ELSEVIER

European Journal of Pharmacology 313 (1996) 11–15

ejp

Systemic administration of dynorphin A-(1–13) markedly improves cycloheximide-induced amnesia in mice

Makoto Ukai *, X. Shan-Wu, Tetsuya Kobayashi, Tsutomu Kameyama

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan

Received 19 February 1996; revised 1 May 1996; accepted 21 June 1996

Abstract

The effects of systemic or intracerebroventricular injection of dynorphin A-(1–13), a κ-selective opioid receptor agonist, on cycloheximide-induced amnesia were investigated by using a step-down-type passive avoidance task in mice. The intracerebroventricular injection of dynorphin A-(1–13) (0.3–3 μg) before training significantly prolonged step-down latency. The systemic administration of dynorphin A-(1–13) (1, 3 and/or 10 mg/kg, i.p.) before training or retention tests markedly inhibited the cycloheximide (30 mg/kg, s.c.)-induced shortening of step-down latency, indicating antiamnesic effects of dynorphin A-(1–13). One and 3 mg/kg doses of ((±)trans-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide, methanesulfonate hydrate (U-50,488H), another κ-selective opioid receptor agonist, significantly inhibited the shortening. The anti-amnesic effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.) were almost completely antagonized by intracerebroventricular administration of the quaternary derivative of the opioid receptor antagonist naltrexone methobromide (0.3 μg), but not by systemic administration of the opioid receptor antagonist (1 mg/kg, s.c.), demonstrating central mediation of the anti-amnesic effects of dynorphin A-(1–13). Furthermore, the κ-selective opioid receptor antagonist, nor-binaltorphimine (2 mg/kg, s.c.), almost completely antagonized the effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.). These results suggest that dynorphin A-(1–13) produces anti-amnesic effects through the blood-brain barrier.

Keywords: Dynorphin A-(1–13); U-50,488H; Cycloheximide; Naltrexone methobromide; Amnesia; (Mouse)

1. Introduction

An increasing body of evidence has demonstrated that endogenous opioid peptides affect learning and memory. For example, post-training administration of β-endorphin is known to cause retrograde amnesia for a variety of tasks in rats (Izquierdo et al., 1980, 1985). In contrast, although β-endorphin has no effects on acquisition, it facilitates retrieval when given prior to the test session (Izquierdo, 1980). Moreover, the δ-selective opioid receptor agonist, [D-Pen^2,D-Pen^5] enkephalin (DPDPE), impairs acquisition of the avoidance response (Schulteis et al., 1988), and conversely deltorphin, a naturally occurring opioid with high selectivity for δ opioid receptors, improves memory consolidation in mice (Pavone et al., 1990).

In contrast, dynorphin A-(1–8), dynorphin A-(1–13) and dynorphin A-(1–17) are naturally occurring opioid peptides distributed in the central nervous system of vertebrates (Cuello, 1983). It is possible that dynorphins are involved in learning and memory processes. In fact, the increase in dynorphin A-(1–8)-like immunoreactivity in the aged hippocampus is associated with a decline in spatial learning ability (Jiang et al., 1989). The post-training or pre-test administration of dynorphin A-(1–17) facilitates or impairs retention performance in rats, depending on the intensities of footshock during acquisition trial (Del Cerro and Borrell, 1990), while dynorphin A-(1–13) has no effects on the inhibitory shuttle avoidance response (Izquierdo et al., 1985). Although the effects of dynorphins on memory processes in normal animals are not consistent (Introini-Collison et al., 1987), dynorphin A-(1–13) attenuates the [D-Ala^2,*N*MePhe^4,Gly-ol] enkephalin-, scopolamine-, pirenzepine-, ischemia- or basal forebrain lesion-induced amnesia in rodents (Itoh et al., 1993a,b,1994; Ukai et al., 1993, 1995a,b).

* Corresponding author. Tel.: 81-52-832-1781; fax: 81-52-834-8780.

PII S0014-2999(96)00484-0

The present study was designed to determine whether dynorphin A-(1–13) affects the cycloheximide-induced impairment of learning and memory processes (Nabeshima et al., 1988), since the effects of cycloheximide are associated with cholinergic and GABA (γ-aminobutyric acid)-ergic neuronal systems which play a major role in learning and memory. It was, moreover, of interest to determine whether systemic administration of opioid peptides affects learning and memory, since a new strategy should be developed for systemic administration of neuropeptides through the blood-brain barrier.

2. Materials and methods

2.1. Animals

Male mice of the ddY strain (Nihon SLC, Shizuoka, Japan), weighing between 30 and 35 g were used in the experiment. The animals were kept in a regulated environment (23 ± 1°C; 50 ± 5% humidity), with a 12-h light/12-h dark cycle (8:00 a.m. and 8:00 p.m.), and were given food and water ad libitum.

2.2. Drugs

Cycloheximide (Sigma Chemical Co., St. Louis, MO, USA), dynorphin A-(1–13) (Peptide Institute, Minoh, Japan), naltrexone methobromide (Boehringer Ingelheim, Ingelheim am Rhein, Germany), nor-binaltorphimine hydrochloride hydrate (Research Biochemicals, Natick, MA, USA) and ((±)*trans*-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide, methanesulfonate hydrate (U-50,488H) (The Upjohn Co., Kalamazoo, MI, USA) were used throughout. Cycloheximide, naltrexone methobromide and nor-binaltorphimine were dissolved in 0.9% saline. When injected i.c.v., dynorphin A-(1–13) and nor-binaltorphimine were dissolved in sterile 0.9% saline (Otsuka Pharmaceutical Co., Tokyo, Japan). The i.c.v. injections were made according to the method of Haley and McCormick (1957). The unilateral injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears. The injection was made with a needle (30-gauge; 4 mm long) attached to a 50-μl microsyringe (705LT; Hamilton Co., Reno, NV, USA). The needle was inserted perpendicularly through the skull and into the lateral ventricle of the brain. The mouse was anesthetized with ether, and solutions were injected in a volume of 5 μl per mouse over a period of 20 s as previously described (Kameyama and Ukai, 1983). The site was checked by injecting a 1:10 dilution of India ink in 0.9% saline. Histological examination revealed particles of the ink in the lateral and third ventricles but not in the others. As previously described, neither insertion of the needle nor injection of 5 μl of 0.9% saline solution had any significant influence on behavioral responses (Kameyama and Ukai, 1983) or cognitive functions (Itoh et al., 1994).

2.3. Passive avoidance learning

2.3.1. Apparatus

The passive avoidance apparatus consisted of a Plexiglas inner box (30 × 30 × 40 cm high) with a grid floor and a sound attenuating wooden outer box (35 × 35 × 90 cm high) with a 15-W light. The grid floor consisted of 30 parallel steel rods (0.3 cm in diameter) placed 1.0 cm apart. A wooden platform (4 × 4 × 4 cm) was placed in the center of the grid floor. Intermittent electroshocks (1 Hz, 0.5 s, 60 V DC) were delivered to the grid floor by an insulated stimulator for 15 s (Nihon Kohden, Tokyo, Japan). As the resistance varied between 100 and 250 KΩ when an animal was placed in a test cage, the mouse received an electric footshock in the range of 0.24–0.6 mA.

2.4. Passive avoidance procedures

2.4.1. Training

Each mouse was placed gently on the wooden platform set in the center of the grid floor. When the mouse stepped down from the platform and placed all its paws on the grid floor, electroshocks were delivered 15 times. The step-down latency was measured. Mice showing a 3–15-s range of step-down latency were used. Thus, animals that did not step down within 15 s were excluded from the data set.

2.4.2. Retention test

Twenty-four hours after training, each mouse was again placed on the platform, and step-down latency was measured up to a maximum cut-off time of 300 s.

2.5. Statistical analysis

The results of the passive avoidance learning were expressed in terms of medians and interquartile ranges and were analyzed using a Kruskal-Wallis multiple range test. Further statistical analyses for individual groups were done with the Mann-Whitney U-test.

3. Results

3.1. Effects of central injection of dynorphin A-(1–13)

Dynorphin A-(1–13) (0.3–3 μg, i.c.v.) produced a marked prolongation of step-down latency in mice pretreated with cycloheximide (30 mg/kg, s.c.) (Fig. 1). A higher dose (12.5 μg, i.c.v.) of dynorphin A-(1–13) failed to affect the cycloheximide (30 mg/kg)-induced short latency to step down, since this dose (12.5 μg) of dynorphin A-(1–13) should have produced marked analgesia.

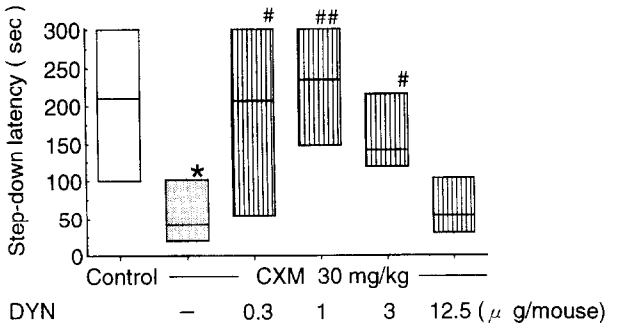

Fig. 1. Effects of dynorphin A-(1–13) (DYN) on the cycloheximide (CXM)-induced impairment of passive avoidance response in mice. DYN (i.c.v.) and CXM (s.c.) were injected 15 and 30 min before training, respectively. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 10$. * $P < 0.01$ vs. control. # $P < 0.05$; ## $P < 0.01$ vs. CXM alone.

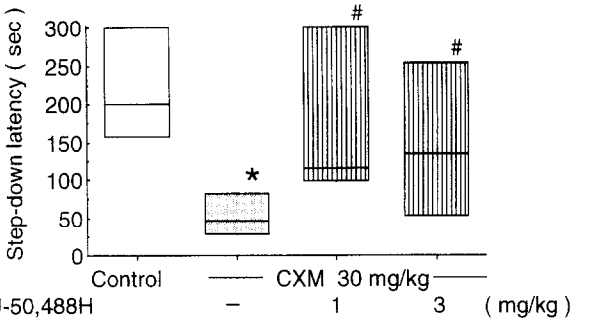

Fig. 3. Effects of U-50,488H on the cycloheximide (CXM)-induced impairment of passive avoidance response in mice. U-50,488H (i.p.) and CXM (s.c.) were injected 15 and 30 min before training, respectively. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 10$. * $P < 0.01$ vs. control, # $P < 0.05$ vs. CXM alone.

3.2. Effects of dynorphin A-(1–13) and U-50,488H on amnesia

Cycloheximide (30 mg/kg, s.c.) produced amnesia as indicated by shorter latency to step down. Dynorphin A-(1–13) (1, 3 and/or 10 mg/kg, i.p.) markedly prolonged step-down latency in mice pretreated with cycloheximide (30 mg/kg, s.c.), when administered 15 min before training (Fig. 2A) or retention tests (Fig. 2B). U-50,488H (1 and 3 mg/kg, i.p.) produced a significant increase in step-down latency in mice pretreated with cycloheximide (30 mg/kg, s.c.) (Fig. 3).

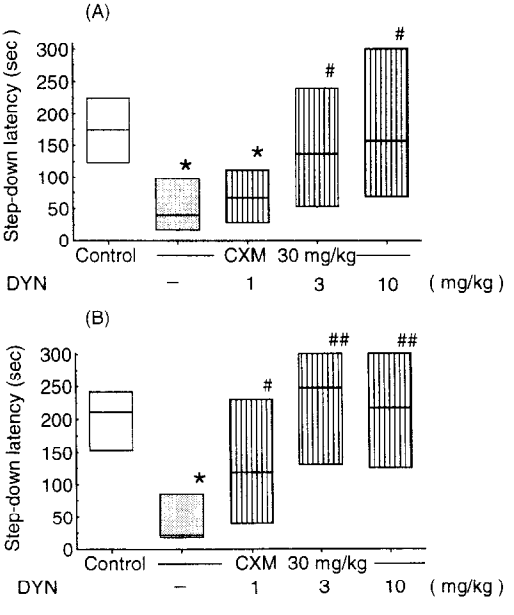

Fig. 2. Effects of dynorphin A-(1–13) (DYN) on the cycloheximide (CXM)-induced impairment of passive avoidance response in mice. DYN (i.p.) was injected 15 min before training (A) or retention tests (B), while CXM (s.c.) was administered 30 min before training. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 10$. * $P < 0.01$ vs. control, # $P < 0.05$; ## $P < 0.01$ vs. CXM alone.

3.3. Effects of naltrexone methobromide on anti-amnesic effects of dynorphin A-(1–13)

Naltrexone methobromide (1 mg/kg, s.c.) alone had no marked effects on step-down latency, while dynorphin A-(1–13) (3 and 10 mg/kg, i.p.) again elicited a marked prolongation of step-down latency in mice pretreated with cycloheximide (30 mg/kg, s.c.) (Fig. 4). The subcutaneous injection of naltrexone methobromide (1 mg/kg) had no marked effects on the antiamnesic effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.) (Fig. 4). The central injection of naltrexone methobromide (0.3 μg, i.c.v.) alone had no marked effects on step-down latency in mice

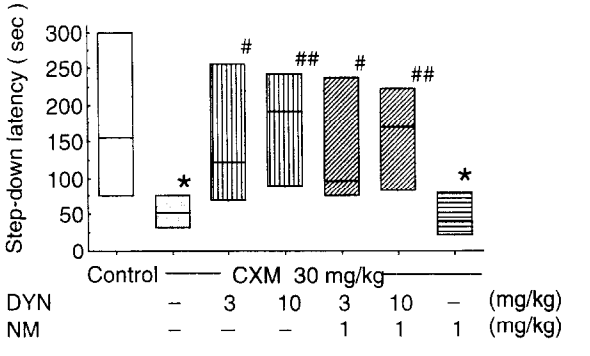

Fig. 4. Effects of systemic injection of naltrexone methobromide (NM) on the anti-amnesic effects of dynorphin A-(1–13) (DYN) in mice treated with cycloheximide (CXM). CXM (s.c.), NM (s.c.) and DYN (i.p.) were injected 30, 20 and 15 min before training, respectively. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 10$. * $P < 0.01$ vs. control; # $P < 0.05$, ## $P < 0.01$ vs. CXM alone.

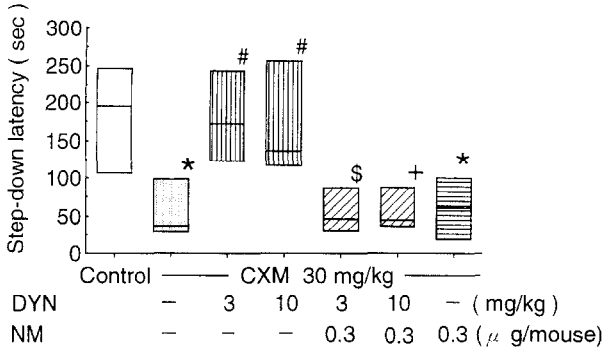

Fig. 5. Effects of intracerebroventricular injection of naltrexone methobromide (NM) on the anti-amnesic effects of dynorphin A-(1–13) (DYN) in mice treated with cycloheximide (CXM). CXM (s.c.), NM (i.c.v.) and DYN (i.p.) were injected 30, 20 and 15 min before training, respectively. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 8–14$. * $P < 0.01$ vs. control; # $P < 0.01$ vs. CXM alone; $ $P < 0.01$ vs. CXM+DYN (3 mg/kg); + $P < 0.05$ vs. CXM+DYN (10 mg/kg).

pretreated with cycloheximide (30 mg/kg, s.c.). Naltrexone methobromide (0.3 μg, i.c.v.) markedly inhibited the anti-amnesic effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.) (Fig. 5).

3.4. Effects of nor-binaltorphimine on anti-amnesic effects of dynorphin A-(1–13)

nor-Binaltorphimine (2 mg/kg, s.c.) alone had no significant effects on step-down latency in mice pretreated with cycloheximide (30 mg/kg, s.c.). The anti-amnesic effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.) were almost completely antagonized by the κ-selective opioid receptor antagonist nor-binaltorphimine (2 mg/kg, s.c.) (Fig. 6).

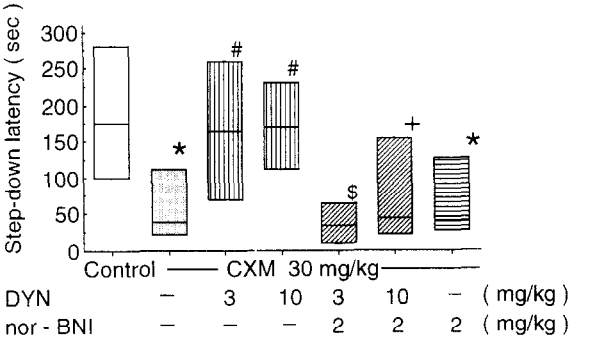

Fig. 6. Effects of nor-binaltorphimine (nor-BNI) on the anti-amnesic effects of dynorphin A-(1–13) (DYN) in mice treated with cycloheximide (CXM). DYN (i.p.), nor-BNI (s.c.) and CXM (s.c.) were injected 15, 20 and 30 min before training, respectively. Each value represents the median and interquartile ranges, which are the distances between the first quartile (25th percentile) and the third quartile (75th percentile). $n = 9$ or 10. * $P < 0.05$ vs. control; # $P < 0.05$ vs. CXM alone; $ $P < 0.05$ vs. CXM+DYN (3 mg/kg); + $P < 0.05$ vs. CXM+DYN (10 mg/kg).

4. Discussion

Although Izquierdo et al. (1985) have shown that dynorphin A-(1–13) does not cause retrograde amnesia for shuttle avoidance or inhibitory avoidance learning, the effects of dynorphin A-(1–17) on memory in normal animals appear to depend on the intensity of reinforcements (Del Cerro and Borrell, 1990). Dynorphin A-(1–17) facilitates the inhibitory avoidance response in rats subjected to weaker footshock when training, while the peptide exerts a deleterious effect in the case of stronger footshock. In contrast the effects of dynorphin A-(1–13) in amnesic animal models seem to be consistent. κ-Selective opioid receptor agonists such as ketocyclazocine and dynorphin A-(1–17) have been shown to improve the impairment of acquisition or consolidation process of memory in adrenalectomized rats (Jefferys et al., 1985). Dynorphin A-(1–13) ameliorates in rodents the scopolamine-, pirenzepine- and basal forebrain lesion-induced memory impairment associated with Alzheimer's disease (Itoh et al., 1993a; Ukai et al., 1993, 1995a,b). Moreover, it is possible that dynorphin A-(1–13) affects the amnesia resulting from the inhibition of protein synthesis, because cholinergic and GABAergic neuronal systems play a major role in the protein synthesis inhibitor cycloheximide-induced amnesia (Nabeshima et al., 1988). In this study, a 30-mg/kg dose of cycloheximide shortened step-down latency in passive avoidance learning, indicating amnesia. The results show that systemic administration of dynorphin A-(1–13) (1, 3 and/or 10 mg/kg) markedly prolonged step-down latency of passive avoidance learning in cycloheximide (30 mg/kg)-pretreated mice. The beneficial effects of dynorphin A-(1–13) were seen when the drug was administered before training or retention tests. Although the use of post-training administration of dynorphin A-(1–13) and U-50,488H would help to more clearly discriminate whether the observed effects on retention performance are due to impairment of acquisition or consolidation processes, we could not provide evidence regarding the effects of post-training administration of κ-opioid receptor agonists on the cycloheximide-induced amnesia. Ukai et al. (1995a) have indicated that the pre-training and pre-retention but not post-training administration of dynorphin A-(1–13) ameliorates the scopolamine-induced amnesia. These findings suggest that κ-selective opioid receptor agonists improve the disturbance of memory in acquisition and retrieval processes.

The effects of systemic administration of dynorphin A-(1–13) could also be mediated via the central nervous system, because intracerebroventricular injection of dynorphin A-(1–13) (0.3–3 μg, i.c.v.) evokes an anti-amnesic effect, and intracerebroventricular but not systemic injection of the quaternary analog of the opioid receptor antagonist, naltrexone methobromide (0.3 μg, i.c.v.), significantly inhibited the anti-amnesic effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.). Moreover, the κ-opioid

receptor antagonist, nor-binaltorphimine (2 mg/kg, s.c.), antagonized the effects of dynorphin A-(1–13) (3 and 10 mg/kg, i.p.), indicating that the effects of systemic administration of dynorphin A-(1–13) are mediated through central κ-opioid receptors. These results are in accordance with evidence that U-50,488H (1 and 3 mg/kg, i.p.), another κ-selective opioid receptor agonist, was also effective to attenuate the cycloheximide (30 mg/kg)-induced amnesia.

The intracerebroventricular and systemic administration of dynorphin A-(1–13) itself has been demonstrated not to influence a variety of behavioral responses (Ukai et al., 1992a,b). Furthermore, it is unlikely that dynorphin A-(1–13) (1, 3 and 10 mg/kg, i.p.; 0.3, 1 and 3 μg, i.c.v.) alters the sensitivity of the mouse to electroshock, because there were no marked changes in behavioral responses to electroshock during training. In particular, if dynorphin A-(1–13) attenuates the sensitivity to electroshock during training, the step-down latency in retention tests should have been shortened. In addition, a 12.5 μg dose (i.c.v.) of the peptide did not affect the cycloheximide (30 mg/kg)-induced amnesia, possibly as a result of analgesia.

To our knowledge, this is the first demonstration that systemic administration of dynorphin A-(1–13) improves amnesia through the mediation of central κ-opioid receptors.

Acknowledgements

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. We are grateful to Boehringer Ingelheim KG and The Upjohn Co. for the generous gifts of naltrexone methobromide and U-50,488H, respectively.

References

Cuello, A.C., 1983, Central distribution of opioid peptides, Br. Med. Bull. 39, 11.

Del Cerro, S. and J. Borrell, 1990, Dynorphin$_{1-17}$ can enhance or impair retention of an inhibitory avoidance response in rats, Life Sci. 47, 1453.

Haley, T.J. and W.G. McCormick, 1957, Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse, Br. J. Pharmacol. 12, 12.

Introini-Collison, I.B. and L. Cahill, C.M. Baratti and J.L. McGaugh, 1987, Dynorphin induces task-specific impairment of memory, Psychobiology 15, 171.

Itoh, J., M. Ukai and T. Kameyama, 1993a, Dynorphin A-(1–13) markedly improves scopolamine-induced impairment of spontaneous alternation performance in mice, Eur. J. Pharmacol. 236, 341.

Itoh, J., M. Ukai and T. Kameyama, 1993b, Dynorphin A-(1–13) potently prevents memory dysfunctions induced by transient cerebral ischemia in mice, Eur. J. Pharmacol. 234, 9.

Itoh, J., M. Ukai and T. Kameyama, 1994, Dynorphin A-(1–13) potently improves the impairment of spontaneous alternation performance induced by the μ-selective opioid receptor agonist DAMGO in mice, J. Pharmacol. Exp. Ther. 269, 15.

Izquierdo, I., 1980, Effect of β-endorphin and naloxone on acquisition, memory, and retrieval of shuttle avoidance and habituation learning in rats, Psychopharmacology 69, 111.

Izquierdo, I., M.A.M.R. De Almeida and V.R. Emiliano, 1985, Unlike β-endorphin, dynorphin$_{1-13}$ does not cause retrograde amnesia for shuttle avoidance or inhibitory avoidance learning in rats, Psychopharmacology 87, 216.

Izquierdo, I., D.O. Souza, M.A. Carrasco, R.D. Dias, M.L. Perry, S. Eisinger, E. Elisabetsky and D.A. Vendite, 1980, β-Endorphin causes retrograde amnesia and is released from the rat brain by various forms of training and stimulation, Psychopharmacology 70, 173.

Jefferys, D., J. Boublik and J.W. Funder, 1985, A κ-selective opioidergic pathway is involved in the reversal of a behavioral effect of adrenalectomy, Eur. J. Pharmacol. 107, 331.

Jiang, H.-K., V. Owyang, J.-S. Hong and M. Gallagher, 1989, Elevated dynorphin in the hipppocampal formation of aged rats: relation to cognitive impairment on a spatial learning task, Proc. Natl. Acad. Sci. USA 86, 2948.

Kameyama, T. and M. Ukai, 1983, Multi-dimensional analysis of behavior in mice treated with morphine, endorphins and [des-tyrosine^1]-γ-endorphin, Pharmacol. Biochem. Behav. 19, 671.

Nabeshima, T., Y. Noda, K. Itoh and T. Kameyama, 1988, Role of cholinergic and GABAergic neuronal systems in cycloheximide-induced amnesia in mice, Pharmacol. Biochem. Behav. 31, 405.

Pavone, F., R. Populin, C. Castellano, G. Kreil and P. Melchiorri, 1990, Deltorphin, a naturally occurring peptide with high selectivity for δ opioid receptors, improves memory consolidation in two inbred strains of mice, Peptides 11, 591.

Schulteis, G., J.L. Martinez, Jr. and V.J. Hruby, 1988, Stimulation and antagonism of opioid δ-receptors produce opposite effects on active avoidance conditioning in mice, Behav. Neurosci. 102, 678.

Ukai, M., T. Kamiya, T. Toyoshi and T. Kameyama, 1992a, Systemic administration of dynorphin A-(1–13) markedly inhibits different behaviors induced by cocaine in the mouse, Neuropharmacology 31, 843.

Ukai, M., T. Kobayashi and T. Kameyama, 1993, Dynorphin A-(1–13) attenuates basal forebrain-lesion-induced amnesia in rats, Brain Res. 625, 355.

Ukai, M., T. Kobayashi, N. Shinkai, X. Shan-Wu and T. Kameyama, 1995a, Dynorphin A-(1–13) potently improves scopolamine-induced impairment of passive avoidance response in mice, Eur. J. Pharmacol. 274, 89.

Ukai, M., N. Shinkai and T. Kameyama, 1995b, κ-Opioid receptor agonists improve pirenzepine-induced disturbance of spontaneous alternation performance in the mouse, Eur. J. Pharmacol. 281, 173.

Ukai, M., T. Toyoshi and T. Kameyama, 1992b, Dynorphin A-(1–13) preferentially inhibits behaviors induced by the D_2 dopamine agonist RU24213 but not by the D_1 dopamine agonist SK&F38393, Pharmacol. Biochem. Behav. 42, 755.