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Dissociation of pharmacological pro- and anti-opioid effects by neuropeptide FF analogs

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Abstract

Neuropeptide FF (NPFF) and its analog 1DMe ([D-Tyr[1],(NMe)Phe[3]]NPFF) have been shown to reverse or potentiate morphine analgesia in rat depending on the supraspinal or spinal site of injection. The properties, in the mouse tail-flick test, of 1DMe and its related compound Nic-1DMe (Nicotinoyl-Pro-1DMe) were investigated after their local (i.c.v. and i.t.) and systemic administration. Whereas Nic-1DMe and 1DMe exhibit the same affinity and selectivity towards $NPFF_1$ and $NPFF_2$ receptors, Nic-1DMe, in contrast to 1DMe, is unable to inhibit morphine-induced analgesia after i.c.v. and i.p. administration. Conversely, after i.t. and i.p. administration, both neuropeptide FF analogs could potentiate morphine analgesia. Differences in disposition parameters between 1DMe and Nic-1DMe are evidenced, suggesting that the two neuropeptide FF analogs could stimulate differentially supraspinal neuropeptide FF receptors. The predominant activation of spinal neuronal pathways by Nic-1DMe could explain the selective pro-opioid action of this compound after i.t., i.c.v. and i.p. administration.

Keywords: Neuropeptide FF; 1DMe ([D-Tyr[1],(NMe)Phe[3]]NPFF); Morphine; Analgesia; (Mouse); Tail flick

1. Introduction

Neuropeptide FF (NPFF, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2) and other peptides possessing the same C-terminal part, PQRFamide, have been identified in the brain of several mammals (Yang et al., 1985; Yang and Martin, 1995; Bonnard et al., 2001). These peptides are issued from a precursor, the gene of which has been cloned (Perry et al., 1997; Vilim et al., 1999). Recently, a second precursor belonging to this family has been described which exhibits also the same PQRFamide sequence (Hinuma et al., 2000). Molecular characterization of two receptors ($NPFF_1$ and $NPFF_2$) recognizing neuropeptide FF with a high affinity has been reported (Bonini et al., 2000; Elshourbagy et al., 2000; Hinuma et al., 2000; Kotani et al., 2001; Liu et al., 2001).

Although the functional significance of neuropeptide FF and related endogenous peptides remains to be determined, many pharmacological data suggest that neuropeptide FF plays an important role in the pain modulation and could act as an endogenous anti-opioid peptide in rodents (Yang et al., 1985; Roumy and Zajac, 1998). Analgesia induced by stress or morphine injection increases following immuno-neutralization of neuropeptide FF (Lake et al., 1991, 1992) and is attenuated by intracerebroventricular (i.c.v.) administration of neuropeptide FF or neuropeptide FF analogs in rat and mouse (Yang et al., 1985; Gicquel et al., 1992, 1994; Gelot et al., 1998a,b). However, after intrathecal administration in rat, neuropeptide FF induces a strong antinociception and markedly enhances the antinociception produced by administration of opioid receptor agonists (Gouardères et al., 1993, 1996; Kontinen and Kalso, 1995; Xu et al., 1999). This effect, reversed by naloxone, could result from an increase of enkephalin release in the spinal cord (Ballet et al., 1999) and, in fact, a recent study suggests that neuropeptide FF acts as a functional δ-opioid autoreceptor antagonist (Mauborgne et al., 2001).

To date, the pro-opioid effects of neuropeptide FF have been reported after i.t. administration only in rat. In contrast,

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the ability of neuropeptide FF and its analog, 1DMe ([D-Tyr[1],(NMe)Phe[3]]NPFF), to reverse morphine analgesia has been largely studied in mouse after i.c.v. administration (Gicquel et al., 1992, 1994; Gelot et al., 1998a,b).

In the present study, we have investigated the pharmacological effects of analogs of neuropeptide FF not only after i.t. and i.c.v., but also after i.p. administration in mouse by using the tail-flick test. These studies were performed with two synthetic neuropeptide FF analogs, 1DMe and Nic-1DMe (Nicotinoyl-Pro-1DMe) which are partially protected against enzymatic breakdown, especially against aminopeptidases (Gicquel et al., 1992). Injected peripherally, these hydrophobic peptides should cross the blood–brain barrier to reach brain neuropeptide FF receptors. Nic-1DMe possesses at its N-terminal part, a nicotinoyl group to increase its lipophily which could be converted by enzymatic oxidation to a water-soluble pyridinium salt resulting in a hydrophilic peptide form. A spacer amino acid residue, proline, separates this nicotinoyl group from 1DMe to enhance its release by specific enzymes (Bodor et al., 1992; Prokai-Tatrai et al., 1996).

We observed that, depending on the route of administration, 1DMe and Nic-1DMe did not exhibit the same pharmacological properties in the mouse tail-flick test. Differences in disposition parameters between both compounds and in their affinity and selectivity towards $NPFF_1$ and $NPFF_2$ receptors have been investigated.

2. Materials and methods

2.1. Chemicals

1DMe ([D-Tyr[1],(NMe)Phe[3]]NPFF) and Nic-1DMe ([Nic-Pro]1DMe) were prepared by manual solid-phase synthesis according to Mazarguil et al. (2001). Nicotinoyl chloride hydrochloride in dichloromethane reacts with peptide resin in the presence of triethylamine. The purity of the final products was assessed by analytical high-pressure liquid chromatography (HPLC) and their integrity was checked by electro-spray mass spectrometry on a TSQ 700 (Finnigan-Mat, San José, CA).

Dilutions of 1DMe were performed in 0.9% NaCl and dilutions of Nic-1DMe in 0.9% NaCl and 10% CH_3OH.

Morphine hydrochloride was obtained from Francopia, France.

2.2. Iodination of 1DME and Nic-1DME

Iodination of 1DMe and Nic-1DMe was performed under conditions which minimize the production of diiodotyrosine derivative (Dossin et al., 2000). Iodination yields were about 95% and 5% for monoiodo- and diiodo-, respectively. [^{125}I]1DMe and [^{125}I]Nic-1DMe were purified by HPLC on a C18 nucleosyl column (250 × 8 mm, Macherey-Nagel) eluted with CH_3OH/H_2O (60/40 for 1DMe and 63/37 for Nic-1DMe) in trifluoroacetic acid 0.1% (v/v) at 2.5 ml/min. The elution times under these conditions are 5 and 6.8 min for 1DMe and monoiodo-1DMe, respectively, and 6.1 and 8.6 min for Nic-1DMe and monoiodo-Nic-1DMe. The identity of the molecule was verified by mass spectrometry (MALDI-TOF-MS) after cold iodination. The radiochemical purity of [^{125}I]1DMe and [^{125}I]Nic-1DMe was superior to 95%. The specific activity was assumed to be identical to that of $Na^{125}I$ (80.5 TBq/mmol, 2175 Ci/mmol). [^{125}I]1DMe and [^{125}I]Nic-1DMe were stored at 4 °C in the presence of 0.1% bovine serum albumin.

2.3. Animals

All the experiments were performed with male Swiss mice (20–32 g) obtained from Depré (Saint-Doulchard, France). The mice were maintained at 21 ± 1 °C under 12 h/12 h light–dark cycle. Food and water were available ad libitum. Animals were tested in accordance with the European Community guidelines for the use of experimental animals. This study was approved by the local committee of biomedical ethics (Comité Régional d'Ethique Midi-Pyrénées, France).

2.4. Drug injections

The procedure for intrathecal (i.t.) injection was adapted from Hylden and Wilcox (1980). Briefly, unanaesthetized mice were restrained manually, while injections were made into the subarachnoid space, at approximately the L-5 L-6 intervertebral space, with a 10 μl luer tip syringe and a 25-gauge needle. All drugs were delivered i.t. in a volume of 5 μl. Penetration through the dura was correlated to a flick of the tail.

The procedure for intracerebroventricular (i.c.v.) injection was adapted from Haley and McCormick (1957). Briefly, solution of peptide and morphine was loaded into a 10-μl syringe. The mouse was hand-held and gently restrained, the skull was puntured perpendicular to the dorsal surface, and 5 μl of the solution was injected into the lateral ventricle over a period of about 5 s. The stereotaxic coordinates for the lateral ventricle were 2 mm posterior to the bregma and 2.5 mm from the interhemispheric line, to a depth of 3 mm. Intraperitoneal (i.p.) injection volumes were 20 μl/g of mouse.

2.5. Nociceptive test

The nociceptive response was assessed by the tail-flick test (D'Amour and Smith, 1941). Mice were restrained in a cylindrical plastic holder and antinociceptive activity of the drugs was measured by changes in the tail-flick latency time using a lamp intensity specified for a predrug latency time of 2–3 s. A cut-off time was set at 8 s to minimize tissue damage and was taken as criterion for complete analgesia. The effects of drugs were evaluated at different times. Data

are expressed as the maximum percentage effect (MPE) calculated as:

$$\text{MPE} = 100 \times [(\text{post} - \text{drug response} - \text{baseline response}) / (\text{cut} - \text{off response} - \text{baseline response})].$$

The results of experiments are presented as the entire time course of the MPE. The area under the curve (AUC) depicting total MPE vs. time was computed by trapezoidal approximation over the total studied period (120 or 180 min).

Data were analyzed by two-way analysis of variance (ANOVA). Post hoc comparisons were made with Newman–Keuls test for multiple comparisons. The level of significance was set at $P<0.05$.

2.6. Binding assays and autoradiographic procedures

Affinities of Nic-1DMe and 1DMe to $NPFF_1$ receptors were measured by binding assays on membranes of HEK293 cells transfected with $hNPFF_1$ receptors using 0.1 nM of [^{125}I]YVP ([^{125}I]YVPNLPQRF-NH_2) as the radioligand and described in details in Gouardères et al. (in press).

Affinities to $NPFF_2$ receptors were measured by quantitative autoradiography in the superficial layers of the dorsal horn of the rat (male Sprague–Dawley, 350 g, Depré) spinal cord, reported to contain only the $NPFF_2$ receptor (Bonini et al., 2000; Gouardères et al., in press), using 0.05 nM of [^{125}I]EYF ([^{125}I]EYWSLAAPQRF-NH_2) (Gouardères et al., 2001).

All binding experiments were performed at 25 °C in Tris–HCl, 50 mM, pH 7.4 in the presence of 0.1% bovine serum albumin, 60 mM NaCl, 25 μM bestatin (Sigma, France) during 120 min for [^{125}I]YVP ($hNPFF_1$, HEK293 membranes) or 150 min for [^{125}I]EYF ($NPFF_2$, prewashed rat spinal cord sections). 1 μM neuropeptide FF was used to reveal the nonspecific labeling of both iodinated ligands.

Peptide affinities were determined by competition experiments using 10–15 peptide concentrations from 10^{-12} to 10^{-5} M as previously described (Gouardères et al., 2001, in press). Displacement curves were fitted according to a one-site model using Prism 2.0 GraphPad software (USA).

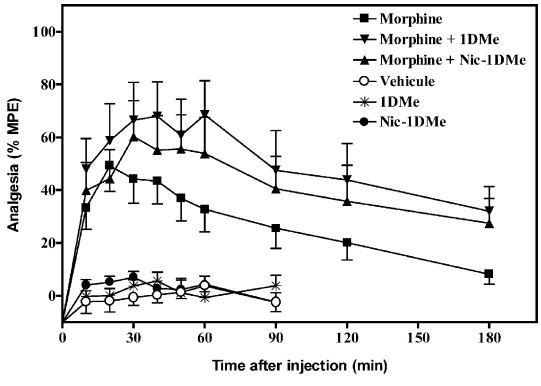

Fig. 1. Effects of i.t. administration of 1DMe and Nic-1DMe on the time course of the antinociception induced by morphine (i.t.) in the mouse tail flick test. A dose of 5 nmol 1DMe (▼) ($n=10$) or Nic-1DMe (▲) ($n=10$) was coinjected intrathecally with 13.3 nmol morphine. Separate animals were injected intrathecally with the peptide (1DMe* or Nic-1DMe ●) ($n=8$) or morphine alone (■) ($n=19$). Data are means ± SEM of MPE. Two-way ANOVA analysis showed significant difference between morphine alone and morphine coinjected with 1DMe or Nic-1DMe ($P<0.05$).

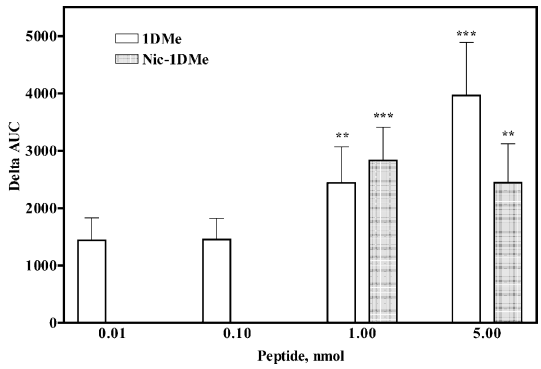

Fig. 2. Effects of different doses of i.t. administered 1DMe and Nic-1DMe on the antinociception induced by morphine (i.t.) in the mouse tail flick test. Data are expressed as differences in area under the curve (AUC) between morphine alone (13.3 nmol) and morphine coinjected with the peptide at the dose indicated, ± SEM. (** $P<0.01$, *** $P<0.001$), significant differences from the action of morphine alone.

2.7. Determination of disposition parameters of [^{125}I] 1DMe and [^{125}I] Nic-1DMe

Fifteen minutes after i.p. injection of 50 μCi of [^{125}I] 1DMe or [^{125}I] Nic-1DMe, 150 μl of blood were rapidly taken and frozen in liquid nitrogen.

The blood was mixed with 500 μl methanol and after centrifugation 15 min at 14,000 rpm, the supernatant was

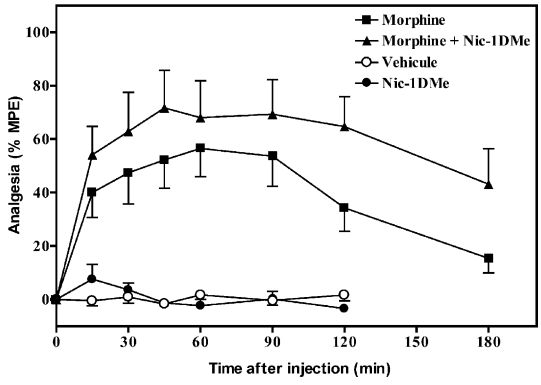

Fig. 3. Effects of i.c.v. administration of Nic-1DMe on the time course of the antinociception induced by morphine (i.c.v.) in the mouse tail flick test. A dose of 32 nmol of Nic-1DMe was coinjected i.c.v. with 2 nmol of morphine (▲) ($n=10$). Separate animals were injected i.c.v. with the peptide (●) ($n=6$) or morphine alone (■) ($n=10$). Data are means ± SEM of MPE. Two-way ANOVA analysis showed significant difference between morphine alone and morphine coinjected with Nic-1DMe ($P<0.05$).

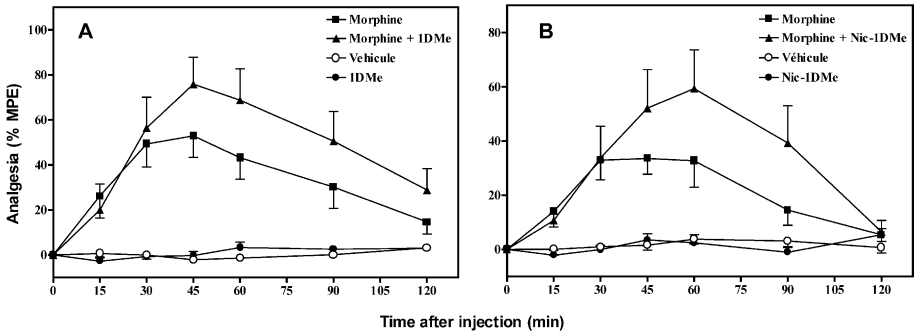

Fig. 4. Effects of i.p. administration of 1DMe (A) and Nic-1DMe (B) on the time course of the antinociception induced by morphine (i.p.) in the mouse tail flick test. A dose of 40 μmol/kg of 1DMe ($n=8$) or 20 μmol/kg of Nic-1DMe ($n=10$) was coinjected i.p. with 13.3 μmol/kg of morphine ($n=15$). Separate animals were injected i.p. with the peptide ($n=10$) or morphine alone ($n=10$). Data are means ± SEM of MPE. Two-way ANOVA analysis showed significant difference between morphine alone and morphine coinjected with the peptide ($P<0.05$).

analyzed by reverse-phase HPLC on a C18 column with a linear gradient of mobile phase A (0.1% trifluoroacetic acid in 10% CH_3OH) and B (0.1% trifluoroacetic acid in 80% CH_3OH) (50/50 to 10/90 during 30 min and 10/90 during 10 min then 50/50 during 10 min). Collected fractions (1.5 ml) were then counted in a γ-counter (Cobra auto-gamma Packard).

[^{125}I] radioactivity remaining in the entire brain was determined after i.c.v. injection of 0.11 μCi in 5 μl of [^{125}I] 1DMe or [^{125}I] Nic-1DMe. Animals were sacrificed 15, 30 or 45 min after i.c.v. injection of the radiolabeled analogs and brains were rapidly dissected and counted in an γ-counter.

3. Results

3.1. Effects of 1DMe and Nic-1DMe after i.t. administration

The effects of 5 nmol 1DMe or Nic-1DMe on the spinal antinociception induced by 13.3 nmol morphine are shown in the Fig. 1. Morphine increased the latency time in a monophasic manner; the response of morphine peaked for 49.2 ± 9.6% at 20 min and declined progressively up to 8.2 ± 3.8% at 180 min. A dose of 5 nmol 1DMe or Nic-DMe alone did not modify the nociceptive threshold.

Coinjection of morphine and either 5 nmol 1DMe or Nic-1DMe resulted in a significant ($P<0.05$) prolongation and enhancement of the effect of morphine. The profile of the response was monophasic similarly to that of morphine, but the maximum effect was greater than this reached by morphine alone (68.5 ± 12.9% at 60 min for 1DMe and 60.3 ± 13.6% at 30 min for Nic-1DMe) and the analgesic effect lasted longer (32.0 ± 9.3% for 1DMe and 27.5 ± 9.4% for Nic-DMe at 180 min).

Injections of lower doses of 1DMe or Nic-1DMe, as illustrated in the Fig. 2, revealed that 1 nmol 1DMe or Nic-DMe produced also a significant increase of the antinociception induced by 13.3 nmol morphine, but 0.01 and 0.1 nmol 1DMe did not modify significantly morphine effects.

3.2. Effects of Nic-1DMe after i.c.v. administration

The effects of 1DMe after i.c.v. injection in mouse are already reported extensively by our laboratory (Gicquel et al., 1992; Gelot et al., 1998a,b). Similarly to 1DMe, i.c.v.-injected Nic-1DMe (0.05 to 0.5 nmol) did not modify (2 nmol) morphine-induced analgesia. However, differences in pharmacological responses induced by 1DMe and Nic-1DMe were revealed by increasing the doses. In contrast to 1DMe (3–25 nmol) which reversed morphine analgesia, Nic-1DMe (3–25 nmol) did not have any effect on morphine analgesia (data not shown). Furthermore, at the highest tested dose, 1DMe (36 nmol) reversed morphine analgesia (data not shown) whereas Nic-1DMe (32 nmol) induced a significant enhancement of the effect of morphine, while by itself, it did not induce any change of the nociceptive threshold (Fig. 3).

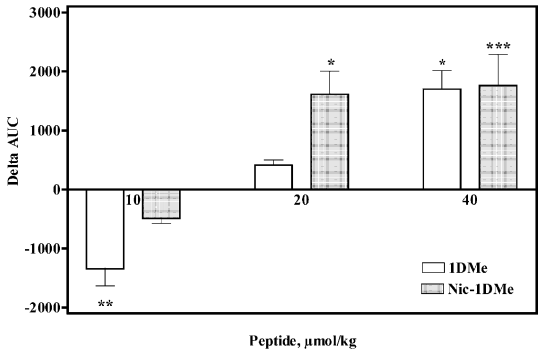

Fig. 5. Effects of different doses of 1DMe (□) and Nic-1DMe (■) injected i.p. on the antinociception induced by morphine (i.p.) in the mouse tail flick test. Data are expressed as differences in area under the curve (AUC) between morphine alone (at a dose giving about 40% of MPE at peak effect) and morphine coinjected with the peptide, at the dose indicated, ± SEM. (* $P<0.05$, *** $P<0.001$), significant differences from the action of morphine alone.

Table 1
Affinity of 1DMe and Nic-1DMe towards neuropeptide FF receptors

Peptide	HEK293, hNPFF$_1$ ([^{125}I]YVP)	Rat dorsal horn, NPFF$_2$ ([^{125}I]EYF)
1DMe	2.01 ± 0.25	0.08 ± 0.01
Nic-1DMe	1.30 ± 0.19	0.07 ± 0.02

Data represent mean K_I values ± SEM. (nM) of three independent experiments, each performed on triplicate samples.
Displacement of [^{125}I]YVP and [^{125}I]EYF specific binding was performed in membranes of cells transfected with hNPFF$_1$ receptor and in the upper dorsal horn of the rat spinal cord containing the NPFF$_2$ receptor, respectively.
[^{125}I]YVP was used at 0.1 nM and [^{125}I]EYF at 0.05 nM, concentrations closed to their respective K_D values (0.18 and 0.04 nM) (Gouardères et al., 2001, in press).
K_I values were calculated by nonlinear regression analysis.

The antinociception induced by Nic-1DMe combined with morphine reached a maximum at 45 min (71.7 ± 14.1%) and was still evident at 180 min (43.1 ± 13.3%), whereas the corresponding value for morphine alone was only 15.4 ± 5.4%. The enhancement of morphine-induced antinociception by Nic-1DMe was also observed when 5 mg/kg (13.3 μmol/kg) morphine was intraperitoneally injected (data not shown).

3.3. Effects of 1DMe and Nic-1DMe after i.p. administration

Coinjected i.p. with 13.3 μmol/kg of morphine, 1DMe (40 μmol/kg) or Nic-1DMe (20 μmol/kg) induced a significant increase of the morphine antinociception, whereas injected alone at the same dose, both peptides did not change the nociceptive threshold (Fig. 4). The maximum of analgesia was reached at 45 min for 1DMe and 60 min for Nic-1DMe and returned to baseline by 120 min.

A lower dose (10 μmol/kg) of 1DMe reduced significantly the antinociceptive effect of morphine (Fig. 5). This anti-opioid effect could not be observed with Nic-1DMe 10 μmol/kg or 5 μmol/kg (data not shown).

3.4. Affinity and selectivity of 1DMe and Nic-1DMe on neuropeptide FF receptors

Nic-1DMe and 1DMe completely inhibited the specific binding of [^{125}I]YVP on NPFF$_1$ receptors in membranes of transfected HEK293 cells as well as the specific binding of [^{125}I]EYF on NPFF$_2$ receptors on rat spinal cord sections with apparent affinities in the nanomolar range (Table 1). Under the same experimental conditions, Nic-1DMe inhibited the specific binding of [^{125}I]EYF to NPFF$_2$ receptors with a calculated K_I value (0.07 nM) very close to that of 1DMe (0.08 nM) (Table 1). Similarly, in NPFF$_1$ receptor-transfected HEK293 cells labeled with [^{125}I]YVP, Nic-1DMe displayed an apparent affinity of 1.30 nM close to that of 1DMe (2.01 nM). Thus, the transformation of the N-terminal part of 1DMe did not modify the affinity for both neuropeptide FF receptors. However, the affinities of Nic-1DMe and 1DMe are approximately 20 times lower for NPFF$_1$ receptors than for NPFF$_2$ receptors (18.6 and 25.1 times, respectively).

3.5. Disposition of ^{125}I-radiolabeled 1DMe and Nic-1DMe

[^{125}I]1DMe or [^{125}I]Nic-1DMe were i.p. injected. Fifteen minutes later, in the blood extracts, different peaks of radioactivity were observed after HPLC separation. A peak coeluting with [^{125}I]1DMe was found for both peptides, whereas for [^{125}I]Nic-1DMe, no radioactivity corresponding to [^{125}I]Nic-1DMe was evidenced (Fig. 6). These features indicate that Nic-1DMe could be metabolized in 1DMe.

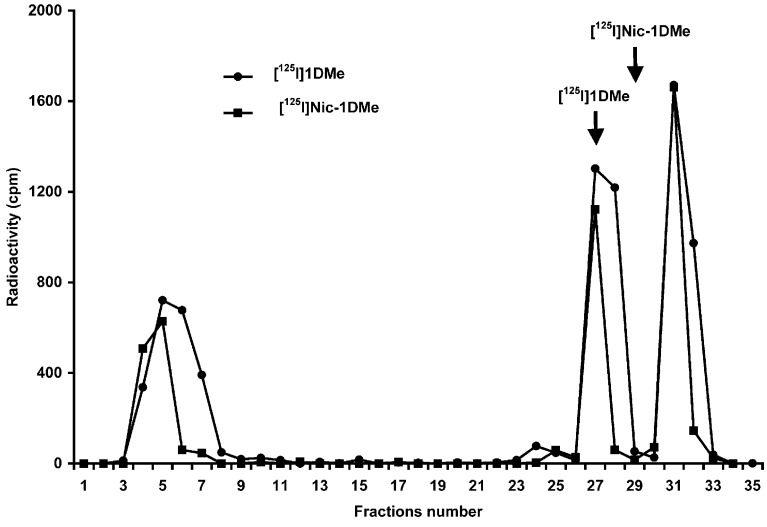

Fig. 6. Chromatogram of the blood radioactivity 15 min after i.p. injection of [^{125}I]1DMe (●) or [^{125}I]Nic-1DMe (■). Radioactivity was extracted from blood and analysed by HPLC. The arrows indicate the elution position of [^{125}I]1DMe and [^{125}I]Nic-1DMe.

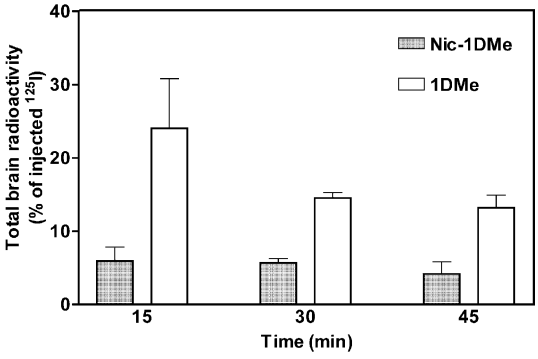

Fig. 7. Kinetic of [^{125}I] radioactivity remaining in the brain after i.c.v. injection of [^{125}I]1DMe or [^{125}I]Nic-1DMe. Data are means ± SEM (n = 3) of radioactivity present in the brain, expressed as percentage of injected radioactivity.

The total radioactivity remaining in the brain 15, 30 and 45 min after i.c.v. [^{125}I]Nic-1DMe administration was about three times lower than after [^{125}I]1DMe injection (4, 2.6 and 3.2 for 15, 30 and 45 min, respectively) (Fig. 7). This result suggests that disposition of the two compounds differed after i.c.v. administration.

4. Discussion

It has been previously shown that subeffective doses of 1DMe injected intrathecally in rat enhanced and prolonged morphine induced analgesia, in the tail-flick and the paw-pressure tests (Gouardères et al., 1993, 1996; Kontinen and Kalso, 1995; Xu et al., 1999). We demonstrate in this study, for the first time in mouse, an increase of morphine analgesia by 1DMe and Nic-1DMe after i.t. injection. The doses of 1DMe required to potentiate morphine analgesia were 100 times higher in mouse (1 nmol) than in rat (0.009 nmol). In the present study, 13.3 nmol morphine promoted 60% of MPE as observed in rat (Gouardères et al., 1996). This value is similar to the ED_{50} value obtained in other studies by using the mouse tail immersion test and the same intrathecal procedure of injection (Tallarida et al., 1999). The comparatively high dose of 1DMe required to produce a clear potentiation of morphine analgesia in mice results probably from the different technique of injection. In fact, in mice, manual injections of 5 μl were performed into the subarachnoid space at the L5–L6 intervertebral space, whereas in rats, a catheter was inserted through the cisterna magna to reach the rostral edge of the lumbar enlargement, corresponding to L1–L2 vertebra at a close distance of the extremity of the spinal cord. The weak distance of diffusion of peptides comparatively to morphine is likely the factor limiting their efficacy after manual injection. Similarly, after i.t. manual injection, very high doses of 1DMe without coinjected morphine are probably required to produce antinociception explaining the absence of such an effect in the range doses used. This is supported by the fact that in rat i.t. injection of 1DMe at the lumbar enlargement level has an antinociceptive effect in the tail-flick test with an ED_{50} value approximating 1 nmol (Gouardères et al., 1996), which is 100 times higher than that required to potentiate morphine antinociception.

After i.t. administration, 1DMe and Nic-1DMe similarly induced a potentiation of morphine antinociception. In contrast, the peptides, i.c.v.-injected, induced different effects, since Nic-1DMe (32 nmol) potentiates morphine antinociception, while 1DMe (3–36 nmol) reduces morphine effects. These differences in activity could result from different binding properties of the analogs towards each $NPFF_1$ and $NPFF_2$ receptors present in the tissue. However, the present study demonstrates that Nic-1DMe and 1DMe exhibit the same affinity for $NPFF_2$ receptors ($K_I \approx 0.1$ nM) as well as for $NPFF_1$ receptors ($K_I \approx 2$ nM). Interestingly, Nic-1DMe and 1DMe did not differ by their absolute affinity for each neuropeptide FF receptor nor by their selectivity between both receptors. This simplifies the interpretation of pharmacological data since in any cases, the differences in biological effects induced by both analogs could not be attributed to their differences in their ability to interact with neuropeptide FF receptors.

It is interesting to note that the affinities of the analogs are 20 times lower for the $NPFF_1$ receptors than for the $NPFF_2$ receptors. Although the relative distribution of $NPFF_1$ and $NPFF_2$ receptors in mouse brain is not yet established, both receptors could be localized differentially as recently reported in the rat brain (Gouardères et al., in press), reflecting the pattern of mRNA distribution in rat where $NPFF_1$ messengers are absent from spinal cord (Liu et al., 2001). In this case, pharmacokinetic parameters of 1DMe and Nic-1DMe after i.c.v. injection could induce a different action on $NPFF_1$ and $NPFF_2$ receptors. The fact that the total radioactivity remaining in the brain 15, 30 and 45 min after i.c.v. [^{125}I]Nic-1DMe administration was about three times lower than after [^{125}I]1DMe injection supports this possibility.

Nic-1DMe was synthesized with the aim to increase the blood–brain barrier passage and to increase central activity after systemic administration in comparison to 1DMe. In fact, we observed that low doses of i.p. administered Nic-1DMe did not modify morphine analgesia, whereas similar doses of 1DMe reversed it. Since Nic-1DMe is more lipophilic than 1DMe (as quantified by its HPLC retention time) and should cross the blood–brain barrier with a higher efficacy, our results suggest that disposition of 1DMe and Nic-1DMe differs. After i.p. administration of [^{125}I]Nic-1DMe, [^{125}I]1DMe and other iodinated metabolites were isolated in the blood, whereas no [^{125}I]Nic-1DMe was detected. This indicates that Nic-1DMe can be transformed in 1DMe. Thus, increasing the doses of i.p. administered Nic-1DMe will reveal an anti-opioid effect due to its transformation in 1DMe. However, such an anti-opioid effect was never observed at any tested doses of i.p. administered Nic-1DMe. In contrast, at the highest dose used, a pro-opioid effect was evidenced.

Other differences in disposition parameters between 1DMe and Nic-1DMe could be evidenced since total radioactivity remaining in the brain after i.c.v. [^{125}I]Nic-1DMe administration was about three times lower than after [^{125}I]1DMe injection.

Clearly, 1DMe and Nic-1DMe did not exhibit the same pharmacological properties since Nic-1DMe, in contrast to 1DMe, did not inhibit morphine analgesia whatever the route of administration. Such a dissociation between pro- and anti-opioid effects cannot be related to differences in the binding properties of the compounds towards $NPFF_1$ and $NPFF_2$ receptors. Our results rather suggest that disposition parameters of the analogs may influence the pro- and anti-opioid pharmacological responses. Furthermore, the present study suggests that spinal and supraspinal neuropeptide FF binding sites differ in their mode of control of opioid functions. In contrast to 1DMe, Nic-1DMe does not induce reversion of morphine analgesia after i.c.v. and i.p. injection, whereas after i.t. administration, both analogs potentiated morphine analgesia. This observation suggests that both compounds similarly act on neuropeptide FF receptors at the spinal level, but produce different effects at the supraspinal level. Since both compounds exhibit the same affinity for $NPFF_2$ receptors as well as for $NPFF_1$ receptors, we propose that, rather the subtype of neuropeptide FF receptor, the specific localization of activated neuronal pathways is responsible for these differences. Supraspinal neuropeptide FF binding sites are clearly involved in the anti-opioid action of neuropeptide FF, whereas spinal neuropeptide FF binding sites are responsible for the pro-opioid effect. Our study suggests that, in contrast to 1DMe, the disposition of Nic-1DMe cannot lead to a selective activation of supraspinal neuropeptide FF binding sites. Thus, Nic-1DMe did not exhibit anti-opioid effects after systemic and cerebral administration.

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References

Ballet, S., Mauborgne, A., Gouardères, C., Bourgoin, A.S., Zajac, J.M., Hamon, M., Cesselin, F., 1999. The neuropeptide FF analogue, 1DMe, enhances in vivo met-enkephalin release from the rat spinal cord. Neuropharmacology 38, 1317–1324.

Bodor, N., Prokai, L., Wu, W.M., Farag, H., Jonalagadda, S., Kawamura, M., Simpkins, J., 1992. A strategy for delivering peptides into the central nervous system by sequential metabolism. Science 257, 1698–1700.

Bonini, J.A., Jones, K.A., Adham, N., Forray, C., Artymyshyn, R., Durkin, M.M., Smith, K.E., Tamm, J.A., Boteju, L.W., Lakhlani, P.P., Raddatz, R., Yao, W.J., Ogozalek, K.L., Boyle, N., Kouranova, E.V., Quan, Y., Vaysse, P.J., Wetzel, J.M., Branchek, T.A., Gerald, C., Borowsky, B., 2000. Identification and characterization of two G protein-coupled receptors for neuropeptide FF. J. Biol. Chem. 275, 39324–39331.

Bonnard, E., Burlet-Schiltz, O., Francés, B., Mazarguil, H., Monsarrat, B., Zajac, J.M., Roussin, A., 2001. Identification of neuropeptide FF-related peptides in rodent spinal cord. Peptides 22, 1085–1092.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Dossin, O., Mouledous, L., Baudry, X., Tafani, J.A., Mazarguil, H., Zajac, J.M., 2000. Characterization of a new radioiodinated probe for the alpha2C adrenoceptor in the mouse brain. Neurochem. Int. 36, 7–18.

Elshourbagy, N.A., Ames, R.S., Fitzgerald, L.R., Foley, J.J., Chambers, J.K., Szekeres, P.G., Evans, N.A., Schmidt, D.B., Buckley, P.T., Dytko, G.M., Murdock, P.R., Tan, K.B., Shabon, U., Nuthulaganti, P., Wang, D.Y., Wilson, S., Bergsma, D.J., Sarau, H.M., 2000. Receptor for the pain modulatory neuropeptides NPFF and NPAF is an orphan G-protein-coupled receptor. J. Biol. Chem. 275, 25965–25971.

Gelot, A., Francés, B., Roussin, A., Latapie, J.P., Zajac, J.M., 1998a. Anti-opioid efficacy of neuropeptide FF in morphine-tolerant mice. Brain Res. 808, 166–173.

Gelot, A., Mazarguil, H., Dupuy, P., Francés, B., Gouardères, C., Roumy, M., Zajac, J.M., 1998b. Biochemical, cellular and pharmacological activities of a human neuropeptide FF-related peptide. Eur. J. Pharmacol. 354, 167–172.

Gicquel, S., Mazarguil, H., Allard, M., Simonnet, G., Zajac, J.M., 1992. Analogs of F8Famide resistant to degradation, with high affinity and in vivo effects. Eur. J. Pharmacol. 222, 61–67.

Gicquel, S., Mazarguil, H., Desprat, C., Allard, M., Devillers, J.P., Simonnet, G., Zajac, J.M., 1994. Structure-activity study of Neuropeptide FF: contribution of N-terminal regions to affinity and activity. J. Med. Chem. 37, 3477–3481.

Gouardères, C., Sutak, M., Zajac, J.M., Jhamandas, K., 1993. Antinociceptive effect of intrathecally administered F8Famide and FMRFamide in the rat. Eur. J. Pharmacol. 237, 73–81.

Gouardères, C., Jhamandas, K., Sutak, M., Zajac, J.M., 1996. Role of opioid receptors in the spinal antinociceptive effects of neuropeptide FF analogues. Br. J. Pharmacol. 117, 493–501.

Gouardères, C., Mollereau, C., Tafani, J.A., Mazarguil, H., Zajac, J.M., 2001. [^{125}I]EYF: a new high affinity radioligand to neuropeptide FF receptors. Peptides 22, 623–629.

Gouardères, C., Quelven, I., Mollereau, C., Mazarguil, H., Rice, S.Q.J., Zajac, J.M., 2002. Quantitative autoradiographic distribution of $NPFF_1$ neuropeptide FF receptor in the rat brain and comparison with $NPFF_2$ receptor by using [^{125}I]YVP and [^{125}I]EYF as selective radioligands. Neuroscience, in press.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intra-cerebral injection of drugs in conscious mouse. Br. J. Pharmacol. 12, 12–15.

Hinuma, S., Shintani, Y., Fukusumi, S., Iijima, N., Matsumoto, Y., Hosoya, M., Fujii, R., Watanabe, T., Kikuchi, K., Terao, Y., Yano, T., Yamamoto, T., Kawamata, Y., Habata, Y., Asada, M., Kitada, C., Kurokawa, T., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y., Fujino, M., 2000. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat. Cell Biol. 2, 703–708.

Hylden, J.L., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Kontinen, V.K., Kalso, E.A., 1995. Differential modulation of a2-adrenergic and m-opioid spinal antinociception by neuropeptide FF. Peptides 16, 973–977.

Kotani, M., Mollereau, C., Detheux, M., Le Poul, E., Brezillon, S., Vakili, J., Mazarguil, H., Vassart, G., Zajac, J.M., Parmentier, M., 2001. Functional characterization of a human receptor for neuropeptide FF and related peptides. Br. J. Pharmacol. 133, 138–144.

Lake, J.R., Hammond, M.V., Shaddox, R.C., Hunsicker, L.M., Yang,

H.Y.T., Malin, D.H., 1991. IgG from neuropeptide FF antiserum reverses morphine tolerance in the rat. Neurosci. Lett. 132, 29–32.

Lake, J.R., Hebert, K.M., Payza, K., Deshotel, K.D., Hausam, D.D., Witherspoon, W.E., Acangeli, K.A.R., Malin, D.H., 1992. Analogs of neuropeptide FF attenuates morphine tolerance. Neurosci. Lett. 146, 203–206.

Liu, Q., Guan, X.M., Martin, W.J., McDonald, T.P., Clements, M.K., Jiang, Q., Zeng, Z., Jacobson, M., Williams Jr., D.L., Yu, H., Bomford, D., Figueroa, D., Mallee, J., Wang, R., Evans, J., Gould, R., Austin, C.P., 2001. Identification and characterization of novel mammalian neuropeptide FF-like peptides that attenuate morphine-induced antinociception. J. Biol. Chem. 40, 36961–36969.

Mauborgne, A., Bourgoin, S., Polienor, H., Roumy, M., Simonnet, G., Zajac, J.M., Cesselin, F., 2001. The neuropeptide FF analogue, 1DMe, acts as a functional opioid autoreceptor antagonist in the rat spinal cord. Eur. J. Pharmacol. 430, 273–276.

Mazarguil, H., Gouardères, C., Tafani, J.A.M., Marcus, D., Kotani, M., Mollereau, C., Roumy, M., Zajac, J.M., 2001. Structure-activity relationships of neuropeptide FF: role of C-terminal regions. Peptides 22, 1471–1478.

Perry, S.J., Huang, E.Y., Cronk, D., Bagust, J., Sharma, R., Walker, R.J., Wilson, S., Burke, J.F., 1997. A human gene encoding morphine modulating peptides related to NPFF and FMRFamide. FEBS Lett. 409, 426–430.

Prokai-Tatrai, K., Prokai, L., Bodor, N., 1996. Brain-targeted delivery of a leucine–enkephalin analogue by retrometabolic design. J. Med. Chem. 39, 4775–4782.

Roumy, M., Zajac, J.M., 1998. Neuropeptide FF, pain and analgesia. Eur. J. Pharmacol. 345, 1–11.

Tallarida, R.J., Stone Jr., D.J., McCary, J.D., Raffa, R.B. 1999. Response surface analysis of synergism between morphine and clonidine. J. Pharmacol. Exp. Ther. 289, 8–13.

Vilim, F.S., Aarnisalo, A.A., Nieminen, M.L., Lintunen, M., Karlstedt, K., Kontinen, V.K., Kalso, E., States, B., Panula, P., Ziff, E., 1999. Gene for pain modulatory neuropeptide NPFF: induction in spinal cord by noxious stimuli. Mol. Pharmacol. 55, 804–811.

Xu, M., Kontinen, V.K., Panula, P., Kalso, E., 1999. Effects of (1DMe) NPYF, a synthetic neuropeptide FF analogue, in different pain models. Peptides 20, 1071–1077.

Yang, H.Y.T., Martin, B.M., 1995. Isolation and characterization of a neuropeptide from brain and spinal cord of rat. Society for Neurosciences Abstract San Diego.

Yang, H.Y.T., Fratta, W., Majane, E.A., Costa, E., 1985. Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. U. S. A. 82, 7757–7761.