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European Journal of Pharmacology 503 (2004) 63–69

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Role of the histamine system in nefopam-induced antinociception in mice

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Received 7 May 2004; received in revised form 8 September 2004; accepted 14 September 2004
Available online 7 October 2004

Abstract

The present study explored the role of the histaminergic system in nefopam analgesia based on the structural relationship between nefopam and diphenhydramine. In vitro binding assays revealed that nefopam possesses moderate affinity for histamine H_1 and H_2 receptor subtypes, with IC_{50} of 0.8 and 6.9 μM, respectively, but no affinity for histamine H_3 receptor subtype until 100 μM. Subcutaneous nefopam administration dose-dependently inhibited pain in acetic acid-induced writhing (1–30 mg/kg) and formalin (1–10 mg/kg) tests in the mouse. Pretreatment with the histamine-depleting agent α-fluoromethylhistidine (α-FMH, 50 mg/kg), the histamine H_1 receptor antagonist pyrilamine (3 or 10 mg/kg), or the histamine H_2 receptor antagonists cimetidine (100 mg/kg) and zolantidine (10 or 30 mg/kg) did not significantly modify nefopam antinociception in both tests. The histamine H_3 receptor agonist *R*(−)α-methylhistamine (RAMH, 10 mg/kg) did not significantly modify the nefopam analgesic activity in the writhing test. At 25 mg/kg, RAMH inhibited nefopam antinociception at 3 mg/kg, but not at 10 mg/kg in the formalin test. However, pretreatment with the histamine H_3 receptor antagonist thioperamide (25 mg/kg) inhibited nefopam antinociception in the writhing test, but not in the formalin test. In conclusion, nefopam analgesic activity is not mediated by histamine H_1 or H_2 receptors, but can be slightly modulated by histamine H_3 receptors in mouse pain tests.

Keywords: Nefopam; Histamine; Nociception; Writhing; Formalin

1. Introduction

Nefopam has shown antinociceptive properties in most noxious and thermal-induced pharmacological tests in rodents (Conway and Mitchell, 1977; Piercey and Schroeder, 1981; Fasmer et al., 1987; Girard et al., 2001; Buritova and Besson, 2002), and is a clinically potent analgesic (Beaver and Feise, 1977; Heel et al., 1980). One mechanism of action that has been proposed to explain its analgesic activity involves the inhibition of monoamines reuptake in the central nervous system (Tresnak-Rustad and Wood, 1981; Rosland and Hole, 1990; Fuller and Snoddy, 1993). The present study explored the role of the histaminergic system in nefopam analgesia.

Chemically, nefopam is derived from the cyclization of diphenhydramine, which has been shown to possess antihistaminic properties in guinea pig-isolated ileum (Klohs et al., 1972).

Histamine is a biogenic amine that acts as a neurotransmitter in mammalian brain and has been shown to play a role in the modulation of pain transmission (Rumore and Schlichting, 1985; Oluyomi and Hart, 1991; Brown et al., 2001; Raffa, 2001; Malmberg-Aiello et al., 1994). Intracerebroventricular injection of histamine induces antinociception or hypernociception depending on the site of injection or on the dose. Low doses of histamine elicit hyperalgesia by acting on presynaptic receptors, while high doses produce antinociception by acting on postsynaptic receptors in rodents (Malmberg-Aiello et al., 1994). These actions of histamine are mediated through three distinct receptors (Arrang, 1994). Histamine H_1 and H_2 receptors are

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doi:10.1016/j.ejphar.2004.09.030

postsynaptically located while histamine H_3 receptors are exclusively presynaptically located and mediate inhibition of histamine release (Brown et al., 2001). Histamine has been shown to modulate analgesia through histamine H_1 receptors (Oluyomi and Hart, 1991; Malmberg-Aiello et al., 1998; Mobarakeh et al., 2000; Farzin et al., 2002), histamine H_2 receptors (Oluyomi and Hart, 1991; Lamberti et al., 1996; Farzin et al., 2002), and histamine H_3 receptors (Lamberti et al., 1996; Farzin et al., 2002).

In the present study, the affinity of nefopam toward the three histamine receptors was evaluated. Also, the effects of various histamine modulators (the histamine-depleting agent α-fluoromethylhistidine, α-FMH), receptor agonist (*R*(−)α-methylhistamine, RAMH), and receptor antagonists (pyrilamine, cimetidine, zolantidine, thioperamide) were examined in two established analgesia mouse models: the acetic acid-induced writhing test that allows studying chemical noxious stimuli (Malmberg-Aiello et al., 1994), and the early licking phase of the formalin test that evokes a short lasting period of acute pain attributed to a direct algogenic effect of formalin on the nociceptors (Hunskaar and Hole, 1987).

2. Materials and methods

2.1. Receptor binding assay

Binding studies were done on guinea pig cerebellum for histamine $H_{1central}$ receptor, guinea pig striatum for histamine H_2 receptor, and rat cerebral cortex for histamine H_3 receptor. Specific ligands were [^{3}H]pyrilamine (0.5 nM, triprolidine 100 μM as nonspecific, 10 min at 25 °C incubation parameters), [^{125}I]APT (0.1 nM, tiotidine 100 μM, 150 min at 22 °C), and [^{3}H](*R*)α-Me-histamine (0.5 nM, (*R*)α-Me-histamine 5 μM, 60 min at 22 °C) for histamine $H_{1central}$ receptor, histamine H_2 receptor, and histamine H_3 receptor, respectively. After incubation, membranes or cells in suspension are filtered (filters GF/B or GF/C, Whatman or Packard) and washed. Radioactivity is measured with a liquid scintillation counter (LS 6000, Beckman or Topcount, Packard). Nefopam was tested at 0.1–1 to 10–100 μM on each receptor, and measures were repeated. IC_{50} values were calculated according to a nonlinear regression model.

2.2. Animals

Male NMRI (Janvier breeding), CD1 (River breeding), or ICR (Harlan breeding) mice were housed appropriately in air-conditioned, temperature (22±2 °C)-, and hygrometry (50±20%)-controlled rooms. The lighting schedule was 12:12 light/dark. Diet (UAR, France) and filtered tap water were available ad libitum. Experiments were run at least 4 days after the animals arrived in the laboratory. Each animal was used only once. All the experiments were carried out in accordance with the recommendations of the International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues Guidelines (Pain 1983;16:109–110).

2.3. Acetic acid-induced writhing

Male CD1 or ICR mice (25–30 g) were used in groups of 10. These mice have been frequently used in the mouse acetic acid-induced writhing test (Moser, 1995; Ormazabal et al., 1999; Alvarez et al., 2000; Pieretti et al., 1999). CD1 and ICR outbred mice have the same origin but are differently labeled according to each breeder. In Charles River and Harlan nomenclatures, the corresponding strains are defined as Crl:CD-1®(ICR)BR and Hsd/ICR(CD-1®), respectively. Writhing was induced by intraperitoneal injection of a 0.6% acetic acid solution (0.1 ml/10 g). The number of abdominal writhings was counted from 5 min after acetic acid injection and during 10 min. Analgesic activity was recorded as the percentage relative to the number of abdominal writhings of a control group. Controls were repeated for each of the pretreatments to control for interday variability inherent to the test and allow valid comparisons. Ten animals were used at each of three to four dose levels to determine the ED_{50} value for a drug.

2.4. Formalin-induced licking

Male NMRI mice (30–35 g) were used in groups of 10. This strain is frequently used in this test (Hunskaar et al., 1985; Fasmer et al., 1987; Millan et al., 1996; Karlsten et al., 1992). Formalin (20 μl at 5%) was injected subcutaneously into the dorsal surface of the right hind paw of each mouse, and the mouse was returned to its cage afterwards. After injection, a mirror was positioned behind the cage and gave an unobstructed view of the hind paw. Immediately after formalin injection, the time spent licking the injected paw was recorded during 10 min, which is the first noxious phase reflecting the direct pain on nociceptors. Each pretreatment had its own control to address interday variability and allow valid comparisons.

2.5. Drugs and treatments

Nefopam hydrochloride was obtained from Biocodex Laboratories as a racemate. S(+)α-FMH (α-fluoromethylhistidine hydrochloride), pyrilamine (mepyramine) maleate, cimetidine, RAMH (*R*(−)α-methylhistamine dihydrochloride), acetic acid, and formalin were purchased from Sigma. Zolantidine dimaleate and thioperamide maleate were purchased from Tocris (France). Drugs were dissolved in distilled water or isotonic saline (NaCl 0.9%) solutions, with the exception of zolantidine, which was dissolved in 1% Tween 80. S(+)α-FMH was injected intraperitoneally (ip) 2

h before nefopam, and the other compounds were administered by subcutaneous (sc) route 15 min before administration of nefopam. Drugs were injected in a volume of 10 ml/kg by both subcutaneous and intraperitoneal routes. Doses of pretreatments were selected from quick pilot studies and/or from available published data in order to avoid perturbating side effects (the only side effect observed was convulsions in the case of thioperamide).

2.6. Statistical methods

Data are expressed as mean±S.E.M. For two groups, Student's *t*-test was done, and for more than two groups, the statistical test used was analysis of variance (ANOVA) followed by comparisons based on Bonferoni or Dunnett methods in order to determine significance differences between groups. A P value less than 0.05 was assumed as the significance level. ED_{50} values with their 95% confidence limits and the significance of the potency ratio between two ED_{50} values were calculated with the Pharm/PCS software according to the method of Litchfield and Wilcoxon (1949).

3. Results

3.1. Receptor binding profile

Binding studies revealed that nefopam displayed IC_{50} values of 0.8 μM on histamine H_1 receptor, 6.9 μM on histamine H_2 receptor, and more than 100 μM for histamine H_3 receptor (Table 1).

3.2. Effect of nefopam alone in analgesia

In the mouse acetic acid-induced writhing test (Table 2), subcutaneous injection of nefopam showed a dose-dependent antinociceptive effect with an ED_{50} of 2.15 mg/kg (0.79–5.85). In the early phase of the mouse formalin test (Table 3), nefopam possessed a dose-dependent analgesic activity at 1, 3, and 10 mg/kg sc, with respective decreases in licking time of 56%, 72%, and 91%. This effect was significant only at the two highest doses.

Table 1
In vitro affinity of nefopam for specific binding sites on membrane preparations

Receptor	Ligand (nM)	Nefopam IC_{50} (μM)	Reference ligand IC_{50} (nM)
$H_{1(central)}$	[^{3}H]Pyrilamine (0.5)	0.8 (0.1–4.0)	Pyrilamine 0.50
H_2	[^{125}I]Aminopotentidine (0.1)	6.9 (1.5–29.4)	Cimetidine 2140
H_3	[^{3}H](R) α-Me-histamine (0.5)	>100	(R)α-Me-histamine 3.0

Table 2
Effects of pretreatments with α-FMH, pyrilamine, cimetidine, zolantidine, RAMH, or thioperamide on the antinociceptive activity of nefopam in the mouse acetic acid-induced writhing test (n=9 or 10)

Pretreatments (mg/kg)	Nefopam (mg/kg)	Abdominal writhings (mean±S.E.M.)	ED_{50} (mg/kg)
	0	32.6±4.8	2.15
	1	20.9±3.2*	(0.79–5.85)
	3	14.6±2.2*	
	10	7.3±2.8*	
	30	2.9±2.5*	
Saline	0	38.4±3.6	
α-FMH (50; ip)	0	25.1±5.3	
α-FMH (50; ip)	0	42.0±5.4	2.21
	1	30.5±3.0	(0.78–6.24)
	3	11.9±3.0*	
	10	12.8±3.5*	
Saline	0	24.4±5.4	
Pyrilamine (3; sc)	0	16.3±4.2	
Pyrilamine (10; sc)	0	13.2±2.7*	
Pyrilamine (3; sc)	0	36.3±3.2	3.56
	1	28.2±5.8	(1.19–10.61)
	3	14.2±3.6*	
	10	13.7±4.3*	
Saline	0	51.6±3.1	
Cimetidine (100; sc)	0	43.1±3.4	
Cimetidine (100; sc)	0	40.8±4.3	6.22
	1	28.9±5.4	(1.78–21.69)
	3	30.0±7.2	
	10	14.7±4.5*	
Saline	0	35.6±3.2	
Zolantidine (10; sc)	0	37.7±3.5	
Saline	0	53.5±3.5	
Zolantidine (20; sc)	0	34.5±5.0*	
Zolantidine (10; sc)	0	49.2±3.7	6.70
	1	32.8±5.6	(1.12–39.84)
	3	32.7±6.8	
	10	20.8±6.4*	
Saline	0	46.5±3.9	
RAMH (10; sc)	0	34.6±6.0	
RAMH (25; sc)	0	24.5±4.8*	
RAMH (10; sc)	0	50.9±4.1	2.30
	1	33.4±7.7*	(0.84–6.29)
	3	23.1±5.5*	
	10	11.5±4.7*	
Saline	0	35.2±2.2	
Thioperamide (25; sc)	0	34.0±5.6	
Thioperamide (25; sc)	0	28.8±4.7	>3
	1	23.3±4.5	
	3	21.0±4.9	

Acetic acid was intraperitoneally injected 30 min after subcutaneous nefopam administration. The number of abdominal writhings (constrictions) was recorded from 5 to 20 min after algogen injection. Pretreatments were performed 2 h before nefopam for α-FMH (ip: intraperitoneal route), and 15 min before nefopam for the other compounds (sc: subcutaneous route). The statistical tests used were Student's *t*-test for two groups and ANOVA for more than two groups at the level of 5%. α-FMH, α-fluoromethylhistidine hydrochloride; RAMH, R(−)α-methylhistamine dihydrochloride.

* P<0.05 for treated groups different from respective control groups.

3.3. Effect of α-FMH on nefopam antinociceptive activity

Intraperitoneal injection of α-FMH, a specific irreversible inhibitor of histidine decarboxylase, alone at 50 mg/kg

Table 3
Effects of various pretreatments on the antinociceptive activity of nefopam in the mouse formalin test (n=8)

Pretreatments	Doses (mg/kg)	Nefopam (mg/kg)	Licking time (mean±S.E.M.)
		0	56.07±10.54
		1	24.55±6.22
		3	15.78±5.39*
		10	4.87±1.54*
α-FMH (ip)	0	0	56.56±8.96
	50	0	53.34±7.86
	50	3	21.02±5.80*
Pyrilamine (sc)	0	0	52.91±9.69
	10	0	53.71±5.44
	10	3	26.11±7.01*
Cimetidine (sc)	0	0	56.16±3.38
	100	0	41.19±6.40
	100	3	20.75±5.58*
Zolantidine (sc)	0	0	55.25±11.62
	30	0	68.58±6.77
	30	3	23.23±4.22*
RAMH (sc)	0	0	87.01±7.81
	25	0	60.56±10.24
	25	3	60.63±8.46
RAMH (sc)	0	0	46.89±7.11
	25	0	57.89±10.06
	25	10	17.76±5.82*
Thioperamide (sc)	0	0	69.34±14.06
	25	0	52.10±7.45
	25	3	16.97±6.17*

Formalin was injected 30 min after subcutaneous nefopam administration. The time spent licking the formalin-injected paw was recorded from 0 to 10 min after algogen injection. Pretreatments were performed 2 h before nefopam for α-FMH (ip: intraperitoneal route) and 15 min before nefopam for the other compounds (sc.: subcutaneous route). The statistical tests used were Student's t-test for two groups and ANOVA for more than two groups at the level of 5%. α-FMH, α-fluoromethylhistidine hydrochloride, RAMH, $R(-)$α-methylhistamine dihydrochloride.

* $P<0.05$ for treated groups different from respective control groups.

did not show any significant antinociceptive activity in both analgesic tests. Pretreatment with α-FMH injected 2 h before nefopam did not modify the antinociceptive effect of nefopam in the writhing test (Table 2) and in the formalin test (Table 3).

3.4. Effect of the histamine H_1 receptor antagonist pyrilamine on nefopam antinociceptive activity

In the acetic acid-induced writhing test, subcutaneous injection of pyrilamine at 3 mg/kg reduced slightly, but not significantly, the number of abdominal writhings. A higher dose of pyrilamine (10 mg/kg) was antinociceptive in the writhing test, but not in the formalin test. Pretreatment with a non-analgesic dose of pyrilamine (3 mg/kg) injected 15 min before nefopam (Table 2) did not significantly modify the ED_{50} of nefopam (3.56 mg/kg) in the acetic acid writhing test. Similarly, a non-analgesic dose of pyrilamine (10 mg/kg) did not alter the antinociceptive effect of nefopam (3 mg/kg) in the formalin test (Table 3).

3.5. Effects of the histamine H_2 receptor antagonists cimetidine and zolantidine on nefopam antinociceptive activity

In the acetic acid-induced writhing test, subcutaneous injections of cimetidine up to 100 mg/kg or zolantidine until 10 mg/kg were devoid of analgesic activity (Table 2). However, a higher dose of zolantidine (20 mg/kg) was antinociceptive in this test. Pretreatment with non-analgesic doses of cimetidine or zolantidine injected 15 min before nefopam (Table 2) did not significantly modify the nefopam ED_{50}s that were 6.22 mg/kg (1.78–21.69) and 6.70 mg/kg (1.12–39.84), respectively. In the formalin test, cimetidine (up to 100 mg/kg) and zolantidine (up to 30 mg/kg) were devoid of analgesic activity. Nefopam antinociceptive effect at 3 mg/kg sc (Table 3) was not reduced by non-analgesic doses of cimetidine or zolantidine.

3.6. Effect of the histamine H_3 receptor agonist RAMH on nefopam antinociceptive activity

In the acetic acid-induced writhing test, subcutaneous injection of RAMH alone until 10 mg/kg was devoid of analgesic activity. However, a higher dose (25 mg/kg) of RAMH was antinociceptive in this test. Pretreatment with a non-analgesic dose of RAMH (10 mg/kg) injected 15 min before nefopam (Table 2) did not modify nefopam ED_{50} (2.30 mg/kg). In the formalin test, RAMH alone until 25 mg/kg was devoid of analgesic activity, but it inhibited nefopam antinociception at 3 mg/kg (Table 3). At a higher dose (10 mg/kg), the nefopam antinociceptive effect was not decreased by RAMH.

3.7. Effect of the histamine H_3 receptor antagonist thioperamide on nefopam antinociceptive activity

In both analgesic tests, subcutaneous injection of thioperamide alone until 25 mg/kg was devoid of analgesic activity (Tables 2 and 3). In the writhing test, pretreatment with a non-analgesic dose of thioperamide (25 mg/kg) injected 15 min before nefopam inhibited the antinociceptive activity of 1 and 3 mg/kg of nefopam (Table 2). A higher dose of nefopam (10 mg/kg) with thioperamide could not be used because it induces toxicological effects (convulsions). In the formalin test, a non-analgesic dose of thioperamide (25 mg/kg) did not change the antinociceptive activity of nefopam at 3 mg/kg (Table 3).

4. Discussion

The non-opioid analgesic nefopam is derived from the histamine H_1 receptor antagonist diphenhydramine (Klohs et al., 1972). However, the antihistaminic activity of nefopam has been assessed to be 90-fold less than that of diphenhydramine in a guinea pig model in vitro (Klohs et al.,

1972). As histamine modulates pain transmission through at least three different receptors (Oluyomi and Hart, 1991; Malmberg-Aiello et al., 1998; Lamberti et al., 1996; Mobarakeh et al., 2000; Farzin et al., 2002), the involvement of the histamine system in nefopam antinociception was evaluated, especially the role of histamine H_1 receptors.

In the present study, the histaminic component of nefopam antinociception was evaluated using both in vitro binding assays with specific histaminergic ligands, and in vivo pretreatment with compounds acting on the histaminergic system in two mouse models of analgesia.

To examine the importance of histamine in nefopam analgesia, we have used α-FMH, a highly specific irreversible inhibitor of brain histidine decarboxylase, which allows the synthesis of histamine from L-histidine in mice (Garbarg et al., 1980). α-FMH was used at a dose (50 mg/kg ip) that had been shown previously to strongly decrease brain histamine level and to deplete histidine decarboxylase activity by 90% in mice (Maeyama et al., 1982), and to antagonize L-histidine antinociception in the mouse acetic acid-induced abdominal writhing test or in the rat paw pressure test (Malmberg-Aiello et al., 1994). Using the same dose and administration route of α-FMH, nefopam antinociceptive activity was not reduced in the mouse acetic acid-induced writhing model or in the early phase of the mouse formalin test. These first results suggest that histamine does not directly mediate nefopam antinociception in both tests.

All three histamine receptors are located throughout the periphery and within the central nervous system (Raffa, 2001), and the involvement of each histamine receptor subtype was evaluated in binding studies and pharmacological analgesic tests.

Histamine H_1 receptors play an important role in both somatic and visceral pain perceptions since mutant mice lacking these receptors showed significantly fewer nociceptive responses in various pain tests (Mobarakeh et al., 2000). A binding assay on histamine H_1 receptors revealed that nefopam possesses moderate affinity for this subtype with IC_{50} of 0.8 μM, which is 1000-fold less than that of pyrilamine. Histamine H_1 receptor antagonists, like dexchlorpheniramine and pyrilamine, have been shown to possess antinociceptive activity in the acetic acid-induced writhing test or in the hot plate test in mice (Ghelardini et al., 1998; Malmberg-Aiello et al., 1998; Farzin et al., 2002). In mouse antinociceptive tests, intracerebroventricular injection of the histamine H_1 receptor agonist HTMT (6-[2-(4-imidazolyl)ethylamino]-N-(4 trifluoro-methylphenyl) heptane carboxamide dimaleate) produced hypernociception in the hot plate and writhing tests, and this effect was inhibited by a non-analgesic dose (20 mg/kg ip) of the histamine H_1 receptor antagonist dexchlorpheniramine (Farzin et al., 2002). In the same way, antinociception induced by intraventricular histamine injection was inhibited by a non-analgesic dose (10 mg/kg ip) of pyrilamine in the tail-flick and hot plate tests in mice (Li et al., 1997). Pyrilamine has been shown to easily penetrate the brain after oral administration (Brown et al., 1986). Pretreatment with non-analgesic doses of pyrilamine at 3 or 10 mg/kg sc did not modify the antinociceptive effect of nefopam either in the acetic acid-induced writhing or the formalin test, respectively. These results suggest that the antinociceptive activity of nefopam is unlikely to involve the histamine H_1 receptor subtype, despite a moderate affinity for this receptor.

Binding assays on histamine H_2 receptors revealed that nefopam possesses moderate affinity for this subtype with an IC_{50} of 6.9 μM, which is near the cimetidine IC_{50}. Two histamine H_2 receptor antagonists, cimetidine, which hardly penetrates into the brain after intraperitoneal injection in rats (Hough et al., 1986), and zolantidine, which readily penetrates into the brain (Calcutt et al., 1988), were used to assess the involvement of this receptor subtype. Intraperitoneal administration of histamine H_2 receptor agonist (dimaprit) or large doses of receptor antagonists (cimetidine, zolantidine) has been shown to possess antinociceptive activity in the acetic acid-induced writhing or hot plate tests in mice (Oluyomi and Hart, 1991). Antinociception induced by intraventricular histamine injection was inhibited by non-analgesic dose (20 mg/kg sc) of zolantidine in tail-flick and hot plate tests in mice (Li et al., 1997). By using non-antinociceptive doses of cimetidine or zolantidine, nefopam ED_{50} was slightly but not significantly increased in the mouse writhing test. Since these histamine receptor antagonists did not modify nefopam antinociception in the mouse formalin test (3 mg/kg), it can be inferred that the histamine H_2 receptors do not mediate nefopam antinociception in both models.

The histamine H_3 receptor has a presynaptic localization and mediates inhibition of histamine release and biosynthesis in histaminergic nerve terminals in the central nervous system (Schwartz et al., 1986). Binding assays on histamine H_3 receptors revealed that nefopam did not show any affinity for this subtype until 100 μM. The selective histamine H_3 receptor agonist *R*(α)-methylhistamine (RAMH) inhibits the release and synthesis of histamine while the selective histamine H_3 receptor antagonist thioperamide enhances them (Garbarg et al., 1989; Oishi et al., 1989). RAMH and thioperamide have been shown to pass the blood–brain barrier after peripheral administration (Arrang et al., 1987; Taylor et al., 1992). Intraperitoneal or intracerebroventricular administration of RAMH induced hyperalgesia in the mouse hot plate and the rat paw pressure tests, and, at a non-hyperalgesic dose (20 mg/kg ip), it completely prevented thioperamide (20 mg/kg sc or ip) antinociception in the mouse hot plate and writhing tests (Malmberg-Aiello et al., 1994). In our study, RAMH at 25 mg/kg sc induced antinociception in the acetic acid writhing test, but not in the formalin test. A non-analgesic dose of RAMH (10 mg/kg) did not modify nefopam antinociception in the writhing test. On the other hand, in the formalin test, a non-analgesic dose of RAMH (25 mg/kg) blocked the antinociceptive effect of nefopam at 3 mg/kg, but not of a

higher dose of nefopam (10 mg/kg). In this case, RAMH could have slightly increased histamine level to produce hyperalgesia that inhibited nefopam antinociception at its lower dose.

Pretreatment with a non-analgesic dose of thioperamide (25 mg/kg sc) did not modify nefopam antinociception in the formalin test, but inhibited nefopam analgesia at 3 mg/kg in the writhing test. In this last test, increasing the nefopam dose to 10 mg/kg in the presence of thioperamide induced toxicological effects like convulsions. To evaluate this last result, we may consider that presynaptic histamine H_3 receptors regulate the release and turnover of histamine, and modulate the release of other neurotransmitters including norepinephrine, dopamine, serotonin, and acetylcholine in brain cortex slices and on histaminergic axon terminals (Vohora et al., 2001; Clapham and Kilpatrick, 1992; Schlicker et al., 1988, 1989, 1993). These results suggest that endogenous histamine can slightly modulate nefopam antinociception indirectly through histamine H_3 receptors in the mouse writhing and formalin tests.

In conclusion, nefopam analgesic activity is not mediated by histamine H_1 or H_2 receptors in mouse pain tests. The histamine H_3 receptors seem to be slightly involved in the modulation of nefopam antinociception, but considering the low affinity of nefopam to histamine H_3 receptors, further studies are required to ascertain the contribution of this mechanism.

Acknowledgment

We thank Prof. R.H. Levy (University of Washington, Seattle, Washington, USA) for his constructive interest in our work and help with the manuscript.

References

Alvarez, I., Andreu, F., Buxens, J., Colombo, M., Dordal, A., Fort, M., Gutierrez, B., Farre, A.J., 2000. Pharmacology of cizolirtine: a new analgesic agent. Methods Find. Exp. Clin. Pharmacol. 22, 211–221.

Arrang, J.M., 1994. Pharmacological properties of histamine receptor subtypes. Cell. Mol. Biol. (Noisy-le-grand) 40, 275–281.

Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., Schwartz, J.C., 1987. Highly potent and selective ligands for histamine H3-receptors. Nature 327, 117–123.

Beaver, W.T., Feise, G.A., 1977. A comparison of the analgesic effect of intramuscular nefopam and morphine in patients with post-operative pain. J. Clin. Pharmacol. 17, 579–591.

Brown, E.A., Griffiths, R., Harvey, C.A., Owen, D.A., 1986. Pharmacological studies with SK and F 93944 (temelastine), a novel histamine H1-receptor antagonist with negligible ability to penetrate the central nervous system. Br. J. Pharmacol. 87, 569–578.

Brown, R.E., Stevens, D.R., Hass, H.L., 2001. The physiology of brain histamine. Prog. Neurobiol. 63, 637–672.

Buritova, J., Besson, J.M., 2002. Effects of nefopam on the spinal nociceptive processes: a c-*fos* protein study in the rat. Eur. J. Pharmacol. 441, 67–74.

Calcutt, C.R., Ganellin, C.R., Griffiths, R., Leigh, B.K., Maguire, J.P., Mitchell, R.C., Mylek, M.E., Parsons, M.E., Smith, I.R., Young, R.C., 1988. Zolantidine (SK and F 95282) is a potent selective brain-penetrating histamine H2-receptor antagonist. Br. J. Pharmacol. 93, 69–78.

Clapham, J., Kilpatrick, G.J., 1992. Histamine H3 receptors modulate the release of [3H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H3 receptor subtypes. Br. J. Pharmacol. 107, 919–923.

Conway, A.C., Mitchell, C.L., 1977. Analgesic studies with nefopam hydrochloride. Arch. Int. Pharmacodyn. Ther. 226, 156–171.

Farzin, D., Asghari, L., Nowrouzi, M., 2002. Rodent antinociception following acute treatment with different histamine receptor agonists and antagonists. Pharmacol. Biochem. Behav. 72, 751–760.

Fasmer, O.B., Berge, O.G., Jorgensen, H.A., Hole, K., 1987. Antinociceptive effects of (±)-, (+)- and (−)-nefopam in mice. J. Pharm. Pharmacol. 39, 508–511.

Fuller, R.W., Snoddy, H.D., 1993. Evaluation of nefopam as a monoamine uptake inhibitor in vivo in mice. Neuropharmacology 32, 995–999.

Garbarg, M., Barbin, G., Rodergas, E., Schwartz, J.C., 1980. Inhibition of histamine synthesis in brain by alpha-fluoromethylhistidine, a new irreversible inhibitor: in vitro and in vivo studies. J. Neurochem. 35, 1045–1052.

Garbarg, M., Trung Tuong, M.D., Gros, C., Schwartz, J.C., 1989. Effect of histamine H3-receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain. Eur. J. Pharmacol. 164, 1–11.

Ghelardini, C., Galeotti, N., Bartolini, A., 1998. No development of tolerance to analgesia by repeated administration of H-1 antagonists. Life Sci. 63, PL 317–PL 322.

Girard, P., Pansart, Y., Coppe, M.C., Gillardin, J.M., 2001. Nefopam reduces thermal hypersensitivity in acute and postoperative pain models in the rat. Pharmacol. Res. 44, 541–545.

Heel, R.C., Brogden, R.N., Pakes, G.E., Speight, T.M., Avery, G.S., 1980. Nefopam: a review of its pharmacological properties and therapeutic efficacy. Drugs 19, 249–267.

Hough, L.B., Glick, S.D., Su, K., 1986. Cimetidine penetrates brain and inhibits non-opiate footshock-induced analgesia. Pharmacol. Biochem. Behav. 24, 1257–1261.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Methods 14, 69–76.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114.

Karlsten, R., Gordh, T., Post, C., 1992. Local antinociceptive and hyperalgesic effects in the formalin test after peripheral administration of adenosine analogues in mice. Pharmacol. Toxicol. 70, 434–438.

Klohs, M.W., Draper, M.D., Petracek, F.J., Ginzel, K.H., 1972. Benzoxacines: a new chemical class of centrally acting skeletal muscle relaxants. Arzneimittel-Forschung 22, 132–133.

Lamberti, C., Bartolini, A., Ghelardini, C., Malmberg-Aiello, P., 1996. Investigation into the role of histamine receptors in rodent antinociception. Pharmacol. Biochem. Behav. 53, 567–574.

Li, B.Y., Nalwalk, J.W., Hough, L.B., 1997. Effects of naltrexone and histamine antagonists on the antinociceptive activity of the cimetidine analog SKF92374 in rats. Brain Res. 748, 168–174.

Litchfield, J.T., Wilcoxon, F., 1949. A simple method for evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Maeyama, K., Watanabe, T., Taguchi, Y., Yamatodani, A., Wada, H., 1982. Effect of alpha-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on histamine levels in mouse tissues. Biochem. Pharmacol. 31, 2367–2370.

Malmberg-Aiello, P., Lamberti, C., Ghelardini, C., Giotti, A., Bartolini, A., 1994. Role of histamine in rodent antinociception. Br. J. Pharmacol. 111, 1269–1279.

Malmberg-Aiello, P., Lamberti, C., Ipponi, A., Bartolini, A., Schunack, W., 1998. Evidence for hypernociception induction following histamine H1 receptor activation in rodents. Life Sci. 63, 463–476.

Millan, M.J., Seguin, L., Honore, P., Girardon, S., Bervoets, K., 1996. Pro- and antinociceptive actions of serotonin (5-HT)1A agonists and

antagonists in rodents: relationship to algesiometric paradigm. Behav. Brain Res. 73, 69–77.

Mobarakeh, J.I., Sakurada, S., Katsuyama, S., Kutsuwa, M., Kuramasu, A., Lin, Z.Y., Watanabe, T., Hashimoto, Y., Watanabe, T., Yanai, K., 2000. Role of histamine H-1 receptor in pain perception: a study of the receptor gene knockout mice. Eur. J. Pharmacol. 391, 81–89.

Moser, P.C., 1995. The effect of 5-HT3 receptor antagonists on the writhing response in mice. Gen. Pharmacol. 26, 1301–1306.

Oishi, R., Itoh, Y., Nishibori, M., Saeki, K., 1989. Effects of the histamine H3-agonist (R)-alpha-methylhistamine and the antagonist thioperamide on histamine metabolism in the mouse and rat brain. J. Neurochem. 52, 1388–1392.

Oluyomi, A.O., Hart, S.L., 1991. Involvement of histamine in naloxone-resistant and naloxone-sensitive models of swim stress-induced antinociception in the mouse. Neuropharmacology 30, 1021–1027.

Ormazabal, M.J., Goicoechea, C., Alfaro, M.J., Sanchez, E., Martin, M.I., 1999. Study of mechanisms of calcitonin analgesia in mice. Involvement of 5-HT3 receptors. Brain Res. 845, 130–138.

Piercey, M.F., Schroeder, L.A., 1981. Spinal and supraspinal sites for morphine and nefopam analgesia in the mouse. Eur. J. Pharmacol. 74, 135–140.

Pieretti, S., Dal Piaz, V., Matucci, R., Giovannoni, M.P., Galli, A., 1999. Antinociceptive activity of a 3(2H)-pyridazinone derivative in mice. Life Sci. 65, 1381–1394.

Raffa, R.B., 2001. Antihistamines as analgesics. J. Clin. Pharm. Ther. 26, 81–85.

Rosland, J.H., Hole, K., 1990. The effect of nefopam and its enantiomers on the uptake of 5-hydroxytryptamine, noradrenaline and dopamine in crude rat brain synaptosomal preparations. J. Pharm. Pharmacol. 42, 437–438.

Rumore, M.M., Schlichting, D.A., 1985. Analgesic effects of antihistaminics. Life Sci. 36, 403–416.

Schlicker, E., Betz, R., Gothert, M., 1988. Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 588–590.

Schlicker, E., Fink, K., Hinterthaner, M., Gothert, M., 1989. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 633–638.

Schlicker, E., Fink, K., Detzner, M., Gothert, M., 1993. Histamine inhibits dopamine release in the mouse striatum via presynaptic H3 receptors. J. Neural Transm. Gen. Sect. 93, 1–10.

Schwartz, J.C., Arrang, J.M., Garbarg, M., Korner, M., 1986. Properties and roles of the three subclasses of histamine receptors in brain. J. Exp. Biol. 124, 203–224.

Taylor, S.J., Michel, A.D., Kilpatrick, G.J., 1992. In vivo occupancy of histamine H3 receptors by thioperamide and (R)-alpha-methylhistamine measured using histamine turnover and an ex vivo labeling technique. Biochem. Pharmacol. 44, 1261–1267.

Tresnak-Rustad, N.J., Wood, M.E., 1981. In vitro biochemical effects of nefopam hydrochloride, a new analgesic agent. Biochem. Pharmacol. 30, 2847–2850.

Vohora, D., Pal, S.N., Pillai, K.K., 2001. Histaminergic H3-receptors as modulators of CNS function. Indian J. Pharmacol. 33, 17–28.