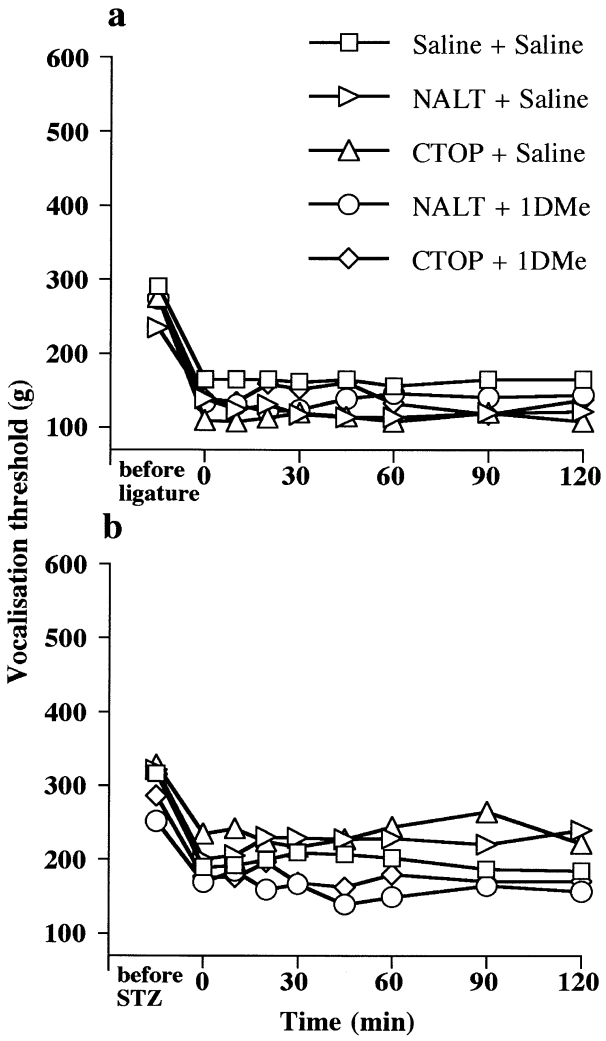

Figure 2 Time course of the effect of CTOP (10 μg rat^{-1}, i.t.) and naltrindole (NALT) (1 μg rat^{-1}, i.t.) on the paw pressure-induced vocalization thresholds after 1DMe in (a) MN (7.5 μg rat^{-1}, i.t.) and (b) D (1 μg rat^{-1}, i.t.) rats. Results are expressed as grams (g) (means ± s.e.mean from 6–7 rats). The absence of an error bar indicates that the value of s.e.mean is smaller than the size of the symbol.

Effect of the combined administration of morphine and 1DMe in D rats

Since the peak effects of morphine (i.v.) (previously reported, Courtiex *et al*., 1994) and of 1DMe (i.t.) appear 10 min after injection, the i.t. administration of 1DMe immediately followed that of i.v. morphine. The two drugs were combined at a fixed ratio determined as follows: $ED_{50\ (morphine)}:ED_{50\ (1DMe)}=4.72$ mg kg^{-1} : 0.0092 mg kg^{-1} (i.e. 513). Coadministration of morphine (i.v.) and 1DMe (i.t.) in the range of 0.1 mg kg^{-1} morphine + 0.06 μg rat^{-1} 1DMe to 4 mg kg^{-1} morphine + 2.4 μg rat^{-1} 1DMe, did not produce any abnormal behaviour. The combination exerted a significant dose-dependent antinociceptive effect from the combined doses of morphine 0.5 mg kg^{-1} + 1DMe 0.3 μg rat^{-1} (Figure 5),

whereas this dose of morphine (0.5 mg kg^{-1}) alone is ineffective (previous results, Courteix *et al*., 1998). The maximal score elevation in the vocalization threshold for the lowest combination doses was +154±62 g (+99±39% of the predrug score), 20 min after injection. With the highest doses, the maximal effects observed were: +319±36 g (+247±32% of the predrug score) and +363±53 g (+203±37% of the predrug score) for morphine 2 mg kg^{-1}+1DMe 1.2 μg rat^{-1} and morphine 4 mg kg^{-1}+1DMe 2.4 μg rat^{-1}, respectively, 20 min after injection.

The isobologram (Figure 6) represents the data for the combination morphine:1DMe. For the ratio morphine:1DMe=513, the total ED_{50} was 0.6774 mg kg^{-1}, representing 0.6760 mg kg^{-1} of morphine plus 0.0014 mg kg^{-1} (i.e. 0.42 μg rat^{-1}) of 1DMe. This point is plotted at (0.6760, 0.0014) on the morphine-1DMe isobologram; likewise, the CL for the total dose is resolved into two components. The theoretical simple additive ED_{50} (ED_{50add}) for the combination 1DMe+morphine, was $[4.72/(0.998+(513\times0.002))]=2.335$ mg kg^{-1} plotted at (2.3304, 0.0047). A graphic depiction on the isobologram shows that the confidence intervals of these two points do not overlap suggesting that the combination represents a superadditive interaction.

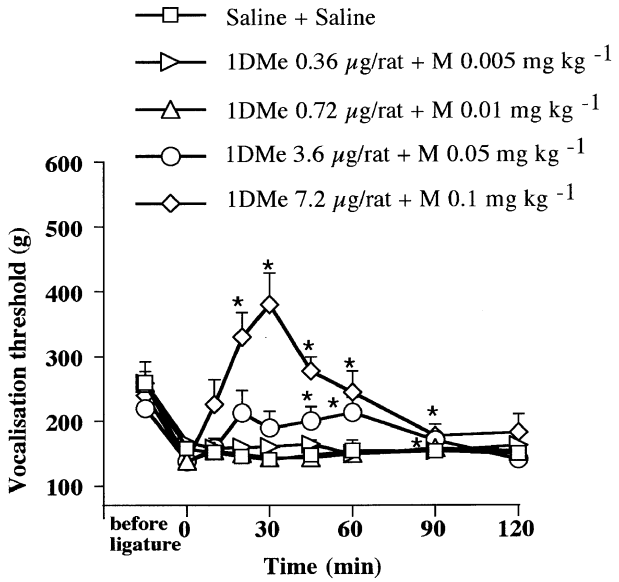

Figure 3 Time course of the effect of the combination of 1DMe and morphine on paw pressure-induced vocalization thresholds in mononeuropathic rats. The following treatments were administered: saline i.t.+saline i.v., or 1DMe (i.t.)+morphine (M) (i.v.) at the following dosages: 0.36 μg rat^{-1}+0.005 mg kg^{-1}, 0.72 μg rat^{-1}+0.01 mg kg^{-1}, 3.6 μg rat^{-1}+0.05 mg kg^{-1}, 7.2 μg rat^{-1}+0.1 mg kg^{-1}. Results are expressed as grams (g) means±s.e.mean from 6–7 rats). The absence of an error bar indicates that the value of s.e.mean is smaller than the size of the symbol. *$P<0.05$, versus corresponding predrug values (time 0).

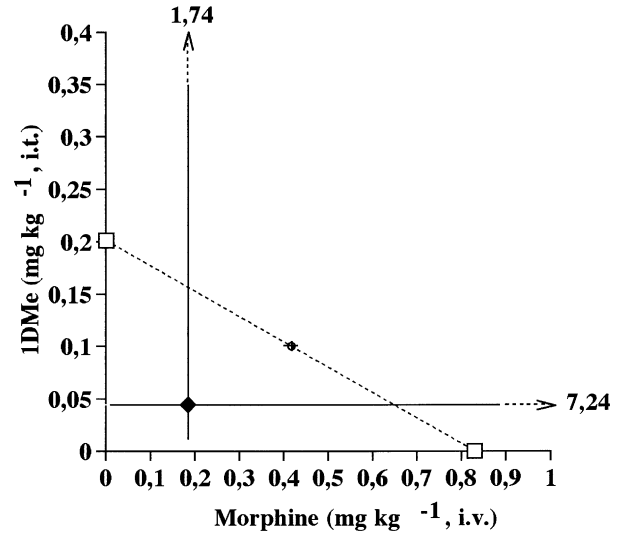

Figure 4 Isobologram for the effect of combination of morphine and 1DMe in attenuating mononeuropathy-induced hyperalgesia. The dashed line represents the theoretical additive interaction. The intercept of the dashed line on the ordinates and abscissae is the observed ED_{50} values for 1DMe and morphine alone, respectively. The solid symbol represents the observed ED_{50} value for the combination morphine:1DMe (80.6:19.4%). The ED_{50} value is represented by the open symbol. The 95% confidence limits for morphine and 1DMe are also resolved into the morphine (abscissa scale) and 1DMe (ordinate scale) components and shown by horizontal and vertical bars, respectively.

Effect of the combined administration of morphine and 1DMe in N rats

In N rats, the coadministration of an ineffective dose of morphine (0.5 mg kg^{-1}, i.v.) with an ineffective dose of 1DMe (1 μg rat^{-1}) did not produce abnormal behaviour and only briefly increased vocalization thresholds at the 20th min after the injection with a maximal score elevation of +64±23 g (+25±9% of the predrug score) (Figure 7).

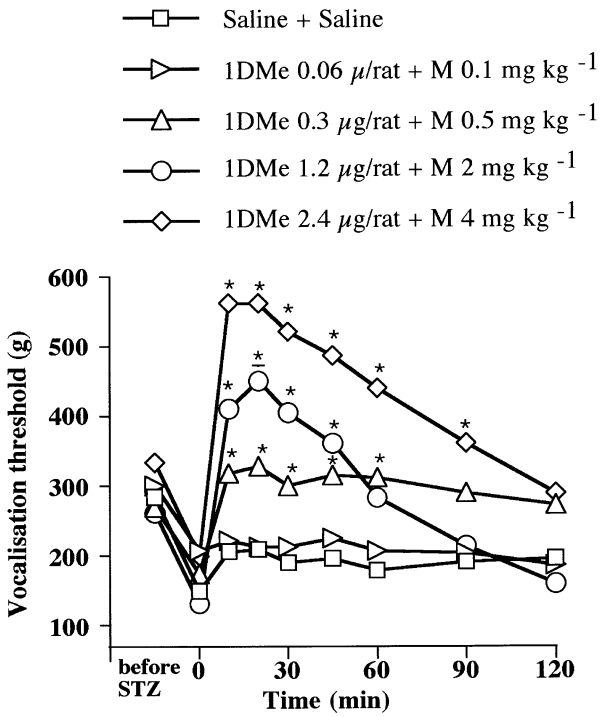

Figure 5 Time course of the effect of the combination of 1DMe and morphine on paw pressure-induced vocalization thresholds in diabetic rats. The treatments administered were: saline i.t.+saline i.v. or 1DMe (i.t.)+morphine (M) (i.v.) at the following dosages: 0.06 μg rat^{-1}+0.1 mg kg^{-1}, 0.3 μg rat^{-1}+0.5 mg kg^{-1}, 1.2 μg rat^{-1}+2 mg kg^{-1}, 2.4 μg rat^{-1}+4 mg kg^{-1}. Results are expressed as grams (g) (means±s.e.mean from 6–11 rats). The absence of an error bar indicates that the value of s.e.mean is smaller than the size of the symbol. *$P<0.05$, versus corresponding predrug values (time 0).

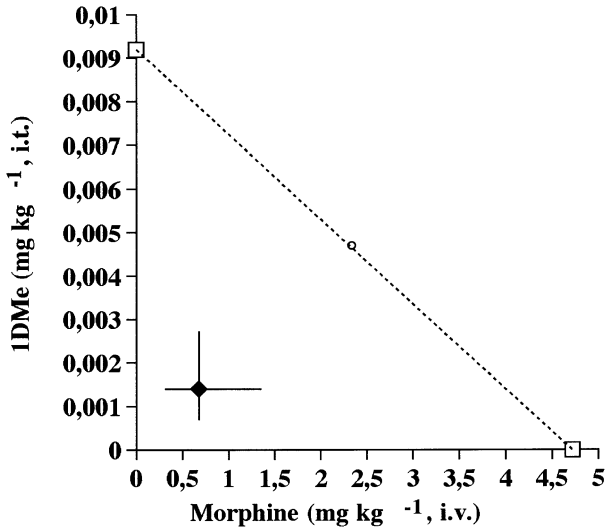

Figure 6 Isobologram for the effect of a combination of morphine and 1DMe in attenuating diabetes-induced hyperalgesia. The dashed line represents the theoretical additive interaction. The intercept of the dashed line on the ordinate and abscissae is the observed ED_{50} values for 1DMe and morphine alone, respectively. The solid symbol represents the observed ED_{50} value for the combination morphine: 1DMe (99.8 : 0.2%). The predicted ED_{50} value is represented by the open symbol. The 95% confidence limits for morphine and 1DMe are resolved into morphine (abscissa scale) and 1DMe (ordinate scale) components and shown by horizontal and vertical bars, respectively.

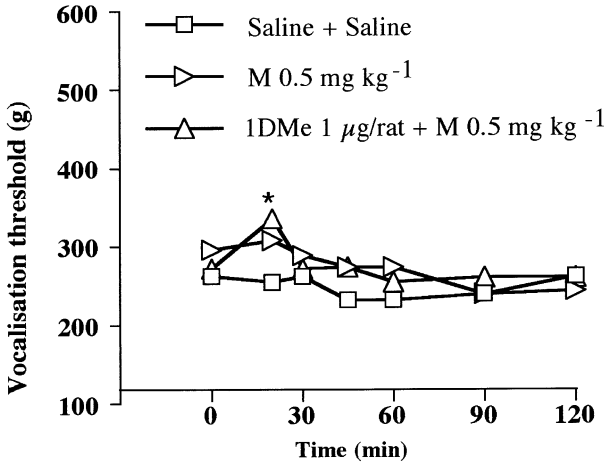

Figure 7 Time course of the effect of the combination of 1DMe (1 μg rat^{-1}, i.t.) and morphine (M) 0.5 mg kg^{-1}, i.v.) or saline i.t. + saline i.v., on paw pressure-induced vocalization thresholds in normal rats. Results are expressed as grams (g) (means ± s.e.mean from 6–7 rats). The absence of an error bar indicates that the value of s.e.mean is smaller than the size of the symbol. *$P<0.05$, versus corresponding predrug values (time 0).

Discussion

The present results indicate that the i.t. administration of the NPFF analogue 1DMe, which is devoid of effect in healthy rats, reverses mechanical hyperalgesia caused by diabetes or sciatic nerve ligature. The lack of activity of 1DMe in N rats agrees with the results obtained using i.t. NPFF (on thermal nociception) by Kontinen & Kalso (1995) but is contrary to the spinal analgesia following mechanical stimulation reported by Gouardères *et al.* (1993; 1996a) in healthy rats. The reasons for this discrepancy are not clear. However (i) the routes of administration are not exactly the same in the reported experiments (pre-implanted catheter) and in the present work (direct i.t. injection) and (ii) the magnitude of the anti-nociceptive effect of 1DMe is lower in the paw pressure test (+40% of threshold elevation) than in the tail-flick test (+100% of threshold elevation) (Gouardères *et al.*, 1996a). Nevertheless, concomitant stimulation of both the pro- and anti-opioid effects of the NPFF system as demonstrated by Million *et al.* (1993), could be responsible for the lack of effect of 1DMe in N rats.

The antinociceptive effect observed after i.t. injection of 1DMe in neuropathic pain models, suggests a new balance between these two opposing effects. The pro-opioid effect of the NPFF analogue is predominant and able to restore normal pain reactions in the two models. This difference between the effect of 1DMe in N and neuropathic animals suggests that low expression of the pro-opioid side of endogenous NPFF, resulting in an imbalance between its pro- and anti-opioid effects, might contribute to the hyperalgesia observed in these models. Moreover, sensitivity to the two components of NPFF is expressed differently according to the etiology of the pathology. For the range of doses used in this study (0.1, 1 and 7.5 μg rat^{-1}), the effect of 1DMe is dose-dependent in MN rats while for the dose of 7.5 μg rat^{-1}, 1DMe completely loses its activity in D rats. These differences lead to the conclusion that 1DMe is more potent in D rats than in MN rats in both its pro- and anti-opioid effects whereas it only exerts its pro-opioid effect, at the doses used, in MN rats.

This demonstration of changes in pharmacological sensitivity due to the etiology of neuropathy agree with the following findings. The neurokinin 1 receptor antagonist, RP-67580 is ineffective in N rats but antinociceptive in D rats (Courteix *et al.*, 1993b) whereas the antinociceptive effect of three NK1 receptor antagonists, CP-96345, SR-140333, RP-67580 is greater in MN than in D rats (Coudoré-Civiale *et al.*, 1998). Furthermore, the cholecystokinin type B (CCK_B) receptor antagonist, CI988, known to inhibit the antagonism of morphine antinociception exerted by endogenous CCK (Xu *et al.*, 1993) has been found to relieve mechanical hyperalgesia in D (Courteix *et al.*, 1997) and to a lesser extent in MN (unpublished data) rats whereas it is devoid of effect in N rats (Courteix *et al.*, 1997).

To ensure that the antinociceptive effect of 1DMe takes place in the endogenous opioid system, the ability of μ- and δ-opioid antagonists to reduce 1DMe-induced antinociception was tested. The loss of antinociceptive effect of 1DMe in the presence of CTOP or naltrindole demonstrates that its effect is mediated through μ- and δ-opioid receptors. Binding experiments have shown that NPFF receptors are distinct from opioid receptors and that NPFF does not directly interact with μ- or δ-opioid receptors (Allard *et al.*, 1989). Forty per cent of NPFF receptors are associated with primary afferents (Gouardères *et al.*, 1996b) that also express opioid receptors suggesting there may be an interaction between these two classes of receptors. Moreover, it has been suggested that NPFF exerts a modulatory role on pain perception through the release of endomorphins (endomorphins 1 and 2, β-endorphin preferentially acting on μ-opioid receptors and enkephalins on δ-opioid receptors) from interneurones in the dorsal horn of the spinal cord (Gouardères *et al.*, 1996a). Whatever the mechanism of action may be, the blockade of either μ- or δ-opioid receptor completely suppresses 1DMe-induced antinociception, and not partially, as expected. This result questions the specificity of the antagoists CTOP and naltrindole in blocking μ- or δ-opioid receptors. However,

several reports suggest that in the range of 0.01 – 1 μg rat^{-1} (i.c.v.) (Calcagnetti & Holtzman, 1991) and 30 μg rat^{-1} (i.t.) (Drower *et al*., 1991), naltrindole fails to attenuate the analgesia produced by DAMGO but antagonizes DPDPE-induced analgesia, providing evidence for the selectivity of NTI at the dose of 1 μg rat^{-1} (i.t.) as a specific δ-opioid receptor antagonist. Similarly the specificity of CTOP at the dose of 10 μg rat^{-1} in successfully antagonizing μ-induced analgesia and not δ-analgesia has been reported by Gouardères *et al*. (1996a). Thus, the expression of 1DMe-induced antinociception requires the availability of both μ- and δ-opioid receptors and the occupancy of δ-opioid receptors with an antagonist influences the μ-mediated effect and *vice versa*. The mechanism underlying this interaction is not known, but physiological and pharmacological experiments demonstrate that some δ-opioid receptors may be linked to μ-opioid receptors (Traynor & Elliott, 1993), these receptors being those responsible for the modulation of μ-antinociception and not for the analgesic effect of the δ-opioid receptor agonist DPDPE (Qi *et al*., 1990) and probably those indirectly involved in the antinociceptive effect of 1DMe in chronic pain conditions in the present study. In the same way, it has been reported that the analgesia induced by classic δ-opioid receptor agonist depends on intact μ-opioid receptors (Sora *et al*., 1997). Thus, a reciprocal modulation of opioid receptors may be postulated.

It has been suggested that the same processes – especially the involvement of anti-opioid systems – which develop during the phenomenon of tolerance could lead to the reduced analgesic efficacy of opioids in neuropathic pain (Hoffmann & Wiesenfeld-Hallin, 1994; Dickenson, 1994; Mao *et al*., 1995). The effect of CCK_B receptor blockade on hyperalgesia or on morphine analgesia has been well described in MN rats (Xu *et al*., 1993). However, the effect of NPFF on morphine analgesia has only been studied after i.c.v. injection in CCI of spinal nerves in rats (Wei *et al*., 1998) and has never been reported in D rats. The co-administration of ineffective doses of 1DMe and morphine in D rats results in significant antinociception; when effective doses were combined, the resulting antinociception was greater than the sum of individual effects but not of longer duration. This superadditive effect was confirmed by isobolographic analysis. In MN rats, the co-administration of ineffective doses of morphine and 1DMe induced antinociception and the use of an ineffective dose of morphine combined with an effective dose of 1DMe potentiated the antinociception observed. The isobolographic analysis suggested that the combination did not produce a superadditive interaction. The results, obtained in MN animals are similar to those obtained in animals with unilateral carrageenan inflammation of the hindpaw, for which no potentiation of morphine-induced antinociception by NPFF was reported (Kontinen *et al*., 1997). The superadditive effect of the combination of 1DMe-morphine in the diabetes-induced neuropathic pain model confirms that, in this pathological condition, 1DMe exerts its pro-opioid effects. The hypothesis, previously advanced to explain its antinociceptive effect, could also underlie the observed synergy. The fact that the reduced efficacy of morphine in D rats can be corrected by increasing the dose (Courteix *et al*., 1998), is consistent with the ability of a pro-opioid agent to exert a synergistic effect with morphine under the same experimental conditions.

The antinociceptive effect of morphine is also potentiated with 1DMe in N rats for which inactive doses of the two drugs produce a weak analgesia. Nevertheless, this potentiation is of short duration. A potentiation by NPFF of morphine-induced antinociception of long duration has been reported in healthy animals (Kontinen & Kalso, 1995; Gouardères *et al*., 1996a). These findings suggest that in normal conditions, the NPFF system needs the stimulation of μ-opioid receptors (with a dose unable to induce analgesia) to exert its pro-opioid effects. Thus in healthy rats, the endogenous equilibrium in favour of the pro-opioid effect of NPFF may be so strong that 1DMe alone is unable to displace it. With the co-administration of 1DMe and morphine, the balance will tip to the pro-opioid effect, giving a weak analgesia.

To conclude, the results reported here demonstrate an antinociceptive effect of 1DMe in neuropathic pain models, with D rats being more sensitive to its biphasic (pro- and anti-opioid) effects than MN rats. The mechanism of action of 1DMe is linked to the availability of μ- and δ-opioid receptors. The interaction of the NPFF system with opioid analgesia has been confirmed by the ability of 1DMe to markedly enhance the antinociceptive effect of morphine in D rats. The demonstration of such a superadditive combination offers both new insight into the controversies surrounding the utility of opioid analgesics in the treatment of neuropathic pain syndromes and new pharmacological tools for neuropathic pain therapy.

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