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# Dynorphin-related peptides cause motor dysfunction in the rat through a non-opiate action

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**1** We compared effects on motor function of four peptides belonging to the dynorphin family – dynorphin-(1–17) (DYN-(1–17)), dynorphin-(1–13) (DYN-(1–13)), dynorphin-(1–8) (DYN-(1–8)) and α-neo-endorphin (αNE).

**2** After intrathecal administration, each of these peptides produced dose-related, flaccid, hindlimb paralysis, with the order of potency being DYN-(1–17) > DYN-(1–13) > αNE ≏ DYN-(1–8).

**3** This motor dysfunction was not reversed or blocked by the opiate receptor antagonist naloxone and was not produced by a variety of other κ-selective agonists.

**4** However, paralysis was produced by des-Tyr-dynorphin (DYN-(2–17)), which does not act at the opioid receptor.

**5** Taken together, the present studies show that dynorphin-related peptides, uniquely amongst opioids, produce motor dysfunction, an action which does not appear to be mediated by opioid receptors.

## Introduction

Dynorphin is a recently recognized endogenous opioid peptide which is found widely distributed within the central nervous system, in addition to its localization in the hypothalamic-pituitary axis (Gramsch, Höllt, Pasi, Mehraein & Herz, 1982; Watson, Khachaturian, Akil, Coy & Goldstein, 1982). It appears, moreover, to be an endogenous ligand for the κ-opioid receptor (Huidobro-Toro, Yoshimura, Lee, Loh & Way, 1981; Chavkin, James & Goldstein, 1982). Dynorphin has a variety of physiological effects, some of which are opiate receptor mediated and others which probably are not (Walker, Moises, Coy, Baldrighi & Akil, 1982). We have recently found that dynorphin-(1–17) (DYN-(1–17)) induces dose-related, partially reversible, hindlimb paralysis following intrathecal administration in the rat, an action not produced by δ- or μ-selective synthetic enkephalins or by β-endorphin (Faden & Jacobs, 1983). Thus, the paralysis produced by dynorphin appears to be relatively unique amongst opioids. It is now known that there exists a family of dynorphin-related peptides, each probably derived from the same pro-hormone, which have different concentrations within the central nervous system and different affinities for the opiate receptor (Kakidani, Furutani, Takahashi, Noda, Morimoto, Hirose, Asai, Inayama, Nakanishi & Numa, 1982; Weber, Evans & Barchas, 1982). In the present studies we compared the effects of DYN-(1–17), dynorphin-(1–13) (DYN-(1–13)), dynorphin-(1–8) (DYN-(1–8)) and α-neo-endorphin (αNE) on motor dysfunction in the hindlimbs following intrathecal administration in the rat. In addition, we have examined the potential role of opioid receptors in mediating the motor dysfunction by studying the ability of naloxone to modify this action of dynorphin, the ability of other κ-receptor agonists to produce this paralysis, and finally of the ability of des-Tyr-dynorphin (DYN-(2–17)) to simulate this effect.

## Methods

Male Sprague-Dawley rats (300 g) were anaesthetized with ketamine hydrochloride (50 mg kg$^{-1}$, i.m.) and sodium pentobarbitone (25 mg kg$^{-1}$, i.p.). Polyethylene tubing (PE 10) was placed in the spinal subarachnoid space and threaded to the level of the lumbar enlargement, using a modification (Faden & Jacobs, 1983) of the method of Yaksh & Rudy (1976). Twenty-four hours later, drugs were infused in a total volume of 23 μl (artificial CSF) over 2 min,

an infusion method which we have previously shown produces no motor changes in saline-treated animals and no forelimb weakness in dynorphin-treated animals (Faden & Jacobs, 1983).

Four separate studies were performed. Study 1 compared the effect of DYN-(1–17), DYN-(1–13), DYN-(1–8) and αNE on hindlimb function after intrathecal administration. The following compounds (Peninsula) were administered: DYN-(1–17) (30 nmol, $n = 9$); DYN-(1–13) (10, 30, 50 and 100 nmol, each $n = 6$); DYN-(1–8) (30, 50 and 100 nmol, $n = 2$, 4 and 5, respectively); and αNE (30, 50 and 100 nmol, each $n = 5$). Six additional animals received an equal volume of artificial CSF alone and served as controls. Study 2 examined the ability of the opiate receptor antagonist naloxone (Endo Laboratories) to modify the paralytic effect of DYN-(1–17) (30–50 nmol, Bachem). Thirteen animals received naloxone i.v. at 15 min after intrathecal DYN-(1–17) (30 nmol): 10 rats received a dose of 2 mg kg$^{-1}$ and three rats a dose of 10 mg kg$^{-1}$. Another 19 rats received naloxone i.v. as pretreatment (5 min before DYN-(1–17) infusion) at doses of 2 mg kg$^{-1}$ ($n = 9$), 10 mg kg$^{-1}$ ($n = 8$) or 20 mg kg$^{-1}$ ($n = 2$). Finally, six animals received naloxone pretreatment intrathecally (100 μg) 15 min

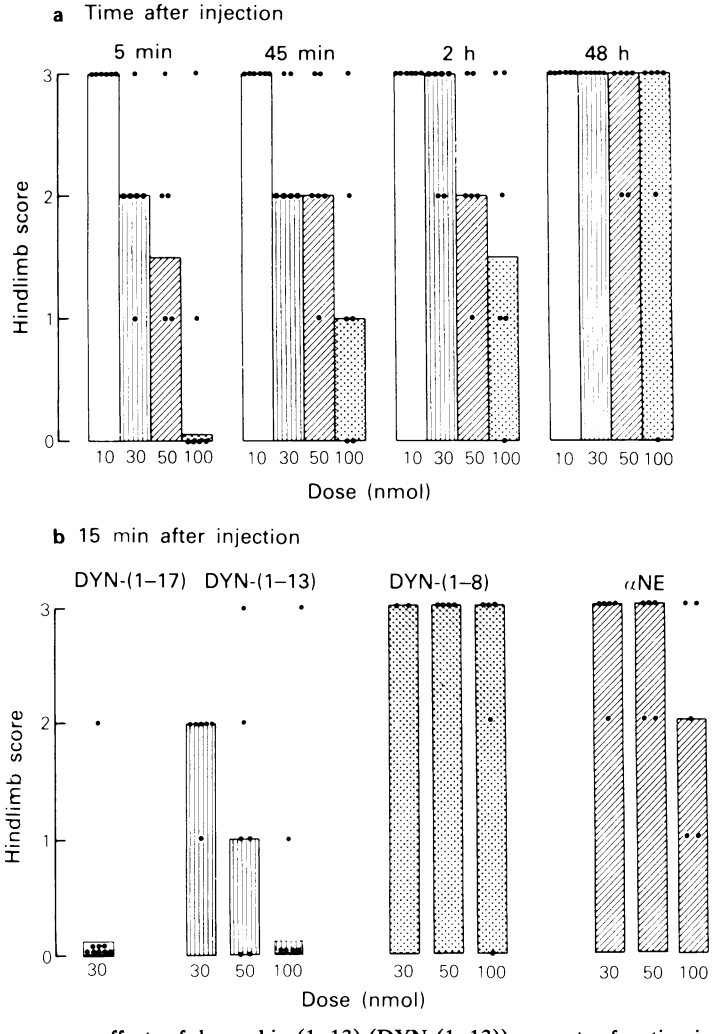


**Figure 1** (a) Dose response effects of dynorphin-(1–13) (DYN-(1–13)) on motor function in the rat following intrathecal administration. DYN-(1–13) produces dose-related, partially reversible paralysis of hindlimb function, with peak effects at approximately 50 nmol. (b) Comparison of effects of DYN-(1–17), DYN-(1–13), DYN-(1–8) and α-neo-endorphin (αNE) motor function in the rat. Each peptide produces hindlimb dysfunction with order of potency being DYN-(1–17) > DYN-(1–13) > αNE ≏ DYN-(1–8).

**Table 1** Effect of naloxone treatment on hindlimb function after intrathecal infusion of dynorphin-(1–17) (DYN-(1–17))

**a** *Post-treatment with intravenous naloxone*† ($n = 13$)

| *Neurological score* | *Pre-naloxone* | *Post-naloxone (2 mg kg$^{-1}$, i.v.)* | *Post-naloxone (10 mg kg$^{-1}$, i.v.)* |
|---|---|---|---|
| 0 | xxxxxxxxxx | xxxxxx | xxx |
| 1 | xxx | xxxx | — |
| 2 | — | — | — |
| 3 | — | — | — |

**b** *Pretreatment with intravenous naloxone*‡ (total $n = 28$)

| *Neurological score* | *Controls* | *Naloxone (2 mg kg$^{-1}$, i.v.)* | *Naloxone (10 mg kg$^{-1}$, i.v.)* | *Naloxone (20 mg kg$^{-1}$, i.v.)* |
|---|---|---|---|---|
| 0 | xxxx | xx | xxxxx | xx |
| 1 | xxx | xxxxx | x | — |
| 2 | x | x | — | — |
| 3 | x | x | xx | — |

**c** *Pretreatment with intrathecal naloxone** (total $n = 15$)

| *Neurological score* | *Controls* | *Naloxone (100 μg, i.t.)* |
|---|---|---|
| 0 | xxxx | xxxx |
| 1 | xx | xx |
| 2 | xx | — |
| 3 | x | — |

†Pre-naloxone scores at 15 min after DYN-(1–17) infusion (30 nmol); post-naloxone scores 15 min later (i.e., 30 min after DYN-(1–17) infusion); x = individual animal scores: 0 = paraplegia; 1 = severe paraparesis; 2 = mild paraparesis; and 3 = normal motor function.
‡Naloxone administered i.v. 5 min before DYN-(1–17) infusion (30 nmol); neurological score at 30 min after DYN-(1–17).
*Naloxone administered i.t. 15 min before DYN-(1–17) infusion (50 nmol); neurological score at 30 min after DYN-(1–17) infusion.

before DYN-(1–17) infusion. Study 3 evaluated the effect of other κ-selective agonists on hindlimb function after intrathecal (i.t.) infusion. These agonists included bremazocine (Romer, Buscher, Hill, Maurer, Petcher, Welle, Bakel & Akkerman, 1980) at a dose of 100 nmol ($n = 3$); MRZ ([(-)5,9-dimethyl - $2^1$ - hydroxy - 2 - (2 - methoxypropyl) - 6,7 - benzomorphan hydrobromide]; Merz & Stockhaus, 1979) at doses of 50 nmol ($n = 2$) and 100 nmol ($n = 3$); and U-50488H (*trans* - 3,4 - dichloro - N - methyl - N - (2 - (1 - pyrrolidinyl) cyclohexyl) - benzine acetamine; Piercey, Lahti, Schroeder, Einspahr & Barsuhn, 1982) at doses of 50 nmol ($n = 2$) and 100 nmol ($n = 6$). Study 4 compared the effect of DYN-(1–17) (Bachem) and DYN-(2–17) (kindly provided by Dr Coy) on hindlimb function following equal-dose i.t. infusions: DYN-(1–17) (30 nmol, $n = 9$; 50 nmol, $n = 9$) and DYN-(2–17) (30 nmol, $n = 10$; 50 nmol, $n = 10$).

Hindlimb neurological function was evaluated over a 48 h period after infusion. Neurological function was graded using a 4-point ordinal scale as follows: 0 = paraplegia; 1 = severe paraparesis; 2 = mild paraparesis with ability to walk; and 3 = normal motor function. Differences in neurological function among the groups were compared utilizing Mann-Whitney U-Tests.

## Results

As we have previously shown for DYN-(1–17), infusion with DYN-(1–13) produced dose-related, flaccid, hindlimb paralysis appearing within the first 10 min post-infusion (Figure 1a). This motor dysfunction was absent at 10 nmol, mild with 30 nmol and moderately severe with doses of 50 and 100 nmol (Figure 1a). In contrast, DYN-(1–17) at a dose of 30 nmol produced complete paralysis in eight of nine animals at 15 min post-injection (Figure 1b). DYN-(1–8) produced no effects at 50 nmol and mild transitory changes at 100 nmol (Figure 1b). Similarly, αNE produced no change at 30 nmol and minimal dose-related changes at 50 and 100 nmol (Figure 1b).

**Table 2** Effect of intrathecal infusions of κ-agonists on hindlimb function in the rat†

| *Neurological score* | *Bremazocine* (100 nmol) | *MRZ* (50 nmol) | *MRZ* (100 nmol) | *U-50488H* (50 nmol) | *U-50488H* (100 nmol) |
|---|---|---|---|---|---|
| 0 | — | — | — | — | — |
| 1 | — | — | — | — | — |
| 2 | — | — | — | — | — |
| 3 | xxx | xx | xxx | xx | xxxxxx |

†Each x represents individual animal score at 30 min after infusion.

Therefore, the order of potency was DYN-(1–17) > DYN - (1–13) ➤ αNE DYN ≈ (1–8). Animals treated with artificial CSF alone showed no change in motor function; thus, the motor changes produced by DYN-(1–17) and DYN-(1–13) were significantly (each $P < 0.05$) different from controls.

Although nociceptive responses were absent (to hindlimb or tail pinch) in animals with severe paraparesis, the effects on pain systems and motor systems were frequently dissociated: some animals showed entirely normal motor function with absent nociceptive responses, whereas others showed motor dysfunction with intact nociceptive responses.

Hindlimb paralysis induced by dynorphin was not modified significantly by the opiate-receptor antagonist naloxone, whether naloxone was given i.v. or i.t., as pretreatment, or i.v. as post-treatment (Table 1). Naloxone doses utilized were quite high: 100 μg i.t., and up to 10–20 mg $kg^{-1}$ i.v. In contrast to the effects of dynorphin peptides, hindlimb paralysis was not produced by other κ-selective agonists administered i.t., even up to doses of 100 nmol (Table 2). Moreover, DYN-(2–17) (des-Tyr-dynorphin), a peptide which is not active at the opiate receptor but which has physiological actions (Walker *et al.*, 1982), produced hindlimb paralysis which was similar to that resulting from DYN-(1–17) (Table 3). Although not quite as potent in this regard as DYN-(1–17), the potency of DYN-(2–17) in eliciting paralysis exceeded that of the other dynorphin-peptides (compare Table 3 and Figure 1b).

## Discussion

Several groups have now shown that dynorphin produces hindlimb paralysis following intrathecal administration in the rat (Hermann, 1982; Przewlocki, Shearman & Herz, 1983; Faden & Jacobs, 1983). Hermann (1982) observed paralysis at doses above 10 nmol and indicated that this motor dysfunction was 'irreversible' at doses above 20 nmol. Przewlocki *et al.* (1983) found motor paralysis in four out of six rats given 4.7 nmol dynorphin, and all rats given 23.3 or 46.6 nmol, but not rats given up to 81.4 nmol αNE or up to 51 nmol DYN-(1–8); they did not mention the duration of the paralysis produced by dynorphin. Neither of these groups quantified the dynorphin-induced motor dysfunction. We have shown (Faden & Jacobs, 1983) that dynorphin-induced paralysis is dose-related and, largely, spontaneously reversible over a 48 h period; motor dysfunction was scored by several observers using a 4-point ordinal scale. At high doses (50 nmol), however, one-third of the animals developed paralysis that was non-reversible.

The present studies demonstrate that dynorphin-induced paralysis is produced by other peptides of the dynorphin family, with the order of potency being DYN-(1–17) > DYN-(1–13) ➤ αNE ≈ DYN-(1–8) (Figure 1). Since dynorphin-related peptides appear to be endogenous ligands for the κ-receptor (Huidobro-Toro *et al.*, 1981; Chavkin *et al.*, 1982), and the κ-receptor appears to be the predominant type of opioid receptor in the spinal cord of the rat

**Table 3** Comparison of intrathecal infusion of dynorphin-(1–17) (DYN-(1–17)) and dynorphin-(2–17) (DYN-(2–17)) on hindlimb function in the rat†

| *Neurological score* | *DYN-(1–17)* (30 nmol) | *DYN-(2–17)* (30 nmol) | *DYN-(1–17)* (50 nmol) | *DYN-(2–17)* (50 nmol) |
|---|---|---|---|---|
| 0 | xxxx | xx | xxxx | xxxx |
| 1 | xxx | x | xx | — |
| 2 | x | xxxxxx | xx | xxxxx |
| 3 | x | x | x | x |

†Each x represents individual animal scores at 30 min after infusion.

(Traynor, Kelley & Rance, 1982), the above observations are consistent with the conclusion that dynorphin-induced paralysis may result from actions mediated by the κ-receptor. On the other hand, DYN-(2–17), which does not act at the opiate receptor (Chavkin & Goldstein, 1981), has potent physiological activity (Walker *et al.*, 1982). To distinguish these alternative mechanisms by which dynorphin might exert its paralytic effects, three parallel studies were performed: (1) to examine whether dynorphin paralysis could be modified by the opiate-receptor antagonist naloxone; (2) to determine whether other κ-selective agonists produced such paralysis; and (3) to observe whether des-Tyr-dynorphin could induce motor dysfunction.

Dynorphin-induced motor dysfunction was not modified by intravenous naloxone, administered either as pretreatment or post-treatment, up to doses of 10–20 mg $kg^{-1}$ (Table 1). Similarly, naloxone administered intrathecally as pretreatment at a dose of 100 μg failed to modify this dynorphin response. These findings are consistent with those of Hermann (1982) who observed that dynorphin paralysis was not reversed by naloxone up to a dose of 32 mg $kg^{-1}$ s.c. In contrast, Przewlocki *et al.* (1983) found that hindlimb paralysis produced by dynorphin at a dose of 4.7 nmol was blocked by naloxone pretreatment at a dose of 10 mg $kg^{-1}$ but not at 1 mg $kg^{-1}$. However, neither Hermann nor we observed significant motor dysfunction with doses at or below 10 nmol.

Other κ-selective opioid agonists (bremazocine, MRZ or U-50488H) failed to produce any motor dysfunction when administered intrathecally at doses up to 100 nmol (Table 2). In contrast, DYN-(2–17) caused hindlimb paralysis, with a potency between that of DYN-(1–17) and DYN-(1–13) (Table 3, Figure 1). Paralysis after DYN-(2–17) was dose-related and persisted up to 48 h after the highest dose (50 nmol); 2 of 10 such animals showed severe irreversible paralysis. Similarly, Przewlocki *et al.* (1983) observed transitory motor dysfunction after relatively high dose infusions (35.1 nmol) of DYN-(2–13). Taken together, these findings strongly support the conclusion that the dynorphin-induced paralysis does not result from actions at opioid receptors. Rather, this motor dysfunction appears to reflect a non-opiate receptor mediated, pharmacological action of this class of peptides, similar to other effects, including posturing and electroencephalographic changes, which have recently been observed after intracerebroventricular administration (Walker *et al.*, 1982).

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