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The involvement of the opioidergic system in the antinociceptive mechanism of action of antidepressant compounds

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1 Debate exists as to the nature of antidepressant-induced antinociception. It is unclear whether antidepressants are inherently antinociceptive, are able to potentiate opioid antinociception or both. We have used the acetic acid induced abdominal constriction assay in mice to investigate antidepressant-induced antinociception.

2 All the antidepressants tested (s.c.) produced dose-dependent protection against acetic acid-induced abdominal constriction. Similarly, morphine and aspirin were also effective antinociceptive agents in this nociceptive assay.

3 Opioid antagonists, naloxone (0.5 mg kg^{-1}, s.c.) and naltrindole (1 mg kg^{-1}, s.c.), shifted the dose-response relationships to the right for each of the antidepressant agents (dothiepin, amitriptyline, sibutramine, (+)-oxaprotiline and paroxetine). In this context the naloxone dose-ratios were 1.95, 3.90, 2.32, 4.50 and 2.65, with naltrindole dose-ratios of 4.36, 17.00, 4.28, 11.48 and 2.65 were obtained, respectively. Naloxone also shifted the morphine dose-response relationship to the right, by a factor of 2.62, whilst naltrindole had no effect upon morphine antinociception. Aspirin antinociception remained unaffected by both opioid antagonists.

4 The enkephalin catabolism inhibitor acetorphan, by itself, produced no activity in this test at a dose of 10 mg kg^{-1} (s.c.). However, at higher doses, acetorphan produced a linear dose-response relationship against acetic acid-induced abdominal constriction.

5 When acetorphan was administered before either the antidepressants or morphine, there was a clear potentiation of the antidepressant- or morphine-induced antinociception. However, acetorphan had no effect on aspirin antinociception.

6 Since neither of the opioid antagonists were able to attenuate, nor was acetorphan able to potentiate, aspirin antinociception, we concluded that the mechanism of antidepressant-induced antinociception is different from that of the non-steroidal anti-inflammatory drugs.

7 These data are consistent with the view that antidepressants may induce endogenous opioid peptide release, as shown by the acetorphan study. In this context, the ability of naltrindole to displace the antidepressant dose-response relationship to the right without affecting morphine antinociception, implicates the δ-opioid receptor and endogenous opioid peptides in antidepressant-induced antinociception.

Keywords: Antidepressant; visceral antinociception; endogenous opioids

Introduction

Depression has been documented as a frequent accompaniment to pain. Indeed, reports of the incidence of depression among pain patients have ranged from 10 to 100% (Pilowsky *et al*., 1977; Turkington, 1980). Furthermore, a wealth of clinical literature advocates the use of antidepressants in the management of certain pain states with (e.g. Feinmann & Harris, 1984; Max *et al*., 1987; Ward *et al*., 1979); and without (Gomersall & Stuart, 1978) co-existing depression.

At the laboratory level, debate exists as to the nature of antidepressant-induced antinociception. It is unclear whether antidepressants are inherently antinociceptive, are able to potentiate opioid antinociception or both. Tura and Tura (1990) demonstrated that amitriptyline possesses intrinsic antinociceptive actions. Botney and Fields (1983) showed a potentiation of morphine antinociception by amitriptyline but produced no effect alone. Ogren and Hölm (1980) were unable to identify any intrinsic antinociceptive activity of antidepressants. Biegon and Samuel (1979, 1980) demonstrated the ability of tricyclic antidepressants to bind to opioid receptors suggesting that antidepressants may have weak opioid-like actions.

The present experimental study addresses the question of antidepressant induced-antinociception, by determining in a dose-dependent manner, antidepressant responses to a chemical stimulus (an algogenic agent; 1% acetic acid). Thus, the antinociceptive profile of five antidepressants: dothiepin, amitriptyline, (+)-oxaprotiline, paroxetine (selective 5-hydroxytryptamine (5-HT) re-uptake inhibitor; SSRI) and sibutramine (5-HT-noradrenaline reuptake inhibitor, SNRI) were determined. The possible involvement of the opioidergic system either directly or indirectly in the antidepressant-induced antinociceptive action in the abdominal constriction assay was investigated from several perspectives. Firstly, the opioid antagonists, naloxone and naltrindole, were used to antagonize the resultant antinociception and secondly, pretreatment with a sub-effective dose of an inhibitor of neutral endopeptidase (EC 3.4.24.11), acetorphan, to determine any enhanced opioid tone from antidepressant-induced antinociception.

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The abdominal constriction assay was chosen as an appropriate paradigm since it detects a range of opioid and opioid-like agonists with great sensitivity. This paradigm yields the clearest distinction between doses which produce antinociception and those which cause motor impairment (Hayes *et al*., 1987).

Methods

Animals

Male GB1 mice (ICI derived strain, bred in our own animal facility) weighed 20 ± 2 g at the beginning of the experiments. Each cage housed 8 mice with the animal unit and laboratory temperature maintained at $22 \pm 1^{\circ}$C, with food and water available *ad libitum*. A 12 hour light/dark cycle was employed with lights on at 08 h 00 min and experiments conducted during the light part of the cycle. Two hours before the commencement of the experiments, the mice were habituated to the test laboratory, with food and water being withdrawn.

Nociceptive test

Groups of eight mice were randomly assigned to one of the 28 treatment groups with appropriate controls. Each of the treatment groups received one of the antidepressant compounds or morphine or aspirin 30 min before 1% acetic acid challenge (10 ml kg^{-1}, i.p.). Twenty one of these treatment groups received either a saline injection or an opioid antagonist after the initial drug treatment. Fourteeen of these treatment groups received naloxone (0.5 mg kg^{-1}) or naltrindole (1 mg kg^{-1}) 5 min or 15 min respectively, before the 1% acetic acid challenge. The seven remaining treatment groups were pretreated with the neutral endopeptidase inhibitor acetorphan (10 mg kg^{-1}) 45 min before administration of the acetic acid.

The opioid antagonists and acetorphan were administered subcutaneously as a contralateral injection to the putative analgesic agent. Following the i.p. injection of acetic acid, animals were placed in individual cages and the number of abdominal constrictions in the ensuing 20 min period was counted. An abdominal constriction was defined as a posture with the abdomen flattened, the back depressed and the hind limbs extended. Each abdominal constriction was taken to have occurred with the adoption of this position and to have terminated with the resumption of the 'normal' position (Millan *et al*., 1994).

Pretreatment times and dosing schedules of the opioid antagonists used were identified from pilot studies with morphine or DPDPE (data not shown). Naloxone (0.5 mg kg^{-1}) and naltrindole (1 mg kg^{-1}) were selected, respectively, from studies by Noble *et al*. (1995) and Narita *et al*. (1993). In the latter case, doses of naltrindole up to 3 mg kg^{-1} were described as specific for δ-opioid receptors. The antinociceptive test was 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).

Drugs

Dothiepin hydrochloride, sibutramine hydrochloride (Knoll Pharmaceuticals, Nottingham, U.K.), amitriptyline hydrochloride (Sigma, Poole, U.K.), (+)-oxaprotiline (Ciba-Geigy, Basle, Switzerland), paroxetine (SmithKline-Beecham, Harlow, U.K.), aspirin (Macarthy, Bristol, U.K.), naloxone hydrochloride (Endo laboratories, New York, U.S.A.), morphine hydrochloride (Vestric, Bristol, U.K.) acetorphan (Endo Laboratories, New York, U.S.A.) were all dissolved in normal apyrogenic saline (0.9 w/v NaCl). Naltrindole hydrochloride (Reckitt and Colman, Hull, U.K.) was dissolved in 0.9% NaCl with the addition of 100 μl of 0.4 M tartaric acid. All drug doses relate to the salt and were injected subcutaneously in a dose volume of 5 ml kg^{-1} body weight.

Data analysis

Dose-response regression lines were analysed by covariance using the MANOVA procedure within SPSS. Further statistical analysis, by use of two-way analysis of variance of the probit derived ED_{50} data, determined which treatment effect(s) were significant. Dose-ratios were also determined from ED_{50} data. All analyses were conducted on the untransformed data. The data are presented as % protection (i.e. a compound producing 100% protection prevents acetic acid-induced abdominal constriction): 100- ([mean number of abdominal constrictions of drug treated\mean number of abdominal constrictions of control] $\times$ 100).

Results

The effect of opioid antagonists on antidepressant-induced antinociception

The selected doses of opioid antagonists used in the present investigation did not produce by themselves any significant effect on acetic acid-induced abdominal constriction. Morphine produced a characteristically steep log dose-response relationship over the relatively narrow dose range examined in the abdominal constriction paradigm. The protection produced by morphine was antagonized by naloxone (0.5 mg kg^{-1}), producing a parallel rightward shift in the morphine dose-response regression line (dose-ratio 2.62, $P<0.01$), but naltrindole did not significantly affect morphine antinociception. Dothiepin produced a steep dose-response relationship to the acetic acid-induced abdominal constriction, but when the opioid antagonists were combined with dothiepin there was a significant shift to the right for the dose-response relationships ($P<0.01$). The dose-ratios were: naloxone 1.95 and naltrindole 4.36. Analysis of covariance revealed these rightward shifts were not parallel (Figure 1a and b). Amitriptyline produced 100% protection against acetic acid-induced writhing at the highest dose examined. The opioid antagonists produced the following rightward shifts in the amitriptyline dose-response curve: naloxone 3.90 and naltrindole 17.00 ($P<0.01$; Figure 2a and b).

The opioid antagonists, naloxone and naltrindole, were used to antagonize the resulting antinociception from the following antidepressants. Sibutramine rightward shift factors were: naloxone 2.32 and naltrindole 4.28 ($P<0.01$); (+)-oxaprotiline with non-parallel rightwards shifts of: naloxone 4.50 and naltrindole 11.48 ($P<0.01$); paroxetine parallel rightward shift factors were: naloxone 2.65 and naltrindole 2.65 ($P<0.01$). Aspirin the only non-steroidal anti-inflammatory drug tested exhibited dose-dependent inhibition of acetic acid-induced abdominal constrictions. Given in combination with the opioid antagonists there was no change in the antinociceptive efficacy of aspirin ($P>0.05$). Table 1 shows the ED_{50} values for all the compounds investigated.

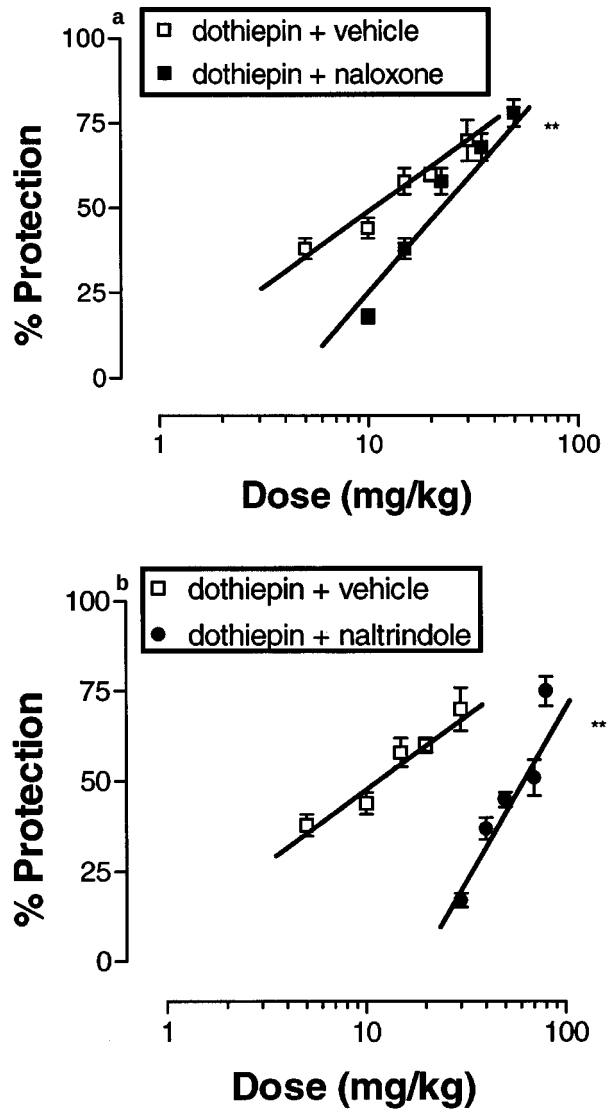

Figure 1 Effect of various doses of dothiepin in the mouse abdominal constriction assay in the presence of opioid antagonists. (a) The effect of naloxone (0.5 mg kg^{-1}, s.c.) on dothiepin antinociception. In the presence of naloxone analysis of covariance revealed a significant non-parallel shift to the right in the dothiepin dose-response relationship. The non-parallel nature indicates that the effect of naloxone is not the same at each dose level of dothiepin investigated. (b) The effect of naltrindole (1 mg kg^{-1}, s.c.) on the dothiepin dose-response relationship. Naltrindole produced a significant shift to the right in the dothiepin dose-response curve. Similar to naloxone, analysis of covariance found the shift not to be parallel indicating that the magnitude of effect of naltrindole varies according to the dose level of dothiepin. Each point represents the mean response of 8 mice; vertical lines show s.e.mean. $^{**}P<0.01$ *versus* dothiepin and vehicle treatment group.

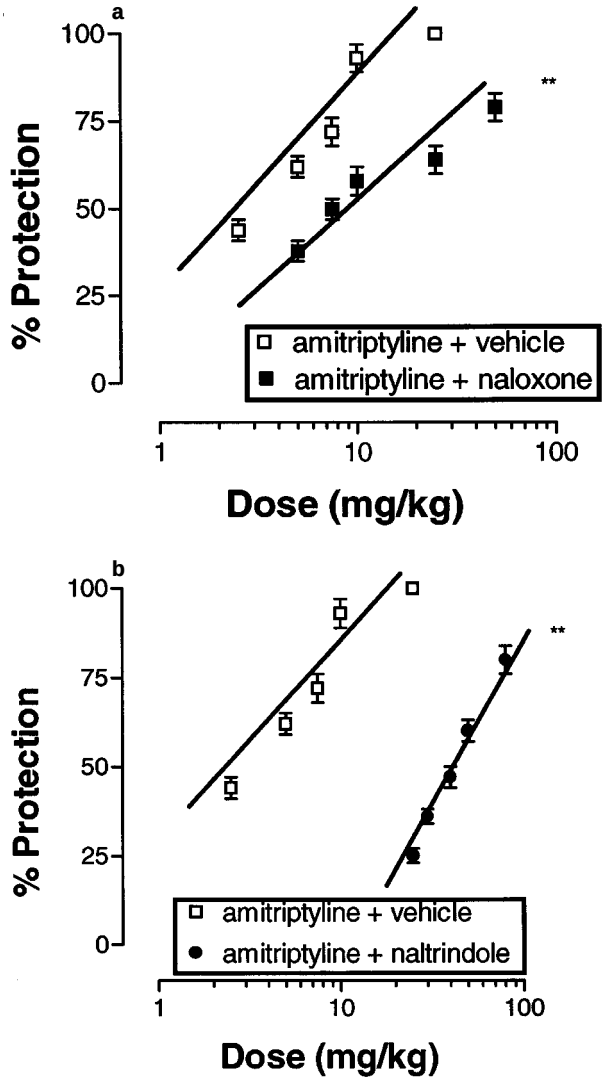

Figure 2 Effect of various doses of amitriptyline in the mouse abdominal constriction assay in the presence of opioid antagonists. (a) The effect of naloxone (0.5 mg kg^{-1}, s.c.) on the amitriptyline dose-response relationship. Naloxone significantly antagonized the amitriptyline dose-response relationship to the right. In contrast to its effect on dothiepin, the effect of naloxone on amitriptyline was similar at the doses examined. Thus analysis of covariance did not demonstrate any significant difference in the slopes of the two regression lines. (b) The effect of naltrindole (1 mg kg^{-1}, s.c.) on the dose-response relationship for amitriptyline. Naltrindole significantly shifted the amitriptyline dose-response regression line to the left in a parallel manner. Each point represents the mean response of 8 mice; vertical lines show s.e.mean. $^{**}P<0.01$ *versus* amitriptyline and vehicle regression line.

The effect of acetorphan on antidepressant-induced antinociception

Acetorphan produced a dose-dependent decrease in the mean number of abdominal constrictions when administered 45 min before intraperitoneal acetic acid (1%; Figure 3a). Consequently, a subeffective dose of 10 mg kg^{-1} was combined with various doses of the antidepressants and morphine. Acetorphan did not potentiate the protection induced by aspirin from acetic acid-induced abdominal constriction ($P>0.05$; data not shown), but enhanced morphine antinociception (Figure 3b). When acetorphan was combined with the antidepressants there was clear potentiation of their antinociceptive activity in all the dose-response curves. The antidepressants exhibited parallel leftward shifts in their dose-response relationships in the presence of acetorphan (only data for dothiepin and amitriptyline are shown; Figure 3c and d).

Discussion

The present study has used a robust nociceptive paradigm which, as previously described in the Introduction, offers good sensitivity and allows the detection of a wide range of opioid agonists. Additionally, this is the first study to use the acetic

Table 1 ED_{50} values (mg kg^{-1}; mean with 95% confidence intervals of 8 mice) for the antidepressant compounds and morphine (s.c.) tested alone and in the presence of opioid antagonists in the mouse abdominal constriction assay ($n=8$)

Drug	*Alone*	*Naloxone*	*Naltrindole*
Dothiepin	11.9 (8.5–16.0)	23.2 (18.5–29.1)	51.9 (41.3–65.5)
Amitriptyline	2.5 (1.7–3.4)	9.9 (7.5–12.8)	43.3 (33.2–56.6)
Sibutramine	4.9 (4.0–5.8)	11.3 (9.5–13.5)	20.9 (17.8–25.4)
(+)-Oxaprotiline	15.0 (10.5–20.0)	67.4 (50.4–95.4)	172.2 (116–291)
Paroxetine	3.8 (2.9–4.9)	10.2 (8.0–13.0)	10.2 (8.0–13.1)
Aspirin	25.5 (22.9–28.1)	24.8 (21.0–28.8)	28.7 (24.0–35.5)
Morphine	0.6 (0.4–0.7)	1.5 (1.1–1.2)	0.6 (0.5–0.8)

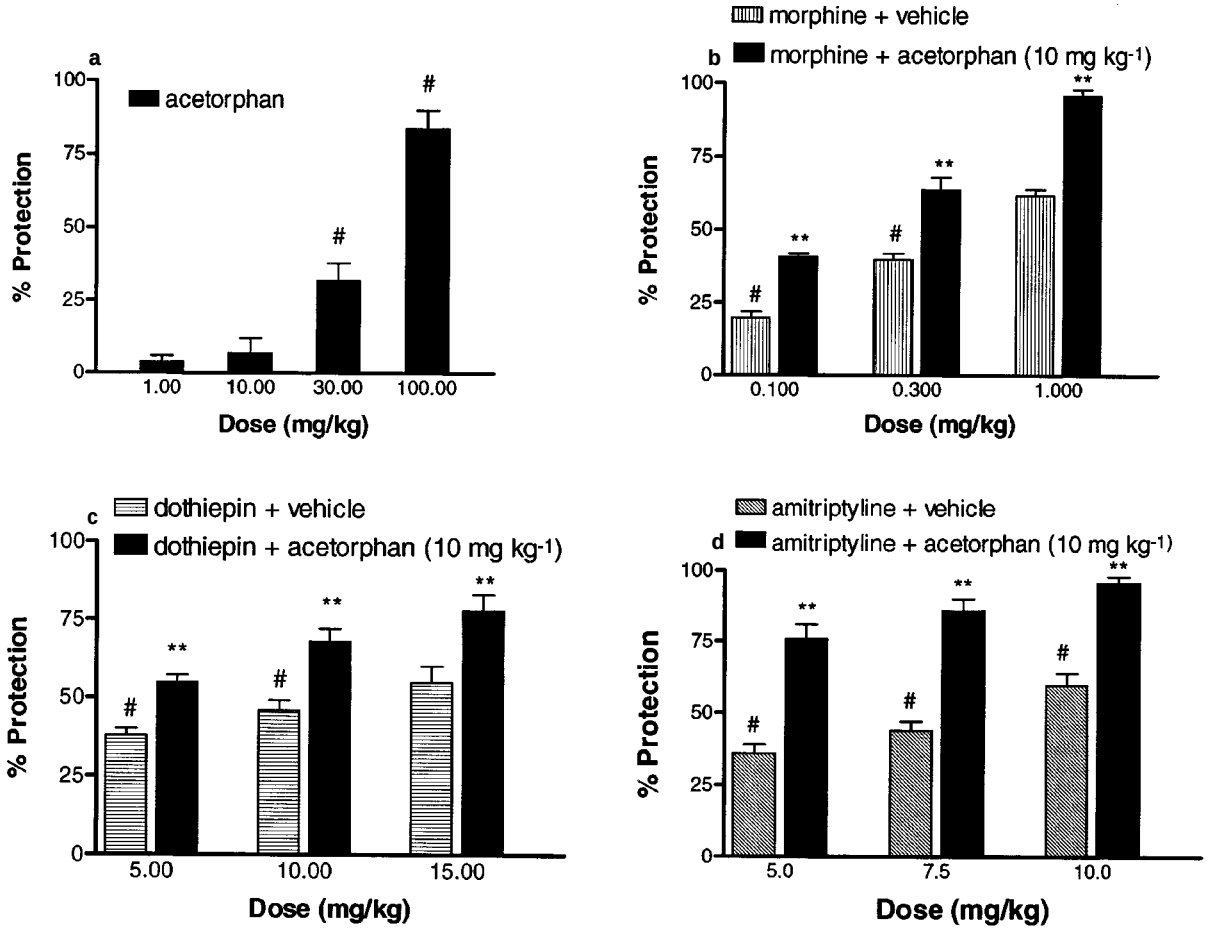

Figure 3 Activity of various agents in the mouse abdominal constriction assay alone and in the presence of a fixed dose of acetorphan (10 mg kg^{-1}). Effect of: (a) acetorphan, (b) morphine, (c) dothiepin and (d) amitriptyline. Each column represents the mean ± s.e.mean of 8 mice. #$P<0.01$ *versus* vehicle control. **$P<0.01$ *versus* appropriate drug + vehicle control. All comparisons of the effects of acetorphan plus antidepressant with antidepressant alone revealed significant increases ($P<0.01$) *vs* antidepressant alone. Doses of agents are shown under the columns.

acid-induced abdominal constriction paradigm to study antidepressant-induced antinociception.

The principal findings from the present study demonstrated that all the antidepressants investigated induced dose-dependent antinociception. This antidepressant-induced antinociception was antagonized by the opioid antagonists, naloxone or naltrindole. Pretreatment with acetorphan potentiated antidepressant-induced antinociception. As anticipated, aspirin, the prototypic non-steroidal anti-inflammatory agent, did not show opioid antagonist reversible antinociception. Aspirin-induced antinociception was not potentiated by acetorphan. Morphine antinociception, in this model, demonstrated naloxone reversible antinociception. Naltrindole at the dose utilized in the present study did not antagonize morphine-induced antinociception. However, acetorphan potentiated morphine antinoception.

Previous work on the antinociceptive properties of the tricyclic antidepressants revealed a lack of consensus as to whether they are intrinsically antinociceptive and/or potentiate opioid antinociception. These studies were conducted with different laboratory nociceptive behavioural assays e.g. tail flick or hot plate. A possible explanation for this lack of consensus may stem from the types of nociceptive assays used.

In this respect, Ögren and Holm (1980) identified a test-specific nature to antidepressant-induced antinociception. Several laboratories have evaluated the antinociceptive profiles of a number of μ- and κ-agonists in various nociceptive behavioural tests with different animal species (Martin *et al.*,

1976; Skingle & Tyers, 1979; 1980; Tyers, 1980). Their findings suggest that a differentiation between μ- and κ-agonists may be obtained with various types of nociceptive stimuli. Various factors underlie noxious test stimulus quality, with a qualitative as opposed to quantitative difference in the discrimination of opioid agonists with respect to nociceptive stimuli having been identified.

Upton *et al*. (1982, 1983) further addressed this differentiation between μ- and κ-agonists. Their results provided good evidence for different functional involvement of subtypes of opioid receptors and neuronal pathways, with respect to the different modalities of noxious stimuli. This work, in accordance with the findings of Tyers (1980), suggested that thermal noxious stimuli preferentially responds to μ-type agonists, while the κ-agonists appear to be more effective against pressure nociception. Hayes and collegues (1987) have extended these studies to include a nociceptive model employing a chemical stimulus and demonstrated that both μ- and κ-opioid agonists responded with equal efficacy to a chemical stimulus. Shaw *et al*. (1988), investigating the effects of partial agonists, found evidence for an involvement of stimulus intensity as a further complicating factor. Thus there is the need for caution when comparing results from several laboratories since the same test is seldom conducted using identical test parameters. Moreover, the use of, for example, a 55°C thermal stimulus yields a model which, although retaining the ability to detect morphine, is insensitive to many analgesic compounds with clinical relevance (Upton *et al*., 1983). We suggest one interpretation of the disarray within the literature as to whether antidepressants are antinociceptive stems from the use of different nociceptive animal models, which is also in agreement with the idea that antidepressants respond differently in different nociceptive assays (Ögren & Holm, 1980).

It is noteworthy that, naltrindole, a specific δ-opioid receptor antagonist which did not antagonize morphine antinociception (see also Portoghese *et al*., 1988) was able to antagonzie antidepressant-induced antinociception. It is also notable that in the present study, naloxone (0.5 mg kg^{-1}, s.c.) attenuated the antinociceptive profile of all the antidepressants. Isenberg & Cicero (1984) commented that many investigators failed to demonstrate any naloxone-reversible component to antidepressant-induced antinociception, though some studies used up to ten times the dose of naloxone required to antagonize the analgesic effects of opioids themselves. Naloxone has been described as having a dual nature, such that it is an opioid antagonist at low doses and an agonist at non-opioid sites at higher doses (Sawynok *et al*., 1979) when the specific nociceptive modality is taken into consideration.

The implication of an opioid antagonist-reversible nature to antidepressant-induced antinociception thus suggests an opioid system involvement. Some antidepressants have been shown to bind to opioid receptors (Biegon & Samuel, 1979; 1980; Isenberg & Cicero, 1984), but whilst the concentrations required to inhibit binding appear to be within pharmacologically relevant brain concentrations (Isenberg & Cicero, 1984), given that the actual binding affinities are extremely low, the fact that some antidepressants bind to opioid receptors does suggest a direct opioid receptor activation as the primary mechanism by which they produce their antinociceptive action. It should be noted that the studies by Biegon and Samuel and Isenberg and Cicero were not designed to identify any agonist action of the antidepressants. However, taking together these studies and our data, showing that antidepressants possess opioid antagonist reversible antinociception, suggests direct agonist-like actions of the antidepressants at opioid receptors.

Sacerdote *et al*. (1987), using the rat tail-flick assay, found that both acute amitriptyline and clomipramine (40 mg kg^{-1}) produced naloxone-reversible antinociception. This group further investigated this apparent opioid-like involvement by measuring β-endorphin levels in the hypothalamus following acute and chronic treatment with these antidepressants, they demonstrated significantly raised levels of β-endorphin. Furthermore, DeFelipe *et al*. (1985) demonstrated increased met-enkephalin like immunoreactivity in the nucleus accumbens and striatum of rats chronically treated with a variety of antidepressants including amitriptyline. In addition, DeFelipe *et al*. (1989) also found that enkephalinase inhibitors potentiated the antidepressant action of several antidepressant compounds. Hameroff *et al*. (1982) found that doxepin not only elevated plasma enkephalin levels but also reduced pain scores in chronic pain patients after six weeks of treatment.

In this context, the second aspect of the present investigation attempted to identify any release of endogenous opioid peptides by augmenting endogenous opioid tone with acetorphan. Thus, with the exception of aspirin, all the compounds tested for antinociceptive activity were potentiated by a sub-antinociceptive dose of acetorphan. These results provide additional support for the hypothesis that both acute and chronic treatment with antidepressants release endogenous opioid peptides. It might, in theory, be expected that enkephalinase inhibitors would produce antinociceptive activity when administered alone, as they should increase any endogenous enkephalinergic tone involved in pain/nociceptive suppression systems. In our present study it was somewhat expected that acetorphan should produce dose-dependent inhibition of abdominal constriction by itself.

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 utilized in this study), and in contrast, when enkephalins are actively being released, enkephalinase inhibitors will both prolong and potentiate these effects. Our data with morphine and acetorphan were somewhat surprising given that morphine acts as a direct μ-opioid receptor agonist. Presently it is unclear the precise mechanism through which acetorphan enhanced morphine antinociception.

Our data provide support for the suggestion that antidepressants activate opioid systems, through both a direct opioid receptor interaction and an indirect action through enhanced release of opioid peptides. Moreover, it is postulated that the direct action of antidepressants on opioid receptors and the endogenous opioid peptides released interact as agonists at both μ- and δ-opioid receptors to inhibit nociceptive transmission, since the activity is antagonized by both naloxone and naltrindole.

In conclusion, the present investigation demonstrated clear intrinsic antinociceptive profiles for the antidepressant compounds in the currently employed nociceptive model, with the recognized analgesics, morphine and aspirin, also having antinociceptive activity, as expected. However, with the exception of morphine, the interesting finding from this study was the opioid-like nature of antidepressant-induced antinociception. Since the antinociceptive action of aspirin was not modified by the opioid antgonists, nor was it potentiated by the neutral endopeptidase inhibitor acetorphan, it is concluded

that the antidepressants were not operating through mechanisms resembling the non steroidal anti-inflammatory agents.

One interpretation of our data is that the antidepressants are operating via a direct and indirect mechanism on opioid receptors and the indirect pathway involves the release of endogenous opioid peptides.

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