ELSEVIER

European Journal of Pharmacology 365 (1999) 149–157

ejp

The involvement of opioidergic and noradrenergic mechanisms in nefopam antinociception

Alex M. Gray *, Mark J. Nevinson, Robert D.E. Sewell

Division of Pharmacology, The Welsh School of Pharmacy, University of Wales, King Edward VII Avenue, Cathays Park, Cardiff CF1 3XF, UK

Received 22 May 1998; revised 6 November 1998; accepted 10 November 1998

Abstract

Nefopam is a clinically effective analgesic agent used to control mild to moderate pain, whose mechanism of action is unknown. We have investigated the antinociceptive activity of nefopam in the mouse abdominal constriction assay and tail immersion test (48°C). Nefopam was found to possess a high degree of potency against acetic acid-induced visceral nociception (ED_{50} 2.5 mg kg^{-1}). In the presence of the opioid receptor antagonists, naloxone or naltrindole, the resulting nefopam dose–response relationships were shifted to the right. Naloxone or naltrindole had no effect upon aspirin (ED_{50} 32.1 mg kg^{-1}) or clonidine (ED_{50} 0.061 mg kg^{-1}) induced antinociception. Acetorphan (10 mg kg^{-1}; s.c.), an inhibitor of neutral endopeptidase (EC 3.4.24.11) was able to potentiate nefopam's antinociceptive activity (ED_{50} 1.5 mg kg^{-1}). The α_2-adrenoceptor antagonist, 2-[2-(2-methoxy-1,4-benzodioxanyl)]imidazoline hydrochloride (RX821002; 1 mg kg^{-1}; s.c.), shifted the dose–response curves for clonidine (ED_{50} 7.1 mg kg^{-1}) and nefopam (ED_{50} 5.3 mg kg^{-1}) to the right in this assay. Additionally, centrally administered RX821002 (1 μg/5 μl/animal; i.c.v.) reduced both clonidine (ED_{50} 7.2 mg kg^{-1}) and nefopam's (ED_{50} 15.5 mg kg^{-1}) efficacy in the abdominal constriction assay. Nefopam (3 and 7.5 mg kg^{-1}; s.c.) produced significant antinociceptive effect in the thermal assay. Aspirin and RX821002 were devoid of any significant activity in the tail immersion test. Nefopam was shown to possess RX821002-reversible antinociceptive activity in both the tail immersion test and the abdominal constriction assay. These data suggest the involvement of an opioidergic and noradrenergic component to nefopam's antinociceptive activity in the mouse abdominal constriction assay and tail immersion test. However, the present results are unable to determine if the opioidergic component of nefopam antinociception is through a direct and/or indirect activation of opioid receptors.

Keywords: Nefopam; Clonidine; Abdominal constriction assay; Tail immersion test; Opioid receptor antagonist; Naltrindole; RX821002

1. Introduction

Nefopam is a clinically effective analgesic agent used to control mild to moderate pain. Chemically, nefopam belongs to the benzoxazocine class of compounds, which are structurally related to the antiparkinson/anticholinergic drug, orphenadrine and the antihistamine, diphenhydramine. Nefopam however, does not display any significant antihistaminic activity and in contrast to orphenadrine, may enhance motor neurone excitability (Heel et al., 1980; Gassel, 1973).

In studies employing a range of various animal models, nefopam has been shown to possess both muscle relaxant and relatively weak anticholinergic actions (Klohs et al., 1972). Additionally, Conway and Mitchell (1977) reported that nefopam had marginal inhibitory effects on eicosinoid activity in vitro. The antinociceptive activity of nefopam has been demonstrated in most standard animal nociceptive models (Piercey and Schroeder, 1980, 1981; Heel et al., 1980).

Nefopam's antinociceptive mechanism(s) of action is largely unknown. It does not appear to possess intrinsic activity at opioid receptors, nor does it interact with the cyclo-oxygenase enzyme system (Heel et al., 1980). There is, however, one pharmacodynamic action that has been identified for nefopam, namely its ability to block the neuronal reuptake of noradrenaline, 5-hydroxytriptamine and to a lesser extent, dopamine (Tresnak-Rustad and

* Corresponding author. PPD Pharmaco, 1400 Perimeter Park Drive, Morrisville, NC 27560, USA. Tel.: +1-919-462-4978; Fax: +1-919-460-1235; E-mail: alex.gray@rtp.ppdi.com

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00837-1

Wood, 1978). In parallel with this activity, some antidepressant-like effects have also been reported in animal models of depression (Basset et al., 1969; Hammerbeck et al., 1974). Thus, nefopam's pharmacology is similar to the tricyclic antidepressants by virtue of its behavioural and monoamine reuptake inhibitory profile. Consequently, the involvement of biogenic amines in nefopam's antinociceptive action has been the subject of several studies. For example, Vonvoigtlander et al. (1983) have shown that pre-treatment with the amine depletor reserpine, abolishes nefopam's antinociceptive effect. Fasmer et al. (1986) using lesioning techniques, have suggested that there is an essential participation of descending 5-HT systems in nefopam antinociception. Furthermore, Fasmer et al. (1987) demonstrated that nefopam antinociception is stereospecific to the enantiomer with the strongest monoamine reuptake inhibitory properties (i.e. (−)-nefopam). Hence, nefopam appears not to resemble opioid analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) in its overall mechanism of action.

We have previously shown that certain tricyclic antidepressants are inherently antinociceptive in the abdominal constriction assay (Gray et al., 1990, 1991). In addition tricyclic antidepressants are able to augment opioid receptor agonist mediated antinociception (Sewell et al., 1979).

Recently, we demonstrated the involvement of opioidergic mechanisms in antidepressant-induced antinociception (Gray et al., 1998). In the present study, we have examined the analgesic effect of nefopam in mice, using the acetic acid-induced abdominal constriction assay and the thermal tail immersion test (48°C). The involvement of the opioidergic system was investigated using the opioid receptor antagonists, naloxone and naltrindole, and the neutral endopeptidase inhibitor, acetorphan (EC 3.4.24.11).

The role of noradrenaline in analgesia remains controversial (Watkins and Mayer, 1986). However, due to increasing evidence for the modulation of nociception by noradrenergic neurones (see Proudfit, 1988), we decided to address the possible involvement of noradrenergic mechanisms in nefopam antinociception through α-adrenoceptors.

The abdominal constriction assay was chosen as an appropriate nociceptive paradigm since it detects a range of opioid receptor and opioid-like agonists with the greatest sensitivity and also the clearest separation between doses causing antinociception and motor impairment (Hayes et al., 1987). The tail immersion technique has minimal liability to cause tissue damage and enables measurements of nociceptive sensitivity to be made on individual animals at frequent intervals and thus, allows the time course of a drug's effects to be obtained. In addition, this technique is sensitive and particularly useful for demonstrating dose-related activity (Sewell and Spencer, 1976).

2. Materials and methods

2.1. Animals

Male ICI GB1 mice (20–30 g) were housed in colony cages and allowed food and water ad libitum up to 2 h prior to the commencement of the experiments. A 12 h/12 h light–dark cycle was used, with all the experiments being performed during the light period of the cycle. Both the animal unit and laboratory temperature were maintained at 21 ± 1°C. The mice were allowed to habituate to the laboratory environment for 2 h before the experiments were initiated.

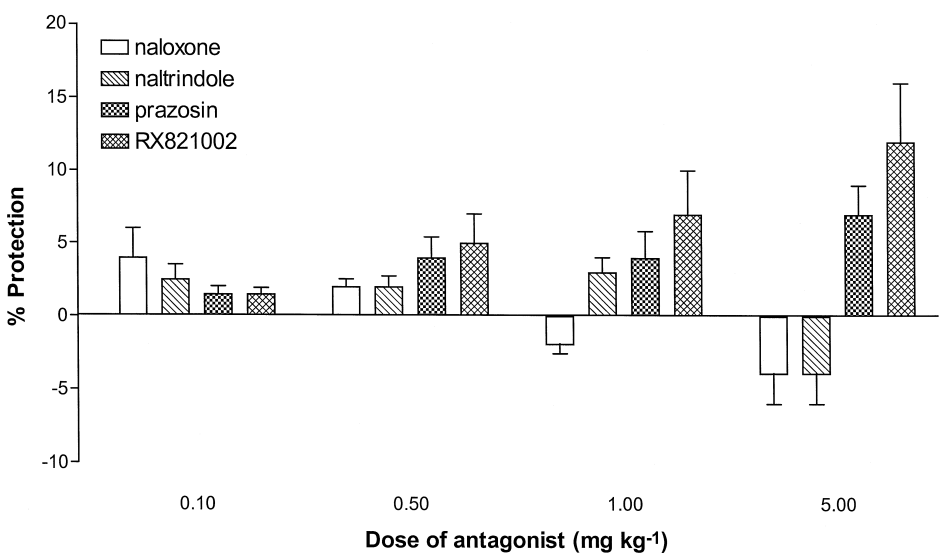

Fig. 1. The effect of the opioid receptor antagonists, naloxone or naltrindole, or noradrenergic receptor antagonists, prazosin or RX821002, on the ability of 1% acetic acid to induce abdominal constrictions (writhes) in mice. Neither of the receptor antagonists at the doses investigated produced any protection against acetic acid-induced writhes ($P > 0.05$; $n = 8$ per treatment group).

2.2. Nociception

2.2.1. Acetic acid-induced abdominal constriction assay

Each mouse was randomly assigned to one of the 19 treatment groups ($n = 8$) and received either nefopam, clonidine or aspirin 30 min prior to a challenge with 1% acetic acid (0.1 ml/10 g; i.p.). The treatment groups in which opioid receptor antagonists were employed received a contralateral subcutaneous injection of this antagonist or saline 25 min after the subcutaneous injection of nefopam, clonidine or aspirin. The α-adrenoceptor antagonists or saline were co-administered as a contralateral injection to nefopam or clonidine 30 min prior to intraperitoneal challenge with acetic acid. In the central administration studies, RX821002 (1 μg/5 μl/animal; i.c.v.) or artificial cerebrospinal fluid (5 μl/animal; i.c.v.) was given 25 min after nefopam or clonidine.

Each mouse was placed individually in a colony cage following acetic acid challenge. Abdominal constrictions were evaluated over the next 20 min after which the mouse was immediately killed. Individual abdominal constrictions were classified as unilateral or bilateral inward rotation of the rear feet, visible wave motions on the margin of the peritoneal cavity and/or abduction of the lower trunk. The number of constrictions from each treatment group of eight was expressed as '% Protection' by application of the following formula: [100 − (mean of drug group/mean of control group)].

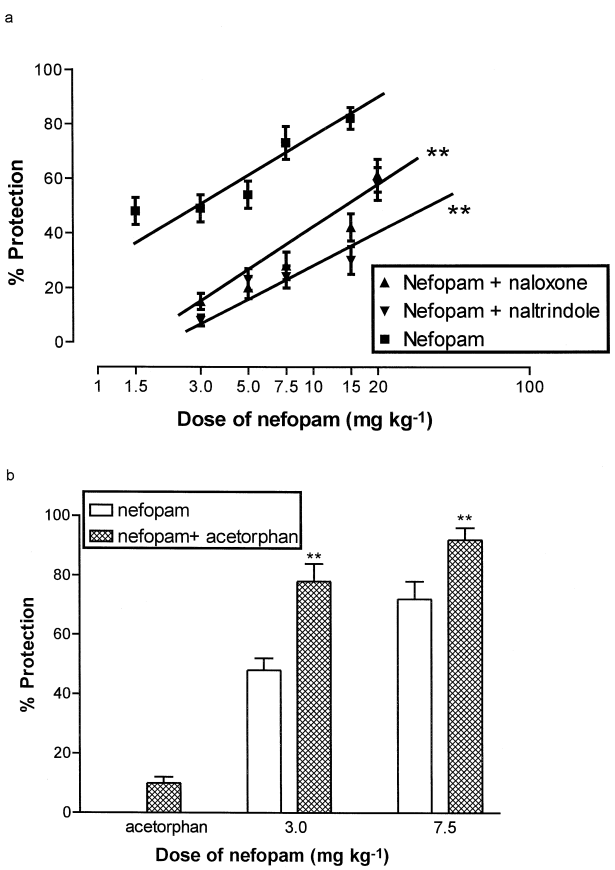

Fig. 2. Effect of nefopam in the acetic acid-induced writhing paradigm. (a) Nefopam was investigated alone and in the presence of the opioid receptor antagonists naloxone (s.c.) and naltrindole (s.c.). Nefopam produced a robust dose response which was shifted significantly to the right following pretreatment with naloxone or naltrindole ($P < 0.01$; $n = 8$ per treatment group). (b) Acetorphan was investigated for its ability to potentiate nefopam antinociception. Acetorphan significantly enhanced nefopam antinociception ($P < 0.01$; $n = 8$ per treatment group).

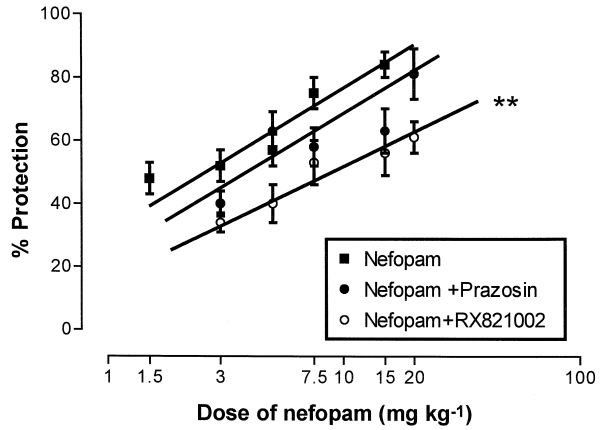

Fig. 3. The noradrenergic receptor antagonists, prazosin and RX821002, were investigated for their ability to antagonise nefopam antinociception in the abdominal constriction assay. Prazosin (1 mg kg^{-1}; s.c.) did not modify the ability of nefopam to reduce the number of acetic acid-induced writhes ($P > 0.05$; $n = 8$). The α_2-adrenoreceptor antagonist, RX821002 (1 mg kg^{-1}; s.c.), shifted the nefopam dose response relationship to the right ($P < 0.01$; $n = 8$).

2.2.2. Tail immersion assay

The tail immersion technique was performed in accordance with that described by Sewell and Spencer (1976). Mice were randomly assigned to one of the six treatment groups ($n = 10$) and were habituated to the testing environment for 1 h in polypropylene restraining containers from which their tail protruded.

Nefopam, aspirin, RX821002, morphine or saline vehicle were investigated for their antinociceptive effects alone following subcutaneous injection. In a separate group of mice, RX821002 was combined with nefopam and was administered as a contralateral injection 5 min before the nefopam injection.

Nociceptive sensitivity (reaction time in s, to the nearest tenth of a s) to tail immersion in a constant temperature water bath, maintained at 48°C by a Grant Instruments Circulator, was determined immediately before, and at successive 20 min intervals after drug (and vehicle) administration for a total test period of 100 min. The nociceptive reaction of mice was taken to be a tail flick or complete withdrawal of the tail when the heat stimulus was applied. A 15 s 'cut-off' time was imposed for all animals that failed to respond to the stimulus after drug treatment in order to avoid the possibility of tissue damage.

Reduced sensitivity in this test was indicated by an increased reaction time to withdrawal of the tail. A quanti-

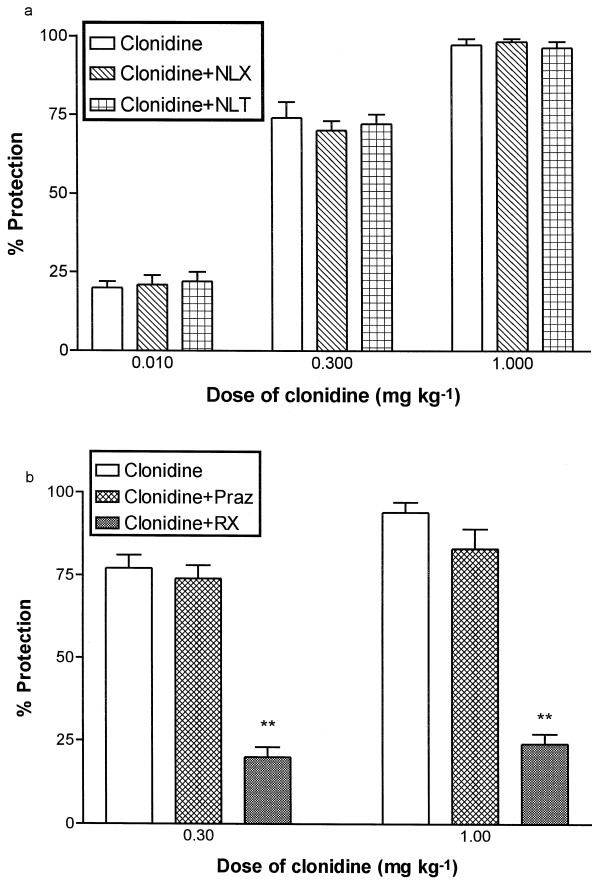

Fig. 4. The effect of clonidine in the abdominal constriction assay. (a) The opioid receptor antagonists naloxone (NLX; 0.5 mg kg^{-1}; s.c.; $n=8$) and naltrindole (NLT; 1 mg kg^{-1}; s.c.; $n=8$) did not affect clonidine-induced antinociception ($P>0.05$). (b) Prazosin (Praz; 1 mg kg^{-1}; s.c.; $n=8$) did not antagonise clonidine antinociception. The α_2-adrenoceptor antagonists, RX821002 (1 mg kg^{-1}; s.c.; $n=8$) significantly antagonised clonidine antinociception in the mouse abdominal constriction assay ($P<0.01$).

tative measure of this change in nociceptive reaction latency was obtained by integrating the experimental duration (100 min) vs. reaction time(s) curves for control and drug treated groups, subsequently expressing the integral from drug treated animals as a percentage increase (antinociceptive effect) of the control integral (Sewell and Spencer, 1976).

The antinociceptive tests were conducted following the ethical guidelines laid out by the committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

2.3. Intracerebroventricular injections

Injections were carried out according to the method of Hayley and McCormick (1957). A 10 μl Hamilton syringe was fitted with a 27 gauge stainless steel needle, which had been cut to a length of 3.25 ± 0.5 mm and polished to a bevelled tip. A volume of 5 μl was injected into the right lateral ventricle via the coronal suture 1.00 mm lateral from the bregma in conscious mice.

2.4. Drugs

Nefopam Hydrochloride (Riker 3M, U.K.), naloxone hydrochloride (Endo Laboratories, U.K.), RX821002 and naltrindole (Reckitt and Coleman, Bristol, U.K.). were dissolved in normal pyrogen-free saline. Prazosin hydrochloride (Sigma U.K.) was dissolved in distilled water and 1% citric acid. The drugs were delivered systemically in a volume of 10 ml kg^{-1}.

2.5. Statistical analysis

The dose response relationships were analysed using analysis of covariance (ANCOVA) within MANOVA of the statistical computer package SPSS/PC+. The ED_{50} values were determined by regression analysis. Additionally, two-way analysis of variance was employed to analyse the ED_{50} data. The shift factors were determined from ED_{50} dose ratios. The tail immersion test data following the conversion to area under the temporal curve was analysed using a one-way analysis of variance followed by the Tukey post hoc test. The level of statistical significance was assumed at the $P<0.05$ level.

3. Results

3.1. Acetic acid-induced abdominal constriction study

3.1.1. Systemic study

The opioid receptor antagonists, naloxone and naltrindole, and the noradrenergic receptor antagonists, pra-

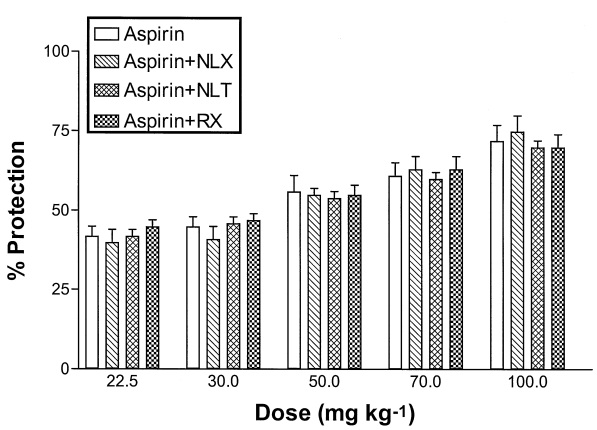

Fig. 5. The effect of aspirin in the abdominal constriction assay. Aspirin produced a shallow dose response to acetic acid-induced abdominal constrictions in mice. The opioid receptors antagonists, naloxone (NLX; 0.5 mg kg^{-1}; s.c.; $n=8$) or naltrindole (NLT; 1 mg kg^{-1}; s.c.; $n=8$), did not affect aspirin-induced protection from acetic acid-induced writhing in mice ($P>0.05$). Furthermore, the α_2-adrenoceptor antagonist, RX821002 (RX; 1 mg kg^{-1}; s.c.) did not affect aspirin antinociception ($P>0.05$; s.c.; $n=8$).

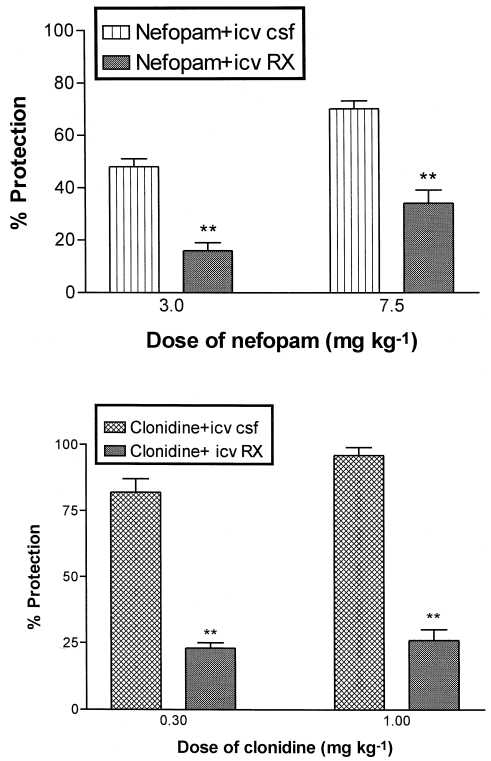

Fig. 6. The effect of intracerebroventricular (icv) RX821002 (RX; 1 μg/5 μl) on (a) nefopam ($n=8$) antinociception in the abdominal constriction test and (b) clonidine ($n=8$) in the abdominal constriction test. RX821002 antagonised the antinociception induced by nefopam and clonidine ($P<0.01$).

zosin and RX821002, were investigated for their activity in the abdominal constriction assay ($n=8$). It can be seen from Fig. 1 that the receptor antagonists were devoid of any inherent activity in this assay ($P>0.05$).

Nefopam produced a dose-dependent inhibition of acetic acid-induced abdominal constrictions in our assay ($P<0.01$; Fig. 2a; $n=8$). The opioid receptor antagonists, naloxone (0.5 mg kg^{-1}) and naltrindole (1 mg kg^{-1}) shifted the dose response relationship for nefopam to the right by the following factors: 3.7 for naloxone and 3.8 for naltrindole ($P<0.01$; Fig. 2a; $n=8$). Nefopam antinociception was also investigated in the presence of the enkephalinase inhibitor, acetorphan. Fig. 2b demonstrates that the dose of acetorphan (10 mg kg^{-1}; s.c.) utilised in the present study was devoid of activity against acetic acid-induced writhes ($P>0.05$; $n=8$). Further, the sub-effective dose of acetorphan potentiated nefopam antinociception ($P<0.01$), shifting the nefopam dose–effect curve to the left by a factor of 0.6 ($P<0.01$; $n=8$).

The α_1-adrenoceptor antagonist, prazosin (1 mg kg^{-1}; s.c.; Fig. 3) was without effect on the antinociceptive capacity of nefopam ($P>0.05$; $n=8$). In contrast, the α_2-adrenoceptor antagonist, RX821002 (1 mg kg^{-1}; s.c.) shifted the nefopam dose–response relationship significantly to the right in this assay. The shift factor for RX821002 was 2.1 ($P<0.01$; Fig. 3; $n=8$).

Clonidine produced protection against acetic acid-induced abdominal constrictions in a dose dependent manner over the dose range examined ($P<0.01$; $n=8$). Moreover, there was no attenuation in protection following administration of naloxone or naltrindole ($P>0.05$; Fig. 4a; $n=8$). However, the administration of RX821002 (1 mg kg^{-1}; s.c.) significantly attenuated clonidine antinociception ($P<0.01$; Fig. 4b; $n=8$). The resultant shift to the right was by a factor of 116.

Aspirin also produced dose-dependent inhibitory activity. When aspirin was administered before the opioid receptor antagonists, naloxone or naltrindole, there was no significant change in aspirin's protective action ($P>0.05$; Fig. 5; $n=8$).

3.1.2. Central administration study

Pilot studies were conducted to identify a dose of RX821002 which would attenuate clonidine antinociception (pilot data not shown). The dose identified (1 μg/5 μl/animal; i.c.v.) shifted clonidine's ED_{50} to the right by a factor of 138 ($P<0.01$; Fig. 6; $n=8$). The same dose of RX821002 (i.e. 1 μg/5 μl/animal; i.c.v.) was used to investigate a supraspinal locus of action for the antinociceptive activity of nefopam. Fig. 6 shows the attenuation of nefopam antinociception following i.c.v. administration of RX821002 (1 μg/5 μl/animal; $P<0.01$; $n=8$).

Table 1
The ED_{50} values (mg kg^{-1} with 95% confidence interval) for nefopam, clonidine and aspirin in the presence of opioid, monoamine receptor antagonists and acetorphan in the mouse acetic acid (1%) abdominal constriction assay

	Nefopam	Clonidine	Aspirin
Alone	2.5 (1.8, 3.3)	0.061 (0.04, 0.07)	32.1 (28, 34)
Naloxone (0.5 mg kg^{-1}; s.c.)	9.38[a] (7.1, 12.4)	0.070 (0.05, 0.08)	33.2 (29, 35)
Naltrindole (1 mg kg^{-1}; s.c.)	9.52[a] (7.2, 12.6)	0.059 (0.05, 0.06)	32.9 (29, 34)
Prazosin (1 mg kg^{-1}; s.c.)	2.10 (1.5, 2.8)	0.057 (0.05, 0.06)	–
RX821002 (1 mg kg^{-1};s.c.)	5.31[a] (4.9, 6.9)	7.1[b] (6.7, 7.4)	30.8 (28, 34)
RX821002 (1 μg/5 μl; i.c.v)	15.5[a] (12.1, 16.2)	7.2[b] (6.8, 7.3)	–
Acetorphan	1.5[a] (1.3, 2.0)	–	–

[a] $P<0.01$ compared to nefopam alone.
[b] $P<0.01$ compared to clonidine alone.

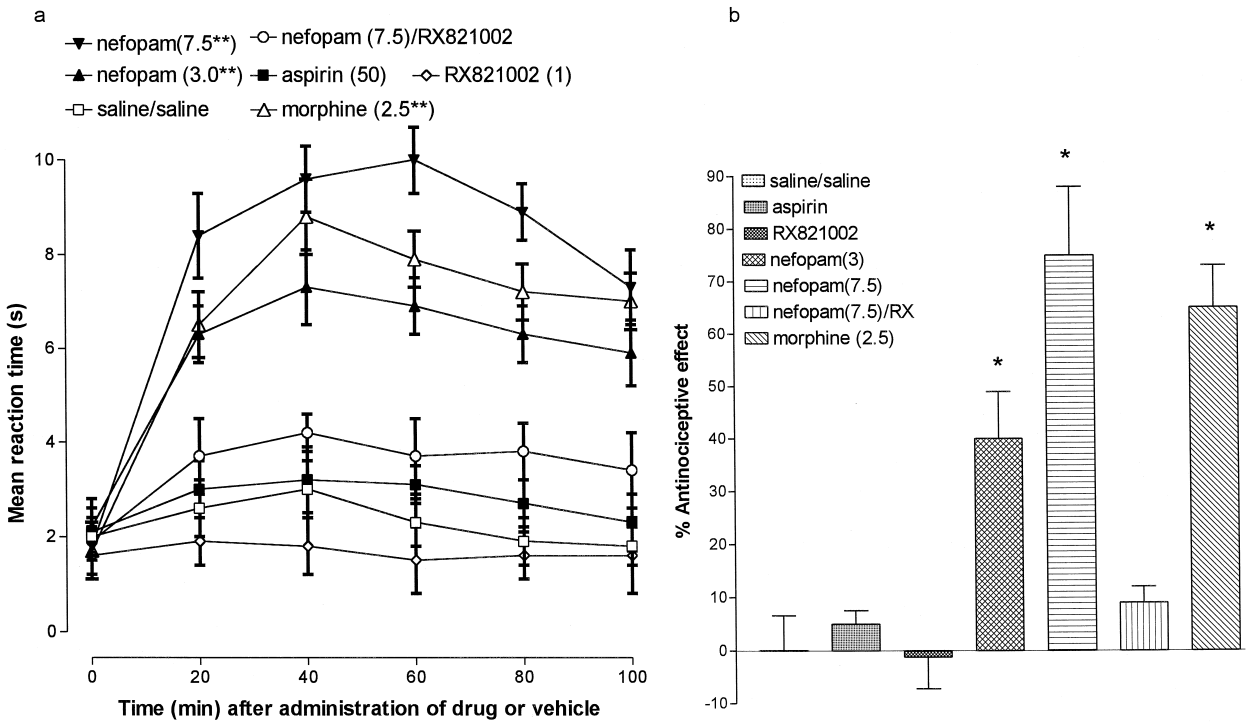

Fig. 7. The effect of nefopam in the mouse tail immersion test (48°C). (a) Temporal profile of nefopam, RX821002, morphine, and the combination of nefopam and RX821002 in the mouse tail immersion test. Each point represents the mean reaction latency (s). The legend contains the dose of the drug investigated (mg kg^{-1}; s.c.) in parenthesis together with statistical significance. ** $P < 0.01$ vs. saline/saline control. Thus both doses of nefopam produced significant increases in tail immersion latency. Morphine, the prototypic opioid receptor agonist, produced significant protection in this assay. However, when nefopam was combined with the α_2-adrenoceptor agonist, RX821002, nefopam was devoid of its antinociceptive capacity. (b) Area under the curve data derived from the temporal profiles for the treatment groups investigated in (a). Both doses of nefopam and morphine produced significant antinociceptive activity. The α_2-adrenoceptor antagonist clearly blocked nefopam (7.5 mg kg^{-1}; s.c.) antinociception.

Analysis of the ED_{50} data shows a shift to the right by a factor of 6. Table 1 details the ED_{50} values with 95% confidence intervals for the above studies.

3.2. Tail immersion study

Nefopam, at the doses examined, produced significant increases in reaction latency in the mouse tail immersion test (48°C). Thus, 3 mg kg^{-1} (s.c.) of nefopam produced a maximal mean reaction latency time of 7.3 ± 0.8 s, 40 min after administration of nefopam which was significantly elevated compared to saline controls. The higher dose of nefopam at this time point produced a mean value of 9.60 ± 0.7 s. The maximal increase in mean reaction latency was 10.0 ± 0.7 s which occurred 60 min after administration of nefopam which was significantly higher than temporally matched saline controls. RX821002 antagonised the ability of nefopam (7.5 mg kg^{-1}) to induce antinociception. Neither aspirin nor RX821002 significantly elevated mean reaction latency over that of controls (Fig. 7a). Morphine induced significant antinociception which reached a maximum 40 min after administration. Fig. 7b displays the temporal data as % antinociceptive effect, showing that both doses of nefopam produced a significant antinociceptive effect comparable to morphine (2.5 mg kg^{-1}) and that nefopam antinociception was antagonised by RX821002.

4. Discussion

Previous studies investigating nefopam antinociception utilised different nociceptive models from the present study. In this respect, it is important to note that Ögren and Holm (1980) identified a test-specific nature to antidepressant-induced antinociception which may also be inherent in the antinociceptive activity of nefopam given that its major pharmacodynamic action is identical to tricyclic antidepressants. Additionally, various factors underlie noxious test stimulus quality, with a qualitative as opposed to quantitative difference in the discrimination of opioid-like agonists having been identified (Upton et al., 1982, 1983; Tyers, 1980; Shaw et al., 1988). Furthermore, methodological problems are evident when investigating nociceptive mechanisms which use behavioural end-points. This observation is clearly demonstrated when one tries to summarise the involvement of serotonergic systems in pain regulation. Indeed, there are many discrepancies and controversies which may, in part, be due to methodological problems in so far as the behavioural end-point criteria is concerned.

Manipulations of neurotransmitter systems may alter motor responses, emotional and autonomic functions (including vasomotor function). Such non-pain related events might influence the results in certain tests of nociception, which invariably rely on motor and/or other responses for identification of an antinociceptive effect.

Lund et al. (1989) identified an ambient room temperature of 24.0–25.0°C as being a contributory factor in tricyclic antidepressant-induced antinociception in the tail flick assay when tail skin temperature was concurrently measured. When a room temperature of 21.0–22.0°C was investigated in the same test, there was no apparent increase in tail flick latency. This finding was attributed to a significant drop in tail skin temperature following desipramine treatment. Thus, Lund et al. (1989) explained this apparent antinociceptive effect as a decrease in tail skin temperature which does not manifest itself when the test procedure is carried out at 21.0–22.0°C.

Thus, the test procedures employed in the present study were carried out at an ambient room temperature of 21.0 ± 1°C, thus eliminating any potential hypothermic effects on tail skin temperature, resulting in a false antinociceptive action of nefopam. The abdominal constriction assay was used since it is capable of detecting a wide range of opioid receptor and opioid-like agonists with the greatest sensitivity and the clearest separation between doses causing antinociception and motor impairment (Hayes et al., 1987).

The present study confirms nefopam's antinociceptive profile in two mouse nociception models. Using the acetic acid abdominal constriction paradigm and the mouse tail immersion test, this study demonstrated that nefopam exhibited naloxone and naltrindole reversible antinociception, an aspect of its pharmacology that many previous studies have failed to note (Conway and Mitchell, 1977; Piercey and Schroeder, 1981).

This study identified further evidence for an opioidergic component to nefopam antinociception by demonstrating the potentiation of nefopam antinociception by acetorphan, an inhibitor of the enzymes responsible for the degradation of the endogenous opioid peptides. Chipkin (1986) postulated that enkephalinase inhibitors would be antinociceptive, only under conditions that activate enkephalin containing endogenous pain suppression systems. Thus, in the absence of any released enkephalin, the enkephalinase inhibitor under investigation would be 'silent' (as demonstrated by the dose utilised in this study), and in contrast, when enkephalins are actively being released, enkephalinase inhibitors will both prolong and potentiate these effects. Similarly, we have shown that several tricyclic antidepressants and paroxetine possess inherent antinociceptive properties, and exhibit naloxone and naltrindole antagonist reversible antinociception (Gray et al., 1990, 1998). In addition, the antinociceptive properties of the tricyclic antidepressants and paroxetine can be potentiated by acetorphan (Gray et al., 1998). In the present study we have also shown that nefopam antinociception is antagonised by both systemic and supraspinal administration of RX821002, a selective α_2-adrenoceptor antagonist.

Nefopam has been shown to possess poor naloxone-displaceable binding to opioid receptors within the hippocampus and other brain areas (Tresnak-Rustad and Wood, 1978). Therefore, it would appear unlikely that direct opioid receptor activation is the primary mechanism by which nefopam produces analgesia. In this context, tricyclic antidepressants have been shown to enhance endogenous opioid peptide immunoreactivity (De Felipe et al., 1985, 1986) and antidepressant activity can be enhanced using neutral endopeptidases (De Felipe et al., 1989). Given that nefopam is similar in its pharmacology to tricyclic antidepressants, it is conceivable that nefopam may also effect opioidergic tone in a manner analogous to the tricyclic antidepressants. This hypothesis is supported by the present study showing the potentiation of nefopam antinociception following the administration of acetorphan.

Results from this study suggest that nefopam also exhibits α_2-adrenoceptor antagonist reversible antinociception. There is a considerable body of evidence indicating an involvement of noradrenergic mechanisms in the control of nociceptive transmission (Yaksh, 1985; Basbaum and Fields, 1984). Accordingly, the α_2-adrenoceptor agonist clonidine is antinociceptive in many assays (Yaksh, 1985). Our data is in accord with this evidence, and further elaborates the graded aspect of this pharmacological property. Four major noradrenergic systems are thought to be involved in modulating nociception: three are antinociceptive and the fourth appears to enhance nociception (see Proudfit, 1988).

Fleetwood-Walker et al. (1985) showed that iontophoretically applied noradrenaline selectively inhibited noxious cutaneous stimuli and these responses were not blocked by the α_1-adrenoceptor antagonist prazosin. Nevertheless, these responses were antagonised by the application of the α_2-adrenoceptor antagonists, yohimbine or idazoxan. Similarly, the effect of iontophoretically applied noradrenaline can be mimicked by clonidine which is also blocked by yohimbine. Furthermore, studies involving lesions of noradrenergic neuronal systems by *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP4) yield a potentiation of clonidine antinociception (Zis and Fibiger, 1975), which may be ascribed to an up-regulation of central α_2-adrenoceptors. In this context, the present study with clonidine and systemic RX821002, is in agreement with current thought on the involvement of noradrenaline in antinociception. Further, our data with i.c.v. RX821002 indicates a central component to nefopam antinociception, which lends further support to the hypothesis of Irikura et al. (1981) that nefopam through a facilitatory action on the descending inhibitory control system originating in supra-spinal structures affects analgesia at the spinal level. However, this hypothesis is at odds with the study by Piercey and Schroeder (1981) who showed that i.c.v. administration of nefopam had a lower ED_{50} in

the hot-plate test than intraspinally administered nefopam. Further, they showed that the ED_{50} for i.c.v. administered nefopam was higher than intraspinally administered nefopam in the tail flick assay. This dichotomy again brings in the debate about neural mechanisms of different nociceptive assays.

Our present findings may suggest that increased intrasynaptic levels of noradrenaline may interact with α_2-adrenoceptors possibly in descending supraspinal noradrenergic systems as one of the major neuronal loci for nefopam antinociception.

5. Conclusion

In conclusion, we suggest that one component mechanism of nefopam antinociception stems from increased levels of intrasynaptic monoamines (Hwang and Wilcox, 1987). The increased intrasynaptic availability of monoamines may result in the activation of α_2-adrenoceptors in addition to the release of opioidergic peptides (Sacerdote et al., 1987). It is not clear at this juncture however, whether the opioidergic release is contingent upon the increased monoamine availability.

References

Basset, J.R., Cairncross, K.D., Hacket, W.B., Story, M., 1969. Studies on the peripheral pharmacology of fenazoxine, a potential antidepressant drug. Br. J. Pharmacol. 37, 69–78.

Basbaum, A.I., Fields, H.L., 1984. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Ann. Rev. Neurosci. 7, 309–338.

Chipkin, R.E., 1986. Inhibitors of enkephalinase: the next generation of analgesics. Drugs of the Future 11, 593–606.

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

De Felipe, M.C., De Caballos, M.L., Gil, C., Fuentes, J.A., 1985. Chronic antidepressant treatment increases enkephalin levels in n. accumbens and striatum of the rat. Eur. J. Pharmacol. 112, 119–122.

De Felipe, M.C., De Caballos, M.L., Fuentes, J.A., 1986. Hypoalgesia induced by antidepressants in mice: a case for opioids and serotonin. Eur. J. Pharmacol. 125, 193–199.

De Felipe, M.C., Jimenez, I., Castro, A., Fuentes, J.A., 1989. Antidepressant action of imipramine and iprindole in mice is enhanced by inhibitors of enkephalin-degrading peptidases. Eur. J. Pharmacol. 159, 175–180.

Fasmer, O.B., Berge, O.-G., Post, C., Hole, K., 1986. Effects of the putative 5-HT_{1a} receptor agonist 8-OH-2-(di-*n*-propylamino) tetralin on nociceptive sensitivity in mice. Pharmacol. Biochem. Behav. 25, 883–888.

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.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., Molony, V., Iggo, A., 1985. An α_2 receptor mediates the selective inhibition by noradrenaline of nociceptive responses of identified dorsal horn neurones. Brain Res. 334, 243–254.

Gassel, M.M., 1973. An objective technique for the analysis of the clinical effectiveness and physiology of the action of drugs in man. In: Desmedt (Ed.), New Developments in Electromyography and Clinical Neurophysiology, Vol. 3. Karger, Basel, pp. 342–359.

Gray, A.M., Sewell, R.D.E., Spencer, P.S.J., 1990. Antidepressant inhibitory activity in the abdominal constriction assay is competitively blocked by opioid antagonists. Br. J. Pharmacol. 101, 554.

Gray, A.M., Nevinson, M.J., Sewell, R.D.E., 1991. Antagonism of nefopam antinociception by naloxone and RX821002. Br. J. Pharmacol. 104, 267.

Gray, A.M., Spencer, P.S.J., Sewell, R.D.E., 1998. The involvement of the opioidergic system in antidepressant-induced antinociception. Br. J. Pharmacol. 124 (4), 669–674.

Hayes, A.G., Sheenan, M.J., Tyers, M.B., 1987. Differential sensitivity of models of antinociception in the rat, mouse and guinea pig to μ- and κ-opioid receptor agonists. Br. J. Pharmacol. 91, 823–833.

Hayley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12, 13–15.

Hammerbeck, D.M., Conway, A.C., Mitchell, C.L., 1974. Pharmacologic evaluation of nefopam hydrochloride (Acupan). Pharmacologist 16, 247.

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

Hwang, A.S., Wilcox, G.L., 1987. Analgesic properties of intrathecally administered heterocyclic antidepressants. Pain 28, 343–355.

Irikura, T., Hirayama, T., Taga, F., 1981. Electrophysiological investigations on the mode of action of nefopam, a novel analgesic agent. Jpn. J. Pharmacol. 31, 815–822.

Klohs, M.W., Draper, M.D., Petracek, F.J., Ginzel, K.H., Re, O.N., 1972. Benzoxazocines: a new chemical class of centrally acting skeletal muscle relaxants. Arzneimittel-Forschung 22, 132–139.

Lund, A., Tjolsen, A., Hole, K., 1989. Desipramine in small doses induces antinociception in the increasing temperature hot-plate test but not the tail-flick test. Neuropharmacology 28 (11), 1169–1173.

Ögren, S.O., Holm, A.C., 1980. Test specific effects of the 5-HT re-uptake inhibitors alaproclate and zimelidine on pain sensitivity and morphine analgesia. J. Neural. Transm. 47, 253–271.

Piercey, M.F., Schroeder, L.A., 1980. A quantitative analgesic assay in the rabbit based on the response to tooth pulp stimulation. Arch. Int. Pharmacol. Ther. 248, 294–304.

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.

Proudfit, H.K., 1988. Pharmacologic evidence for the modulation of nociception by noradrenergic neurons. Prog. Brain Res. 77, 357–370.

Sacerdote, P., Brini, A., Mantegazza, P., Panerai, A.E., 1987. A role for serotonin and beta-endorphin in the analgesia induced by some tricyclic antidepressant drugs. Pharmacol. Biochem. Behav. 26, 153–158.

Sewell, R.D.E., Spencer, P.S.J., 1976. Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. Neuropharmacology 15, 683–688.

Sewell, R.D.E., Lee, R.L., Spencer, P.S.J., 1979. Interactions between amines, analgesics and enkephalins: new approaches to the management of pain. Pharmacol. Med. 1, 23–38.

Shaw, J.S., Rourke, J.D., Burns, K.M., 1988. Differential sensitivity of antinociceptive tests to opioid agonists and partial agonists. Br. J. Pharmacol. 95, 578–584.

Tresnak-Rustad, N.J., Wood, M.E., 1978. In vitro biochemical effects of nefopam hydrochloride, a new analgesic agent. Biochem. Pharmacol. 30, 2847–2850.

Tyers, M.B., 1980. A classification of opioid receptors that mediate antinociception in animals. Br. J. Pharmacol. 69, 503–512.

Upton, N., Sewell, R.D.E., Spencer, P.S.J., 1982. Differentiation of potent μ- and κ-opioid agonists using heat and pressure antinociceptive profile and combined potency analysis. Eur. J. Pharmacol. 78, 421–429.

Upton, N., Sewell, R.D.E., Spencer, P.S.J., 1983. Analgesic actions of μ- and κ-opioid agonists in rats. Arch. Int. Pharmacodyn. 262, 199–207.

Vonvoigtlander, P.F., Lewsi, R.A., Neff, G.L., Triezenberg, H.J., 1983. Involvement of biogenic amines with mechanisms of novel analgesics. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 7, 651–656.

Watkins, L.R., Mayer, D.J., 1986. Multiple endogenous opiate and non-opiate analgesia systems: evidence of their existence and clinical implications. Annals of the New York Academy of Sciences 467, 273–299.

Yaksh, T.L., 1985. Pharmacology of spinal adrenergic systems which modulate nociceptive processing. Pharmacol. Biochem. Behav. 22 (5), 845–858.

Zimmermann, M., 1983. Ethical guidelines for investigation of experimental pain in conscious animals. Pain 16, 109–110.

Zis, A.P., Fibiger, H.C., 1975. Functional evidence for postsynaptic supersensitivity of central noradrenergic receptors after denervation. Nature 256, 659–660.