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Antinociceptive effects of (±)-, (+)- and (−)-nefopam in mice

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The antinociceptive activity of (±)-, (+)- and (−)-nefopam in mice has been examined using the hot-plate, formalin and tail-flick tests. Nefopam was administered by the intraperitoneal, intracerebroventricular (i.c.v.) and intrathecal (i.th.) routes. Intraperitoneal injection of (±)-nefopam (10–20 mg kg^{-1}) had powerful analgesic effects in the hot-plate and formalin tests. In the tail-flick test it produced a weak, but significant elevation of the response latencies. In spinalized animals, however, the effect was abolished, indicating that nefopam prolonged the tail-flick latencies by activation of descending pain-modulating pathways. (±)-Nefopam (5–20 μg) elicited analgesia in the hot-plate test after i.c.v. or i.th. injection. These findings suggest that nefopam has both a spinal and a supraspinal site of action. (+)-Nefopam was significantly more potent than (−)-nefopam after both systemic and central administration.

Nefopam is an effective analgesic in man (Heel et al 1980) and its analgesic activity can also be demonstrated in some tests of nociception in animals (Conway & Mitchell 1977; Piercey & Schroeder 1981). The antinociceptive effect involves neither activation of opiate receptors nor inhibition of prostaglandin synthesis (Conway & Mitchell 1977). Pretreatment with reserpine, however, blocks the drug's effect, indicating an involvement of biogenic amines (Vonvoigtlander et al 1983). It has been reported that nefopam produces its analgesic activity by activation of supraspinal structures (Piercey & Schroeder 1981).

We have compared the analgesic activity of (±)-, (+)- and (−)-nefopam administered systemically and centrally. The hot-plate, formalin and tail-flick tests were employed to measure different aspects of antinociception.

MATERIALS AND METHODS

Animals and drugs

Male albino NMRI mice (30–40 g) were housed in colony cages with free access to food and water. Testing took place in the middle of the light period of a 12/12 h light/dark cycle. All animals were tested once only. Racemic nefopam and the (+)- and (−)-enantiomers were dissolved in 0·9% NaCl.

Injection procedures and surgery

Nefopam was administered intraperitoneally (i.p.), intrathecally (i.th.) or intracerebroventricularly (i.c.v.). Intraperitoneal injection volume was 10 mL kg^{-1}. The i.th. injection technique was according to Hylden & Wilcox (1980). Drugs were administered in a volume of 5 μL. Intracerebroventricular injections were made using a modification of the procedure described by Haley & McCormick (1957). The injection site was 2 mm from the midline in a line drawn through the anterior base of the ears. A gauge 26 cannula was inserted to a depth of 3·5 mm and a volume of 5 μL injected.

Spinal transections were performed under combined pentobarbitone (23 mg kg^{-1}) and chloral hydrate (100 mg kg^{-1}) anaesthesia. One lamina was removed with a dental burr at the level of Th7–8 and the spinal cord was cut with fine scissors. The animals were tested 3 weeks after surgery.

Testing of nociception

Hot-plate testing was performed using a modification of the method of Woolfe & MacDonald (1944). An IITC Inc. Mod. 35-D Analgesiameter was set to a temperature of 55 ± 0·2 °C. The response criterion was licking of a hindpaw. Cut-off time was 45 s. The animals were tested 15 min after i.p., and 10 min after i.th. or i.c.v., injections.

The formalin test was adapted from the method described by Dubuisson & Dennis (1977). One hour before testing the animals were placed individually in standard cages (30 × 12 × 13 cm), which served as observation chambers. Twenty μL of 1% formalin was injected into the dorsal surface of the left hindpaw. The mice were observed for 5 min after the injection of formalin, and the amount of time spent

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licking the injected hindpaw was recorded. Nefopam was administered 15 min before injection of formalin.

For tail-flick testing (D'Amour & Smith 1941) an IITC Inc. Mod. 33 Analgesiameter was used. The animals were tested 15 min after i.p. nefopam.

Results are given as mean ± s.e.m.

Statistical analysis

Student's *t*-test, analysis of variance (ANOVA) and the Newman-Keul test were used to determine significant differences between groups ($P < 0.05$).

RESULTS

Analgesic profile of (±)-nefopam

Intraperitoneal administration of 20 mg kg^{-1} of (±)-nefopam produced clear analgesic effects in both the hot-plate and the formalin tests (Fig. 1). In the hot-plate test, mean latency was elevated 300% above controls ($P < 0.01$, Student's *t*-test). In the formalin test the amount of licking was reduced by 70% ($P < 0.01$, Student's *t*-test). The same dose of nefopam significantly increased the tail-flick latencies in intact animals (3·6 ± 0·3 vs 2·7 ± 0·2, mean ± s.e.m., $P < 0.05$, Student's *t*-test). However, in mice with spinal cord transection, nefopam had no effect in the tail-flick test (3·1 ± 0·1 vs 3·2 ± 0·1).

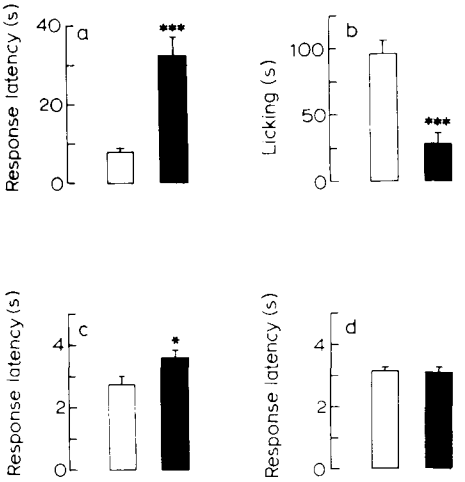

FIG. 1. The effects of 20 mg kg^{-1} of (±)-nefopam in the (a) hot-plate (n = 8), (b) formalin (n = 8) and (c, d) tail-flick (n = 9–11) tests. The tail-flick test was performed using both (c) intact mice and (d) mice with spinal cord transections. Results are presented as mean ± s.e.m. $^*P < 0.05$, $^{***}P < 0.001$, Student's *t*-test. Key: □ vehicle, ■ nefopam.

Effects of (±)-, (+)- and (−)-nefopam in the hot-plate test

Racemic nefopam and the (+)- and (−)-enantiomers were administered in the dose range of 0·63–20 mg kg^{-1} (Fig. 2). It was evident that (+)-nefopam had the stronger analgesic effects. At low doses (2·5–5·0 mg kg^{-1}) (+)-nefopam also seemed to be more effective than the racemate. Application of ANOVA (3 × 6 design) demonstrated significant differences between drugs ($F(2,108) = 15.3$; $P < 0.001$) and between doses ($F(5,108) = 27.6$; $P < 0.001$). There was also a significant interaction between drugs and doses ($F(10,108) = 2.29$; $P < 0.02$). Furthermore, (+)-nefopam was significantly different from both (−)-nefopam ($F(1,72) = 27.9$; $P < 0.001$) and (±)-nefopam ($F(1,72) = 7.97$; $P < 0.01$) (2 × 6 ANOVA). In the (−)-nefopam group there was some elevation of latencies at the highest doses, and the difference between doses was significant ($F(5,36) = 4.08$; $P < 0.01$, one-way ANOVA).

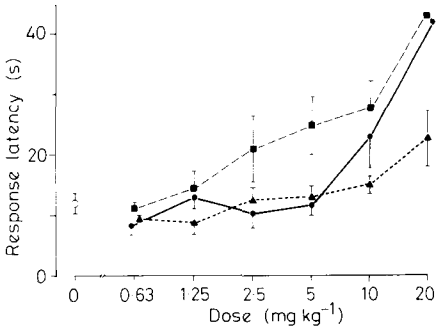

FIG. 2. The analgesic effects of (±)-, (+)- and (−)-nefopam in the hot-plate test (n = 7). Results are presented as mean ± s.e.m. Key: ○ vehicle, ■ (+)-nefopam, ▲ (−)-nefopam, ● (±)-nefopam.

Effects of (±)-, (+)- and (−)-nefopam (1·25–20 mg kg^{-1}) in the formalin test

The dose-response curves from the formalin test are shown in Fig. 3. At the highest dose clear analgesic effects were found with all the compounds while only (+)- and (±)-nefopam were effective at lower doses. (+)-Nefopam tended to be more potent than the racemate as shown by the leftward displacement of the dose-response curve. Statistical analysis, using data from all doses (3 × 5 ANOVA), demonstrated significant differences between the drugs ($F(2,80) = 6.26$; $P < 0.01$) and between doses ($F(4,80) = 22.0$, $P < 0.001$), and also a significant interaction between drugs and doses ($F(8,80) = 2.54$, $P < 0.02$). (−)-Nefopam was significantly different from (+)-

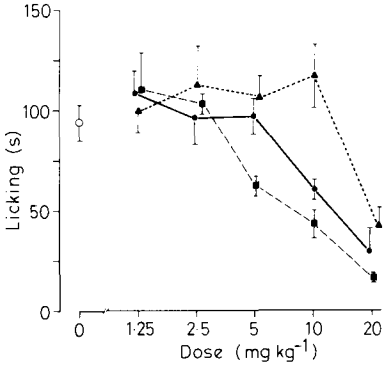

FIG. 3. The analgesic effects of (±)-, (+)- and (−)-nefopam in the formalin test (n = 6–7). Results are presented as mean ± s.e.m. Key: as Fig. 2.

nefopam ($F(1,53) = 11{\cdot}7$, $P < 0{\cdot}01$, 2 × 5 ANOVA), and from (±)-nefopam ($F(1,53) = 4{\cdot}39$, $P < 0{\cdot}05$). The difference between (+)- and (±)-nefopam was not statistically significant ($F(1,54) = 2{\cdot}05$, $P < 0{\cdot}1$).

Effects of (±)-, (+)- and (−)-nefopam (2·5–20 mg kg⁻¹ i.p.) in the tail-flick test

The results of tail-flick testing in intact mice are shown in Fig. 4. (+)-Nefopam tended to have a stronger effect than (−)-nefopam. However, statistical analysis showed that the differences were not significant ($F(2,103) = 3{\cdot}05$, $P < 0{\cdot}05$, 3 × 5 ANOVA). Analysis of dose-response data for each enantiomer separately (one-way ANOVA) showed that only for (+)-nefopam was there a significant difference between doses ($F(4,43) = 4{\cdot}47$, $P < 0{\cdot}01$).

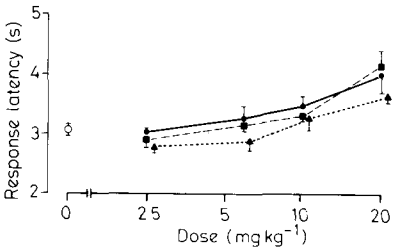

FIG. 4. The effects of (±)-, (+)- and (−)-nefopam in the tail-flick test (n = 9–10). Results are presented as mean ± s.e.m. Key: as Fig. 2.

Central administration of (±)-nefopam

Latencies to hindpaw lick were recorded in the hot-plate test after i.th. or i.c.v. administration of 5 and 20 μg of the racemate. The results are in Fig. 5. Clear analgesic effects were found after both i.th. and i.c.v. injections ($F(2,23) = 3{\cdot}71$, $P < 0{\cdot}05$ for the i.th. experiment and $F(2,24) = 13{\cdot}5$, $P < 0{\cdot}01$ for the i.c.v. experiment, one-way ANOVA). The mean response latency after i.th. administration of 20 μg (0·7 mg kg⁻¹) was 160% longer than the control latency. This was a stronger effect than after i.p. injection of 10 mg kg⁻¹ (90% prolongation). Injection of 40 μg i.th. did not give a stronger effect than 20 μg (data not shown). Intracerebroventricular administration of 5 μg (0·2 mg kg⁻¹) and 20 μg (0·7 mg kg⁻¹) prolonged the latencies by 140 and 240% respectively, compared with controls. Testing for nociception was not conducted with doses higher than 20 μg i.c.v., as injection of 40 μg produced convulsions in 5 out of 9 animals.

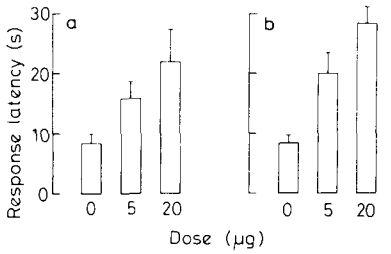

FIG. 5. The analgesic effects in the hot-plate test after (a) intrathecal and (b) intracerebroventricular administration of (±)-nefopam (n = 8–9). Results are presented as mean ± s.e.m.

Central administration of (±)-, (+)- and (−)-nefopam

Twenty μg of each drug was administered i.th. and response latencies were recorded in the hot-plate test. The results are shown in Fig. 6. The difference between the groups was significant ($F(3,30) = 5{\cdot}52$, $P < 0{\cdot}01$, one-way ANOVA). (+)-Nefopam had a stronger effect than either (±)- ($P < 0{\cdot}05$) or (−)-nefopam ($P < 0{\cdot}05$, Newman-Keuls).

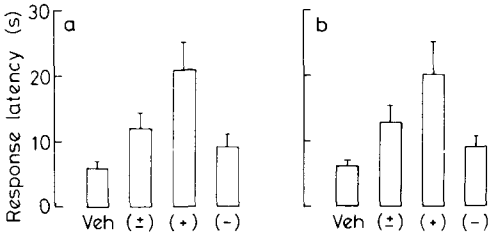

FIG. 6. The analgesic effects in the hot-plate test after (a) intrathecal (i.th.) and (b) intracerebroventricular (i.c.v.) administration of (±)-, (+)- and (−)-nefopam (n = 8–9). A dose of 20 μg of each drug was injected i.th. and 5 μg i.c.v. Results are presented as mean ± s.e.m.

The results obtained after i.c.v. administration of 5 μg of each drug were similar to those from the i.th. experiment. The difference between the groups was significant ($F(3,31) = 4{\cdot}01$, $P < 0{\cdot}02$, one-way ANOVA). (+)-Nefopam had a significantly stronger effect than (−)-nefopam ($P < 0{\cdot}05$, Newman-Keuls).

DISCUSSION

The present study confirms previous findings showing analgesic activity of nefopam in the mouse hot-plate test (Conway & Mitchell 1977; Piercey & Schroeder 1981). Strong analgesic effects were also found in the formalin test, but nefopam had only a weak effect in the tail-flick test. Furthermore, in agreement with the findings of Piercey & Schroeder (1981), it was observed that nefopam has analgesic activity after i.c.v. injection of doses insufficient to give analgesia systemically. It was also shown that nefopam has analgesic activity after i.th. administration, which is in contrast to the results of Piercey & Schroeder. The present findings therefore indicate that nefopam has both a spinal and a supraspinal site of action. After systemic and central injections, the (+)-form of nefopam was found to be the more potent enantiomer.

Piercey & Schroeder (1981) found that nefopam was able to give only a moderate analgesic effect in the hot-plate test after i.p. administration. In the present study, however, we found it to produce a pronounced analgesia in this test. In one experiment, 5 out of 7 animals reached the predetermined cut-off value of 45 s after 20 mg kg^{-1} of (±)-nefopam (mean control latency was 12·0 s). After 20 mg kg^{-1} of the (+)-enantiomer 6 out of 7 animals reached cut-off values. One reason for this discrepancy may be that in the study by Piercey & Schroeder, licking of any paw was regarded as the response criterion, while in the present study only hindpaw lick was used. Berge et al (1983) have previously demonstrated that forepaw lick may be an unreliable index of analgesic activity in the rat hot-plate test.

The acceptance of forepaw lick as a criterion may also explain why Piercey & Schroeder (1981) failed to find any effect of nefopam in the hot-plate test after i.th. administration. The amount of drug reaching the lower cervical spinal cord after injection at the lumbar level is probably both small and variable. Piercey & Schroeder also reported that doses in excess of 30 μg i.th. caused hindlimb paralysis. No motor problems were observed in the present study after doses of up to 40 μg.

In contrast to other studies (Conway & Mitchell 1977; Piercey & Schroeder 1981) a significant effect in the tail-flick test in intact animals was found. No effect, however, was found in spinalized animals, which indicates that the analgesic activity of nefopam in the spinal cord is produced by activation of descending pain-modulating systems. This conclusion is supported by Irikura et al (1981) who studied spinal potentials evoked by splanchnic nerve stimulation in intact and spinalized cats. Nefopam inhibited these evoked potentials in intact animals, but had no inhibitory effect in spinal animals.

The convulsions which were observed after administration of 40 μg (±)-nefopam i.c.v. were not described by Piercey & Schroeder after doses of up to 100 μg. In the present study a volume of 5 μL was injected, while Piercey & Schroeder used only 2 μL. A greater injection volume would, presumably, increase the amount of drug reaching structures distant from the injection site.

This could possibly indicate that explosive motor behaviour is caused by an action of nefopam on structures at some distance from the injection site. The convulsions may be related to motor hyperactivity seen after high systemic doses (Schror 1979).

In conclusion, the present study indicates that nefopam has both a spinal and a supraspinal site of action, and that (+)-nefopam is more potent as an analgesic than the (−)-enantiomer.

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