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Role of catecholamines and serotonin receptor subtypes in nefopam-induced antinociception

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Abstract

The non-opiate analgesic nefopam has been shown to inhibit monoamines uptake, but little is known about receptor subtypes effectively involved in its analgesic effect. In vitro binding assays yielded the following measures of affinity (IC_{50}): serotonergic 5-HT_{2C} (1.4 μM), 5-HT_{2A} (5.1 μM), 5-HT_3 (22.3 μM), 5-HT_{1B} (41.7 μM), 5-HT_{1A} (64.9 μM), adrenergic α_1 (15.0 μM) and dopaminergic D_1 (100 μM). Subcutaneous nefopam administration dose-dependently inhibited pain in acetic acid-induced writhing (1–30 mg kg^{-1}) and formalin (1–10 mg kg^{-1}) tests in the mouse. Pretreatments with adrenergic α_1 (prazosin) and α_2 (yohimbine), and serotonergic 5-HT_{1B} (GR127935) receptor antagonists significantly increased the nefopam ED_{50} in the writhing test. The serotonergic 5-HT_{2C} (RS102221) and the dopaminergic D_2 (sulpiride) receptor antagonists inhibited nefopam antinociception in the formalin test. However, in both tests, nefopam analgesic activity was not modified by the following receptor antagonists: dopaminergic D_1 (SCH23390), serotonergic 5-HT_{1A} (NAN-190, WAY100635), 5-HT_{2A} (R96544, ketanserin), 5-HT_3 (tropisetron), and 5-HT_4 (SDZ205557). In conclusion, nefopam analgesic activity could be modulated by the adrenergic α_1 and α_2 receptors, the dopaminergic D_2 receptors, and the serotonergic 5-HT_{1B} and 5-HT_{2C} receptor subtypes.

Keywords: Nefopam; Catecholamines; Serotonergic; Writhing; Formalin

1. Introduction

Nefopam is a non-opiate clinically potent analgesic [1–4] that has shown antinociceptive properties in several noxious and thermal-induced pharmacological tests in rodents [5–9], but whose mechanism of action is not fully understood. Although recent studies suggest that nefopam interacts with the glutamatergic system [10–12], earliest mechanistic studies have suggested that its analgesic activity involves inhibition of catecholamines and serotonin reuptake in the central nervous system [13–15]. These effects lead to an increase in noradrenaline, dopamine and serotonin levels in the synaptic cleft. These neuromediators have been shown to modulate the pain transmission pathway [16–20] through, at least, adrenergic α_1 [21,22] and α_2 [21,23], dopaminergic D_1 [24] and D_2 [17,18], and serotonergic 5-HT_{1A} [25–27], 5-HT_{1B} [28], 5-HT_{2A} [29,30], 5-HT_{2C} [31], 5-HT_3 [29,32,33] and 5-HT_4 [34,35] receptor subtypes.

The role of monoamines in nefopam-induced antinociception has been examined in several rodent models. Nefopam antinociception was blocked by reserpine, a monoamine depletor that reduces noradrenaline, dopamine and serotonin pools in neuronal vesicles, in the mouse writhing test [36,37], and in the mouse hot plate test [38]. Moreover, depletion of brain serotonin with *para*-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, prevented nefopam analgesia in the mouse formalin test [39], but did not inhibit nefopam antinociceptive effect in the mouse writhing test [36]. However, selective depletion of central serotonin by intracerebroventricular injection of 5,7-dihydroxytryptamine did not reduce the analgesic activity of nefopam in the rat hot plate [38].

The present study pursued further the monoamine hypothesis using a two-fold approach. Firstly, it explored the affinity of nefopam on monoamines receptor subtypes, in in-vitro binding experiments. Secondly, the effects of pretreatment by various monoamine receptor antagonists (prazosin, yohimbine, SCH23390, sulpiride, methysergide, NAN-190, WAY100635, R96544, ketanserin, GR127935, RS102221, tropisetron, SDZ205557) were examined in two different and

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established analgesia mouse models. In the acetic acid-induced writhing test, systemic intraperitoneal administration of acetic acid induces reflex reactions through abdominal writhings resulting from an inflammatory visceral pain that allows the evaluation of chemical stimuli [40]. In the early licking phase of the formalin test, local subcutaneous injection of formalin in one hind-paw induces licking and biting behaviour directed towards the injected paw that evokes a short lasting period of non-inflammatory acute pain attributed to a direct algogenic effect on the nociceptors [41,42].

2. Materials and methods

2.1. Receptor binding assay

Binding studies were done on rat cerebral cortex for α_1, α_2, 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A} receptors, rat striatum for D_1 and D_2 receptors, pig choroïd plexus for 5-HT_{2C} receptor, N1E-115 cells for 5-HT_3 receptor (CEREP, France). Specific ligands were [^{3}H]prazosin (0.25 nM, prazosin 0.5 μM as non-specific, 60 min at 25 °C incubation parameters) and [^{3}H]RX821002 (0.5 nM, adrenaline 100 μM, 30 min at 22 °C) for adrenergic α_1 and α_2 receptors subtypes respectively; [^{3}H]SCH23390 (0.3 nM, SCH23390 10 μM, 45 min at 25 °C) and [^{3}H]YM-09151-2 (0.1 nM, butaclamol 10 μM, 60 min at 25 °C) for dopaminergic D_1 and D_2 receptors subtypes respectively; and [^{3}H]8-OH-DPAT (0.5 nM, 8-OH-DPAT 10 μM, 30 min at 25 °C), [^{125}I]CYP (0.15 nM, serotonin 10 μM, 90 min at 37 °C), [^{3}H]ketanserin (0.5 nM, ketanserin 1 μM, 15 min at 37 °C), [^{3}H]mesulergine (1 nM, serotonin 1 μM, 30 min at 37 °C) and [^{3}H]BRL43694 (1 nM, metoclopramide 100 μM, 180 min at 4 °C) for serotonergic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors subtypes respectively. After incubation, membranes or cells in suspension were filtered (filters GF/B or GF/C, Whatman or Packard) and washed. Radioactivity was measured with a liquid scintillation counter (LS 6000, Beckman or Topcount, Packard). Nefopam was tested at 0.1–1–10–100 μM on each receptor, and measures were repeated. IC_{50} values were calculated according to a non-linear 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 (SAFE, 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 IASP (International Association for the Study of Pain) Committee for Research and Ethical Issues Guidelines (Pain 1983;16:109–10).

2.3. Acetic acid-induced writhing

Male CD1 or ICR mice (25–30 g) were used in groups of 10. Writhing was induced by an 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 inter-day 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. The series of experiments reported were performed through a 1-year period and that factor introduced some variability for nefopam ED_{50} in this model. However, for each adrenergic, dopaminergic or serotonergic system, the same mouse strain was used, and experiments were realized at the same time.

2.4. Formalin-induced licking

Male NMRI mice (30–35 g) were used in groups of 10. 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 inter-day variability and allow valid comparisons. In this model, there was much less time associated variability than in the writhing model. However, for each adrenergic, dopaminergic or serotonergic system, experiments were always realized at the same time.

2.5. Drugs and treatments

Nefopam hydrochloride was obtained from Biocodex as a racemate. Reserpine; prazosin hydrochloride; yohimbine hydrochloride; sulpiride; SCH23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride); PCPA (DL-*para*-chlorophenylalanine), methysergide maleate; NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide); WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate); ketanserin tartrate; tropisetron (3-tropanyl-indole-3-carboxylate) methiodide; SDZ-205557 (4-amino-5-chloro-2-methoxybenzoic acid 2-(diethylamino)ethyl ester hydrochloride); acetic acid and formalin were purchased from Sigma. GR127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride); R96544 ((2R,4R)-5-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]ethyl]-1-methyl-3-pyrrolidinol hydrochloride); and RS102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride) were purchased from Tocris (France). Drugs were dissolved in distilled water or isotonic saline (NaCl 0.9%) solutions, with the exception of reserpine, PCPA, methysergide, NAN-190, and RS102221 that were dissolved in a 1% solution of tween 80. Compounds were administered by subcutaneous (s.c.) route 15 min before the