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## Modulation of $\mu$ -mediated antinociception by $\delta$ agonists in the mouse: selective potentiation of morphine and normorphine by [D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]enkephalin

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The effect of the  $\delta$ -selective agonist [D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]enkephalin (DPDPE) on the antinociception produced by intracerebroventricular (i.c.v.) administration of the  $\mu$  agonists morphine, [D-Ala<sup>2</sup>,NMePhe<sup>4</sup>,Gly-ol<sup>5</sup>]enkephalin (DAGO), [NMePhe<sup>3</sup>,D-Pro<sup>4</sup>]morphiceptin (PLO17),  $\beta$ -endorphin, phenazocine, etorphine and sufentanil was studied in mice. Only the antinociceptive effects of morphine and normorphine were modulated by i.c.v. coadministration of a dose of DPDPE which did not produce any significant antinociception alone. Both the morphine and normorphine dose-response lines were displaced to the left in the presence of DPDPE. The  $\delta$ -selective antagonist ICI174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH) (where Aib is  $\alpha$ -aminoisobutyric acid) blocked the modulation of morphine antinociception by DPDPE. ICI 174,864 alone failed to produce either a significant increase or decrease of morphine, phenazocine, etorphine or  $\beta$ -endorphin antinociception. The results of the present study provide support for the hypothesis that the enkephalins may function to modulate antinociception produced at the  $\mu$  receptor; such modulation may come about via the existence of an opioid  $\mu$ - $\delta$  receptor complex. The  $\mu$  receptors existing in such a complex may be selectively activated by morphine and normorphine, but not the other  $\mu$  agonists studied here. Thus, the enkephalins may function both to directly initiate, as well as to modulate, some forms of supraspinal  $\mu$  receptor-mediated antinociception.

Opioid antinociception;  $\mu$  Receptors;  $\delta$  Receptors; (Intracerebroventricular, Mouse)

### 1. Introduction

As the diverse nature of opioid receptor subtypes has been recognized, a great deal of work has attempted to correlate opioid effects with specific receptors. One of the most studied effects is the production of antinociception in rodents.

Many tests of antinociception have utilized heat as the noxious stimulus. There is now a significant body of evidence supporting the view that the antinociceptive response to a thermal stimulus can be mediated at supraspinal sites by both opioid  $\mu$  and  $\delta$  receptors in the mouse (Porreca et al., 1984; Heyman et al., 1987; Mathiasen et al., 1987; Porreca et al., 1987; Takemori and Portoghese, 1987). Although these studies in the mouse have provided evidence for a role of the  $\delta$  receptor in the direct mediation of antinociception, data also exist which suggest that the enkephalins, endogenous

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ligands for the  $\delta$  receptor (Lord et al., 1977), may also act indirectly to influence the antinociceptive processes that are mediated by  $\mu$  receptors.

Previous studies have demonstrated a modulation of the antinociception produced by the  $\mu$ -preferring prototype agonist, morphine, by compounds with selectivity for the  $\delta$  receptor. Intracerebroventricular (i.c.v.) administration of sub-antinociceptive doses of [Leu<sup>5</sup>]enkephalin and [Leu<sup>5</sup>]enkephalin analogs have been shown to increase the potency of morphine in producing antinociception as well as the development of morphine tolerance and dependence in the mouse (Vaught and Takemori, 1979; Barrett and Vaught, 1982; Vaught et al., 1982). Additionally, sub-antinociceptive doses of the highly  $\delta$ -selective cyclic enkephalin analog [D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]enkephalin (DPDPE) (Mosberg et al., 1983; Galligan et al., 1984; James and Goldstein, 1984; Porreca et al., 1984) given i.c.v. have also been shown to increase morphine antinociceptive potency in the mouse (Porreca et al., 1987). In contrast sub-antinociceptive doses of [Met<sup>5</sup>]enkephalin analogs attenuate morphine antinociception (Lee et al., 1980; Vaught et al., 1982).

Based on observations of the modulation of morphine antinociception by  $\delta$  agonists, it has been hypothesized that some supraspinal  $\mu$  and  $\delta$  receptors may exist in an opioid receptor complex (Vaught et al., 1982). Further evidence for such a complex has been provided by mathematical analyses of radioligand binding data in brain membrane preparations from the rat (Rothman and Westfall, 1982a,b; 1983; Demoliou-Mason and Barnard, 1986; Rothman et al., 1987) and mouse (Barrett and Vaught, 1983). Furthermore, studies in vitro using the mouse vas deferens bioassay have also provided suggestions for a  $\mu$ - $\delta$  receptor complex (Sanchez-Blazquez et al., 1983). Finally, evidence in vivo for a  $\mu$ - $\delta$  receptor complex is not limited to systems mediating antinociception. A  $\mu$ - $\delta$  receptor complex has also been suggested to be involved in the reversal of endotoxic shock in the rat (Holaday and D'Amato, 1983; D'Amato and Holaday, 1984).

The present study has investigated further the supraspinal modulation of  $\mu$ -mediated antinociception in vivo by DPDPE. Additionally, this

study has addressed the possibility that agonists with equal or near-equal affinity for the opioid  $\mu$  and  $\delta$  receptor may be 'self-modulating' (Vaught et al., 1982). Previous studies of  $\mu$ - $\delta$  interactions generally used morphine as the  $\mu$  agonist. Inasmuch as (a) morphine is  $\mu$  preferring, but does not display a great deal of selectivity for the  $\mu$  receptor (Mosberg et al., 1983), and (b) no specific endogenous ligand for the  $\mu$  receptor has yet been identified, a series of agonists with varying selectivities for the  $\mu$  receptor were studied: these included morphine, normorphine, [D-Ala<sup>2</sup>,NMe-Phe<sup>4</sup>,Gly-ol<sup>5</sup>]enkephalin (DAGO), [NMePhe<sup>3</sup>,D-Pro<sup>4</sup>]morphiceptin (PLO17), etorphine, phenazocine, sufentanil and  $\beta$ -endorphin (human).

## 2. Materials and methods

### 2.1. Animals

Male, ICR mice (20-50 g, Harlan) were used in all experiments. Animals were housed in groups of five in a temperature controlled room with a standard 12 h light-dark cycle (lights on at 7:00 a.m.). Food and water were available continuously.

### 2.2. Injection techniques

Compounds were delivered into the lateral cerebral ventricle using a modification of the method of Haley and McCormick (1957) as previously described (Porreca et al., 1984). The mice were lightly anesthetized with ether, an incision made in the scalp and bregma located. Compounds were injected directly through the skull at a point 2 mm caudal and 2 mm lateral to bregma at a depth of 3 mm using a Hamilton (Reno, NV) microliter syringe with a 26-gauge needle. All i.c.v. injections were made in a volume of 5  $\mu$ l.

### 2.3. Test of antinociception

Antinociceptive responses were determined using warm (55°C) water as the noxious stimulus; the latency to tail withdrawal was taken as the endpoint according to the method of Janssen et al.

(1963). Prior to agonist administration, the tail of each mouse was immersed in the water and the latency to a rapid flick recorded (control latency). Animals not flicking their tails within 5 s were eliminated from the study. This procedure was repeated 20 min after i.c.v. administration of all compounds; this was the time of peak agonist effect as determined from time-response curves (DAGO, morphine and DPDPE; Heyman et al., 1986) (normorphine, PLO17, phenazocine, etorphine, sufentanil and  $\beta$ -endorphin; present study; data not shown). Animals not flicking their tails within 15 s were removed from the nociceptive stimulus and assigned a maximal antinociceptive score of 100% in order to avoid tissue damage. Antinociception was expressed as: % antinociception =  $100 \times (\text{test latency} - \text{control latency}) / (15 \text{ s} - \text{control latency})$ . All testing was done in unanesthetized mice.

#### 2.4. Modulation of antinociception

Following the determination of the i.c.v. dose-response curves for the agonists, a dose of DPDPE which produced barely detectable antinociception (0-5%) was chosen by extrapolation of the DPDPE dose-response line. This sub-effective dose, 1.6 nmol, did not produce significant antinociception when given alone. In order to determine the modulatory effects of DPDPE on the antinociception produced by the various  $\mu$  agonists, DPDPE (1.6 nmol) was coadministered in the same i.c.v. injection with graded doses of the  $\mu$  agonists as described previously (Vaught and Takemori, 1979). Testing took place 20 min after injection.

#### 2.5. Antagonist study

The  $\delta$ -selective antagonist N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH, where Aib is  $\alpha$ -aminoisobutyric acid (ICI 174,864) (4 nmol) (Cotton et al., 1984) was coadministered in the same i.c.v. injection as the agonists and antinociception was determined 20 min after injection. This dose of ICI 174,864 was previously demonstrated to significantly antagonize the analgesic effects of higher doses of i.c.v. DPDPE (Heyman et al., 1987).

#### 2.6. Chemicals

DPDPE was synthesized as described previously (Mosberg et al., 1983). PLO17 and human  $\beta$ -endorphin (Peninsula Laboratories, Inc., Cambridge, MA), as well as DPDPE, were dissolved in distilled water, frozen in aliquots and lyophilized, and redissolved immediately before use. Morphine sulfate (Mallinckrodt Inc., St. Louis, MO), normorphine HCl, etorphine HCl, phenazocine HBr and sufentanil HCl (all generously provided by Dr. Alan Cowan, Department of Pharmacology, Temple University School of Medicine) were dissolved in distilled water just prior to administration.

#### 2.7. Statistics

The dose of DPDPE chosen for the modulation of  $\mu$ -mediated antinociception was determined from the regression line of the individual data points using the computer program of Tallarida and Murray (1986) (procedure 8), and extrapolating to obtain a sub-antinociceptive dose. A minimum of 10 mice were studied at each dose level. The antinociception produced by individual doses of each agonist in the absence and presence of 1.6 nmol DPDPE were compared using a Student's t-test for grouped data. The data are presented as the mean and the error bars are the S.E.

### 3. Results

When coadministered in the same i.c.v. injection as morphine, DPDPE (1.6 nmol) consistently and significantly increased the antinociception produced by graded doses of morphine as previously reported (Porreca et al., 1987). An example illustrating the increase of morphine antinociception (3 nmol) produced by 1.6 nmol of DPDPE is shown in fig. 1. This dose of DPDPE (1.6 nmol) produced minimal antinociception when given alone. The increase of morphine antinociception by DPDPE was prevented by coadministration of the  $\delta$  antagonist ICI 174,864 (4 nmol) (fig. 1). ICI 174,864 failed to produce antinociception when given alone, and neither increased nor decreased

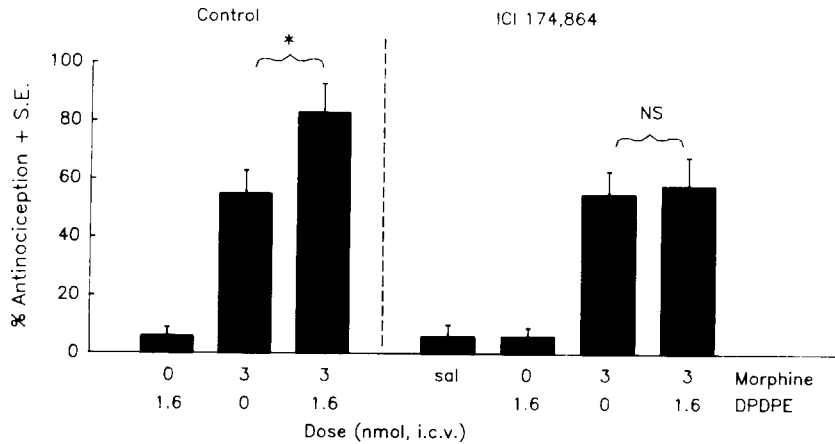


Fig. 1. DPDPE-induced modulation of morphine antinociception and antagonism of modulation by ICI 176,864 (4 nmol) following administration of compounds in the same i.c.v. injection. The bars represent the mean and S.E. of the agonist effects; asterisks indicate a significant difference ( $P < 0.05$ ) when groups were compared to morphine antinociception alone.

morphine antinociception at this dose (4 nmol) (Heyman et al., 1987; Porreca et al., 1987). Similar to its effect on morphine, DPDPE increased the antinociceptive potency of normorphine (fig. 2). In contrast, DPDPE coadministration did not affect the antinociception produced by the peptide  $\mu$  agonists DAGO (fig. 3a) and PLO17 (fig. 3b), and the non-peptide  $\mu$  agonists etorphine (fig. 3c), phenazocine (fig. 3d) and sufentanil (fig. 3e). Furthermore,  $\beta$ -endorphin antinociception was unaltered by DPDPE (fig. 3f). Additionally, coadministration of ICI 174,864 at a dose (4 nmol) which was effective in antagonizing DPDPE anti-

nociception had no significant effect on the antinociceptive effects of i.c.v. etorphine (fig. 4a), phenazocine (fig. 4b) or  $\beta$ -endorphin (fig. 4c).

#### 4. Discussion

The aim of the present study was to investigate and characterize the effects of coadministration of a highly selective  $\delta$  agonist, DPDPE, with antinociception initiated by several  $\mu$  agonists. As no endogenous ligand has yet been established for the  $\mu$  receptor, a variety of structurally diverse  $\mu$  agonists were chosen and studied for possible antinociceptive interactions with DPDPE. Evidence from previous studies suggests that  $\delta$  agonists given directly into the brain can modulate the antinociceptive responses to morphine. [Leu<sup>5</sup>] enkephalin, [Leu<sup>5</sup>]enkephalin analogs and the  $\delta$ -selective agonist DPDPE have been shown to increase morphine antinociceptive potency (Vaught and Takemori, 1979; Barrett and Vaught, 1982; Vaught et al., 1982; Porreca et al., 1987) while [Met<sup>5</sup>]enkephalin and [Met<sup>5</sup>]enkephalin analogs attenuate morphine antinociception (Lee et al., 1980; Vaught et al., 1982). Based on these observations, a model suggesting a  $\mu$ - $\delta$  receptor complex has been proposed to explain  $\mu$ - $\delta$  interactions in vivo (Vaught et al., 1982). Such a model suggests two possible mechanisms for  $\mu$ - $\delta$  interac-

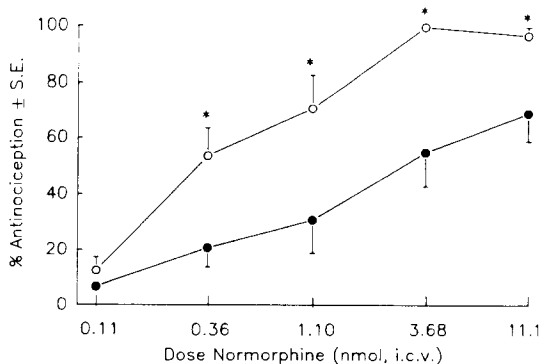


Fig. 2. DPDPE-induced modulation of normorphine antinociception. Dose-response curves for i.c.v. normorphine antinociception in the absence (closed circles) and presence (open circles) of a sub-agonist dose of DPDPE (1.6 nmol); asterisks indicate a significant difference ( $P < 0.05$ ).

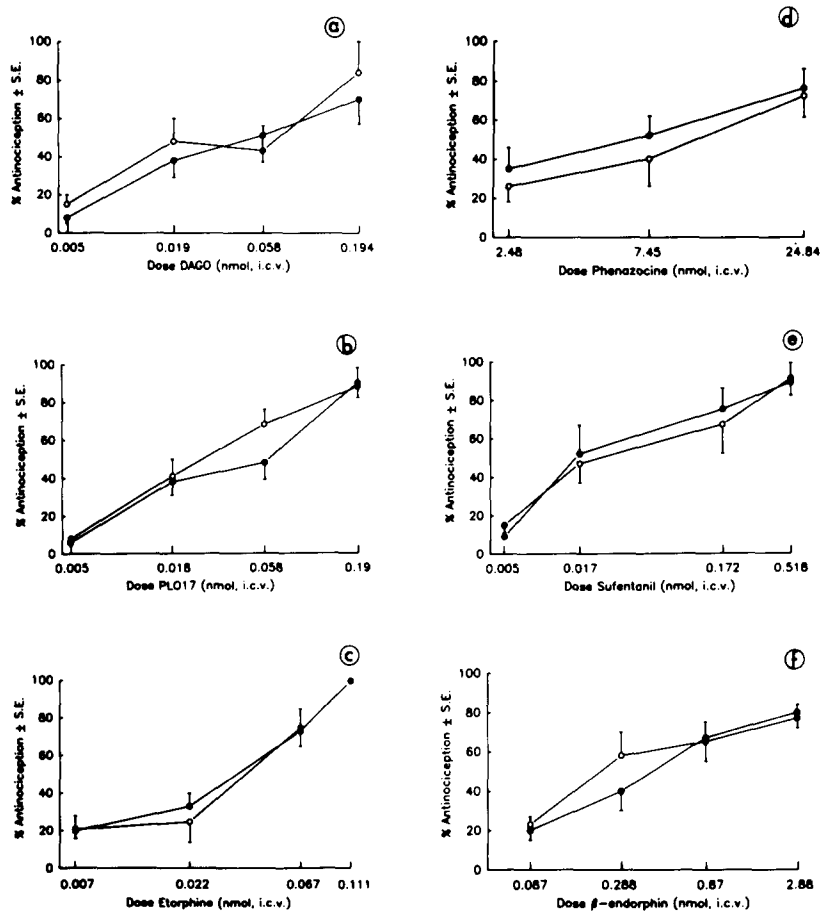


Fig. 3. DPDPE fails to increase i.c.v. DAGO, PLO17, etorphine, sufentanil, phenazocine and  $\beta$ -endorphin antinociception. Dose-response curves for i.c.v. (a) DAGO, (b) PLO17, (c) etorphine, (d) phenazocine, (e) sufentanil and (f)  $\beta$ -endorphin in the absence (closed symbols) and presence (open symbols) of DPDPE (1.6 nmol).

tions: one possibility is the allosteric modification of one receptor by occupation of the other. Radioligand binding studies have provided some evidence for such a mechanism and have further suggested that the allosteric modulation is bidirectional (Rothman and Westfall, 1982a,b; Barrett and Vaught, 1983; Rothman and Westfall, 1983; Demoliou-Mason and Barnard, 1986). A second possibility is that occupation of the  $\delta$  receptor by the enkephalins or enkephalin analogs may alter the  $\mu$  receptor conformation in order to enhance or inhibit the coupling of the  $\mu$  receptor to the antinociceptive effector system.

Our results show that a dose of DPDPE which does not produce significant antinociception when

given alone, increased i.c.v. morphine antinociceptive potency. The increase of i.c.v. morphine antinociception by DPDPE is in agreement with previous reports from our laboratory (Porreca et al., 1987). In order to determine if the observed increase in morphine antinociception was due to an interaction of DPDPE with the  $\delta$  receptor, the modulatory dose of DPDPE was challenged with the selective  $\delta$  antagonist ICI 174,864. The increase of i.c.v. morphine antinociception associated with DPDPE was prevented by  $\delta$  receptor blockade with ICI 174,864. This finding suggests that the modulation of morphine antinociception results from the action of DPDPE at the  $\delta$  receptor. It is important to note that ICI 174,864 had

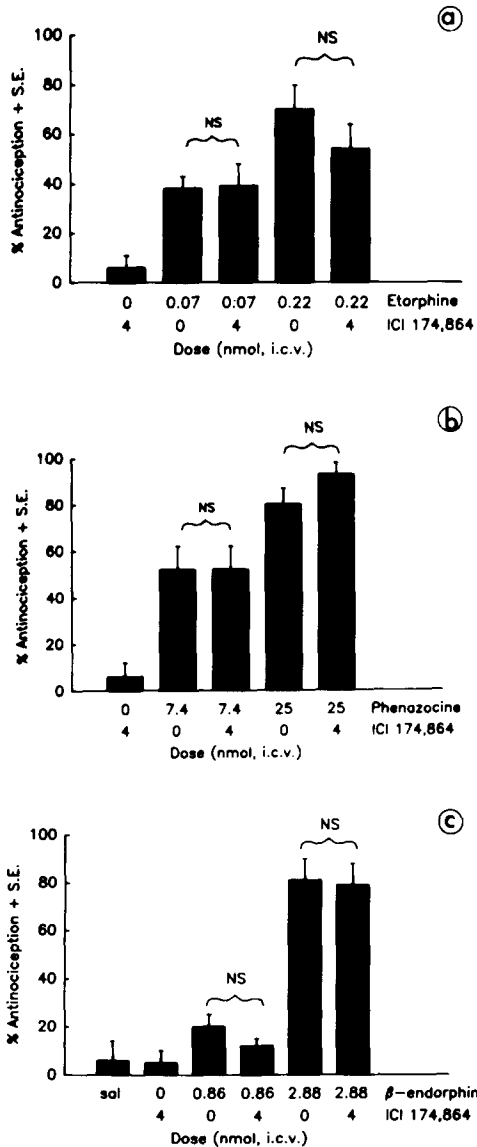


Fig. 4. Antinociceptive effects of i.c.v. etorphine (a), phenazocine (b) and  $\beta$ -endorphin (c) in the absence and presence of ICI 174,864 (4 nmol). The bars represent the mean and S.E. NS indicates no significant difference ( $P < 0.05$ ).

no antinociceptive effects alone, and did not directly antagonize or potentiate morphine antinociception (Heyman et al., 1987; Porreca et al., 1987; present study). This result correlates well with the findings of Vaught et al. (1982) who showed that [Met<sup>5</sup>]enkephalin could counteract the increase of morphine antinociception pro-

duced by [Leu<sup>5</sup>]enkephalin in mice, and that [Leu<sup>5</sup>]enkephalin could prevent the attenuation of morphine antinociception produced by [Met<sup>5</sup>]enkephalin. These findings were interpreted as indicating that the modulatory effects may occur through a common opioid ( $\delta$ ) receptor. Additionally, Lee et al. (1980) have shown that the same dose of [Met<sup>5</sup>]enkephalin antagonized morphine antinociception in naive and morphine-tolerant mice. As a higher dose of [Met<sup>5</sup>]enkephalin was not required to attenuate morphine antinociception in morphine-tolerant mice, it was suggested that [Met<sup>5</sup>]enkephalin does not compete with morphine for the  $\mu$  receptor to reduce morphine antinociception, but does so via a distinct ( $\delta$ ) receptor. Further, Porreca et al. (1987) have shown that a higher dose of i.c.v. DPDPE is not required to produce a similar degree of increase of morphine antinociception in morphine-tolerant mice, again suggesting that the enkephalins and enkephalin analogs produce their modulatory effects via an action at a receptor ( $\delta$ ) distinct from that activated by morphine ( $\mu$  receptor). As an increase in the dose of DPDPE is not required for modulation of morphine antinociception in morphine-tolerant mice, and modulation is reversed by ICI 174,864, it seems reasonable to conclude that the modulation of morphine antinociception occurs by actions of DPDPE at the  $\delta$  receptor.

It could be argued that DPDPE increases morphine antinociception by simply displacing morphine from the  $\delta$  receptor, thereby increasing the relative concentration of morphine available at the  $\mu$  receptor. If this hypothesis were correct, then the  $\delta$  agonist DPDPE, and the  $\delta$  antagonist ICI 174,864 would (as a result of higher affinity for the  $\delta$  receptor) both be expected to displace morphine from binding to the  $\delta$  site and consequently raise the relative concentration of morphine available at the  $\mu$  receptor, resulting in potentiation of antinociception. This was not the case, however, as the  $\delta$  antagonist ICI 174,864, at a dose which antagonized  $\delta$ -mediated effects, had no antagonist effect on morphine antinociception and did not increase the effects of morphine (Heyman et al., 1987).

In addition to the interaction of DPDPE and morphine, the modulatory effect of DPDPE on

the antinociception produced by several other structurally diverse  $\mu$  agonists was also studied in the present investigation. Similar to its effect on morphine, DPDPE also increased i.c.v. normorphine antinociceptive potency. In contrast, DPDPE had no effect on the antinociception elicited by i.c.v. DAGO, PLO17, phenazocine, sufentanil or etorphine. Furthermore, DPDPE failed to increase  $\beta$ -endorphin antinociception, which is in agreement with a previous study which showed that [Leu<sup>5</sup>]enkephalin did not increase  $\beta$ -endorphin antinociception (Vaught et al., 1982). While the ability of DPDPE to modulate morphine and normorphine antinociception supports further the concept of opioid  $\mu$ - $\delta$  receptor interactions via an opioid receptor complex, our results suggest that, of the  $\mu$  compounds examined in the present study only morphine and normorphine are selective for actions at this receptor complex. It should be noted that the antinociceptive effects of levorphanol were modulated by [Leu<sup>5</sup>]enkephalin (Vaught and Takemori, 1979), suggesting that other  $\mu$  agonists may also fall into the modulated category.

The issue of self-modulation was introduced by Vaught et al. (1982) to explain the discrepancies between the affinity of certain agonists for the  $\mu$  receptor in vitro and their antinociceptive potencies in vivo. Traditionally there has been a strong correlation between affinity for the  $\mu$  receptor in vitro and antinociceptive potency in tests where heat is used as the noxious stimulus. Discrepancies between affinity and potency began to arise with the advent of enkephalin analogs. Vaught et al. (1982) demonstrated clearly that although the affinity of morphine for the  $\mu$  receptor is greater than that of the enkephalin analog [D-Ala<sup>2</sup>,D-Leu<sup>5</sup>]enkephalin (DADLE), DADLE is a much more potent antinociceptive agent than is morphine. The discrepancy between  $\mu$  affinity and antinociceptive potency of DADLE was attributed to the affinity of DADLE for the  $\delta$  receptor. Inasmuch as the affinity of DADLE for the  $\delta$  receptor is only 12-fold greater than its affinity for the  $\mu$  receptor (Vaught et al., 1982), it is conceivable that DADLE is capable of acting at both the  $\mu$  and  $\delta$  receptor of the receptor complex. By activating both receptors of the  $\mu$ - $\delta$  receptor com-

plex, the presence of DADLE at the  $\delta$  site of the receptor complex would thus 'modulate' its own  $\mu$ -initiated antinociception. The antinociceptive effects of  $\beta$ -endorphin, therefore, could also be attributed to self-modulation based upon the similar affinities of  $\beta$ -endorphin for the  $\mu$  and  $\delta$  receptor (Vaught et al., 1982). If compounds are indeed self-modulating, then no further increase in effect by DPDPE would be expected.

While the concept of self-modulation is alluring, it does not explain the lack of effect of DPDPE on DAGO, PLO17 and sufentanil antinociception in the current investigation. If the measured antinociceptive effect of the  $\mu$  compounds were a self-modulated effect, it is reasonable to expect that the possibility for self-modulation increases as the affinity for the  $\delta$  receptor increases. Such an increase would, thus, decrease the  $\mu/\delta$  affinity ratio which is an index of compound selectivity. Conversely, as the  $\mu$  affinity of an agonist increased ( $\mu/\delta$  increased), its ability for self-modulation would decrease, and its susceptibility to DPDPE would increase. The rank order of  $\delta$  affinity (taken from literature sources), from highest to lowest, of the  $\mu$  agonists used in the present study is as follows: etorphine <sup>a</sup> > phenazocine <sup>a</sup> >  $\beta$ -endorphin <sup>a</sup> > morphine <sup>b, c</sup> > sufentanil <sup>a</sup> > DAGO <sup>c</sup> > normorphine <sup>a</sup> > PLO 17 <sup>c</sup> (date from; <sup>a</sup> Magnan et al., 1982; <sup>b</sup> Mosberg et al., 1983; <sup>c</sup> personal communication, Dr. Henry I. Yamamura).

To address the possibility of self-modulation, we attempted to antagonize the antinociceptive effects of i.c.v. etorphine, phenazocine and  $\beta$ -endorphin with the  $\delta$  antagonist ICI 174,864 as these  $\mu$  agonists possess the greatest affinity for the  $\delta$  receptor among the compounds tested, and therefore, were most likely to be self-potentiating. A dose of 4 nmol ICI 174,864 given i.c.v. has been shown previously to essentially abolish the antinociceptive effects of i.c.v. DPDPE while having no effect on i.c.v. morphine antinociception (Heyman et al., 1987). If indeed, etorphine, phenazocine or  $\beta$ -endorphin were self-potentiating, this same dose of ICI 174,864 would be expected to produce some reduction or attenuation in the antinociceptive effect. Such a reduction would be due to the elimination of the  $\delta$  (potentiating)

component of these agonists. Figure 4 reveals, however, that ICI 174,864 had no effect on the antinociceptive effects of i.c.v. etorphine, phenazocine or  $\beta$ -endorphin. Thus, self-modulation does not appear to explain the lack of effect of DPDPE on etorphine, phenazocine or  $\beta$ -endorphin antinociception. Based on the thinking that self-modulation comes from the action of an agonist at both the  $\mu$  and  $\delta$  receptor of the receptor complex, and that decreasing the  $\delta$  affinity of a  $\mu$  agonist increases the possibility that a compound will be modulated by DPDPE, the antinociceptive effects of DAGO, PLO17 and sufentanil in addition to morphine and normorphine, should also have been modulation by DPDPE. This was not the case, however, as DPDPE affected neither DAGO, PLO17 or sufentanil antinociception.

An alternative explanation for the differential effects of DPDPE on  $\mu$ -mediated antinociception is that not all  $\mu$  agonists are capable of activating a proposed 'complexed'  $\mu$  receptor. If this is the case, the present data imply that differences may exist between hypothesized complexed and non-complexed  $\mu$  receptors, i.e.  $\mu$  receptor subtypes. The concept of  $\mu$  receptor subtypes has been suggested previously. For example, Pasternak and coworkers (Pasternak, 1980) have provided considerable evidence based on radioligand studies and pharmacological studies *in vivo* and *in vitro* (for review, see Pasternak and Wood, 1986) supporting the existence of  $\mu$  receptor subtypes. Additional evidence for  $\mu$  receptor subtypes has been provided by other investigators as well (Rothman et al., 1984; Sheldon et al., 1987; Rothman et al., 1987; Heyman et al., submitted; Bowen et al., 1988). It is interesting to note that the specific  $\mu$  agonists which are modulated by i.c.v. DPDPE in the present study (i.e. morphine and normorphine) are the same as those modulated by DPDPE in the rat using inhibition of the micturition reflex as the endpoint (Sheldon et al., *in press*). The similarity in profile of modulated  $\mu$  agonists across species and effect not only supports the concept of  $\mu$ - $\delta$  receptor complex, but also the existence of  $\mu$  receptor subtypes with characteristics which differ based on whether they reside in, or outside of the hypothesized receptor complex.

The criteria for activation of the complexed  $\mu$

receptor are not inherently obvious. Whilst there is some evidence for distinct  $\mu$  receptors in the guinea pig ileum (Takemori and Portoghesi, 1985) which may be selectively activated by peptide or non-peptide agonists (Ward et al., 1986), such a distinction may not apply to the present data as neither the peptides DAGO, PLO17 or  $\beta$ -endorphin, nor the non-peptides phenazocine, etorphine, or sufentanil were modulated by DPDPE. Regardless of physicochemical commonalities amongst the  $\mu$  agonists used, which could provide insight into the structure-activity relationship of complexed vs. non-complexed  $\mu$  receptors, the differential modulation of the various  $\mu$  agonists is strongly suggestive of the existence of  $\mu$  receptor subtypes. Recently reported radioligand binding and autoradiography studies (Rothman et al., 1987; Bowen et al., 1988) have provided evidence that both supports the concept of a  $\mu$ - $\delta$  receptor complex and of  $\mu$  receptor subtypes, and additionally suggests that differences exist between the  $\mu$  receptors in, and outside of the receptor complex. These  $\mu$  receptor subtypes have been termed  $\mu_{\text{complexed}}$  ( $\mu_{\text{cx}}$ ) and  $\mu_{\text{non-complexed}}$  ( $\mu_{\text{ncx}}$ ) (Rothman et al., 1987). Differences have also been suggested between  $\delta$  receptors with exist in ( $\delta_{\text{cx}}$ ) and outside ( $\delta_{\text{ncx}}$ ) the hypothesized receptor complex (Bowen et al., 1988) based on the differential coupling of the  $\delta$  receptors to guanine nucleotide binding proteins in rat brain membranes (see Heyman et al., 1988, for further discussion).

The present study also continues to support the concept of opioid receptor interactions *in vivo*. Blockade of  $\mu$ - $\delta$  interactions by the  $\delta$ -selective antagonist ICI 174,864 suggests that the modulation stems from action of DPDPE at the  $\delta$  receptor. The  $\mu$  receptor in the complex may be a specific  $\mu$  receptor subtype which is activated only by some agonists represented by morphine and normorphine, to date. While we do not know the identity of the hypothesized  $\mu_{\text{cx}}$  receptor, it appears that it is not the  $\mu_1$  receptor proposed by Pasternak (1980). A recent study from our laboratory (Heyman et al., submitted) has shown that DPDPE continues to increase morphine antinociception in the presence of naloxonazine, a proposed  $\mu_1$  antagonist (Hahn et al., 1982). Supple-



mentary investigation using various pharmacological agents to disrupt the receptor complex is needed to further elucidate the  $\mu$ - $\delta$  interactions, and the identity of  $\mu$  receptor residing in the complex.

In conclusion, the current data suggest that in addition to playing a direct role in the production of antinociception, the enkephalins appear to play an additional modulatory role in antinociceptive processes. This modulatory role may provide a new inroad to the development of antinociceptive agents which could prove useful in cases where traditional  $\mu$  agonists such as morphine are insufficient.

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