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Long-lasting antinociceptive effects of a novel dynorphin analogue, Tyr-D-Ala-Phe-Leu-Arg ψ (CH_2NH) Arg-NH_2, in mice

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1 Tyr-D-Ala-Phe-Leu-Arg ψ (CH_2NH) Arg-NH_2 (SK-9709) is a dynorphin derivative in which the peptide bond was replaced with a ψ (CH_2NH) bond. In the present study, the antinociceptive effects of SK-9709 were determined in an acetic acid-induced writhing test and a hot-plate test.

2 In the acetic acid-induced writhing test, significant antinociceptive effects were observed after subcutaneous (s.c.), intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection of SK-9709, with maximal effects at 120, 30 and 15 min, respectively. The antinociceptive effects were dose-dependent and ED_{50} values (range of 95% confidence limits) after s.c., i.c.v. and i.t. injection were 1.36 (0.61–3.02) μmol kg^{-1}, 2.11 (1.18–3.79) and 0.79 (0.61–1.03) nmol per mouse, respectively.

3 The effects of SK-9709 (s.c., i.c.v. and i.t.) were reversed by the opioid receptor antagonist naloxone (1.36 μmol kg^{-1}, s.c.). The effects of SK-9709 (s.c.) were also reversed by the selective μ-opioid receptor antagonist β-funaltrexamine (4.7 nmol per mouse, i.c.v.), and κ-opioid receptor antagonist nor-binaltorphimine (4.9 nmol per mouse, i.t.).

4 In the hot-plate test, the antinociceptive effect of SK-9709 (s.c., i.c.v. and i.t.) was also dose-dependent with the maximal peak effect at 120, 15 and 15 min similarly to the acetic acid-induced writhing test. The antinociceptive effects were dose-dependent and ED_{50} values (range of 95% confidence limits) after s.c., i.c.v. and i.t. injection were 39.1 (5.4–283.0) μmol kg^{-1}, 6.5 (4.0–10.7) and 7.4 (5.0–11.0) nmol per mouse, respectively.

5 These findings indicated that systemically administered SK-9709 produced long-lasting antinociceptive effects and these effects were mediated by both supra-spinal μ- and spinal κ-opioid receptors.

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Keywords: SK-9709; nor-binaltorphimine; acetic acid-induced writhing test; hot-plate test; μ-opioid receptor; κ-opioid receptor

Abbreviations: β-FNA, β-funaltrexamine; DAMGO, [D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; E-2078, [N-methyl-Tyr^1, N-methyl-Arg^7-D-Leu^8] dynorphin A-(1–8) ethylamide; nor-BNI, nor-binaltorphimine; % MPE, percentage of the maximum possible effect; SK-9709, Tyr-D-Ala-Phe-Leu-Arg ψ (CH_2NH) Arg-NH_2; U-50,488H, trans-(±)3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]-cyclohexyl)benzeneacetamide methanesulphonate salt

Introduction

Dynorphin A-(1–17) is an endogenous neuropeptide acting on κ-opioid receptors (Chavkin *et al*., 1982). This peptide was first isolated from the pituitary and gut (Goldstein *et al*., 1979), and high concentrations were detected in the dorsal horn of the spinal cord where primary processing of primary afferent nociceptive information is known to occur (Millan *et al*., 1986). Dynorphin A peptides show analgesic properties and may be useful in the management of pain (Hooke *et al*., 1995; Smith & Lee, 1988). However, natural dynorphin A peptides are susceptible to enzymatic degradation *in vitro* (Goldstein *et al*., 1979; Young *et al*., 1987). Studies have also indicated that this peptide and its congeners may attenuate the behavioural symptoms of the opioid withdrawal syndrome in morphine-dependent rodents (Takemori *et al*., 1992; 1993) and improve learning and memory deficits in mice and rats (Hiramatsu *et al*., 1995; 1996). Therefore, it may be useful as a therapeutic agent for the treatment of dependency and learning and memory impairments in man (Takemori *et al*., 1992; 1993; Hiramatsu *et al*., 1995; 1996; 1998). Dynorphin A peptides undergo proteolytic cleavage in a biological matrix to form a variety of biotransformation products (Chou *et al*., 1996). Corbett *et al*. (1982) suggested that dynorphin A-(1–8) and dynorphin A-(1–9) were ligands for not only the κ-opioid receptors but also the μ-opioid receptors.

To stabilize dynorphin A peptides against enzymatic degradation, a group of researchers synthesized a variety of dynorphin A fragments (Tachibana *et al*., 1988). In the search for a stable dynorphin fragment, the degradation of dynorphin A in mouse serum and mouse brain homogenate was examined, and the Arg^6-Arg^7 and Pro^{10}-Lys^{11} bonds, in addition to the well-known Tyr^1-Gly^2 bond, were found to be cleaved in the brain (Tachibana *et al*., 1988). The substitution of either Gly^2 by D-Ala^2 or L-Arg by D-Arg protects dynorphin A against enzymatic degradation, but also reduces

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κ-selectivity (Chavkin & Goldstein, 1981; Gairin *et al*., 1988). In addition, the large molecular weight of dynorphin could be a disadvantage, as it may prevent passage through the blood–brain barrier when given systemically. Due to these disadvantages, few dynorphin fragments have demonstrated sufficient analgesic or other pharmacological effects. Tachibana *et al*. (1988) evaluated several synthesized dynorphin A fragments and studied their stability, receptor-binding properties and biological activities, and finally arrived at [N-methyl-Tyr[1], N-methyl-Arg[7]-D-Leu[8]] dynorphin A-(1–8) ethylamide, designated as E-2078. Studies showed that E-2078 was stable against enzymatic degradation (Nakazawa *et al*., 1990; Yu *et al*., 1997a) and crossed the blood-brain barrier in rhesus monkeys (Yu *et al*., 1997b). E-2078 bound to κ-opioid receptors similarly to dynorphin A-(1–17) (Yoshino *et al*., 1990), and exhibited a more potent analgesic effects than morphine (Nakazawa *et al*., 1991). This enhanced analgesic action was attributed to its stability against enzymatic degradation (Nakazawa *et al*., 1990).

The newly synthesized peptide Tyr-D-Ala-Phe-Leu-Arg ψ (CH_2NH) Arg-NH_2 (SK-9709) may also be a very stable fragment. Replacement of the peptide bond by the ψ (CH_2NH) bond has been shown to be an effective modification for the design of antagonists of various biologically active peptides. This modification confers higher resistance to enzymatic degradation (Ambo *et al*., 1995). This peptide was also designed based on the supposition that a 6-amino acid peptide may pass through the blood–brain barrier and that it would therefore be effective.

In the present study, we examined the antinociceptive effects of SK-9709, in comparison with morphine as a μ-opioid receptor agonist, U-50,488H as a selective κ-opioid receptor agonist and E-2078 as a potent dynorphin A fragment, following s.c., i.c.v. and i.t. injection, using the acetic acid-induced writhing test as a chemical stimulus model and the hot-plate test as a heat stimulus model.

Methods

Animals

Seven- to eight-week-old male ddY mice and guinea-pigs (Japan SLC, Shizuoka, Japan) were housed in transparent plastic cages in a regulated environment (24±1°C, 55±5% humidity), under a 12 h light–dark cycle (light on 0800–2000 h), with free access to food and water.

Drugs and injections

Tyr-D-Ala-Phe-Leu-Arg ψ (CH_2NH) Arg-NH_2 (SK-9709) was synthesized in our laboratory (Prof Y. Sasaki, Tohoku College of Pharmacy, Sendai, Japan). E-2078 ([N-methyl-Tyr[1], N-methyl-Arg[7]-D-Leu[8]] dynorphin A-(1–8) ethylamide) was kindly supplied by Eizai Co. Ltd (Ibaragi, Japan). Morphine hydrochloride (Shionogi, Osaka, Japan), U-50,488H (trans-(±)3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]-cyclohexyl) benzeneacetamide methanesulphonate salt (Sigma, St. Louis, MO, U.S.A.), naloxone hydrochloride (Endo Lab., U.S.A.), β-funaltrexamine (β-FNA, Research Biochemicals International, MA, U.S.A.), nor-binaltorphimine (nor-BNI, Research Biochemicals International), DAMGO ([D-Ala[2], (Me)Phe[4],Gly(ol)[5]] enkephalin) and DPDPE ([D-Pen[2],D-Pen[5]] enkephalin) were used. Drugs were dissolved in 0.9% saline and administered subcutaneously (s.c.) in a volume of 0.1 ml per 10 g body weight, intracerebroventricularly (i.c.v.) or intrathecally (i.t.) in a volume of 5 μl per mouse. I.c.v. and i.t. injections were performed by the methods of Haley & McCormick (1957) and Hylden & Wilcox (1980), respectively. Control mice were given 0.9% saline as vehicle alone.

Acetic acid-induced writhing test

The writhing test was conducted 30, 60, 120, 180 and 240 min after s.c. injection, or 15, 30, 60 and 120 min after i.c.v. or i.t. injection. Mice were treated with 0.7% acetic acid solution (i.p.) 10 min before writhing test, and then numbers of writhing responses were counted for 10 min. Antinociception was quantified as per cent inhibition using the following formula:

$$\%\ \text{Inhibition} = [(\text{control responses} - \text{test responses})/\text{control responses}] \times 100 \quad (1)$$

% Inhibition was calculated for each dosage. Dose-response curves were generated using at least three doses of test drugs. ED_{50} values were determined by log–probit analysis, and 95% confidence limits were determined using the method of Litchfield & Wilcoxon (1949).

Hot-plate test

A mouse was placed on a steel plate maintained 55±0.5°C and the time (in seconds) taken until it licked its hind paw or jumped was recorded as the response latency. Each mouse was tested twice, with 15-min intervals, before drug injection. Mice that showed control latencies of less than 10 s were used. The cut-off time was 15 s. The antinociceptive effect after injection of the drugs was calculated per animal with the following formula:

$$\%\ \text{MPE (percentage of the maximum possible effect)} = [(\text{latency with test drugs} - \text{control latency})/(\text{cut off latency} - \text{control latency})] \times 100 \quad (2)$$

Each mouse was tested 30, 60, 120, 180 and 240 min after s.c. injection of SK-9709 and E-2078. In the case of i.c.v. or i.t. injection of SK-9709, response latency was evaluated 15, 30, 45 and 60 min after injection. Other compounds were evaluated 15, 30, 45, 60, 75 and/or 90 min after s.c., i.c.v. and i.t. injection. Per cent MPE of each animal was then calculated.

Receptor binding assay

The receptor binding assay was performed by the method described previously (Fujita *et al*., 1990; Ambo *et al*., 1995). [^{3}H]-U-69,593, [^{3}H]-DAMGO and [^{3}H]-DPDPE were used as κ-, μ- and δ-opioid radioligands, respectively. Binding assays for μ- and δ-opioid receptors were carried out by incubating aliquots of the crude rat brain synaptosomal fraction (600 μg protein ml^{-1}) in an assay

mixture containing 500 μg of bovine serum albumin, 50 μg of bacitracin, 10 μg of bestatin, 20 μg of soybean trypsin inhibitor, and 2 nM radioligand in a final volume of 500 μl (50 mM Tris-HCl buffer at pH 7.4). The κ-opioid receptor-binding assay was performed with guinea-pig brain homogenate. Non-specific binding was determined in the presence of excess unlabelled ligand. After incubation for 60 min at 25°C, the content of each tube was filtered through GF/B filters (pre-soaked in 0.1% polyethyleneimine). Filters were counted in a Beckman liquid scintillation counter after overnight extraction with liquid scintillation fluid. The IC_{50} values were obtained from log dose-displacement curves. Competitive inhibition constants (K_i) were derived from the IC_{50} values based on the equations of Cheng & Prusoff (1973). The K_d values of [^{3}H]-U-69,593, [^{3}H]-DAMGO and [^{3}H]-DPDPE used are 0.46, 0.73 and 1.00 nM, respectively.

Statistical analysis

All data were expressed as means ± s.e.mean and analysed by one-way analysis of variance (ANOVA). Further statistical analyses for *post hoc* comparisons were performed with Bonferroni's test. The criterion for statistical significance was $P<0.05$ in all evaluations.

Results

Antinociceptive effects in the acetic acid-induced writhing test

In the acetic acid-induced writhing test, control mice showed about 20 writhing responses during the 10-min observation period from 10 min after injection of 0.7% acetic acid solution. A significant antinociceptive effect was observed after s.c. injection of SK-9709, and the peak effect was seen at 120 min. The antinociceptive effect persisted until 180 min and returned to the control level at 240 min (Figure 1A). When SK-9709 was administered i.c.v., the peak effect was observed at 30 min, and returned to normal by 120 min (Figure 1B). A similar time course was observed after i.t. injection, with the peak effect at 15 min, and the antinociceptive effect gradually returning to the control level (Figure 1C).

The dose-related antinociceptive effects were evaluated after s.c., i.c.v. and i.t. injection of SK-9709. A significant effect was obtained when SK-9709 was administered s.c. at a dose of 1.93 μmol kg^{-1} (Figure 2A). Significant effects were obtained after i.c.v. and i.t. injection of SK-9709 at doses of 2.5 and 5.0 nmol per mouse (Figure 2B), and 0.94 and 1.25 nmol per mouse (Figure 2C), respectively. The ED_{50} values (range of 95% confidence limits) after s.c., i.c.v. and i.t. injection were 1.36 (0.6–3.02) μmol kg^{-1}, 2.11 (1.18–3.79) and 0.79 (0.61–1.03) nmol per mouse, respectively (Table 1).

The antinociceptive effects were compared with those of morphine as a μ-opioid receptor agonist, U-50,488H as a selective κ-opioid receptor agonist and E-2078 as a dynorphin A-(1–8) fragment after s.c., i.c.v. and i.t. injection. The order of potencies of the antinociceptive effects was E-2078 > morphine > = U-50,488H and SK-9709 for s.c. injection, morphine > E-2078 > > SK-9709 > > U-50,488H for i.c.v. injection, and E-2078 > morphine > > SK-9709 > U-50,488H for i.t. injection (Table 1). SK-9709 was less potent than morphine with relative values of 0.81 (s.c), 0.03 (i.c.v) and 0.01 (i.t.), but was more potent than U-50,488H (Table 1).

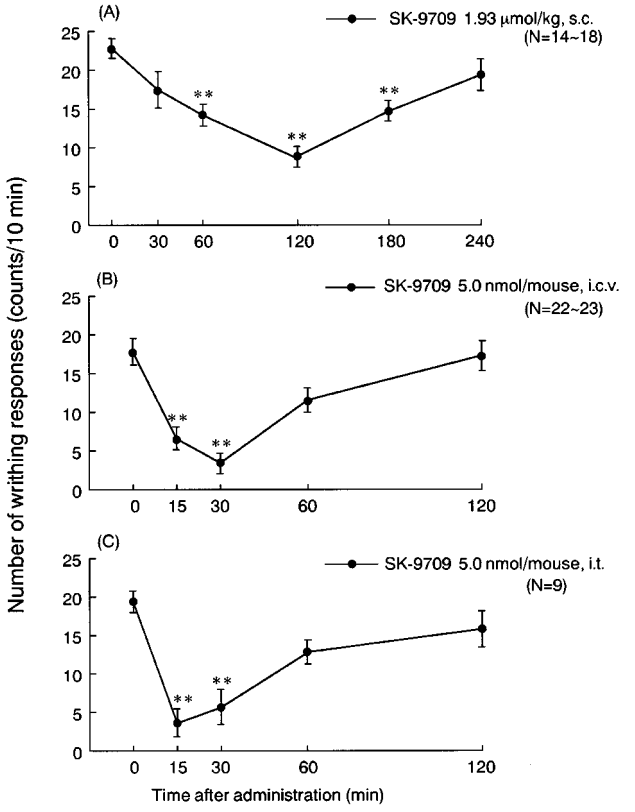

Figure 1 Time course of antinociceptive effects of SK-9709 after s.c. (A), i.c.v. (B) and i.t. (C) injection in the acetic acid-induced writhing test. Mice were treated with SK-9709 (1.93 μmol kg^{-1}) (A) subcutaneously (s.c.) 30, 60, 120, 180 and 240 min before, (B) intracerebroventricularly (i.c.v.) or (C) intrathecally (i.t.) with SK-9709 (5.0 nmol per mouse) 15, 30, 60 and 120 min before testing. Acetic acid (0.7 %) was injected 10 min before writhing test, and then writhing responses were counted for 10 min. Responses of control mice are shown as data at time 0. Each value is the means ± s.e.mean. The numbers of mice used are shown in parentheses. Significance levels; *$P<0.05$, **$P<0.01$ *vs* corresponding time point of control group (Bonferroni's test).

Antagonism of SK-9709-induced antinociception by naloxone in the acetic acid-induced writhing test

To evaluate whether the antinociceptive effects of SK-9709 were mediated *via* opioid receptors, the non-selective opiate antagonist naloxone was used. It has been reported that naloxone (1.36 μmol kg^{-1}) acts mainly on μ-opioid receptors. Antinociceptive effects of SK-9709 after s.c. and i.c.v. injection were almost completely reversed by s.c. injection of naloxone (1.36 μmol kg^{-1}) (Figure 3A,B). On the other hand, naloxone did not antagonize the antinociceptive effect of intrathecally administered SK-9709 (Figure 3C). These results indicated that μ-opioid receptors in the spinal cord were not important for the antinociceptive effects of SK-

9709. This raises the question of how SK-9709 produces anti-nociceptive effects when injected intrathecally.

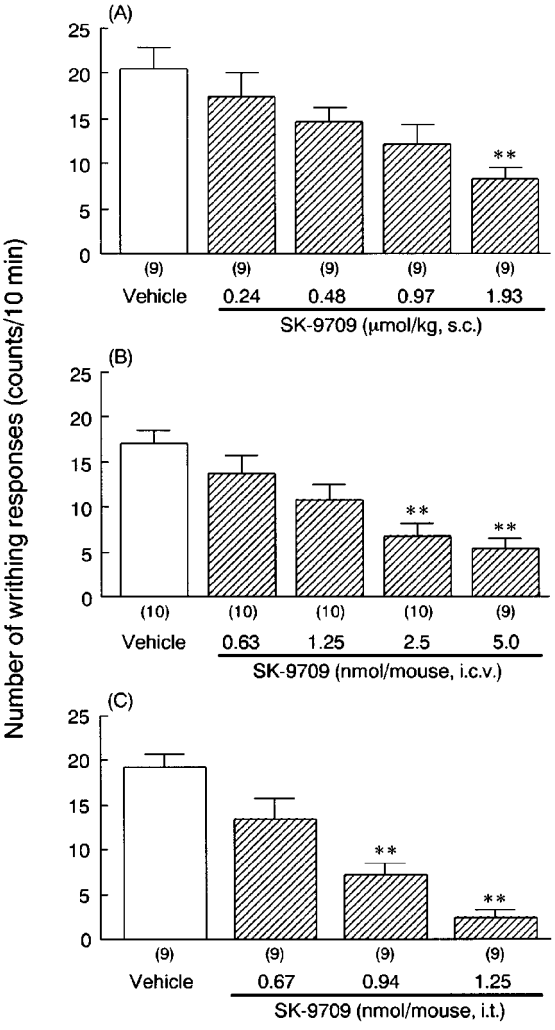

Figure 2 Antinociceptive effects of SK-9709 after s.c. (A), i.c.v. (B) and i.t. (C) injection in the acetic acid-induced writhing test. Mice were treated with SK-9709 (0.24, 0.48, 0.97 and 1.93 μmol kg^{-1}) (A) subcutaneously (s.c.) 120 min before, (B) intracerebroventricularly (i.c.v.) SK-9709 (0.63, 1.25, 2.5 and 5.0 nmol per mouse) 30 min before or (C) intrathecally (i.t.) with SK-9709 (0.67, 0.94 and 1.25 nmol per mouse) 15 min before testing. Acetic acid (0.7%) was injected 10 min before writhing test, and then writhing responses were counted for 10 min. Each value is the mean $\pm$ s.e.mean. The numbers of mice used are shown in parentheses. Significance levels; $^{**}P<0.01$ *vs* vehicle-treated group (Bonferroni's test).

Antagonism of SK-9709-induced antinociception by β-funaltrexamine and nor-binaltorphimine in the acetic acid-induced writhing test

To further elucidate the mechanism of the antinociceptive effects of SK-9709, the selective μ-opioid receptor antagonist β-FNA and selective κ-opioid receptor antagonist nor-BNI were used. I.c.v. injection of β-FNA (4.7 nmol per mouse) completely reversed the antinociceptive effect of s.c. injection of SK-9709 (1.93 μmol kg^{-1}) (Figure 4A) similarly to the effect of naloxone. On the other hand, the antinociceptive effect of SK-9709 (s.c.) was completely reversed by i.t. injection of nor-BNI (4.9 nmol per mouse) (Figure 4B), while naloxone showed no such effect (Figure 3).

Antinociceptive effects of SK-9709 in the hot-plate test

In the hot-plate test, control mice showed licking of the hind paw or jumping responses about 1.5–6.5 s after being placed on a steel plate maintained at a temperature of 55 $\pm$ 0.5°C. The time course of the antinociceptive effects was evaluated after s.c., i.c.v. and i.t. injection of SK-9709. SK-9709 (s.c.) dose-dependently prolonged the response latency and the effects were significant at 19.3 and 57.9 μmol kg^{-1} (Figure 5A). The peak effect was observed at 120 min and returned to the control level by 240 min (Figure 5A). When administered i.c.v., SK-9709 also showed dose-dependent antinociceptive effects, and its peak effect was seen from 15–30 min after the injection and then returned to the control level (Figure 5B). The effect of a higher dose (15.0 nmol per mouse) persisted even at 75 min (Figure 5B). On the other hand, i.t. injection of SK-9709 showed a less pronounced effect. Only the higher dose of SK-9709 showed significant effects, and its effect was short acting compared to i.c.v. injection (Figure 5C). The ED_{50} values (range of 95% confidence limits) after s.c., i.c.v. and i.t. injection were 39.1 (5.4–283.0) μmol kg^{-1}, 6.5 (4.0–10.7) and 7.4 (5.0–11.0) nmol per mouse, respectively (Table 2).

The antinociceptive effect was compared with those of morphine, U-50,488H and E-2078 after s.c., i.c.v. and i.t. injections. The order of potencies of the antinociceptive effects was E-2078 > morphine > SK-9709 > U-50,488H for s.c. injection, E-2078 > > morphine > SK-9709 > > U-50,488H for i.c.v. injection, and E-2078 > > SK-9709 > morphine > U-50,488H. SK-9709 was less potent than morphine with relative potency values of 0.52 (s.c), 0.37 (i.c.v) and 1.27 (i.t.), but was more potent than U-50,488H (Table 2).

Table 1 ED_{50} values (95% confidence limits) and relative potencies of opioid drugs in the acetic acid-induced writhing test

Drugs	μmol kg^{-1} *s.c.*	*Relative potency*	nmol mouse^{-1} *i.c.v.*	*Relative potency*	nmol mouse^{-1} *i.t.*	*Relative potency*
SK-9709	1.36 (0.61–3.02)	0.81	2.11 (1.18–3.79)	0.03	0.79 (0.61–1.03)	0.01
E-2078	0.46 (0.28–0.78)	2.39	0.016 (0.007–0.033)	0.44	0.001 (0.0004–0.0029)	10.0
Morphine	1.10 (0.67–1.83)	1	0.007 (0.003–0.019)	1	0.01 (0.005–0.02)	1
U-50,488H	1.29 (0.72–2.32)	0.85	15.8 (7.2–34.7)	0.0004	3.83 (2.31–6.34)	0.003

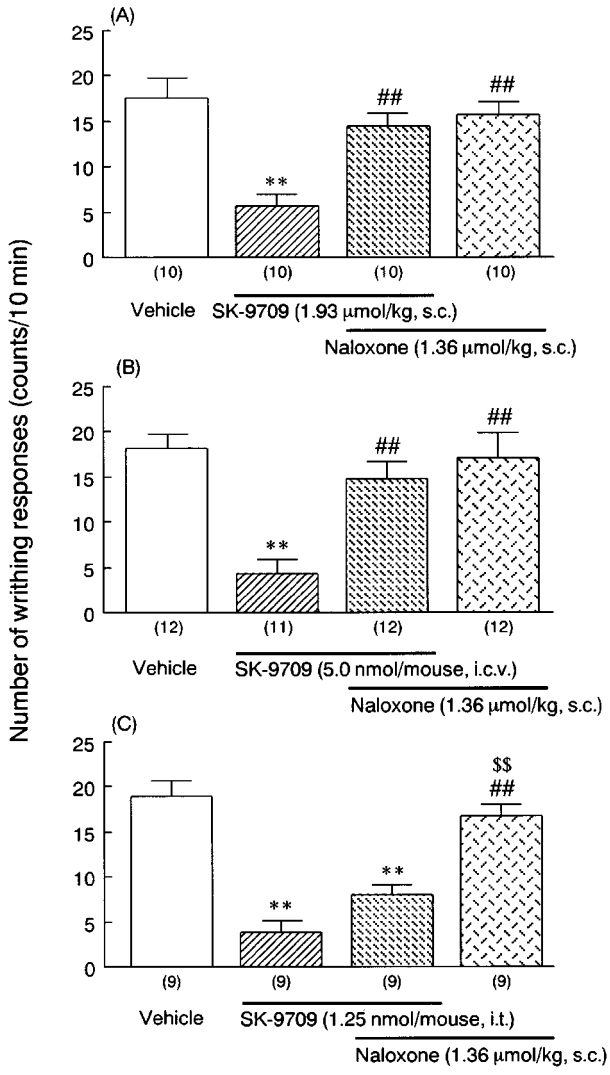

Figure 3 Antinociceptive effects of SK-9709 after s.c. (A), i.c.v. (B) and i.t. (C) injection alone or in combination with naloxone in the acetic acid-induced writhing test. Mice were treated (A) subcutaneously (s.c.) with SK-9709 (1.93 μmol kg^{-1}) 120 min before, (B) intracerebroventricularly (i.c.v.) with SK-9709 (5.0 nmol per mouse) 30 min before or (C) intrathecally (i.t.) with SK-9709 (1.25 nmol per mouse) 15 min before testing. Naloxone (1.36 μmol kg^{-1}, s.c.) was injected 30 min before testing. Acetic acid (0.7%) was injected 10 min before writhing test, and then writhing responses were counted for 10 min. Each value is the mean$\pm$s.e.mean. The numbers of mice used are shown in parentheses. Significance levels; $^{**}P<0.01$ *vs* vehicle-treated group, ## $P<0.01$ *vs* SK-9709 alone, \$\$ $P<0.01$ *vs* SK-9709+naloxone (Bonferroni's test).

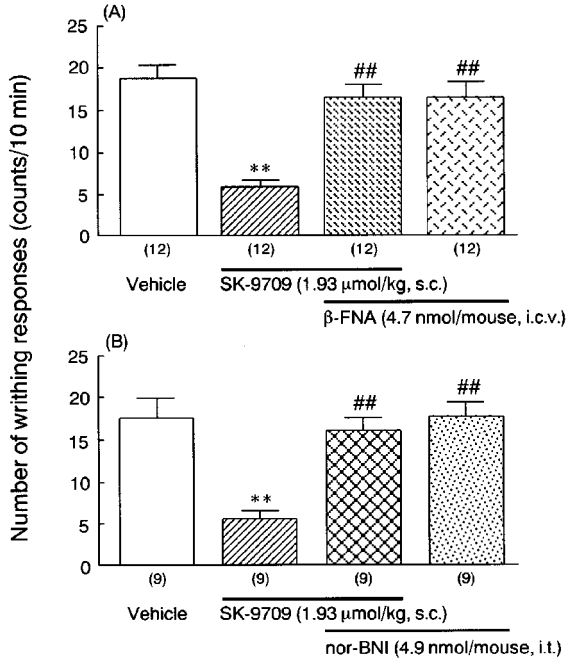

Figure 4 Antinociceptive effects of SK-9709 alone and in combination with β-funaltrexamine (β-FNA) (A) or nor-binaltorphimine (nor-BNI) (B) in the acetic acid-induced writhing test. Mice were treated with SK-9709 (1.93 μmol kg^{-1}, s.c.) 120 min before testing. (A) β-FNA (5.1 nmol per mouse, i.c.v.) and nor-BNI (4.9 nmol per mouse, i.t.) were injected 24 h and 25 min before testing, respectively. Acetic acid (0.7 %) was injected 10 min before writhing test, and then writhing responses were counted for 10 min. Each value is the mean$\pm$s.e.mean. The numbers of mice used are shown in parentheses. Significance levels; $^{**}P<0.01$ *vs* vehicle-treated group, ## $P<0.01$ *vs* SK-9709 alone (Bonferroni's test).

Receptor binding assay

In the receptor binding assay, the K_i values of SK-9709 for κ- and μ-opioid receptors were 2.99 ± 0.38 and 45.5 ± 21.5, whereas the K_i values of dynorphin A-(1-13) for these receptors were 0.047 ± 0.014 and 0.81 ± 0.27, respectively. The ratios of $K_i(\mu)/K_i(\kappa)$ values for SK-9709, dynorphin A-(1–13) and E-2078 were 15.2, 17.3 and 4.18, respectively (Table 3).

Discussion

In the search for a stable dynorphin fragment, Tachibana *et al.* (1988) synthesized and tested more than 70 fragments of dynorphin for affinities to μ-, δ- and κ-opioid receptors, as well as analgesic activities, and finally found [N-methyl-Tyr^1, N-methyl-Arg^7-D-Leu^8] dynorphin A-(1–8) ethylamide, which was designated as E-2078. E-2078 had antinociceptive effects and no major biotransformed products were obtained after incubation with mouse serum or rhesus monkey blood (Yu *et al.*, 1997a). In agreement with these previous reports, our results demonstrated that E-2078 showed potent and long-lasting antinociceptive effects even after systemic injection in both the acetic acid-induced writhing and hot-plate tests. Interestingly, the newly synthesized dynorphin A fragment SK-9709 also showed long-lasting antinociceptive effects, although its potency was less than that of E-2078. It has been demonstrated that the replacement of peptide bonds by ψ (CH_2NH) bonds is an effective modification for the design of antagonists of various biologically active peptides (Schiller *et al.*, 1993). Ambo *et al.* (1995) reported that this modification conferred higher resistance to enzymatic degradation. This resistance against enzymatic degradation significantly increases the possibility that the peptide will cross the blood–brain barrier. In the present study, the antinociceptive effects of systemically administered SK-9709 were dose-dependent with the maximal peak effect at 120 min in the acetic acid-induced writhing test and hot-plate test.

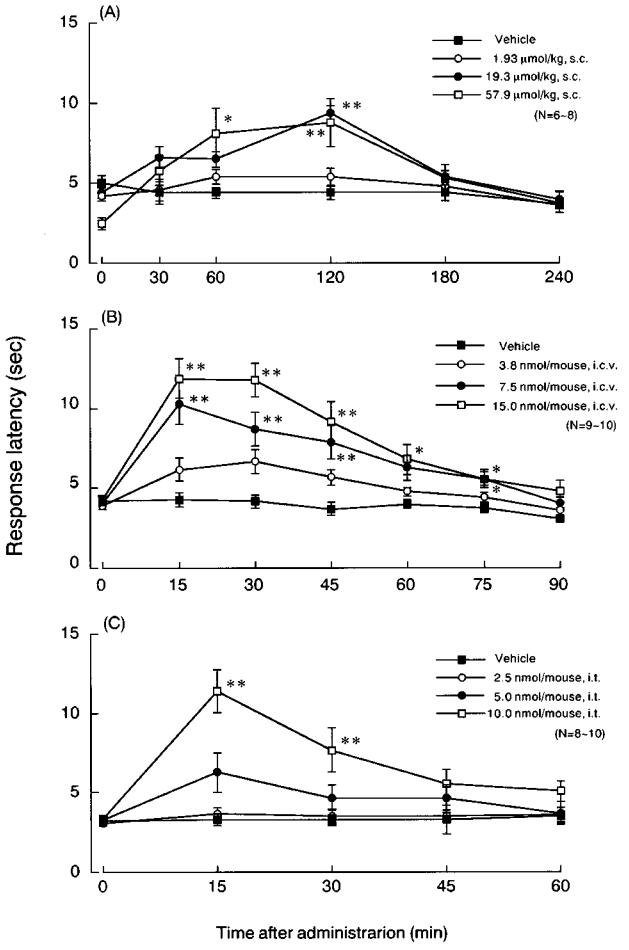

Figure 5 Time course of antinociceptive effects of SK-9709 after s.c. (A), i.c.v. (B) and i.t. (C) injection in the hot-plate test. Mice were treated (A) subcutaneously (s.c.) with SK-9709 (1.93, 19.3 and 57.9 μmol kg^{-1}), (B) intracerebroventricularly (i.c.v.) with SK-9709 (3.8, 7.5 and 15.0 nmol per mouse) or (C) intrathecally (i.t.) with SK-9709 (2.5, 5.0 and 10.0 nmol per mouse) before testing. Responses of control mice are shown as data at time 0. Each value is the mean$\pm$s.e.mean. Significance levels; $^*P<0.05$, $^{**}P<0.01$ *vs* corresponding time point of control group (Bonferroni's test).

This time course was similar to that of E-2078, which produced long-lasting antinociceptive effects. On the other hand, the antinociceptive effects induced by i.c.v. and i.t. injection of SK-9709 showed rather rapid onset, whereas the peak effects of SK-9709 (s.c.) occurred at 120 min. Since it has been reported that the delayed peak effects of systemic E-2078 suggested slow penetration across the blood–brain barrier (Terasaki *et al*., 1989) and the presence of E-2078 in the brain indicated that E-2078 had crossed the blood–brain barrier (Yu *et al*., 1997b), SK-9709 may also slowly penetrate the blood–brain barrier and act on the central nervous system. Furthermore, SK-9709 is shorter and this characteristic is advantageous for penetration of the blood–brain barrier. As further direct evidence for central actions of systemically applied SK-9709, its antinociceptive effects observed following s.c. injection were completely reversed by i.c.v. injection of β-FNA or i.t. injection of nor-BNI. These doses of antagonists are sufficient to block the analgesic responses of morphine and U-50,488H as reported previously (Jiang *et al*., 1995; Takemori *et al*., 1988). The general concept that some peptides can cross the blood–brain barrier as intact molecules has gained acceptance in recent years (Banks *et al*., 1992). Studies have shown in both *in vitro* and *in vivo* systems that peptides can cross the blood–brain barrier using a variety of direct techniques such as brain microdialysis, specific radioimmunoassay and high-performance liquid chromatography (Banks & Kastin, 1990). Thus, systemically administered SK-9709 exerted its antinociceptive effects by acting on the opioid receptors located in the central nervous system.

The N-terminal Tyr^1 of opioid peptides, including dynorphin A-(1–17) is important for the opioid activity. The Arg^6-Arg^7 bond, in addition to the well-known Tyr^1-Gly^2 bond, were cleaved in the brain (Tachibana *et al*., 1988). In addition, the substitution of either Gly^2 by D-Ala^2 or L-Arg by D-Arg protected dynorphin A against enzymatic degradation, but also reduced κ-opioid receptor selectivity (Chavkin & Goldstein, 1981; Gairin *et al*., 1988). Shorter peptide lengths decreased the selectivities and affinities of dynorphin A fragments for κ-opioid receptors and increased those for μ-opioid receptors (Corbett *et al*., 1982). SK-9709 is supposed to be stable against enzyme degradation because of replacement of the Arg-Arg peptide bond by a ψ (CH_2NH) bond and the replacement of Gly^2-Gly^3 with D-Ala. In the binding assay, the K_i values of SK-9709 for κ- and μ-opioid receptors were 2.99 and 45.5, whereas the K_i values of dynorphin A-(1–13) for these receptors were 0.0471 and 0.813, respectively (Table 3). The ratios of $K_i(\mu)/K_i(\kappa)$ values for SK-9709 and dynorphin A-(1–13) were 15.2 and 17.3, respectively (Table 3). These observations suggested that SK-9709 binds not only to κ-opioid receptors, but also to μ-opioid receptors similarly to E-2078 but with lower affinity. Therefore, to examine whether the effects of SK-9709 were mediated *via* opioid receptors, we used naloxone as a non-selective opioid receptor antagonist. The antinociceptive effects of SK-9709 after s.c. and i.c.v. injection were completely antagonized by systemic injection of naloxone. On the other hand, the antinociceptive effect after intrathecal injection of SK-9709 was not reversed by naloxone. Second, we tested what types of opioid receptors were involved in the effects of SK-9709; the antinociceptive effects of SK-9709 after s.c. injection were antagonized by i.c.v. injection of β-FNA, a selective μ-opioid receptor antagonist, and by intrathecal injection of nor-BNI, a selective κ-opioid receptor antagonist. It has been reported that κ-opioid receptor agonists including dynorphin A showed antinociceptive effects only acting at the spinal level (Hayes *et al*., 1983), while the ligands for μ-opioid receptors acted at the supra-spinal level (Tachibana *et al*., 1988). Taken together, our results indicated that the antinociceptive effects of SK-9709 were mediated by both supra-spinal μ- and spinal κ-opioid receptors.

In the acetic acid-induced writhing test, the order of potencies of antinociceptive effects was E-2078 > morphine > SK-9709 > U-50,488H. The antinociceptive effects of SK-9709 relative to those of morphine (arbitrarily assigned a value of 1.0) were 0.81 (s.c), 0.03 (i.c.v) and 0.01 (i.t.). This result indicated that only systemic injection of SK-9709 has an effect comparable to that of morphine. In the hot-plate test, the potencies of SK-9709 were similar to those of morphine, and thermal stimulus was blocked more efficiently

Table 2 ED_{50} values (95% confidence limits) and relative potencies of opioid drugs in the hot-plate test

Drugs	μmol kg^{-1} *s.c.*	*Relative potency*	nmol mouse^{-1} *i.c.v.*	*Relative potency*	nmol mouse^{-1} *i.t.*	*Relative potency*
SK-9709	39.1 (5.4–283.0)	0.52	6.5 (4.0–10.7)	0.37	7.4 (5.0–11.0)	1.27
E-2078	8.35 (3.78–18.5)	2.46	0.068 (0.038–0.122)	35.6	0.037 (0.017–0.079)	255.4
Morphine	20.5 (12.0–35.0)	1	2.42 (1.29–4.54)	1	9.45 (5.6–15.9)	1
U-50,488H	88.1 (37.2–208.7)	0.23	88.2 (53.6–145.1)	0.027	64.5 (25.0–166.4)	0.14

Table 3 K_i values and relative selectivities of opioid drugs in the receptor binding assay

Peptides	*[^{3}H]-U-69,593* K_i, nM	*[^{3}H]-DAMGO* K_i, nM	*[^{3}H]-DPDPE* K_i, nM	$\kappa/\mu/\delta$
SK-9709	2.99±0.38	45.5±21.5	1045±335	1 : 15.2 : 350
Dynorphin A-(1-13)	0.047±0.014	0.81±0.27	–	1 : 17.3 :
E-2078	1.01±0.19	4.22±1.46	–	1 : 4.18 : –

Values are shown as means ±s.e.mean.

by intrathecal administration of SK-9709 than morphine. Furthermore, compared with the chemical stimulus test, SK-9709 was about 10 fold more potent than morphine when injected intracerebroventricularly in the hot-plate test (0.03 *vs* 0.37). These results, therefore, indicated that systemic injection of SK-9709 was more effective for thermal stimulus. Intravenously administered E-2078 was approximately equipotent to morphine in the tail-pinch assay in mice (Yoshino *et al*., 1990), and they proposed that this systemic effectiveness of E-2078 was due to its stability against enzymatic degradation (Nakazawa *et al*., 1990; Yoshino *et al*., 1990). Therefore, subcutaneously administered SK-9709 also showed potent antinociceptive effects.

In addition to activities at κ-opioid receptors, the endogenous κ-opioid receptor agonist dynorphin A-(1–17) and its fragments have effects that are not associated with opioid receptors (Faden, 1992; Faden & Jacobs, 1984; Lai *et al*., 1998; Moises & Walker, 1985; Shukla & Lemaire, 1994). With these 'non-opioid' effects, it has been suggested that dynorphins may act at the NMDA receptor complex, because dynorphins inhibited the binding of [^{3}H]-glutamate and [^{3}H]-MK-801 to NMDA receptors in membranes. This effect of dynorphin was not sensitive to opioid antagonists, implying that it was not acting at the opioid receptors (Massardier & Hunt, 1989; Vanderah *et al*., 1996). We have recently shown that [des-tyrosine^1] dynorphin A-(2–13) improved carbon monoxide (CO)-induced memory dysfunction. Naturally, however, the anti-amnesic effects of dynorphin A-(2–13) on delayed amnesia induced by CO exposure were not blocked by administration of nor-BNI (Hiramatsu & Inoue, 2000).

The mechanism of 'non-opioid' effects may include: (1) release of excitatory amino acids, which may lead to central sensitization; (2) direct agonist activity of dynorphins on NMDA receptors causing neuronal excitability; and (3) other mechanisms mediating some neuronal and/or endocrine systems. Microdialysis studies have demonstrated localized, dose-dependent release of glutamate and aspartate in the hippocampus not only by dynorphin A-(1–17) but also by dynorphin A-(2–17) (Faden, 1992).

Dynorphin A-(1–17) and A-(2–17) are effective in potentiating NMDA currents, while the specific κ-opioid receptor agonist U-50,488H, cannot mimic the action of dynorphin A-(1–17) in periaqueductal gray cells (Caudle & Dubner, 1998; Lai *et al*., 1998). Dynorphins have been suggested to act directly at NMDA receptor channels (Lai *et al*., 1998) because dynorphin displaces the binding of [^{3}H]-MK-801 binding in rat brain membranes (Caudle & Isaac, 1988; Massardier & Hunt, 1989). Our preliminary data showed that both dynorphin A-(1–13) and A-(2–13) improved (+)-MK-801-induced impairment of spontaneous alternation, and that the effects of dynorphin A-(1–13) were partially antagonized by nor-BNI in mice (data not shown).

Hooke *et al*. (1995) reported that dynorphin A-(1–13) and A-(2–17) showed antinociceptive activity in the writhing assay of peripheral analgesia, and that this activity was 'non-opioid' in nature. Dynorphin A-(2–17) was as effective as dynorphin A-(1–13) in inhibition of the writhing response. Further, the ED_{50} values of both dynorphin A-(1–13) and A-(2–17) were unaffected by the presence of 10 to 50 μmol kg^{-1} naloxone, or 20 μmol kg^{-1} nor-BNI. It has also been reported that high doses of dynorphin A-(2–17) (20 μg per rat = 10 nmol per rat) produce neurological deficits, motor dysfunctions, and flaccid hind limb paralysis in the rat (Caudle & Isaac, 1988; Walker *et al*., 1982). Further, Laughlin *et al*. (1997) reported that a single intrathecal injection of dynorphin A-(1–17) and dynorphin A-(2–17) (3 nmol) induced long-lasting allodynia and hyperalgesia in mice. These effects were blocked by the NMDA antagonist (+)-MK-801, but not the opioid antagonist naloxone. Although we did not observe any motor dysfunction or hind limb paralysis in mice during the test, higher doses of dynorphin derivatives (20.0 nmol per mouse, i.t.) induced hind limb paralysis. Since we did not test the existence of allogynia, we cannot completely exclude the possibility that SK-9709 also acts 'non-opioid' sites showing anti-nociceptive effects and other neurological phenomena.

In summary, the results of our study suggested that systemically administered SK-9709 produced long-lasting antinociceptive effects in the acetic acid-induced writhing test

and hot-plate test, and these effects were mediated by both supra-spinal μ- and spinal κ-opioid receptors. The peripherally administered peptide could exert behavioural effects by acting on the central nervous system. Mnemonic effects of κ-opioid receptor agonists including dynorphin A are thought to be mediated primarily by central mechanisms (Hiramatsu *et al*., 1995; 1996; 1998), whereas analgesic or antinociceptive effects of opioids can be either centrally or peripherally mediated, depending on the experimental situation (Gmerek *et al*., 1986; Stein *et al*., 1996). These studies suggested that SK-9709 may be a suitable candidate for further pharmacological and neurobiological studies, as well as being a potentially useful therapeutic agent both as an analgesic and for the treatment for learning and memory deficits. Future investigations are required to elucidate the effects of SK-9709 in more detail.

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References

AMBO, A., ADACHI, T. & SASAKI, Y. (1995). Synthesis and opioid activities of [D-Leu[8]] dynorphin (1-8) fragments containing a reduced peptide bond, ψ (CH_2NH). *Chem. Pharm. Bull.,* **43,** 1547–1550.

BANKS, W.A., AUDUS, K.L. & DAVIS, T.P. (1992). Permeability of the blood-brain barrier to peptides: an approach to the development of therapeutically useful analogs, peptide. *Peptide,* **13,** 1289–1294.

BANKS, W.A. & KASTIN, A.J. (1990). Peptide transport systems for opiates across the blood-brain barrier. *Am. J. Physiol.,* **259,** E1–E10.

CAUDLE, R.M. & DUBNER, R. (1998). Ifenprodil blocks the excitatory effects of the opioid peptide dynorphin 1-17 on NMDA receptor-mediated currents in the CA3 region on the guinea pig hippocampus. *Neuropeptides,* **32,** 87–95.

CAUDLE, R.M. & ISAAC, L. (1988). A novel interaction between dynorphin (1-13) and an N-methyl-D-aspartate site. *Brain Res.,* **443,** 329–332.

CHAVKIN, C. & GOLDSTEIN, A. (1981). Specific receptor for the opioid peptide dynorphin: Structure-activity relationships. *Proc. Natl. Acad. Sci., U.S.A.,* **78,** 6543–6547.

CHAVKIN, C., JAMES, I.F. & GOLDSTEIN, A. (1982). Dynorphin is a specific endogenous ligand of the κ opioid receptor. *Science,* **215,** 413–415.

CHENG, Y. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.,* **22,** 3099–3108.

CHOU, J.Z., CHAIT, B.T., WANG, R. & KREEK, M.J. (1996). Differential biotransformation of dynorphin A (1-17) and dynorphin A (1-13) peptides in human blood, ex vivo. *Peptides,* **17,** 983–990.

CORBETT, A.D., PATERSON, S.J., MCKNIGHT, A.T., MAGNAN, J. & KOSTERLITZ, H.W. (1982). Dynorphin$_{1\text{-}8}$ and dynorphin$_{1\text{-}9}$ are ligands for the κ-subtype of opiate receptor. *Nature,* **299,** 79–81.

FADEN, A.I. (1992). Dynorphin increases extracellular levels of excitatory amino acids in the brain through a non-opioid mechanism. *J. Neurosci.,* **12,** 425–429.

FADEN, A.I. & JACOBS, T.P. (1984). Dynorphin-related peptides cause motor dysfunction in the rat through a non-opiate action. *Br. J. Pharmacol.,* **81,** 271–276.

FUJITA, H., SASAKI, Y., KOHNO, H., OHKUBO, Y., AMBO, A., SUZUKI, K. & HINO, M. (1990). Studies on analgesic oligopeptides. VI. Further studies of synthesis and biological properties of tripeptide alkylamides, Tyr-D-Arg-Phe-X. *Chem. Pharm. Bull.,* **38,** 2197–2200.

GAIRIN, J.E., GOUT, R., MEUNIER, J.C. & CROS, J. (1988). [D-Pro[10]]-dynorphin (1-11) is a kappa-selective opioid analgesic in mice. *J. Pharmacol. Exp. Ther.,* **245,** 995–1001.

GMEREK, D.E., COWAN, A. & WOODS, J.H. (1986). Independent central and peripheral mediation of morphine-induced inhibition of gastrointestinal transit in rats *J. Pharmacol. Exp. Ther.,* **236,** 8–13.

GOLDSTEIN, A., TACHIBANA, S., LOWNEY, L.I., HUNKAPILLER, M. & HOOD, L. (1979). Dynorphin-(1-13), an extraordinarily potent opioid peptide. *Proc. Natl. Acad. Sci., U.S.A.,* **76,** 6666–6670.

HALEY, T.J. & MCCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmacol.,* **12,** 12–15.

HAYES, A.G., SKINGLE, M. & TYERS, M.B. (1983). Antinociceptive profile of dynorphin in the rat. *Life Sci.,* **33,** 657–660.

HIRAMATSU, M. & INOUE, K. (2000). Des-tyrosine[1] dynorphin, A-(2-13) improves carbon monoxide-induced impairment of learning and memory in mice. *Brain Res.,* **859,** 303–310.

HIRAMATSU, M., MORI, H., MURASAWA, H. & KAMEYAMA, T. (1996). Dynorphin A-(1-13) improves galanin-induced impairment of memory accompanied by blockage of reductions in acetylcholine release in rats. *Br. J. Pharmacol.,* **118,** 255–260.

HIRAMATSU, M., MURASAWA, H., NABESHIMA, T. & KAMEYAMA, T. (1998). Effects of U-50,488H on scopolamine-, mecamylamine- and dizocilpine-induced learning and memory impairment in rats. *J. Pharmacol. Exp. Ther.,* **284,** 858–867.

HIRAMATSU, M., SASAKI, M. & KAMEYAMA, T. (1995). Effects of dynorphin A-(1-13) on carbon monoxide-induced delayed amnesia in mice studied in a step-down type passive avoidance task. *Eur. J. Pharmacol.,* **282,** 185–191.

HOOKE, L.P., HE, L. & LEE, N.M. (1995). Dynorphin A modulates acute and chronic opioid effects. *J. Pharmacol. Exp. Ther.,* **273,** 292–297.

HYLDEN, J.K. & WILCOX, G. (1980). Intrathecal morphine in mice: A new technique. *Eur. J. Pharmacol.,* **67,** 313–316.

JIANG, Q., SEYED-MOZAFFARI, A., SEBASTIAN, A., ARCHER, S. & BIDLACK, J.M. (1995). Preventing morphine antinociceptive tolerance by irreversible mu opioid antagonists before the onset of their antagonism. *J. Pharmacol. Exp. Ther.,* **273,** 680–688.

LAI, S.L., GU, Y. & HUANG, L.Y. (1998). Dynorphin uses a non-opioid mechanism to potentiate N-methyl-D-aspartate currents in single rat periaqueductal gray neurons. *Neurosci. Lett.,* **247,** 115–118.

LAUGHLIN, T.M., VANDERAH, T.W., LASHBROOK, J., NICHOLS, M.L., OSSIPOV, M., PORRECA, F. & WILCOX, G.L. (1997). Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors. *Pain,* **72,** 253–260.

LITCHFIELD, S.T. & WILCOXON, F. (1949). A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Ther.,* **96,** 99–113.

MASSARDIER, D. & HUNT, P.F. (1989). A direct interaction of dynorphin-(1-13) with the N-methyl-D-aspartate (NMDA) receptor. *Eur. J. Pharmacol.,* **170,** 125–126.

MILLAN, M.J., MILLAN, M.H., CZLONKOWSKI, A., HOLLT, V., PILCHER, C.W., HERZ, A. & COLPAERT, F.C. (1986). A model of chronic pain in the rat: response of multiple opioid systems to adjuvant-induced arthritis. *J. Neurosci.,* **6,** 899–906.

MOISES, H.C. & WALKER, J.M. (1985). Electrophysiological effects of dynorphin peptides on hippocampal pyramidal cells in rat. *Eur. J. Pharmacol.,* **108,** 85–98.

NAKAZAWA, T., FURUYA, Y., KANEKO, T., YAMATSU, T., YOSHINO, H. & TACHIBANA, S. (1990). Analgesia produced by E-2078, a systemically active dynorphin analog, in mice. *J. Pharmacol. Exp. Ther.,* **252,** 1247–1254.

NAKAZAWA, T., FURUYA, Y., KANEKO, T. & YAMATSU, T. (1991). Spinal κ receptor-mediated analgesia of E-2078, a systemically active dynorphin analog, in mice. *J. Pharmacol. Exp. Ther.,* **256,** 76 – 81.

SCHILLER, P.W., WELTROWSKA, G., NGUYEN, T.M., WILKES, B.C., CHUNG, N.N. & LEMIEUX, C. (1993). TIPP[ψ]: a highly potent and stable pseudopeptide delta opioid receptor antagonist with extraordinary delta selectivity. *J. Med. Chem.,* **36,** 3182 – 3187.

SHUKLA, V.K. & LEMAIRE, S. (1994). Non-opioid effects of dynorphins: possible role of the NMDA receptor. *Trends Pharmacol. Sci.,* **15,** 420 – 424.

SMITH, A.P. & LEE, N.M. (1988). Pharmacology of dynorphin. *Annu. Rev. Pharmacol. Toxicol.,* **28,** 123 – 140.

STEIN, C., PFLUGER, M., YASSOURIDIS, A., HOELZL, J., LEHRBERGER, K., WELTE, C. & HASSAN, A.H. (1996). No tolerance to peripheral morphine analgesia in presence of opioid expression in inflamed synovia *J. Clin. Invest.,* **98,** 793 – 799.

TACHIBANA, S., YOSHINO, H., ARAKAWA, Y., NAKAZAWA, T., KANEKO, T., YAMATSU, K. & MIYAGAWA, H. (1988). Design and synthesis of metabolically stable analogues of dynorphin-A and their analgesic characteristics. In *Biowarning System in the Brain* ed. Takagi, H., Oomura, Y., Ito, M. & Otsuka, M. pp 101 – 109, Tokyo: University of Tokyo Press.

TAKEMORI, A.E., HO, B.Y., NAESETH, J.S. & PORTOGHESE, P.S. (1988). Nor-binaltorphimine, a highly selective kappa-opioid antagonist in analgesic and receptor binding assays. *J. Pharmacol. Exp. Ther.,* **246,** 255 – 258.

TAKEMORI, A.E., LOH, H.H. & LEE, N.M. (1992). Suppression by dynorphin A-(1-13) of the expression of opiate withdrawal and tolerance in mice. *Eur. J. Pharmacol.,* **221,** 223 – 226.

TAKEMORI, A.E., LOH, H.H. & LEE, N.M. (1993). Suppression by dynorphin A and [des-Tyr^1]dynorphin A peptides of the expression of opiate withdrawal and tolerance in morphine-dependent mice. *J. Pharmacol. Exp. Ther.,* **266,** 121 – 124.

TERASAKI, T., HIRAI, K., SATO, H., KANG, Y.S. & TSUJI, A. (1989). Absorptive-mediated endocytosis of a dynorphin-like analgesic peptide, E-2078, into the blood-brain barrier. *J. Pharmacol. Exp. Ther.,* **251,** 351 – 357.

VANDERAH, T.W., LAUGHLIN, T., LASHBROOK, J.M., NICHOLS, M.L., WILCOX, G.L., OSSIPOV, M.H., MALAN, T.P. & PORRECA, F. (1996). Single intrathecal injections of dynorphin A or des-tyr-dynorphins produce long-lasting allodynia in rats : blockade by MK-801 but not naloxone. *Pain,* **68,** 275 – 281.

WALKER, J.M., MOISES, H.C., COY, D.H., BALDRIGHI, G. & AKIL, H. (1982). Nonopiate effects of dynorphin and des-Tyr-dynorphin. *Science,* **218,** 1136 – 1138.

YOSHINO, H., NAKAZAWA, T., ARAKAWA, Y., KANEKO, T., TSUCHIYA, Y., MATSUNAGA, M., ARAKI, S., IKEDA, M., YAMATSU, K. & TACHIBANA, S. (1990). Synthesis and structure-activity relationships of dynorphin A-(1-8) amide analogues. *J. Med. Chem.,* **33,** 206 – 212.

YOUNG, E.A., WALKER, J.M., HOUGHTEN, R. & AKIL, H. (1987). The degradation of dynorphin A in brain tissue in vivo and in vitro. *Peptides,* **8,** 701 – 707.

YU, J., BUTELMAN, E.R., WOODS, J.H., CHAIT, B.T. & KREEK, M.J. (1997a). Dynorphin A (1-8) analog, E-2078, is stable in human and rhesus monkeys. *J. Pharmacol. Exp. Ther.,* **280,** 1147 – 1151.

YU, J., BUTELMAN, E.R., WOODS, J.H., CHAIT, B.T. & KREEK, M.J. (1997b). Dynorphin A (1-8) analog, E-2078, crosses the blood-brain barrier in rhesus monkeys. *J. Pharmacol. Exp. Ther.,* **282,** 633 – 638.

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