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Comparison of pharmacological activities of Neuropeptide FF_1 and Neuropeptide FF_2 receptor agonists

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Abstract

The pharmacological effects of Neuropeptide FF (NPFF) analogs exhibiting different selectivities towards Neuropeptide FF_1 ($NPFF_1$) and Neuropeptide FF_2 ($NPFF_2$) receptors were investigated after supraspinal administration in mice. Injected into the third ventricle, VPNLPQRF-NH_2, which is selective for Neuropeptide FF_1 receptor induced a hypothermia while EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 which are selective for Neuropeptide FF_2 receptor, did not. Furthermore, EFWSLAAPQRF-NH_2 significantly increased the body temperature when compared to saline treated mice, indicating that Neuropeptide FF_1 receptor could be responsible for hypothermia while Neuropeptide FF_2 mediated an hyperthermic effect. After administration into the lateral ventricle, 1DMe ([D.Tyr^1,(N.Me)Phe^3]NPFF), a weakly selective Neuropeptide FF_2 receptor agonist, exerted a clear anti-opioid effect in the tail flick test. The selective Neuropeptide FF_1 receptor agonist VPNLPQRF-NH_2 did not induce significant anti-opioid actions but rather increased, dose-dependently, morphine analgesia while EFWSLAAPQRF-NH_2, the highest selective Neuropeptide FF_2 receptor analog, induced the same pharmacological effect as VPNLPQRF-NH_2 at comparable doses. These features indicate that the pro- and anti-opioid actions are not strictly related to the selectivity towards Neuropeptide FF_2 or Neuropeptide FF_1 receptor. Our data demonstrate that Neuropeptide FF_1 and Neuropeptide FF_2 receptors differently modulate nervous system functions.

Keywords: Neuropeptide FF; Receptor; Morphine; Analgesia; (Mouse)

1. Introduction

Neuropeptide FF (NPFF, FLFQPQRF-NH_2) represents a neurotransmitter system acting as a modulator of endogenous opioid functions (Zajac and Gouardères, 2000). Two precursors (Hinuma et al., 2000; Perry et al., 1997; Vilim and Ziff, 1995) containing the same C-terminal PQRF-NH_2 sequence have been cloned in mammals, a proNPFF$_A$ containing FLFQPQRF-NH_2 (NPFF) and SLAAPQRF-NH_2 (NPSF) sequences and a proNPFF$_B$ containing LPLRF-NH_2 and LPQRF-NH_2 (called also RFamide related peptide precursor (Hinuma et al., 2000)). Several peptides processed from these precursors have been isolated in the rodent central nervous system (Bonnard et al., 2001, 2003). Several arguments justify that the longest form of peptides corresponding to the classical processing rules of precursor maturation are likely to be the physiologically active neurotransmitters in rat brain (Roumy et al., 2000).

The pharmacological effects of Neuropeptide FF analogs result from their interactions with two G protein-coupled receptors, Neuropeptide FF_1 and Neuropeptide FF_2, recently cloned and characterized in mammals (Bonini et al., 2000; Elshourbagy et al., 2000; Hinuma et al., 2000; Kotani et al., 2001). Neuropeptide FF_1 and Neuropeptide FF_2 receptors are about 50% identical and are most similar to neuropeptide Y and orexin receptors. The pharmacological and functional profiles of human Neuropeptide FF_1 and Neuropeptide FF_2 receptors, stably expressed in CHO cells, have been recently compared (Mollereau et al., 2002). Each Neuropeptide FF receptor recognizes peptides derived from both precursors

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with affinities in the nanomolar range, however with a slight preference of pro-$NPFF_A$ peptides for Neuropeptide FF_2 receptors and of pro-$NPFF_B$ peptides for Neuropeptide FF_1 receptors. These receptors are Gi/o-protein coupled when expressed in several cell lines (Bonini et al., 2000; Kotani et al., 2001). At this time, the endogenous intracellular signalling pathways activated by Neuropeptide FF receptors in neurones are still unknown, while high Neuropeptide FF receptor agonists concentrations could stimulate adenylate cyclase activity in membranes of mouse olfactory bulb (Gherardi and Zajac, 1997). At the cellular level, Neuropeptide FF receptor agonists exhibit anti-opioid effects since they attenuate the magnitude of the inhibitory effect of nociceptin and of mu-opioid receptor agonist on the conductance of N-type Ca2+ channels (Rebeyrolles et al., 1996; Roumy and Zajac, 2001).

Neuropeptide FF has been designated as a morphine modulatory peptide on the basis of its ability to influence opioid analgesia within the spinal cord and brain (Panula et al., 1996; Roumy and Zajac, 1998). In rodents, Neuropeptide FF receptor agonists exhibit either anti-opioid activities or potentiate opioid analgesia when injected intracerebroventricularly or intrathecally, respectively (see references in Roumy and Zajac, 1998). After intracerebroventricular (i.c.v.) administration, Neuropeptide FF reverses morphine antinociception in the tail flick test, both in rat and mouse (Dupouy and Zajac, 1995; Gelot et al., 1998a; Yang et al., 1985) and produces an abstinence syndrome in naive and morphine-tolerant rats (Malin et al., 1990). Conversely, in rat, intrathecal injection of Neuropeptide FF receptor agonists produces a strong and long lasting antinociception and potentiates in mouse the morphine-induced analgesia (Gouardères et al., 1996; Quelven et al., 2002). These Neuropeptide FF antinociceptive effects are related to the release of Met-enkephalin-like immunoreactive material in the spinal cord (Ballet et al., 1999; Mauborgne et al., 2001).

We have recently demonstrated that both Neuropeptide FF_1 and Neuropeptide FF_2 receptors induce anti-opioid actions at the cellular level (Roumy et al., 2003) suggesting that the pro-opioid effects observed in functional pharmacology reflect a circuitry-induced control of opioid functions rather than a specific effect mediated by one Neuropeptide FF receptor type. The question remains however, among the different pharmacological activities of Neuropeptide FF receptor agonists, whether each receptor type is particularly associated with one neuronal function since Neuropeptide FF_1 and Neuropeptide FF_2 receptors are distributed very differently in the central nervous system (Gouardères et al., 2004). The recent availability of selective ligands for each Neuropeptide FF receptors should help to investigate such question (Mollereau et al., 2002). In order to precise the implication of each Neuropeptide FF receptor in the hypothermic and anti-opioid effects of Neuropeptide FF, we have investigated the pharmacological effects of analogs presenting different selectivities towards Neuropeptide FF_1 and Neuropeptide FF_2 receptors.

2. Materials and methods

2.1. Chemicals

1DMe ([D.Tyr^1,(N.Me)Phe^3]NPFF), EFWSLAAPQRF-NH_2, SPAFLFQPQRF-NH_2 and VPNLPQRF-NH_2 were synthesized using an automated peptide synthesizer (Applied Biosystems model 433A).

Morphine hydrochloride was obtained from Francopia (France).

2.2. Animals

All experiments were performed with male Swiss mice (22–32 g) obtained from Depré (Saint-Doulchard, France). Mice were maintained at 21±1 °C under 12 h/12 h light–dark cycle with food and water available ad libitum. Animals were tested according to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. This study was approved by the local committee of biomedical ethics (Comité Régional d'Ethique Midi-Pyrénées, France).

2.3. Intracerebroventricular (i.c.v.) injections

The procedure for i.c.v. injection was adapted from Haley and McCormick (1957). Briefly, a 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 third ventricle (thermoregulation) or the lateral ventricle (antinociception) over a period of about 5 s. The stereotaxic coordinates for the injection into the third ventricle were 2 mm anterior to the interaural line, on the interhemispheric line, to a depth of 2.5 mm. For the injection into the lateral ventricle, the coordinates were 2 mm posterior to the bregma and 2.5 mm from the interhemispheric line, to a depth of 3 mm.

2.4. Temperature measurement

The animals were restrained in a cylindrical plastic holder. Rectal temperature was measured with a thermistor probe (Ellab Instrument, Copenhagen) inserted to a depth of 2.5 cm into the rectum. Body temperature was recorded before injection and then at 10-min intervals for 60 min. Changes in body temperature after injection and before drug administration were calculated for each animal. The experiments were performed between 10:00 and 14:00 h.

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 the activity of the drugs was measured by changes in the tail flick latency time using a

Table 1
Affinities (K_i) of Neuropeptide FF analogs on human Neuropeptide FF_1 and Neuropeptide FF_2 receptors

	Neuropeptide FF_1 K_i (nM)	Neuropeptide FF_2 K_i (nM)	$S_{1/2}$
FLFQPQRF-NH_2 (NPFF)	2.82±0.06	0.21±0.03	13
[D.Tyr^1,(N.Me)Phe^3]NPFF	1.09±0.03	0.18±0.04	6
VPNLPQRF-NH_2	0.6±0.1	17.4±1.7	0.03
EFWSLAAPQRF-NH_2	20.8±0.8	0.21±0.01	94
SPAFLFQPQRF-NH_2	2.62±0.5	0.047±0.003	56

Data are mean±S.D. determined previously (Gouardères et al., 2002; Mollereau et al., 2002). $S_{1/2}$=K_i Neuropeptide FF_1 receptor/K_i Neuropeptide FF_2 receptor represents the index of selectivity.

lamp intensity specified for a pre-drug 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:

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

2.6. Statistical analysis

2.6.1. Temperature measurement

Statistical analyses were performed on the time course of changes in body temperature before injection (baseline) and every 10 min until 60 min after injection of NaCl or peptide for each animal. Data were analyzed by two-way analysis of variance (ANOVA), the factors being treatment (peptide versus NaCl) and time.

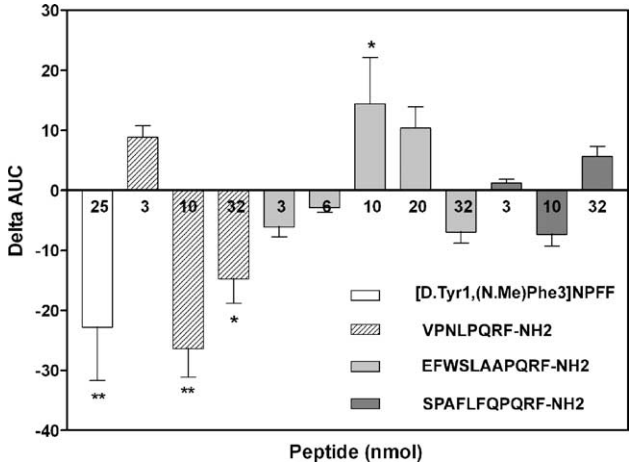

Fig. 1. Effects of different doses of [D.Tyr^1,(N.Me)Phe^3]NPFF, VPNLPQRF-NH_2, EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 injected i.c.v. in the third ventricle on changes in body temperature in mouse. Data are expressed as differences in area under the curve (AUC) between peptides at the dose indicated and NaCl, ±S.E.M. during 60 min (*P<0.05, **P<0.01, significant differences from the action of NaCl with two-way ANOVA followed by Bonferroni's post-hoc test). The statistical analysis was performed on time course peptide effects as described the legend of the Fig. 2.

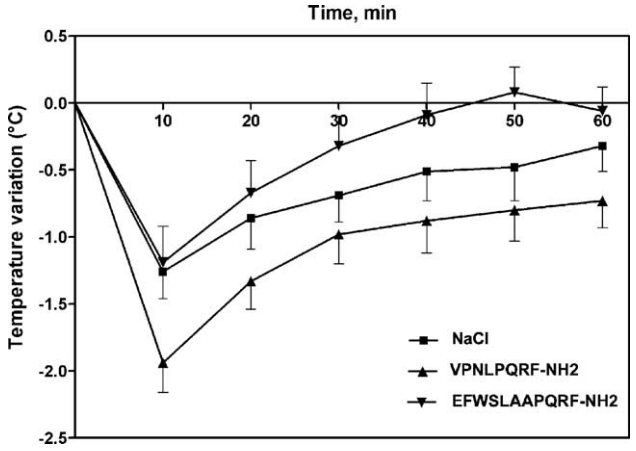

Fig. 2. Time course of the change in body temperature induced by VPNLPQRF-NH_2 and EFWSLAAPQRF-NH_2 injected i.c.v. in the third ventricle. The rectal temperature was recorded after injection of 10 nmol of VPNLPQRF-NH_2 (n=9) or EFWSLAAPQRF-NH_2 (n=10). Independent animals were injected with NaCl (n=9). Data are means±S.E.M. of change in body temperature before injection and every 10 min after injection. The baseline temperatures were 38.0±0.11, 38.1±0.06 and 37.9±0.12 °C in NaCl, VPNLPQRF-NH_2 and EFWSLAAPQRF-NH_2 group, respectively. Two-way ANOVA, followed by Bonferroni's post-hoc test, shows significant difference between NaCl and peptide (P<0.01 for VPNLPQRF-NH_2 and EFWSLAAPQRF-NH_2).

2.6.2. Nociceptive test

Statistical analyses were performed on the time course of MPE after injection of morphine or peptide during 90 min. Data were analyzed by two-way analysis of variance (ANOVA), the factors being treatment (peptide plus

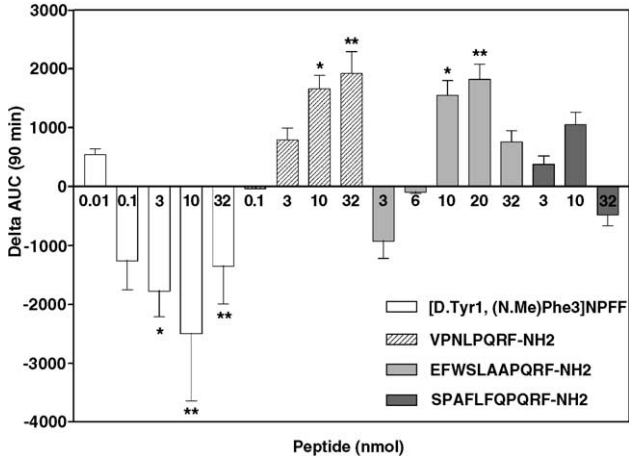

Fig. 3. Effects of different doses of [D.Tyr^1,(N.Me)Phe^3]NPFF, VPNLPQRF-NH_2, EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 injected i.c.v. on the antinociception induced by morphine (i.c.v.) in the mouse tail flick test. Data are expressed as mean (±S.E.M.) differences in area under the curve (AUC) between morphine alone (1.5 nmol) and morphine coinjected with the peptide, at the dose indicated during 90 min. (*P<0.05, **P<0.01) significant differences from the action of morphine alone with two way ANOVA, followed by Bonferroni's post-hoc test. The statistical analysis was performed on time course peptide effects as detailed in the legend of the Figs. 4 and 5.

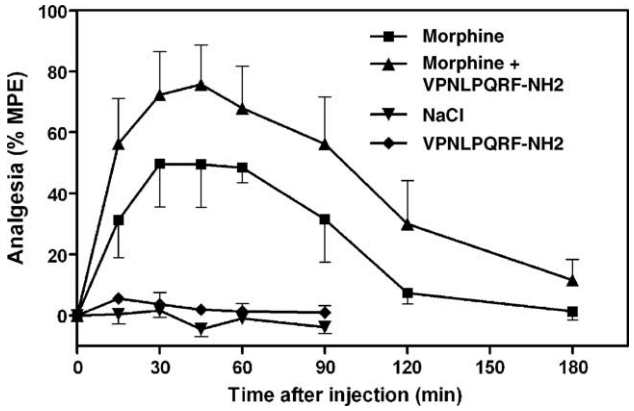

Fig. 4. Time course of the effects of i.c.v. administration of VPNLPQRF-NH_2 on the antinociception induced by 1.5 nmol of morphine (i.c.v.) in the mouse tail flick test. 1.5 nmol of morphine was injected i.c.v. alone (n=10) or coinjected with 32 nmol of VPNLPQRF-NH_2 (n=9). Separate animals were injected i.c.v. with VPNLPQRF-NH_2 (32 nmol) (n=7) or NaCl (n=7). Data are means±S.E.M. of MPE. Two-way ANOVA analysis, followed by Bonferroni's post-hoc test, showed significant difference between morphine alone and morphine coinjected with 32 nmol of VPNLPQRF-NH_2 (P<0.001).

morphine versus morphine) and time. Post-hoc comparisons were made with Bonferroni's test.

3. Results

The selectivity and the affinity to Neuropeptide FF_1 and Neuropeptide FF_2 receptors of the different ligands used in this study ([D.Tyr^1,(N.Me)Phe^3]NPFF, VPNLPQRF-NH_2, EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 are presented in the Table 1.

3.1. Effects of [D.Tyr^1,(N.Me)Phe^3]NPFF, VPNLPQRF-NH_2, EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 i.c.v. injection on body temperature

As previously described (Desprat and Zajac, 1997; Frances et al., 2001) [D.Tyr^1,(N.Me)Phe^3]NPFF (25 nmol) administered into the third ventricle induced a significant hypothermia compared to NaCl (Fig. 1). VPNLPQRF-NH_2 (3 nmol) did not modify significantly the body temperature but higher doses (10–32 nmol) induced a significant hypothermia (Fig. 1) as compared to saline treatment. The maximal hypothermic effect of 10 nmol VPNLPQRF-NH_2 occurred 10 min after injection (−2.0±0.2 °C versus −1.3±0.2 °C for NaCl) (Fig. 2).

EFWSLAAPQRF-NH_2 did not induce hypothermia at the doses tested (3, 6, 10, 20 and 32 nmol) (Fig. 1). In contrast, 10 nmol EFWSLAAPQRF-NH_2 significantly increased body temperature as compared to NaCl. The effect was maximal 50 min after injection (0.1±0.2 °C versus −0.5±0.2°C for NaCl) (Fig. 2).

No significant effect on body temperature compared to NaCl was observed after i.c.v. administration of SPAFLFQPQRF-NH_2 (3, 10 and 32 nmol) (Fig. 1).

3.2. [D.Tyr^1,(N.Me)Phe^3]NPFF, VPNLPQRF-NH_2, EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 and morphine-induced analgesia

A dose of 1.5 nmol morphine into the lateral ventricle was used to induce a 50% analgesia (Figs. 3–5) allowing investigation of both potentialisation and reversion of morphine effects.

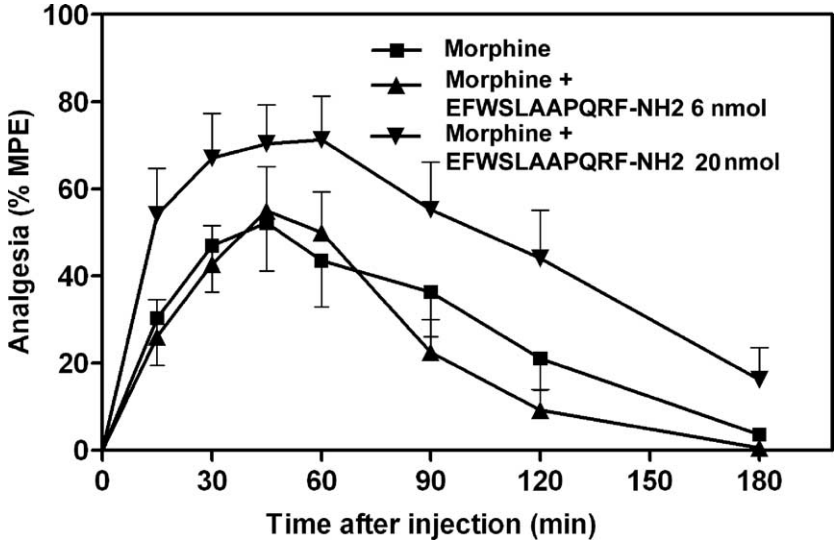

Fig. 5. Time course of the effects of i.c.v. administration of 6 and 20 nmol of EFWSLAAPQRF-NH_2 on the antinociception induced by 1.5 nmol of morphine (i.c.v.) in the mouse tail flick test. 1.5 nmol of morphine was injected i.c.v. alone (n=10) or coinjected with 6 (n=10) or 20 (n=7) nmol of EFWSLAAPQRF-NH_2. Data are means±S.E.M. of MPE. Two-way ANOVA analysis, followed by Bonferroni's post-hoc test, showed significant difference between morphine alone and morphine coinjected with 20 nmol of EFWSLAAPQRF-NH_2 (P<0.001).

[D.Tyr^1,(N.Me)Phe^3]NPFF administered into the lateral ventricle, dose-dependently reversed morphine-induced analgesia in the mouse tail flick test (Fig. 3). This effect was significant for doses larger than 3 nmol as previously described (Gicquel et al., 1992, 1994).

VPNLPQRF-NH_2 (0.1 and 3 nmol) did not significantly change morphine analgesia but larger doses (10–32 nmol) induced a significant increase of morphine analgesia (Figs. 3 and 4). The maximal analgesia was observed 45 min after injection (75.7±13.0% for morphine plus 32 nmol VPNLPQRF-NH_2 versus 49.5±14.0% for morphine alone) and at 120 min the analgesia was 30.0±14.2% versus 11.3±3.6% for morphine. Injected alone, VPNLPQRF-NH_2 (32 nmol) did not change nociceptive threshold.

I.c.v. administration of EFWSLAAPQRF-NH_2 (10–20 nmol) alone did not modify antinociceptive threshold but significantly increased morphine induced analgesia, whereas administration of 3, 6 or 32 nmol did not change it significantly (Fig. 3). With 20 nmol, the effect of EFWSLAAPQRF-NH_2 was maximal at 60 min (Fig. 5) 71.3±9.9% versus 43.5±10.6% for morphine alone and the effect was still observed at 120 min; 44.1±11.0% versus 21.1±7.3% for morphine.

After i.c.v. administration of SPAFLFQPQRF-NH_2 (3–32 nmol) morphine-induced analgesia was not significantly modified (Fig. 3).

Although EFWSLAAPQRF-NH_2 i.c.v. injection (10–20 nmol) increased analgesia induced by 1.5 nmol of morphine, EFWSLAAPQRF-NH_2 (6 nmol) significantly reduced analgesia induced by a higher dose of morphine (2.5 nmol) inducing 75% analgesia (Fig. 6). After EFWSLAAPQRF-NH_2 and morphine coinjection, the maximal analgesic effect was 54.2±12.7% at 60 min versus 71.0±14.0% for morphine alone.

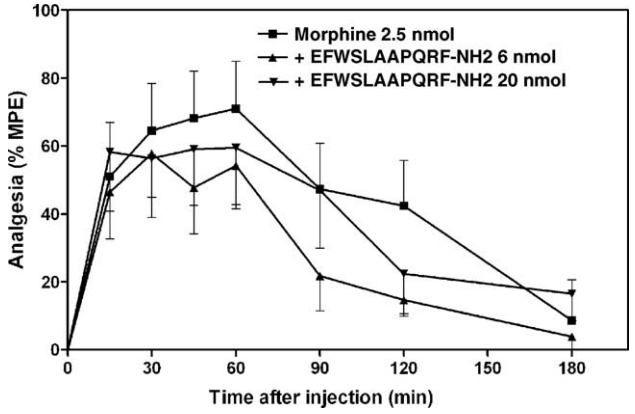

Fig. 6. Time course of the effects of i.c.v. administration of 6 and 20 nmol of EFWSLAAPQRF-NH_2 on the antinociception induced by 2.5 nmol of morphine (i.c.v.) in the mouse tail flick test. A dose of 6 (n=9) or 20 (n=9) nmol of EFWSLAAPQRF-NH_2 was coinjected i.c.v. with 2.5 nmol of morphine. Separate animals were injected i.c.v. with morphine (2.5 nmol) alone (n=9). Data are means±S.E.M. of MPE. Two-way ANOVA analysis, followed by Bonferroni's post-hoc test, showed significant difference between morphine alone and morphine coinjected with 6 nmol of EFWSLAAPQRF-NH_2 (P<0.05).

4. Discussion

The major findings of this study are that Neuropeptide FF receptor agonists exhibiting different selectivities towards Neuropeptide FF_1 and Neuropeptide FF_2 receptors, possess different pharmacological activities on body temperature and on opioid analgesia.

In comparison to saline-treated mice, VPNLPQRF-NH_2 injected into the third ventricle which is selective for Neuropeptide FF_1 receptor induces an hypothermia as [D.Tyr^1,(N.Me)Phe^3]NPFF (Desprat and Zajac, 1997; Frances et al., 2001) while EFWSLAAPQRF-NH_2 and SPAFLFQPQRF-NH_2 which are selective for Neuropeptide FF_2 do not. Taking into account the absolute affinity towards Neuropeptide FF_1 receptors of these ligands (Table 1), it appears that [D.Tyr^1,(N.Me)Phe^3]NPFF (Ki=1.09 nM) and VPNLPQRF-NH_2 (0.6 nM) exhibit the highest affinity and induce hypothermia while EFWSLAAPQRF-NH_2 (20.8 nM) displays a lower affinity to Neuropeptide FF_1 sites and does not produce an hypothermic effect. Similarly, the rank order of selectivity (Ki_1/Ki_2) (Mollereau et al., 2002) seems to indicate that the selectivity to Neuropeptide FF_1 sites ([D.Tyr^1,(N.Me)Phe^3]NPFF (6) and VPNLPQRF-NH_2 (0.03)) is associated with an hypothermic response while EFWSLAAPQRF-NH_2 (94) and SPAFLFQPQRF-NH_2 (56) which are highly selective for Neuropeptide FF_2 receptors do not produce a significant hypothermia. Thus, insofar as the affinity measured in in vitro assays reflects correctly the physiological activation of Neuropeptide FF receptors, these results suggest that Neuropeptide FF_1 receptor are responsible for the hypothermia induced by Neuropeptide FF receptor agonists compared to saline control. The fact that EFWSLAAPQRF-NH_2 induced a hyperthermic response when compared with saline control, probably reveals the ability of Neuropeptide FF_2 receptors to increase the body temperature. According to this hypothesis, SPAFLFQPQRF-NH_2 should produce two independent effects which tend to counterbalance each other.

The decrease in body temperature mediated by Neuropeptide FF_1 receptor could be correlated with the restricted localization of these receptors in rodent brain. Several regions could be involved in the control of body temperature by Neuropeptide FF receptors such as the preoptic area and the anterior hypothalamus (Ishiwata et al., 2001) in which Neuropeptide FF receptors are expressed in rodents (Gouardères et al., 2004). Interestingly, the preoptic area possesses a clear role in the thermoregulatory system in rodents and is one of the rare areas exhibiting Neuropeptide FF_1 receptors. However, other areas as the ventromedial hypothalamus and the septum also are involved in hypothermia (Adler et al., 1988) and are easily accessible through administration into the third ventricle and exhibit Neuropeptide FF_1 receptors.

Several studies performed with mice have demonstrated that stress induced by injection and rectal probe insertion produces hyperthermia (for review, see Olivier et al., 2003). In the present study, NaCl i.c.v. injection produced a

hypothermic effect. Therefore, the modification of the body temperature under our experimental conditions, seems not related to stress.

With respect to opioid analgesia, the pharmacological effects of Neuropeptide FF receptor agonists are more complex since both anti- and pro-opioid activities are observed depending on the administration sites (Roumy and Zajac, 1998). However, after injection into the lateral ventricle or generally after supraspinal administration, [D.Tyr^1,(N.Me)Phe^3]NPFF only exerts an anti-opioid effect (Roumy and Zajac, 1998) and SQAFLFQPQRF-NH_2 which has good selectivity towards Neuropeptide FF_2 receptors, mimics the anti-opioid activity of [D.Tyr^1,(N.Me)-Phe^3]NPFF in the tail flick test in mice, though with lesser potency (Gelot et al., 1998b). Similarly in rat, after microinfusion into the dorsal raphe nucleus and the nucleus parafascicularis of the thalamus which possess Neuropeptide FF_2 receptors, [D.Tyr^1,(N.Me)Phe^3]NPFF does not modify the animal response in the tail-immersion test but significantly reverses analgesia induced by coinjected morphine (Dupouy and Zajac, 1997). In the present study, the only available selective Neuropeptide FF_1 receptor agonist (VPNLPQRF-NH_2) has no significant anti-opioid actions but rather increases dose-dependently, morphine analgesia at doses at which [D.Tyr^1,(N.Me)Phe^3]NPFF is anti-opioid in the tail flick test (Gelot et al., 1998b). EFWSLAAPQRF-NH_2, a highly selective Neuropeptide FF_2 receptor analog, induces the same pharmacological effect as VPNLPQRF-NH_2 at comparable doses and in contrast, [D.Tyr^1,(N.Me)Phe^3]NPFF and VPNLPQRF-NH_2 exert opposite actions. These features clearly indicate that the pro- and anti-opioid actions do not depend directly or not only upon the selectivity of the compounds used. It should be observed that at high doses, EFWSLAAPQRF-NH_2 loses its effect, suggesting the existence of bell-shaped dose–response curve corresponding to two different effects of Neuropeptide FF_1 and Neuropeptide FF_2 receptors only observed for analogs with a sufficient selectivity. However in this case, pro- and anti-opioid effects could not be attributed to a simple relationship between one receptor type and one effect.

Liu et al. reported that rat NPSF(1-37) (possessing a PLRFamide C-terminal), which is more active on Neuropeptide FF_1 than on Neuropeptide FF_2 receptors in vitro (Liu et al., 2001), exhibits like Neuropeptide FF and at lower doses, supraspinal anti-opioid activity in the hot plate and formalin tests in the rat. This result is in apparent contradiction with our study showing that VPNLPQRF-NH_2 (with a PQRFamide end, like Neuropeptide FF), which is also selective towards Neuropeptide FF_1 receptors is inactive by itself but potentiates morphine-induced analgesia in the tail flick test when injected i.c.v. in mice. However, NPSF(1-37) is not as selective as claimed by the authors who used the cellular activity as the sole criterion of selectivity. In fact, taking into account the binding affinities, NPSF(1-37) exhibits a poor selectivity.

At the cellular level, both Neuropeptide FF_2 and Neuropeptide FF_1 receptors activation produce an anti-opioid effect (Roumy et al., 2003). Paradoxically, such a cellular anti-opioid activity accounts for the potentiation of opioid analgesia induced by intrathecal injection of [D.Tyr^1,(N.-Me)Phe^3]NPFF (Gouardères et al., 1996) since Ballet et al. have shown that [D.Tyr^1,(N.Me)Phe^3]NPFF enhances the Met-enkephalin outflow in in vivo perfused spinal cord (Ballet et al., 1999). In fact, the Neuropeptide FF analog prevents the delta agonist-induced reduction of Met-enkephalin release and increases enkephalin level by acting on the presynaptic delta-opioid autoreceptor (Mauborgne et al., 2001). This complex situation could correspond at the pharmacological level, to a facilitation of enkephalin-mediated activity as well as the contrary depending on the presence and the activity of Neuropeptide FF receptors on pre- or post-synaptic enkephalinergic neurones. One putative simplification to this model, would be the exclusive participation of one Neuropeptide FF receptor type at the pre- or post-synaptic level. That it is not so, is suggested by the fact that the pharmacological activity of one agonist is not defined by its selectivity towards only a Neuropeptide FF receptor type.

However, our data also indicate that an important variable in determining a pro- or an anti-opioid effect is the degree of opioid receptor stimulation. This is reminiscent of our previous work (Gelot et al., 1998a) revealing that in tolerant mice in which the opioid system is hardly stimulated, the potency of [D.Tyr^1,(N.Me)Phe^3]NPFF in reversing morphine-induced analgesia at peak effects, increased by a 100-fold factor in the tail flick test. A refined analysis revealed that [D.Tyr^1,(N.Me)Phe^3]NPFF produced its effect through an U-shaped dose–response relationship in tolerant mice indicating that [D.Tyr^1,(N.Me)-Phe^3]NPFF is not only more active in tolerant mice but also that interactions between Neuropeptide FF and opioid are clearly modified in these animals. Thus, a high dose of morphine could trigger an immediate compensatory increase in the release of endogenous Neuropeptide FF which increases the activity of administered EFWSLAAPQRF-NH_2. The pro-opioid effect of EFWSLAAPQRF-NH_2 could be observed at very high doses which could not be reached experimentally.

Cholecystokinin, another anti-opioid peptide, shows also complex dose–response relationships in reversing morphine analgesia in the tail flick test (Barbaz et al., 1989), a complexity explained by the existence of two types of cholecystokinin receptors in the brain. Similarly, our data could be interpreted in terms of a dual effect mediated by each Neuropeptide FF receptor, the manifestation of each effect depending on the intensity of opioid receptor stimulation. Such an interpretation is however difficult to prove in the lack of compounds exhibiting a total selectivity.

In summary, Neuropeptide FF_1 and Neuropeptide FF_2 receptors modulate differently several physiological functions as the control of body temperature and pain's

perception controlled by the opioid system. Secondly, our data evidence complex interactions between Neuropeptide FF and opioid system since the pro- or anti-opioid activities in mice do not depend strictly upon one Neuropeptide FF receptor type.

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