British Journal of Pharmacology (2000) 129, 1668–1672 

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# Antihyperalgesic effects of $\delta$ opioid agonists in a rat model of chronic inflammation

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**1** Opioid receptors in the brain activate descending pain pathways to inhibit the nociceptive response to acute noxious stimuli. The aim of the present study was to clarify the role of supraspinal opioid receptors in modulating the nociceptive response to persistent inflammation in rats.

**2** Subcutaneous administration of 50 $\mu$l of complete Freund's Adjuvant (CFA) into the plantar surface of the hindpaw induced a significant decrease in paw withdrawal latency to thermal stimuli ($P<0.01$) at 24 h post-injection.

**3** Intracerebroventricular (i.c.v.) administration of the $\mu$ opioid receptor agonists, DAMGO and morphine, and the $\delta$ opioid receptor agonists, deltorphin II and SNC80, significantly reversed the hyperalgesic response associated with peripheral inflammation in a dose-dependent manner ($P<0.0001$).

**4** The $\mu$ and $\delta$ agonists also significantly attenuated the antinociceptive response to acute thermal stimulation in rats ($P<0.001$). However, deltorphin II and SNC80 were less potent, and in the case of SNC80 less efficacious, in modulating the response to acute thermal nociception in comparison to hyperalgesia associated with persistent inflammation.

**5** These results indicate that $\mu$ and $\delta$ opioid receptors in the brain modulate descending pain pathways to attenuate the nociceptive response to acute thermal stimuli in both normal and inflamed tissues. The heightened response to $\delta$ agonists in the hyperalgesia model suggests that $\delta$ opioid receptors in the brain are promising targets for the treatment of pain arising from chronic inflammation.

*British Journal of Pharmacology* (2000) **129,** 1668–1672

**Keywords:** $\mu$ opioid receptor; $\delta$ opioid receptor; chronic inflammation; hyperalgesia; antinociception; Freund's Adjuvant

**Abbreviations:** AP, anterior-posterior; CFA, complete Freund's Adjuvant; DAMGO, [D-Ala$^2$, N-Me-Phe$^4$, Gly-ol$^5$]-enkephalin; DOR-1, cloned $\delta$ opioid receptor; DV, dorsal-ventral; ML, medial-lateral

## Introduction

It has been proposed that opioid-induced disinhibition of neurons in the periaqueductal gray (PAG) activates spinally projecting neurons in the rostroventral medulla (RVM) to attenuate nociceptive signals originating from sites in the dorsal horn (Basbaum & Fields, 1984). This model is supported by autoradiographic and immunocytochemical studies demonstrating the expression of $\mu$ and $\delta$ opioid receptors in the PAG and RVM (Mansour *et al*., 1987; Kalyuzhny *et al*., 1996). *In vivo*, the modulation of nociceptive transmission in the cord by descending inputs from the brainstem, and the effect of opiates in this paradigm, have been demonstrated using acute measures of nociception such as the tail flick assay (Rossi *et al*., 1994). However, chronic pain following tissue damage leads to persistent functional changes in the nervous system (Dubner & Ruda, 1992). Accordingly, intra-plantar injection of inflammatory agents such as complete Freund's adjuvant (CFA) causes increased firing of peripheral afferents in the spinal cord leading to hyperexcitability of dorsal horn nociceptive neurons and consequent hyperalgesia in response to mechanical or thermal stimuli (Hargreaves *et al*., 1988). In turn, this elevated nociceptive input in the spinal cord appears to trigger increased neuronal activity in descending pain pathways originating in the brain (Schaible *et al*., 1991; Ren & Dubner, 1996). It is unclear to what extent the increased activity in descending pathways associated with peripheral hyperalgesia is susceptible to modulation by exogenous opioids.

The antinociceptive effects of $\mu$ opioid agonists such as morphine and DAMGO ([D-Ala$^2$, N-Me-Phe$^4$,Gly-ol$^5$]-enkephalin) in the brain have been well established in various acute pain assays. These compounds have also proven to be effective in models of chronic inflammatory pain, although it is unclear to what extent these anti-hyperalgesic effects were mediated at supraspinal sites (Joris *et al*., 1990; Ho *et al*., 1997; Zhou *et al*., 1998). In contrast, studies of the supraspinal antinociceptive effects of $\delta$ agonists have produced conflicting results in rats, perhaps reflecting differences in the type of acute pain tests used (Negri *et al*., 1991; Ossipov *et al*., 1995). To the best of our knowledge, the anti-hyperalgesic efficacy of $\delta$ agonists administered directly into the brain of conscious animals has not been previously demonstrated.

In the present study, thermal hyperalgesia associated with CFA-induced persistent inflammation of the rat hind paw was evaluated using the plantar test (Hargreaves *et al*., 1988). This experimental paradigm appears to be highly predictive of thermal hyperalgesia in humans (Montagne-Clavel & Oliveras, 1996). The effects of $\mu$ and $\delta$ opioid agonists in the thermal hyperalgesia assay were compared to their effects in the tail flick assay of nociception.

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## Methods

### *Preparation of animals*

Animals were handled in strict adherence to the guidelines established by the Canadian Council for Animal Care. Male Sprague-Dawley rats (250–300 g) were anaesthetized with 80 mg $kg^{-1}$ body weight ketamine-xylazine solution (i.p.; RBI, Natick, MA, U.S.A.) and placed in a stereotaxic device. Each animal was then implanted with a 23 gauge cannula extending into the right lateral ventricle (i.c.v.; coordinates from bregma, AP: 0.8 mm, ML: 1.5 mm, DV: 3.5 mm). The guide cannula was fixed in place with dental cement. Rats were allowed three or more days to recover from the surgery prior to random allocation into treatment groups. Pre-habituation to the i.c.v. injection procedure was effected by administering 10 μl of 0.9% saline solution *via* the indwelling cannula 24 h prior to experimentation.

### *Inflammation*

Rats were briefly anaesthetized by inhalation of isofluorane (5% saturation in $O_2$, flow rate of 800–900 ml $min^{-1}$). Inflammation was produced by the subcutaneous injection of 20, 50 or 100 μl of complete Freund's Adjuvant (CFA; Sigma, St. Louis, MO, U.S.A.) into the plantar surface of the right hind paw. Only rats designated for testing in the thermal hyperalgesia assay were treated with CFA.

### *Plantar test*

Thermal hyperalgesia was assessed in unrestrained rats using a procedure adapted from published reports (Hargreaves *et al*., 1988). Rats ($n=6-8$ per group) were placed in opaque, plastic chambers ($13\times24\times13$ cm) positioned on a glass surface. Animals were allowed to habituate in this environment for 20 min prior to testing. Paw withdrawal latency in response to radiant heat was measured using the plantar test apparatus (Ugo Basile, Comerio, Italy). The heat source was positioned beneath the plantar surface of the affected hind paw and activated. The digital timer connected to the heat source automatically recorded the response latency for paw withdrawal to the nearest tenth of a second. A cut-off time of 22 s was used to prevent tissue damage. The paw withdrawal latency of each rat was measured three times at each test interval and the median score recorded. The effects of opioid agonists on paw withdrawal latency were measured 24 h after the injection of CFA. Control (saline-injected) and dose treatment groups were tested in parallel for each drug. Paw withdrawal latencies were converted to % anti-hyperalgesia using the following equation:

$$\%\ \text{anti-hyperalgesia} = [(\text{drug} - \text{CFA}) \div (\text{baseline} - \text{CFA}] \times 100 \quad (1)$$

where 'drug' represents the response latency for each treatment group in response to opioid agonist. 'CFA' represents the average paw withdrawal score for all groups of rats prior to opiate agonist treatment and 'baseline' represents the average of all baseline scores prior to CFA treatment.

### *Tail flick assay*

The antinociceptive effects of opioid agonists were measured using the tail flick apparatus (IITC Inc., Woodland Hills, CA, U.S.A.). Rats were positioned on a flat surface and held gently by the experimenter. Tail withdrawal latencies were recorded in response to heat from a light beam focused on the dorsal surface of the tail (approximately 2 cm from the tip). A digital timer automatically recorded response latencies to the nearest tenth of a second. The light beam intensity was adjusted to produce a baseline latency of 3–5 s. The recommended cut-off time of 12 s was used to prevent tissue damage. On the day of testing, two baseline responses were recorded 5 and 15 min prior to injection of drug to habituate the rats to the testing procedure. The antinociceptive effects of opioid agonists were measured 15, 30, 45 and 60 min after drug treatment. Control (saline-injected) and dose treatment groups were tested in parallel for each drug. Tail flick response latencies were converted to per cent of maximum possible effect (% MPE) according to the formula:

$$\%\text{MPE} = [(\text{post-drug latency} - \text{control}) \div (\text{cut-off latency} - \text{control})] \times 100 \quad (2)$$

### *Drug administration*

The opioid agonists (DAMGO and deltorphin II supplied by RBI, Natick, MA, U.S.A.; SNC-80 supplied by Tocris Cookson Inc., Ballwin, MO, U.S.A.; morphine sulphate supplied by BDH, Toronto, ON, Canada) were dissolved in 0.9% saline solution and administered to rats *via* the guide cannula (i.c.v.) immediately prior to behavioural testing. All opioid drug solutions were injected in a volume of 10 μl using a 50 μl Hamilton syringe attached to a catheter (15 cm) constructed from PE20 polyethylene tubing and terminating in a 30-gauge needle. Solution was injected slowly over a period of 60 s and the needle was left within the guide cannula for an additional 30 s after the injection. In all cases, additional rats were treated concomitantly with 0.9% saline solution as a control for the drug treatment paradigm.

### *Statistical analyses*

Data are presented as mean ± standard error of the mean (s.e.m.). Differences between treatment groups were analysed by one-way or two-way analysis of variance (ANOVA) with DOSE and TIME as between-subject and within-subject factors, respectively. *Post-hoc* analyses were performed with Dunnett's multiple comparison test where appropriate. $ED_{50}$ values were determined by linear regression analysis of the dose response curves. All analyses were performed using GraphPad Prism software (San Diego, CA, U.S.A.).

## Results

### *Thermal hyperalgesic response to CFA*

Intra-plantar injection of 20, 50 or 100 μl volumes of CFA caused localized erythema and oedema in the affected hind paw. The degree of erythema and oedema appeared to increase in relation to the injection volume (data not shown). There were no obvious changes in weight gain, grooming or social interactions following CFA treatment over the duration of the 48 h test period. The largest and most clear dose-dependent decrease in paw withdrawal latency occurred at 24 h (Figure 1). Rats treated with the highest dose of CFA (100 μl) also exhibited spontaneous paw licking and decreased weight bearing for the affected paw at this test interval. Therefore, subsequent experiments measuring the effects of opioid

agonists were performed on rats pre-treated with 50 μl CFA (i.pl.) 24 h prior to drug testing.

### *Anti-hyperalgesic effects of opioid agonists*

The effects of the δ agonists deltorphin II and SNC80 in the thermal hyperalgesia model are presented in Figure 2. Dose-response curves derived from these data are presented together with the corresponding data for the μ agonists DAMGO and morphine (Figure 3A). The peak antihyperalgesic effects for DAMGO, morphine and SNC80 occurred at the 20-min test interval for all doses, whereas the peak effects for deltorphin II occurred at the 40-min test interval. Each opioid agonist reversed thermal hyperalgesia by >90%. There was a significant effect of drug treatment (i.c.v.) for each compound (DAMGO – $F_{(3,60)}=9.085$, $P<0.0001$; morphine – $F_{(3,26)}=20.3$, $P<0.0001$; deltorphin II – $F_{(4,90)}=48.4$, $P<0.0001$; SNC80 – $F_{(3,75)}=25.77$, $P<0.0001$). There was no significant difference between groups for both the baseline scores and the pre-drug CFA scores in all four experiments. There were no clear decreases in locomotor activity or other signs of sedation for any of the compounds tested.

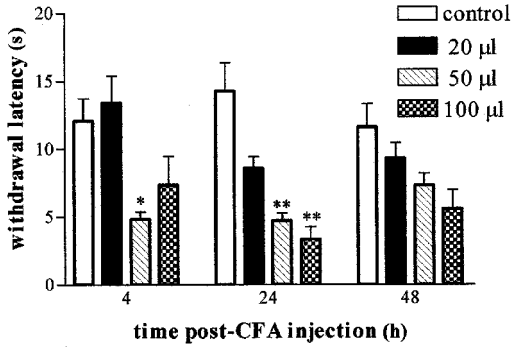


**Figure 1** Dose-related effects of CFA (i.pl.) on paw withdrawal latency following exposure to radiant heat. Only response latencies for the injected paw were measured. * and ** represent significant differences between the control (saline-injected) group and the CFA-treated groups ($P<0.05$ and $P<0.01$ respectively; Dunnett's test). Each bar represents the mean ± s.e.mean. response of 6–9 rats.

### *Effects of opioid agonists in the tail flick assay*

The peak antinociceptive effects for DAMGO, morphine and SNC80 occurred at the 15-min test interval for all doses, whereas the peak effects for deltorphin II occurred at the 30-min test interval. These data are presented in dose-response format (Figure 3B). Treatment (i.c.v.) with DAMGO, morphine, and deltorphin II significantly increased response latencies in the tail flick assay to >90% of MPE at the highest doses. In comparison, SNC80 significantly increased response latencies to a sub-maximal level in the tail flick assay over the dose range tested ($E_{max}=60\%$ of MPE). There was a significant effect of drug

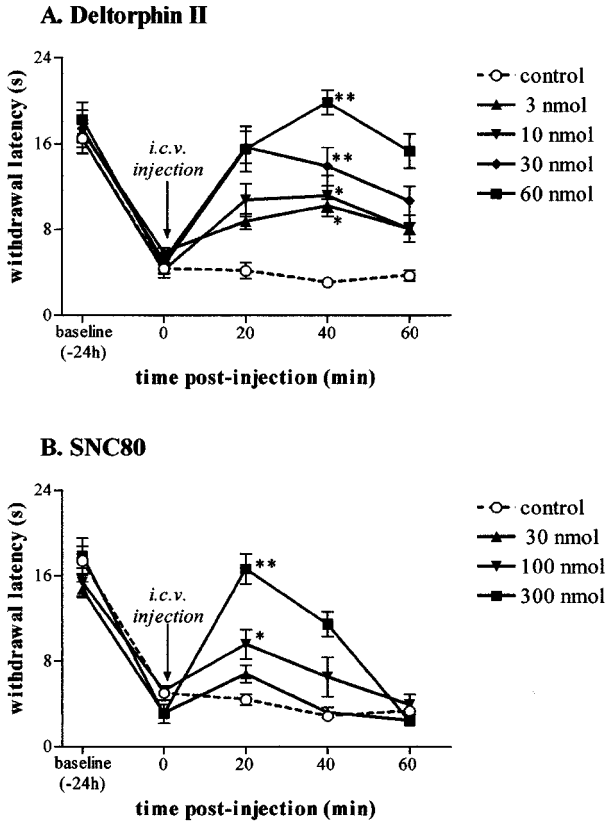


**Figure 2** Anti-hyperalgesic effects of (A) deltorphin II and (B) SNC-80. Baseline paw withdrawal latencies were measured for all rats prior to administration of CFA (50 μl i.pl.). The effects of δ agonists were measured 24 h after CFA treatment. * and ** represent significant differences between the control group and the drug treatment groups ($P<0.05$ and $P<0.01$ respectively; Dunnett's test). Each curve represents the mean ± s.e.mean response of 6–8 rats.

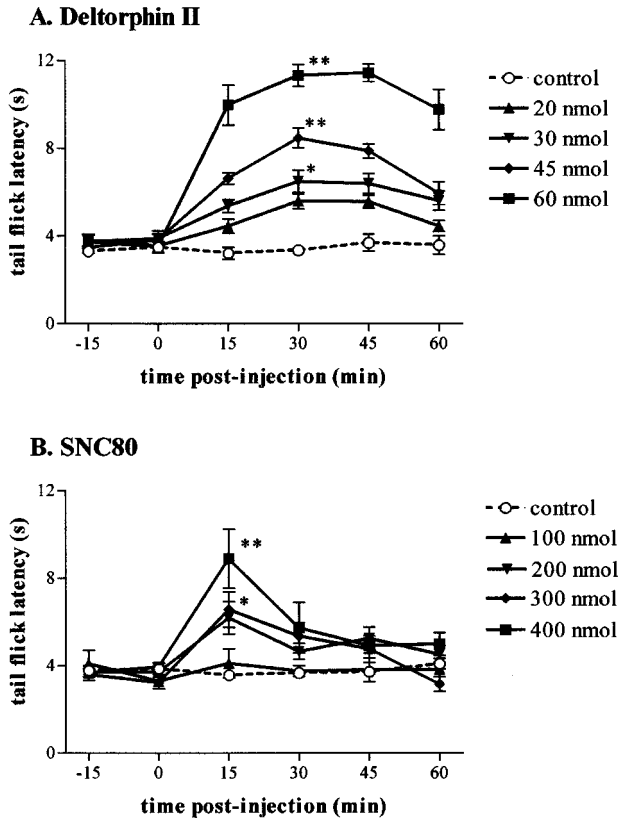


**Figure 3** Antinociceptive effects of (A) deltorphin II and (B) SNC80. Baseline tail flick latencies were measured for all rats prior to the administration of drug. * and ** represent significant differences between the control group and the drug treatment groups ($P<0.05$ and $P<0.01$ respectively; Dunnett's test). Each curve represents the mean ± s.e.mean response of 7–12 rats.

**Table 1** Comparison of the antinociceptive potency of opioid agonists in the plantar test and tail flick assays

| | *Plantar test* ($D_{50}$, nmoles) | *Tail flick* ($ED_{50}$, nmoles) | *Potency ratio* |
|---|---|---|---|
| DAMGO | 0.10 | 0.11 | 1.1 |
| Morphine | 5.6 | 9.8 | 1.7 |
| Deltorphin II | 11 | 37 | 3.4 |
| SNC80 | 120 | 340 | 2.9 |

$D_{50}$ and $ED_{50}$ values were determined by linear regression analysis of the dose-response curves presented in Figure 4.

treatment for each compound (DAMGO $F_{(3,120)}=21.2$, $P<0.0001$; morphine $F_{(3,84)}=30.6$, $P<0.0001$; deltorphin II $F_{(4,144)}=34.6$, $P<0.0001$; SNC80 $F_{(4,136)}=11.1$; $P<0.0001$). $D_{50}$ and $ED_{50}$ values for the opioid agonists in the plantar test and tail flick assay are presented and compared in Table 1.

## Discussion

The main findings of this study are that δ agonists are effective anti-hyperalgesics when administered directly into the brain. Moreover, deltorphin II and SNC80 had improved potency in rats with persistent peripheral inflammation compared to normal rats tested in the tail flick assay. Our findings complement those of previous reports suggesting that δ agonists reverse peripheral hyperalgesia following administration directly into the inflamed tissue (Zhou *et al*., 1998) or intrathecal space (i.t.; Hylden *et al*., 1991; Ho *et al*., 1997). Increased potency of δ agonists (i.t.) has been demonstrated in rats with unilateral hindpaw inflammation (Hylden *et al*., 1991), but these findings were not corroborated in other published reports (Ho *et al*., 1997). The lesser potency for δ agonists administered i.c.v. and tested in acute pain assays is consistent with the data presented in previous reports (Negri *et al*., 1991; Ossipov *et al*., 1995). Although δ opioid receptor subtypes have been postulated (e.g. Mattia *et al*., 1991; Vanderah *et al*., 1994), antisense studies suggest that supraspinal antinociception in response to deltorphin II and SNC80 is predominantly mediated by the cloned δ opioid receptor (DOR-1; Fraser *et al*., 2000). The potency difference for δ agonists (i.c.v.) in chronic versus acute pain models suggests a more prominent role for DOR-1 in supraspinal pain processing centres (Kalyuzhny *et al*., 1996) as a consequence of the enhanced neuronal activity in descending pain pathways following peripheral inflammation (Ren & Dubner, 1996).

The antinociceptive effects of the μ agonists DAMGO and morphine in the tail flick assay are consistent with those described in previous reports (Rossi *et al*., 1994). We are not aware of any previous reports demonstrating the supraspinal effects of μ agonists in models of chronic inflammation. However, μ agonists have been shown to have anti-hyperalgesic effects following peripheral (Joris *et al*., 1990) or intrathecal administration (Hylden *et al*., 1991). In general, the anti-hyperalgesic potency of μ agonists in rats with unilateral inflammation of the hindpaw is much greater than the antinociceptive activity observed in normal animals. The enhanced potency of systemically active μ opioids in animals with peripheral inflammation has been ascribed to the activity of these drugs at sites proximal to the inflamed tissue or changes in spinal systems that would specifically affect the function of μ opioid receptors (Stanfa & Dickenson, 1995). However, the findings of the present study indicate that these reported changes at peripheral or spinal sites are not accompanied by μ-specific changes in supraspinal pain processing since μ agonists inhibited the nociceptive thresholds in chronic and acute pain models with similar potency.

In summary, supraspinal δ opioid receptors have an enhanced role in inhibiting nociceptive signals following chronic inflammation and thus represent promising targets for the treatment of clinical hyperalgesia. In contrast, supraspinal μ opioid receptors have a similar role in inhibiting nociceptive signals associated with both acute and chronic pain states.

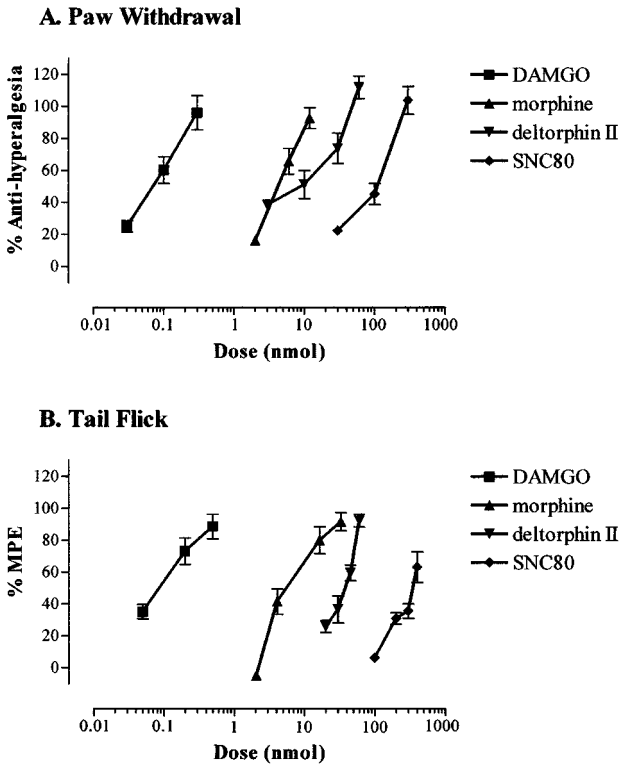


**Figure 4** Opioid agonists (i.c.v.) have similar response profiles in anti-hyperalgesic and antinociceptive models. (A) Thermal hyperalgesia was measured using the Hargreave's assay. Per cent anti-hyperalgesia was determined relative to the baseline paw withdrawal response to radiant heat prior to CFA treatment. Each data point represents the peak anti-hyperalgesic response to drug, which occurred at 20 min post-injection for DAMGO, morphine and SNC80 and 40 min post-injection for deltorphin II. (B) Antinociception was measured in the tail flick assay. Per cent MPE was determined relative to the pre-determined cut-off for the test apparatus. The dose-response curves represent the peak antinociceptive response to drug at 15 min post-injection for DAMGO, morphine and SNC80, and 30 min post-injection for deltorphin II. Each data point represents the mean ± s.e.mean response of 6–12 rats.

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*(Received September 23, 1999*
*Revised January 1, 2000*
*Accepted January 18, 2000)*