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Differential Modulation by [D-Pen^2, D-Pen^5]Enkephalin and Dynorphin A-(1–17) of the Inhibitory Bladder Motility Effects of Selected *Mu* Agonists *in Vivo*[1]

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ABSTRACT

The possibility that the *delta* agonist, [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and the putative endogenous *kappa* agonist, dynorphin A-(1–17) could differentially modulate the effects of a group of chemically diverse *mu* agonists was evaluated using inhibition of volume-induced contractions of the rat urinary bladder as a model of central nervous system opioid receptor function *in vivo*. Intracerebroventricular administration of equieffective doses of the *mu* agonists [D-Ala^2, NMPhe^4, Gly-ol]enkephalin (DAMGO), [N-MePhe^3, D-Pro^4]enkephalin (PL017), morphine, normorphine, sufentanil, etorphine, phenazocine, meperidine and methadone inhibited spontaneous bladder contractions for approximately 20 to 30 min. Low doses of DPDPE or dynorphin A-(1–17) failed to affect spontaneous bladder contractions; higher doses of DPDPE (greater than 15.5 nmol) and dynorphin A-(1–17) (*i.e.*, greater than 3.7 nmol), inhibited bladder contractions. When coadministered i.c.v., DPDPE displaced the morphine dose-response line to the left and also potentiated the effects of normorphine and etorphine. In contrast, DPDPE failed to alter the actions of equieffective doses of DAGO, PL017, meperidine, methadone, phenazocine or sufentanil. The potentiation of the effects of morphine by DPDPE were prevented by i.c.v. coadministration of the *delta* antagonist, ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH); at the dose tested, the *delta* antagonist had no agonist effects alone and did not antagonize the effects of morphine directly. Furthermore, the agonist effects of morphine were potentiated by several different doses of DPDPE. Administration of i.c.v. dynorphin A-(1–17) produced a rightward displacement of the morphine dose-response line and also antagonized the effects of normorphine. Dynorphin A-(1–17) did not alter the actions of equieffective doses of DAMGO, PL017, sufentanil, meperidine, phenazocine or methadone. The antagonism of morphine effects by dynorphin A-(1–17) was prevented by coadministration of the *kappa* antagonist, nor-binaltorphimine. This antagonist did not directly antagonize the agonist effects of morphine at the dose tested. Furthermore, different doses of dynorphin A-(1–17) were effective in antagonizing morphine effects. Thus, the modulatory profiles of a variety of *mu* agonists by either DPDPE or dynorphin A-(1–17) were very similar, with the only observed exception being modulation of etorphine. The modulation of some *mu* agonists by actions at either *delta* or *kappa* receptors demonstrate differences between morphine, normorphine and the other *mu* agonists tested. These results appear to support the concept of *mu* receptor subtypes *in vivo*, as well as the hypothesis of functionally coupled opioid receptors.

The discovery of specific opiate receptors within the central nervous system of mammals has led to a great deal of work attempting to discover the physiological importance of opioid systems. In spite of evidence suggesting that the opiate receptors exist as separate macromolecules with anatomically distinct localization and pharmacology (Martin *et al.*, 1976; Mansour *et al.*, 1987), evidence has also begun to accumulate which suggests that in some cases, opiate receptors may exist in physically (Goodman *et al.*, 1980; Bowen *et al.*, 1981; Rothman and Westfall, 1982a,b) or functionally (Vaught and Takemori, 1979; Lee *et al.*, 1980) coupled states (Vaught *et al.*, 1982).

Much of the evidence supporting receptor interaction stemmed from early observations which showed that the analgesic effect of morphine could be either potentiated or antagonized by the coadministration of Leu-enkephalin (Vaught and Takemori, 1979) or Met-enkephalin (Lee *et al.*, 1980), respectively. More recently, evidence for receptor interactions has been reported using approaches using both *in vivo* and *in vitro* techniques. Holaday and colleagues (Holaday and D'Amato, 1983; D'Amato and Holaday, 1984) have suggested that a *mu-delta* receptor complex may be involved in the reversal of endotoxic shock in the rat. Furthermore, mathematical analyses of radioligand binding surfaces in preparations of rat brain (Rothman and Westfall, 1982a,b, 1983; Demoliou-Mason and Barnard, 1986), have revealed two types of *delta* binding sites,

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ABBREVIATIONS: DAMGO, [D-Ala^2, NMPhe^4, Gly-ol]enkephalin; DPDPE, [D-Pen^2-D-Pen^5]enkephalin; PL017, [N-MePhe^3, D-Pro^4]enkephalin; nor-BNI, nor-binaltorphimine.

which can be differentiated in competition studies with *mu*-selective ligands. Such studies reveal one *delta* site that is altered competitively by *mu* ligands and a second *mu*-noncompetitive *delta* binding site. Finally, functional studies *in vitro* using the mouse vas deferens bioassay (Sanchez-Blasquez *et al.*, 1983) also support receptor interactions (see Holaday *et al.*, 1985, for review).

Recently, Dray and colleagues (Dray and Metsch, 1984a,b) have established that central *mu* and *delta*, but not *kappa*, receptors are involved in the modulation of motor activity of the rat urinary bladder preparation. Intracerebroventricular administration of *mu* agonists such as morphine and DAMGO, as well as DPDPE cause a rapid suppression of bladder activity resulting from the central micturition reflex; the duration of inhibition of the micturition contractions was taken as a measure of the compounds agonist effect in this model. The central effect of DPDPE, but not that of morphine and DAMGO, was antagonized by ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH), a selective *delta* receptor antagonist (Cotton *et al.*, 1984), suggesting distinct *mu* and *delta* mediation of these effects (Dray and Nunan, 1984). In contrast, i.c.v. administration of compounds generally classified as *kappa* agonists such as U50,488H (Von Voigtlander *et al.*, 1983) produce little or no effect in this system, but appear to readily modulate morphine and normorphine activity. Agonists such as ethylketocyclazocine and dynorphin A-(1-13) can be shown to produce agonist effects in this preparation, but only at very high doses (Dray and Metsch, 1984b). Thus, the rat urinary bladder model seemed an ideal preparation to pursue the possibility of opioid receptor interactions *in vivo*.

Our previous work has demonstrated that compounds usually classified as opioid *kappa* agonists such as U50,488H (Von Voigtlander *et al.*, 1983), tifluadom (Roemer *et al.*, 1982) and ethylketocyclazocine (Martin *et al.*, 1976) when studied at doses that do not produce measurable effects in the bladder model, can antagonize the suppression of volume-induced micturition contractions of the rat bladder produced by selected opiate *mu* agonists such as morphine and normorphine (Sheldon *et al.*, 1987). These compounds, however, failed to alter the effects produced by other agonists (also generally classified as acting at *mu* receptors) such as DAMGO, PL017, meperidine and phenazocine (results with etorphine and sufentanil were equivocal). The data were interpreted to suggest the possibility of *mu* receptor subtypes within the central nervous system which could be activated by morphine and normorphine and modulated by *kappa* agonists, with other *mu* receptor types not associated with *kappa* receptor modulation. In support of this concept, reports have appeared in the literature showing cases in which opioid *mu* effects are differentially modulated by *kappa* agonists. For example, the increase in striatal dopamine metabolism and respiratory depression associated with morphine are antagonized by *kappa* agonists, whereas the reduction in cortical and hippocampal acetylcholine turnover produced by morphine are unaffected by these agents (Wood, 1984). Furthermore, U50,488H has been shown to antagonize the hyperactivity, but not the antinociception, produced by morphine in the mouse (Browne, 1986).

Collectively, these reports have demonstrated that 1) *mu* agonist effects can, in some cases, be antagonized by compounds generally classified as *kappa* agonists and 2) *mu* agonist effects can, in some cases, be potentiated or antagonized by *delta* agonists. As no endogenous *mu* receptor ligand has been identified, it was the purpose of this study to determine if the specific *mu* agonists modulated by *kappa* agonists (Sheldon *et al.*, 1987) are the same as those modulated by *delta* agonists. The compounds chosen as modulators included the putative endogenous *kappa* agonist, dynorphin A-(1-17) (Goldstein *et al.*, 1978; Chavkin and Goldstein, 1981) as well as a stabilized and highly selective enkephalin analog, DPDPE, as a substitute for the endogenous agonist at the *delta* receptor (Mosberg *et al.*, 1983; Porreca *et al.*, 1984; Heyman *et al.*, 1987). Our reasoning was that if the hypothesized *mu-delta-kappa* complex were to exist in rat brain, then agonists at the *mu* site of this complex should be modulated both by *delta* and *kappa* agonists.

Materials and Methods

Compounds. Normorphine hydrochloride, sufentanil, etorphine hydrochloride, phenazocine hydrobromide, meperidine hydrochloride, naloxone hydrochloride, ethylketocyclazocine methanesulfonate and tifluadom were kindly donated by Dr. Alan Cowan (Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA). Morphine sulfate (Mallinckrodt Inc., St. Louis, MO), DAMGO, PL017 and dynorphin A-(1-17) were purchased from Bachem Inc. (Torrence, CA). DPDPE was synthesized and generously donated by Dr. Henry Mosberg (Department of Chemistry, University of Michigan, Ann Arbor, MI). ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH) and nor-BNI were purchased from commercial sources (Cambridge Research Biochemicals, Atlantic Beach, NY and Research Biochemicals Inc., Wayland, MA, respectively). Drugs were dissolved in deionized water before use and stored frozen (−20°C); all compounds retained full activity under these procedures.

Rat bladder preparation. Opioid interactions *in vivo* were studied using the rat urinary bladder preparation as described by Dray (1985). Female Sprague-Dawley rats (200–250 g, Hilltop Laboratories, Scottsdale, PA) were anesthetized initially with ketamine hydrochloride (100 mg/kg i.p., Parke-Davis and Company, Detroit, MI) and supplemented with urethane (1.2 g/kg i.p.) as needed. Body temperature was maintained at 37°C by means of a warm-water blanket. The urinary bladder was catheterized *via* the urethra using polyethylene tubing (PE-50, American Scientific, McGraw Park, IL). The bladder was slowly filled in 0.2 ml increments with warm saline *via* the catheter until spontaneous contractions occurred as a result of the central micturition reflex. Intravesicular pressure was measured isometrically using a Statham pressure transducer and was recorded continually on a chart recorder. Once initiated, rhythmic contractions of the bladder persisted spontaneously for many hours.

Experimental protocols. Drugs were administered i.c.v. in volumes of 1 to 6 μl *via* a Hamilton syringe fitted with a 26-gauge needle using a stereotaxic device and the following injection coordinates: 2 mm posterior to bregma, 2 mm lateral from midline and 4 mm deep from the skull surface. Agonist effects of each compound were assessed in individual rats and quantified as the duration of blockade of spontaneous bladder contractions in minutes; measurements were made from the time of complete cessation of contractions to the onset of the first bladder contraction. In studies designed to evaluate the modulation of *mu* agonist activity by DPDPE, an initial (control) response to the *mu* agonist was obtained. After a minimum recovery of 1 hr, the modulating compound or saline was given concurrently with the *mu* agonist. Potentiation was indicated by a significantly greater shutdown of bladder contractions when compared with control responses. In studies with the *delta* antagonist, ICI 174,864, this compound was given concurrently with DPDPE and morphine. To evaluate modulation of *mu* agonist activity by dynorphin A-(1-17), an initial (control) response to the *mu* agonist was obtained, the animal was allowed to recover for at least 1 hr and dynorphin A-(1-17) or saline was given 15 min before re-evaluation of the *mu* agonist. Antagonism was indicated by 1) an inability of the *mu* agonist to depress spontaneous bladder contractions or 2) a statistically significant reduction in the duration of agonist-

induced bladder shutdown when compared to the control response. In studies with nor-BNI (Takemori *et al.*, 1988), this *kappa* antagonist (Takemori *et al.*, 1988) was given concurrently with dynorphin A-(1-17). The modulation of *mu* agonist activity by DPDPE and dynorphin A-(1-17) was evaluated in 6 to 12 rats.

Statistics. Significant differences between treated and control responses were evaluated using a paired Student's *t* test at a confidence level of 95%; comparisons between animals in different groups were made using the Student's *t* test at the same CL.

Results

Agonist studies. Administration of *mu* agonists resulted in an inhibition of bladder contractions due to the central micturition reflex. The group of *mu* agonists were evaluated after i.c.v. administration of doses producing approximately equal suppression of bladder motility (about 20 min). The compounds were given at the following doses (most to least potent): sufentanil (0.002 nmol), etorphine (0.004 nmol), DAMGO (0.02 nmol), PL017 (0.03 nmol), morphine (0.08 nmol), normorphine (3.1 nmol), phenazocine (14.9 nmol), meperidine (176 nmol) and methadone (17.3 nmol). These doses were chosen to result in an approximately equal inhibition of bladder contractions. Administration of low doses of DPDPE (4.6 nmol) produced no observable changes in either the frequency or amplitude of bladder contractions. Higher doses (*i.e.*, greater than 15.5 nmol) produced marked and dose-related agonist effects with suppression of bladder activity for a duration of approximately 16.8 ± 1.3 min. Similarly, dynorphin A-(1-17) did not produce agonist effects at low doses (0.23 nmol) but did show dose-related inhibition of bladder motor activity at doses higher than 3.7 nmol. At the dose tested, ICI 174,864 (4.4 nmol) and nor-BNI (15 nmol) did not produce any agonist effects.

Modulation studies. The agonist effects of morphine and normorphine were consistently (7 of 7 and 8 of 10 animals tested, respectively) potentiated by a subagonist dose of DPDPE (4.6 nmol) (Fig. 1A, normorphine; table 1). Experiments with DPDPE modulation of etorphine produced somewhat inconsistent results, showing potentiation of 4 of 7 animals (table 1). In contrast, the agonist effects of DAMGO, PL017, meperidine, phenazocine, sufentanil and methadone were unaffected by DPDPE (fig. 1B, DAMGO; table 1). It should be noted that the modulating dose of DPDPE was chosen by extrapolation of the DPDPE agonist dose-response line in an effort to obtain occupation of receptors without production of a measureable effect.

Administration of ICI 174,864 at a dose (4.4 nmol) which antagonized agonist doses of DPDPE (Dray and Nunan, 1985; fig. 7), did not affect the suppression of bladder contractions produced by *mu* agonists, in agreement with the results of previous experiments with morphine and DAMGO (Dray and Nunan, 1984). Although the direct agonist effect of morphine was unchanged, the potentiation of the bladder effects of this agonist by DPDPE was prevented by coadministration of the selective *delta* antagonist, ICI 174,864 (Cotton *et al.*, 1984) (fig. 2; table 1). Administration of DPDPE produced a leftward displacement of the i.c.v. morphine dose-response curve (fig. 3). Additionally, the effects of a single dose of morphine (0.08 nmol) were significantly potentiated by three different doses of DPDPE which did not produce inhibition of the micturition contractions when given alone (fig. 4).

The agonist effects of morphine and normorphine were antagonized consistently and completely by doses of dynorphin A-(1-17) which did not produce agonist effects alone (fig. 5A, morphine; table 2). The dose-response line for morphine was displaced to the right by dynorphin A-(1-17) (fig. 6). In contrast, dynorphin A-(1-17) did not affect the agonist effects of DAMGO, PL017, meperidine, phenazocine, methadone or sufentanil (fig. 5B, PL017; table 2), a profile identical to that seen with DPDPE modulation of these *mu* agonists. Dynorphin A-(1-17) also did not antagonize agonist doses of DPDPE (fig. 7; table 2).

Administration of three doses of dynorphin A-(1-17) produced significant antagonism of morphine effects at the two highest dynorphin A-(1-17) doses, and an attenuation at the lowest dose (fig. 8). Furthermore, whereas administration of nor-BNI alone, at this dose (15 nmol), did not antagonize the effects of morphine, the antagonism of morphine by dynorphin was blocked in the presence of this *kappa* antagonist (fig. 9).

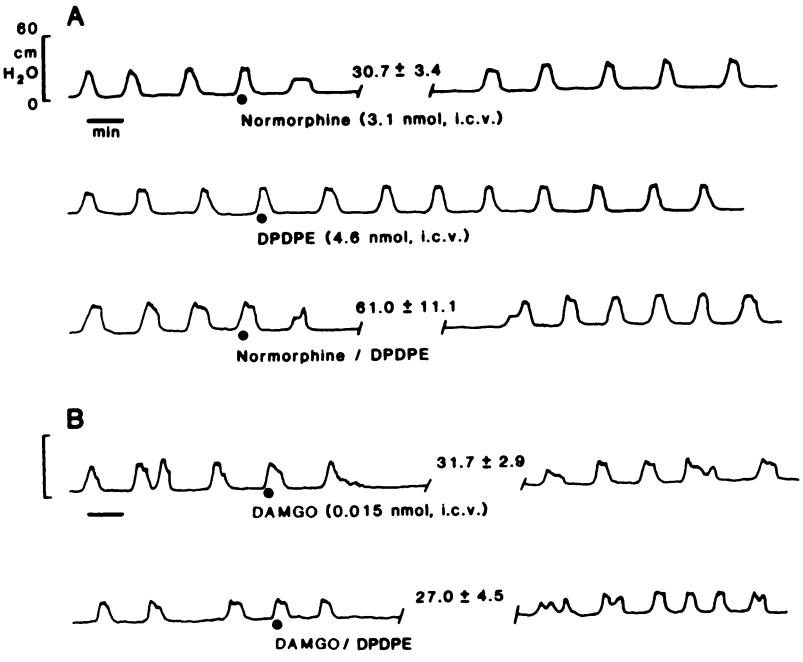

Fig. 1. Inhibition of bladder activity by i.c.v. administration of normorphine (3.1 nmol; A, top trace) and lack of effect of i.c.v. DPDPE (4.6 nmol; A, middle trace). Coadministration of normorphine and DPDPE resulted in a potentiation of the normorphine effect (A, bottom trace). In contrast, the effects of i.c.v. DAMGO (0.015 nmol; B, top) are not affected by coadministration of DPDPE (B, bottom).

TABLE 1

Effect of i.c.v. *mu*-agonists on suppression of bladder motility given alone or in the presence of i.c.v. DPDPE

Agonist	Dose	*n*	Response (mean ± S.E.)
	nmol		
Morphine	0.8	22	27.8 ± 2.2
DPDPE	4.6	20	0
Morphine + DPDPE	0.8/4.6	7	74.6 ± 6.0*
ICI 174,864	4.4	11	0
Morphine + ICI 174,864	0.8/4.4	8	27.4 ± 8.7
Morphine + DPDPE + ICI 174,864	0.8/4.6/4.4	11	23.5 ± 6.5
Normorphine	3.1	12	30.7 ± 3.4
Normorphine + DPDPE	3.1/4.6	10	61.0 ± 11.1*
Etorphine	0.004	14	16.9 ± 1.8
Etorphine + DPDPE	0.004/4.6	7	39.9 ± 7.5*
DAMGO	0.02	14	31.7 ± 2.9
DAMGO + DPDPE	0.02/4.6	7	27.0 ± 4.5
PLO17	0.03	14	17.9 ± 1.9
PLO17 + DPDPE	0.03/4.6	6	25.0 ± 7.3
Phenazocine	14.9	18	29.1 ± 1.5
Phenazocine + DPDPE	14.9/4.6	6	38.7 ± 10.3
Meperidine	176.2	11	20.8 ± 3.8
Meperidine + DPDPE	176.2/4.6	7	16.9 ± 3.5
Methadone	17.3	12	23.1 ± 2.1
Methadone + DPDPE	17.3/4.6	6	22.3 ± 4.7
Sufentanil	0.002	14	24.1 ± 1.6
Sufentanil + DPDPE	0.002/4.6	6	20.5 ± 5.0

* $P < .05$; Student's *t* test for paired data.

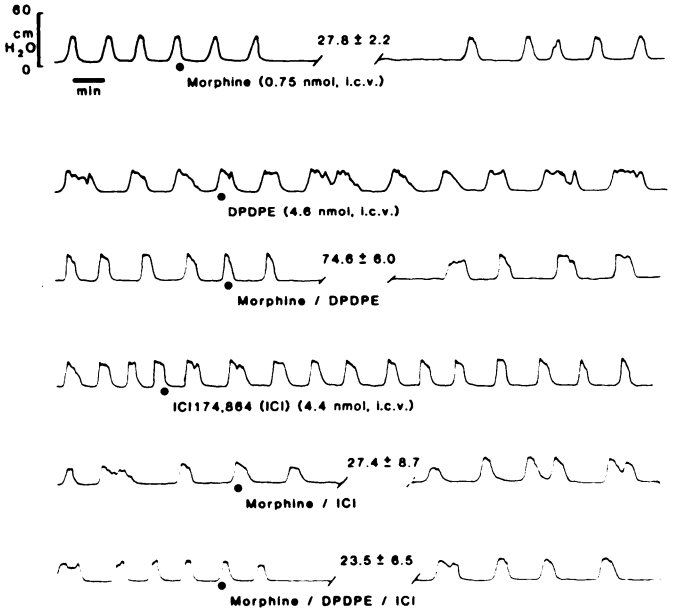

Fig. 2. Inhibition of bladder activity by i.c.v. morphine (0.75 nmol) and potentiation by coadministration of DPDPE. ICI 174,864 (ICI; 4.4 nmol) has no agonist effects when given alone. When ICI is given together with coadministration of morphine and DPDPE, the potentiation of i.c.v. morphine effect is prevented.

Surprisingly, the agonist effects of etorphine were potentiated significantly by dynorphin A-(1–17) (table 2).

Discussion

Previous studies using the micturition reflex of the anesthetized rat as a model of opioid function have indicated the

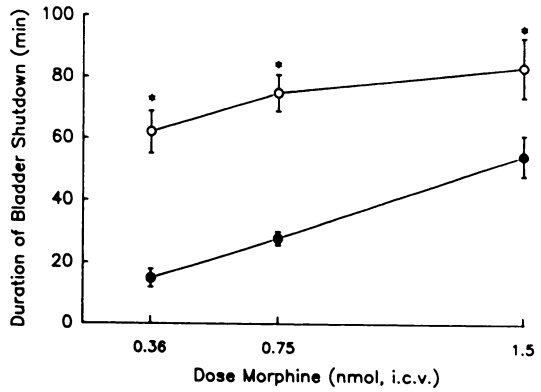

Fig. 3. Dose-response line for i.c.v. morphine inhibition of urinary bladder contractions in the absence (●) and in the presence of a subagonist dose of coadministered i.c.v. DPDPE (4.6 nmol). Asterisks indicate significance at $P < .05$ by the Student's *t* test.

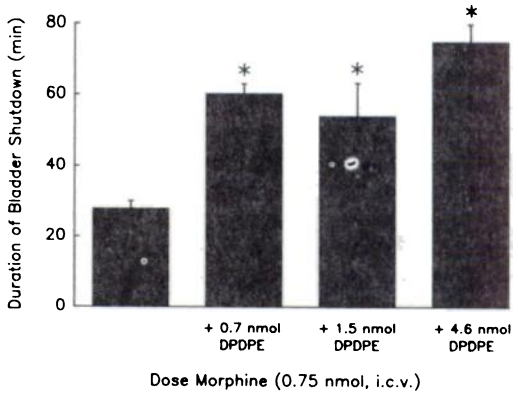

Fig. 4. Effects of i.c.v. morphine (0.75 nmol) on inhibition of bladder contractions in the absence and presence of different i.c.v. doses of DPDPE. Asterisks indicate significance at $P < .05$ by the Student's *t* test.

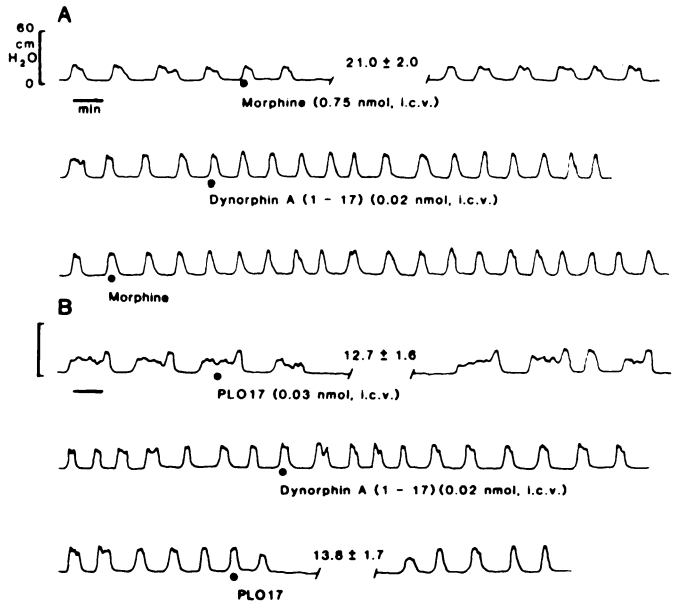

Fig. 5. Inhibition of bladder activity by i.c.v. morphine (0.75 nmol; A, top trace), absence of agonist effects of i.c.v. dynorphin A-(1–17) (0.02 nmol; A, middle trace) and antagonism of morphine effect when given 15 min after dynorphin A-(1–17) (A, bottom trace); lack of antagonism of PL017 (0.03 nmol; B, top trace) by pretreatment with dynorphin A-(1–17) (B, bottom trace).

TABLE 2

Effect of i.c.v. dynorphin A-(1–17) pretreatment on suppression of rat bladder activity induced by *mu* and *delta* agonists

Kappa Agonist	Compound	*n*	Control[a]	Treated[b]
			min ± *S.E.*	*min* ± *S.E.*
Dynorphin A	Morphine	5	21.8 ± 1.6	2.4 ± 2.4*
	Normorphine	6	24.2 ± 3.2	0*
	DAMGO	6	36.5 ± 5.9	38.2 ± 7.3
	PL017	6	12.7 ± 1.7	13.8 ± 1.7
	Phenazocine	6	26.7 ± 2.0	30.5 ± 3.4
	Meperidine	5	14.2 ± 5.0	20.6 ± 3.2
	Methadone	6	23.5 ± 3.9	23.7 ± 3.9
	Sufentanil	6	25.7 ± 2.8	17.2 ± 2.6
	Etorphine	6	22.7 ± 2.2	31.0 ± 3.4*
	DPDPE	5	16.8 ± 1.3	15.6 ± 2.3

[a] Duration of bladder shutdown.
[b] Duration of bladder shutdown after *kappa*-agonist.
* $P < .05$, Student's *t* test for paired data.

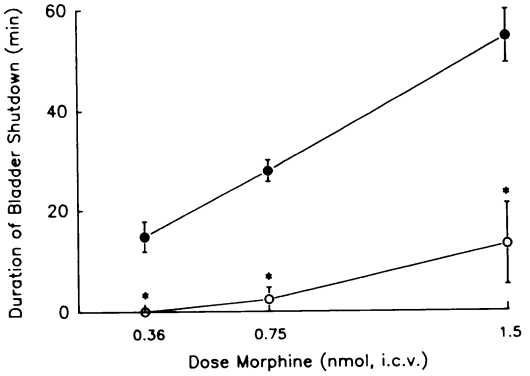

Fig. 6. Dose-response line for inhibition of bladder contractions by i.c.v. morphine in the absence (●) or in the presence of a pretreatment dose of i.c.v. dynorphin A-(1–17) (0.5 nmol). Asterisks indicate significance at $P < .05$ by the Student's *t* test.

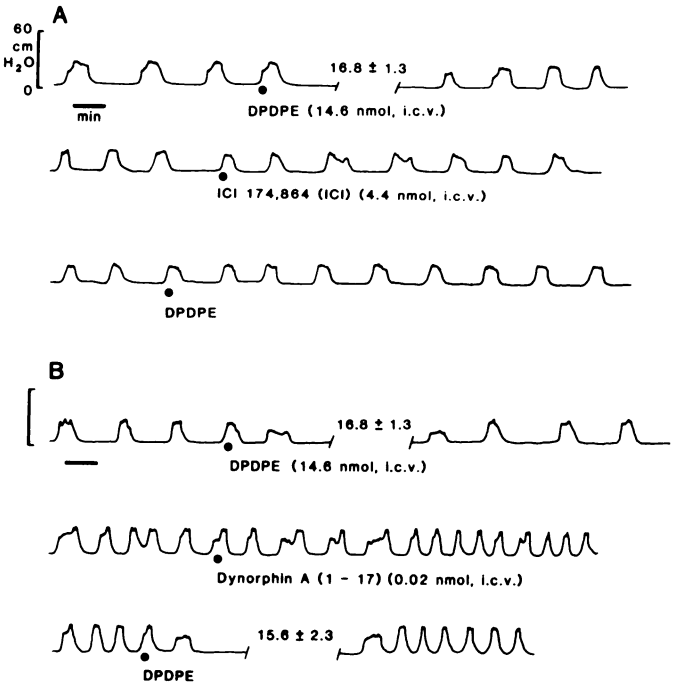

Fig. 7. Inhibition of bladder activity by i.c.v. DPDPE (14.6 nmol; A, top), lack of effect of ICI 174,864 (ICI) (4.4 nmol; A, middle trace) and antagonism of subsequent (+15 min) administration of DPDPE (A, bottom trace); failure of i.c.v. dynorphin A-(1–17) to antagonize the effects of DPDPE (B, bottom trace).

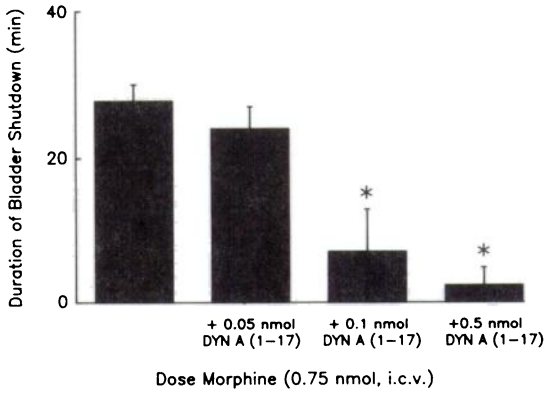

Fig. 8. Effects of i.c.v. morphine (0.75 nmol) on inhibition of bladder contraction in the absence or in the presence of three pretreatment doses of dynorphin (DYN) A-(1–17). Asterisks indicate significance at $P < .05$ by the Student's *t* test.

involvement of supraspinal *mu* and *delta*, but not *kappa*, opioid receptors in the direct central control of the volume-induced micturition reflex (Dray and Metsch, 1984a,b). These, and other (Dray and Nunan, 1984) studies have consistently shown that *mu* and *delta* agonists are efficacious in suppressing bladder motility, whereas most *kappa* agonists fail to have significant effects, until high doses are used (Dray and Metsch, 1984b; Sheldon *et al.*, 1987). In contrast to the results with ethylketocyclazocine and tifluadom, however, U50,488H, a compound believed to have high selectivity for the *kappa* receptor (Von Voigtlander *et al.*, 1983; James and Goldstein, 1984) does not produce a consistent inhibition of bladder motility even when given at very high doses (Dray and Metsch, 1984b; Sheldon *et al.*, 1987; present study). The present work has extended our previous observations on interactions between synthetic *kappa* agonists and *mu* agonists by using dynorphin A-(1–17), a compound thought to be an endogenous ligand for the opioid *kappa* receptor (Chavkin and Goldstein, 1981; Goldstein *et al.*, 1979). Our results indicate that, in this specific model, dynorphin A-(1–17) is more similar in profile to ethylketocyclazocine and tifluadom than to U50,488H, as large doses can suppress bladder motility. It seems possible that the agonist effects produced by ethylketocyclazocine, tifluadom and dynorphin A-(1–17) may be related to the relative lack of selectivity of these compounds for the *kappa* receptor. In this regard, ethylketocyclazocine, tifluadom and dynorphin A-(1–17) have all been shown to have significant affinity for the opioid *mu* site (Magnan *et al.*, 1982; Pfeiffer and Herz, 1982; Wood, 1984; Garzon *et al.*, 1983).

Recent work has demonstrated that, at doses below those producing agonist effects, compounds classified as *kappa* preferring can prevent the activity of some *mu* agonists both at supraspinal (Sheldon *et al.*, 1987) and spinal (Sheldon *et al.*, 1988) sites. The finding that *kappa* agonists can modulate the effects of morphine was not unexpected. Previous reports had indicated that, in some instances where there was no measureable agonist effect, compounds classified as *kappa* agonists could antagonize the effects of *mu* agonists. For example, Browne (1986) found that U50,488H antagonized the hyperactivity, but not the antinociception, associated with morphine in the mouse. Additionally, Wood and colleagues (Wood *et al.*, 1982; Wood, 1984) have shown that some *mu* agonist effects,

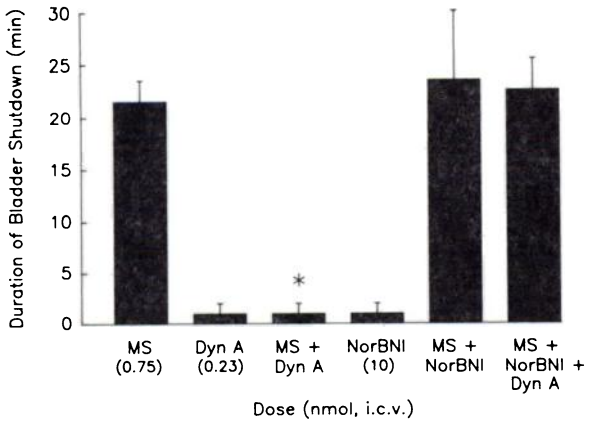

Fig. 9. Effects of i.c.v. morphine (MS; 0.75 nmol) on inhibition of bladder contraction alone, or after pretreatment with dynorphin (Dyn) A-(1–17) (0.5 nmol) or after pretreatment with Dyn A-(1–17) plus nor-BNI (15 nmol). Dyn A-(1–17) and nor-BNI did not produce significant inhibition of bladder contractions at these doses. Dyn A-(1–17) pretreatment significantly antagonized the effects of MS ($P < .05$, paired t test). The agonist effects of MS were unaltered in the presence of nor-BNI alone, however, the antagonism of MS by Dyn A-(1–17) was prevented in the presence of nor-BNI.

as well as *delta* agonist effects, are antagonized by *kappa* agonists. These investigators interpreted their data to suggest that *kappa* agonists were specific antagonists of a subtype of *mu* receptor (mu_2) (see Pasternak and Wood, 1986 for review). Thus, *kappa* agonists were suggested to be antagonists of the mu_2 site as well as antagonists of the *delta* site (Wood, 1984; Pasternak and Wood, 1986).

The issue of whether the modulation by dynorphin A-(1–17) of *mu* agonists such as morphine occurs *via* the *kappa* receptor, or possibly by actions at the *mu* receptor, can be addressed through several observations. First, in the present studies the morphine dose-response line was displaced to the right with decreased maximum effect by dynorphin A-(1–17), a pattern consistent with a noncompetitive interaction. Second, several doses of dynorphin were each able to attenuate the morphine effect, suggesting that dynorphin was indeed producing its effects by interacting with a specific receptor. Finally, administration of the *kappa* antagonist, nor-BNI (Takemori *et al.*, 1988), blocked the modulatory effects of dynorphin A-(1–17), but not the direct agonist effect of morphine at the antagonist dose tested. These findings all support an interaction of dynorphin A-(1–17) at the *kappa*, rather than the *mu*, receptor.

The present study demonstrates the modulatory profile of dynorphin A-(1–17) to be consistent with earlier observations made with this model, using synthetic *kappa* agonists such as U50,488H, tifluadom and ethylketocyclazocine; these synthetic *kappa* compounds and dynorphin A-(1–17), were found to antagonize the inhibition of the micturition reflex produced by i.c.v. administration of some, but not all, *mu* agonists (Sheldon *et al.*, 1987; present study). It should be noted that the present findings are supported specifically under the conditions of these experimental conditions. It may be possible that when given at higher doses, the nonmodulated *mu* agonists might be subject to the effects of these modulating substances [*i.e.*, dynorphin A-(1–17) or DPDPE]. Although this possibility cannot be excluded, it should be noted that the *mu* agonists tested were all given at equieffective doses.

The similarity in profile demonstrated for the putative endogenous *kappa* ligand in this model, dynorphin A-(1–17) (Goldstein *et al.*, 1979; Chavkin and Goldstein, 1981), suggests the possibility of a physiological modulatory interaction between *kappa-mu* receptors. Significantly, dynorphin A-(1–13) has been shown to antagonize the analgesic effects of morphine and of β-endorphin (Friedman *et al.*, 1981; Tulunay *et al.*, 1981). Furthermore, dynorphin A-(1–13) has been shown to antagonize analgesic effects produced by vasopressin in the mouse (Tulunay *et al.*, 1982), a finding which in light of the colocalization of these peptides in certain parts of the brain (Hollt *et al.*, 1980), provides additional evidence for a modulatory role of the endogenous *kappa* agonist in physiological processes (Tulunay *et al.*, 1982).

Our results with dynorphin A-(1–17) are in good agreement with previous work with the synthetic *kappa* agonist, showing that dynorphin A-(1–17) antagonized morphine and normorphine consistently, but did not affect the agonist actions of the *mu* agonists, DAGO, PL017, meperidine, phenazocine, and methadone and sufentanil. Thus, the *mu*-antagonist profile of dynorphin A-(1–17) is in complete agreement with those of tifluadom and ethylketocyclazocine, and differs from that of U50,488H only in that the latter agonist partially antagonized sufentanil and etorphine (Sheldon *et al.*, 1987). Furthermore, the *mu*-antagonist profile of dynorphin A-(1–17) agrees with that seen for U50,488H after administration by the intrathecal route, with only morphine and normorphine being antagonized (Sheldon *et al.*, 1988). Additionally, it is noteworthy that dynorphin A-(1–17) did not alter the agonist actions of the *delta* agonist, DPDPE, also in agreement with results of studies which demonstrated that U50,488H, tifluadom and ethylketocyclazocine did not antagonize this *delta* agonist (Sheldon *et al.*, 1987).

It is interesting to speculate on the mechanisms involved in the selective antagonism of morphine and normorphine by both synthetic and endogenous ligands for the *kappa* receptor. Although the *mu* agonists tested had differing potency, they were given at equieffective doses and appeared equiefficacious in this model. The relative selectivities of these agonists for the *mu* site, however, as well as the selectivity of the putative *kappa* agonists for the *kappa* receptor, differed substantially (Magnan *et al.*, 1982; Pfeiffer and Herz, 1982; Wood, 1984). Curiously, in both the present study and in our previous work (Sheldon *et al.*, 1987) the agonist effects of etorphine were potentiated by dynorphin A-(1–17) and by ethylketocyclazocine, respectively. Of the *mu* agonists examined, etorphine had the highest affinity for *kappa* binding sites (Magnan *et al.*, 1982). It seems possible, then, that by binding to *kappa* sites, ethylketocyclazocine and dynorphin A-(1–17) may reduce binding of etorphine to the *kappa* receptor, thus resulting in a relative increase in the concentration of this agonist at the *mu* receptor, resulting in an increased effect. The specificity of the effects observed and the occasional discrepancies may reflect the complex binding and pharmacokinetic properties of these diverse *mu* and *kappa* agonists. The reasons for the discrepancy observed with etorphine are unclear and await further investigation.

Selective antagonism of *mu* agonists by four different *kappa* agonists, however, might be explained by the possibility of subtypes of *mu* receptors, through which a group of chemically diverse *mu* agonists can produce identical effects. Thus, the *kappa* agonists tested act to prevent the actions of agonists at one population of *mu* receptors (*i.e.*, those sensitive, for example, to morphine and normorphine) whereas not affecting a second population of *mu* receptors (*i.e.*, those activated, for

example, by DAGO, PL017, meperidine, phenazocine, methadone and sufentanil). This possibility of *kappa* agonists being antagonists of certain populations of *mu* receptors (*i.e.*, mu_2 receptors) as well as antagonists of *delta* receptors, has been suggested by Pasternak and Wood (1986). Inconsistent with that hypothesis, however, is the observation of a failure to modulate agonist effects of DPDPE by either synthetic (Sheldon *et al.*, 1987, 1988) *kappa* agonists or dynorphin A-(1–17) (present study). That the effects of DPDPE are mediated through *delta* receptors in this endpoint is indicated by the selective antagonism of DPDPE, but not *mu* agonists, by ICI 174,864 (Dray and Nunan, 1984), as well as by the dose-response nature of the modulatory effect. In this regard, DPDPE displaced the morphine dose-response line to the left and several doses of DPDPE each potentiated the effects of morphine. Alternative explanations of the mechanisms involved in the modulation of *mu* agonist effects may stem from suggestions of complexed opioid receptors.

The concept of physical and functional coupling between opioid receptors has been suggested based on patterns of interactions between ligands having various types of selectivity (see Holaday *et al.*, 1985 for review). Thus, *kappa* agonists have been suggested to be antagonists of not only *mu* agonists, and of *delta* agonists (*e.g.*, Pasternak and Wood, 1986) but also of *delta* antagonists (Holaday and D'Amato, 1983; D'Amato and Holaday, 1984; Long *et al.*, 1984). Additionally, evidence from radioligand binding experiments suggest the possibility of interactions between *mu* and *kappa* receptors. For example, dynorphin A-(1–13) has been shown to inhibit opiate receptor binding in a noncompetitive manner *in vitro* (Garzon *et al.*, 1982) and Demoliou-Mason and Barnard (1986) have demonstrated an interaction between *mu* and *kappa* ligands in the rat brain. The suggestion of an opioid receptor complex is extended by the literature which demonstrates functional and physical interactions between *mu* and *delta* receptors (*e.g.*, Vaught and Takemori, 1979; Lee *et al.*, 1980; Barrett and Vaught, 1982; Vaught *et al.*, 1982; Rothman and Westfall, 1982a,b, 1983; Rothman *et al.*, 1983, 1984, 1987; Sheehan *et al.*, 1986; Porreca *et al.*, 1987; Heyman *et al.*, 1989a,b). From these collective observations, the possibility of a *mu-delta-kappa* receptor complex has been suggested (Holaday *et al.*, 1985).

From these suggestions, reports of potentiation of *mu* agonist effects by *delta* agonists (Vaught and Takemori, 1979; Lee *et al.*, 1980; Porreca *et al.*, 1987; Heyman *et al.*, 1989a,b) and our observations of antagonism of some, but not all, *mu* agonists by *kappa* agonists, we wondered if those *mu* agonists antagonized by *kappa* agonists were the same compounds that might be potentiated by *delta* agonists. As we had demonstrated previously in other endpoints that DPDPE can produce its effects at the *delta* receptor (Dray and Nunan, 1984; Heyman *et al.*, 1987; Porreca *et al.*, 1987) and that the modulatory effects of this synthetic analog were identical to those seen with Leu-enkephalin in antinociception (Vaught *et al.*, 1979; Porreca *et al.*, 1987), this compound was chosen for possible modulation of the group of *mu* agonists used in the present study.

Our results show that of the compounds studied in the present work, only the effects of morphine and normorphine are significantly potentiated by DPDPE (table 1). However, it is likely that further studies may reveal that other *mu* agonists might also fall into the *delta* modulated group in this model, as observations by Vaught and Takemori (1979) showed that the antinociceptive effects of levorphanol in mice were potentiated by Leu-enkephalin. The present work shows further that the potentiation but not the direct agonist effects of morphine and normorphine can be prevented by the selective *delta* antagonist, ICI 174,864 (Cotton *et al.*, 1984). The blockade of the modulatory effects of DPDPE, by ICI 174,864 strongly supports the concept of modulation occurring through a *delta* recptor. Additionally, it seems particularly significant that the agonist effects of DAMGO, PL017, meperidine, phenazocine, sufentanil, methadone or etorphine were unaffected by DPDPE. The selective modulation, although opposite in overall effect, is identical to that seen with dynorphin A-(1–17). It is also relevant to note that recent experiments examining DPDPE modulation of antinociception produced by a group of *mu* agonists showed that only the antinociception produced by morphine and normorphine, but not that induced by DAMGO, PL017, etorphine, phenazocine, sufentanil or meperidine could be potentiated (Heyman *et al.*, 1989b). The potentiation of morphine antinociception by DPDPE could be prevented by coadministration of ICI 174,864, again suggesting the direct involvement of the *delta* receptor in this modulatory process (Heyman *et al.*, 1989b). The concept of a *mu-delta-kappa* complex may be supported in two species by these observations on antinociception and inhibition of the micturition reflex. Thus, a functional, and possibly physical, complex may exist in which the effects of an endogenous *mu* agonist are modulated either positively or negatively by endogenous agonists at the *delta* and *kappa* sites, respectively. As the endogenous *mu* agonist remains to be identified, our use of many exogenous compounds may shed light on the nature of the *mu* receptors involved in physiological regulatory processes. The *mu* sites in this putative opioid receptor complex may be activated selectively by compounds such as morphine and normorphine, whereas *mu* sites existing independently from the putative complex may be activated by other types of agonists such as those examined here. Interestingly, observations of possible differences in *mu* receptors have also been made in isolated tissue preparations such as the guinea pig isolated ileum (Takemori and Portoghese, 1985; Ward *et al.*, 1986) and selective activation of these putative *mu* subtypes of recptors by peptide and nonpeptide *mu* agonists has been suggested (Ward *et al.*, 1986). In light of recent suggestions that the endogenous *mu* ligand may indeed be morphine or a morphine-like alkaloid (*e.g.*, Weitz *et al.*, 1987), the selective modulation of morphine and normorphine by four putative *kappa* agonists, and by DPDPE, seems particularly interesting.

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