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# κ-Opioid receptor agonists improve pirenzepine-induced disturbance of spontaneous alternation performance in the mouse

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**Abstract**

We investigated the effects of κ-opioid receptor agonists such as dynorphin A-(1–13) and U-50,488H on the muscarinic $M_1$-selective receptor antagonist pirenzepine (3 μg, i.c.v.)-induced impairment of spontaneous alternation performance in the mouse. Although dynorphin A-(1–13)(1–5.6 μg, i.c.v.) or U-50,488H ((±)*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide, methanesulfonate hydrate) (0.1–1 mg/kg, i.p.) alone did not influence either spontaneous alternation performance or total arm entries, pirenzepine (3 μg, i.c.v.) impaired spontaneous alternation performance without producing any significant change in total arm entries. In contrast, dynorphin A-(1–13) (3 and 5.6 μg, i.c.v.) and U-50,488H (0.3 and 1 mg/kg, i.p.) ameliorated the pirenzepine (3 μg, i.c.v.)-induced impairment of spontaneous alternation performance. The ameliorating effects of dynorphin A-(1–13)(3 μg, i.c.v.) and U-50,488H (0.3 mg/kg, i.p.) were almost completely reversed by pretreatment with nor-binaltorphimine (4 μg, i.c.v.), a κ-opioid receptor antagonist. These results suggest that the stimulation of κ-opioid receptors improves memory dysfunctions resulting from the blockade of muscarinic $M_1$ receptors.

*Keywords:* Alternation performance, spontaneous; Dynorphin A-(1–13); U-50,488H; κ-Opioid receptor; Mouse

## 1. Introduction

Muscarinic receptors have been classified into at least $M_1$ and $M_2$ subtypes (Potter et al., 1984). An $M_1$ subtype of muscarinic receptor is distributed throughout the hippocampus and cortex (Levey et al., 1991; Wall et al., 1991) and is thought to play an essential role in memory processes. Although the $M_1$-selective receptor antagonist pirenzepine administered intracranially has been reported to disrupt memory (Hagan et al., 1987; Messer et al., 1987), the effects of pirenzepine on memory processes have not been characterized in detail.

The density of κ-opioid receptors has been demonstrated to be increased in the postmortem brain of Alzheimer's disease patients with dementia (Hiller et al., 1987) and in the hippocampus of amnesic rats after lesioning the basal forebrain with ibotenic acid (Ofri et al., 1992), suggesting that the release of dynorphins is decreased in the brain of Alzheimer's disease patients. In particular, the κ-opioid receptor agonist dynorphin A-(1–17) facilitates the retention of inhibitory avoidance responses (Del Cerro and Borrell, 1990). Because the post-training i.p. administration of dynorphin A-(1–13) impairs the habituation of exploration and the retention of inhibitory avoidance (Introini-Collison et al., 1987), the effects of κ-opioid receptor agonists on learning and memory are inconsistent. In contrast, dynorphin A-(1–13) attenuates impairments of passive avoidance response evoked by basal forebrain lesion with ibotenic acid (Ukai et al., 1993). Moreover, it has been reported that dynorphin A-(1–13) improves the scopolamine-induced impairment of spontaneous alternation performance and passive avoidance responses (Itoh et al., 1993a; Ukai et al., 1995b). However, the effects of κ-opioid receptor agonists on the cholinergic system in terms of receptor subtypes such as muscarinic $M_1$ and $M_2$ are still unknown.

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The present study was designed to examine the effects of the κ-opioid receptor agonists dynorphin A-(1–13) and U-50,488H on the muscarinic $M_1$ receptor antagonist pirenzepine-induced impairment of spontaneous alternation performance related to spatial working memory (Sarter et al., 1988; Itoh et al., 1993a).

## 2. Materials and methods

### 2.1. Animals

Male mice of the ddY strain (Nihon SLC Co., Hamamatsu, Japan), weighing between 30 and 40 g, were used. They were housed in groups of eight under standard conditions (22 ± 2° C, 50 ± 10% humidity, light-dark cycle with the light on between 8:00 and 20:00 h) with free access to food and water. The mice were used for the experiments following adaptation to laboratory conditions for at least 1 week and were naive to each of the tests used in the present study. The experiments were conducted between 10:00 and 18:00 h in a sound-attenuated room.

### 2.2. Drugs

Pirenzepine dihydrochloride, nor-binaltorphimine (Research Biochemicals, Natick, MO, USA) and dynorphin A-(1–13) (Peptide Institute, Minoh, Japan) were dissolved in isotonic saline solution (Otsuka Pharmaceutical Co., Tokyo, Japan) and administered into the lateral ventricle (i.c.v.) of the mouse brain according to the method of Haley and McCormick (1957) in an injection volume of 5 μl/mouse over 1 min through an injection needle. The mice were lightly anesthetized with ether. Stereotaxic instruments were not employed. The site was checked by injecting a 1:10 dilution of ink in the lateral and 3rd ventricles but not in the others. Severe tissue damage was never seen in the brain. Because the brief etherization during i.c.v. injection almost completely prevents stress-related events, neither insertion of the needle nor injection of 5 μl of isotonic saline solution actually had a significant influence on behavior such as spontaneous alternation performance in normal mice as previously described. U-50,488H ((±)*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide, methanesulfonate hydrate (Upjohn Co., Kalamazoo, MI, USA) was dissolved in 0.9% saline solution and administered i.p. in an injection volume of 0.1 ml/10 g body weight. Doses were expressed in terms of the base.

### 2.3. Apparatus

Spontaneous alternation performance was assessed in a black-painted Y-maze which was 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top and positioned at an equal angle.

### 2.4. Procedures

The testing procedure was based on that of Sarter et al. (1988). Following vehicle or drug injection, each of

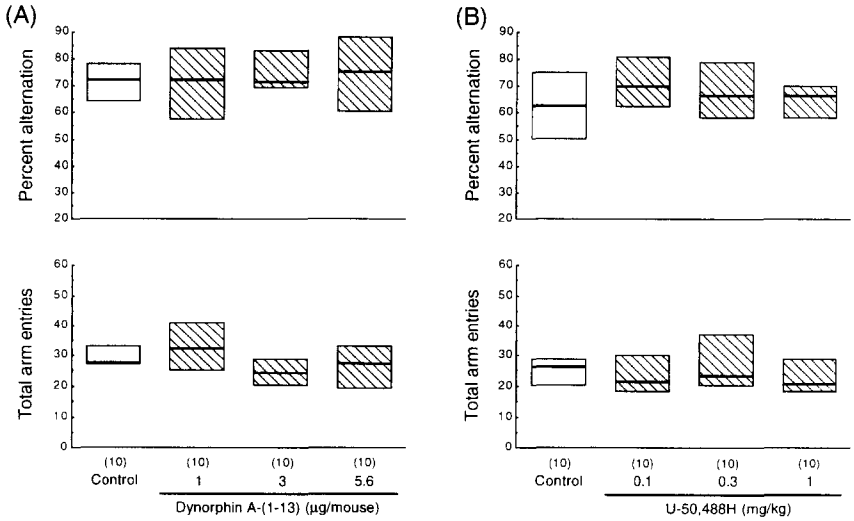


Fig. 1. Effects of dynorphin A-(1–13) (A) and U-50,488H (B) on percent alternation and total arm entries in normal mice. Each value represents the mean ± S.E. Dynorphin A-(1–13) (i.c.v.) and U-50,488H (i.p.) were administered 15 and 30 min before behavioral measurements, respectively. The number of mice used is shown in parentheses.

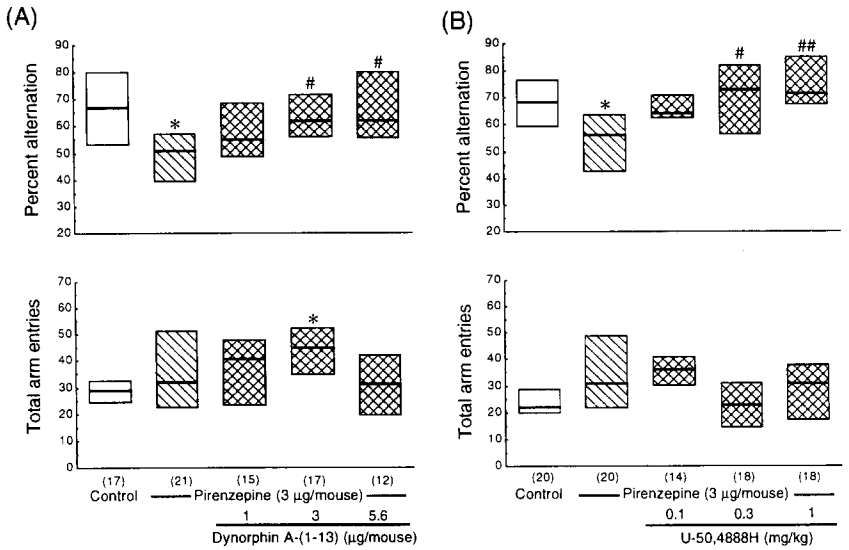


Fig. 2. Effects of dynorphin A-(1–13) (A) and U-50,488H (B) on the pirenzepine-induced change in percent alternation and total arm entries in mice. Each value represents the mean ± S.E. Pirenzepine (i.c.v.), dynorphin A-(1–13) (i.c.v.) and U-50,488H (i.p.) were administered 10, 15 and 30 min before behavioral measurements, respectively. The number of mice used is shown in parentheses. $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ vs. pirenzepine alone.

the mice was placed at the end of one arm and allowed to move freely through the maze for an 8-min test session. The sequence of arm entries was recorded manually. An alternation was defined as the entry into all three arms on consecutive choices. The number of maximum alternations was then the total number of arms entered minus 2, and the percent alternation was calculated as (actual alternations/maximum alternations) × 100. Mice which exhibited arm entries less than 8 times during the test were eliminated from the study because the data obtained from these mice were not considered to reflect precise alternations. Piren-

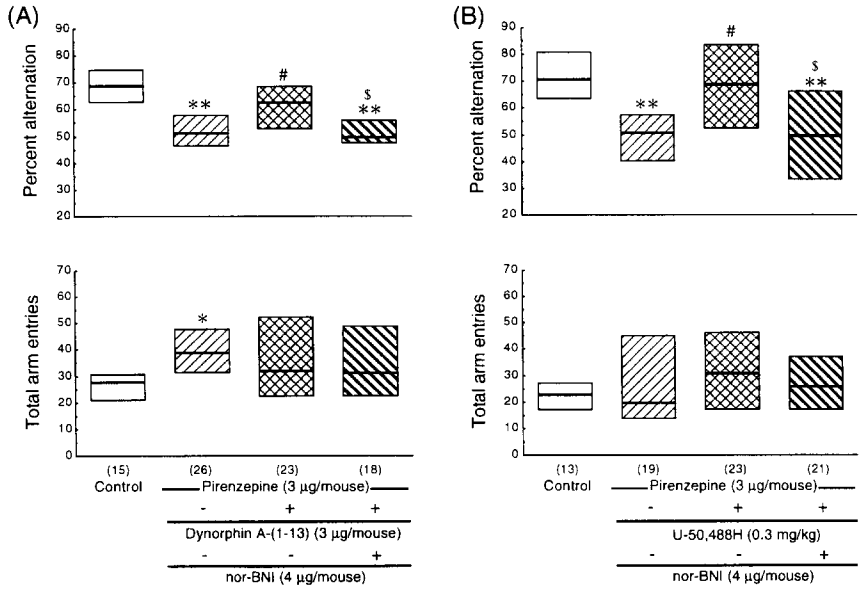


Fig. 3. Effects of dynorphin A-(1–13) (A), U-50,488H (B) and their combinations with nor-binaltorphimine (nor-BNI) on the pirenzepine-induced impairment of percent alternation and total arm entries in mice. Each value represents the mean ± S.E. Pirenzepine (i.c.v.), dynorphin A-(1–13) (i.c.v.), U-50,488H (i.p.) and nor-binaltorphimine (i.c.v.) were administered 10, 15, 30 and 60 min before behavioral measurements, respectively. The number of mice used is shown in parentheses. $^{**}P < 0.01$ vs. control, $^{\#}P < 0.05$ vs. pirenzepine alone, $^{\$}P < 0.05$ vs. pirenzepine plus dynorphin A-(1–13) or U-50,488H.

zepine, dynorphin A-(1–13), U-50,488H and nor-binaltorphimine were injected 10, 15, 30 and 60 min before measurements, respectively.

### 2.5. Statistical analysis

The results were expressed as the means ± S.E.M. and analyzed by a Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA). Further statistical analysis for post hoc comparisons was done with a Bonferroni's non-parametric multiple comparison test (two-tailed) (Wallenstein et al., 1980). The criterion for statistical significance was $P < 0.05$ in all statistical evaluations.

## 3. Results

### 3.1. Effects of κ-opioid receptor agonists on spontaneous alternation performance

Dynorphin A-(1–13) (1–5.6 μg) or U-50,488H (0.1–1 mg/kg) alone did not change percent alternation (Kruskal-Wallis analysis: $H = 0.19$, $P > 0.05$ for dynorphin A-(1–13); $H = 1.62$, $P > 0.05$ for U-50,488H) (Fig. 1A,B) or total arm entries (Kruskal-Wallis analysis: $H = 4.47$, $P > 0.05$ for dynorphin A-(1–13); $H = 1.58$, $P > 0.05$ for U-50,488H) (Fig. 1A,B).

### 3.2. Effects of κ-opioid receptor agonists on pirenzepine-induced impairment

Pirenzepine (3 μg) significantly decreased percent alternation (Kruskal-Wallis analysis: $H = 15.69$, $P < 0.05$ for dynorphin A-(1–13); $H = 19.22$, $P < 0.01$ for U-50,488H) (Fig. 2A,B) without producing any marked change in total arm entries (Kruskal-Wallis analysis: $H = 9.06$, $P > 0.05$ for dynorphin A-(1–13); $H = 2.38$, $P > 0.05$ for U-50,488H) (Fig. 2A,B). Dynorphin A-(1–13) (3 and 5.6 μg) significantly inhibited the pirenzepine (3 μg)-induced decrease in percent alternation ($P < 0.05$) (Fig. 2A). Pirenzepine (3 μg) plus dynorphin A-(1–13) (3 μg) significantly increased total arm entries as compared with controls ($P < 0.05$) (Fig. 2A). U-50,488H (0.3 and 1 mg/kg) significantly inhibited the pirenzepine (3 μg)-induced reduction of percent alternation ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 2B)

### 3.3. Effects of nor-binaltorphimine on κ-opioid receptor agonist-induced effects

The κ-opioid receptor antagonist nor-binaltorphimine (4 μg) alone did not influence percent alternation or total arm entries in normal mice (data not shown). Nor-binaltorphimine (4 μg) almost completely antagonized the effects of dynorphin A-(1–13) (3 μg) and U-50,488H (0.3 mg/kg) on the pirenzepine (3 μg)-induced reduction of percent alternation (Kruskal-Wallis analysis: $H = 22.95$, $P < 0.05$ for dynorphin A-(1–13); $H = 18.13$, $P < 0.05$ for U-50,488H) (Fig. 3A,B) without influencing total arm entries (Kruskal-Wallis analysis: $H = 9.37$, $P > 0.05$ for dynorphin A-(1–13); $H = 11.59$, $P > 0.05$ for U-50,488H)(Fig. 3A,B).

## 4. Discussion

There are several reports that intracranial or systemic injection of the muscarinic $M_1$-selective receptor antagonist pirenzepine impairs passive avoidance response (Caulfield et al., 1983), spatial alternation behavior (Bymaster et al., 1993), water maze performance (Hunter and Roberts, 1988) and radial arm maze performance (Sala et al., 1991). In the present study, pirenzepine (3 μg) impaired spontaneous alternation performance without affecting locomotor activity, as indexed by total arm entries, suggesting that the blockade of muscarinic $M_1$ receptors plays an inhibitory role in spontaneous alternation performance.

Dynorphin A-(1–8) injected into the dorsal hippocampus of normal rats impairs both spatial working memory in a radial maze win-stay task and acquisition of reference memory in a water maze (McDaniel et al., 1990). In contrast, dynorphin A-(1–17) facilitates the retention of inhibitory avoidance (Del Cerro and Borrell, 1990). However, the present study indicates that dynorphin A-(1–13) and U-50,488H failed to affect spontaneous alternation performance associated with spatial working memory in normal animals. These results are consistent with the report that dynorphin A-(1–13) has no effects on inhibitory or shuttle avoidance responses in normal rats (Izquierdo et al., 1985).

Dynorphin A-(1–13)(3 and 5.6 μg) and U-50,488H (0.3 and 1 mg/kg) improved the pirenzepine-induced impairment of spontaneous alternation performance. Furthermore, the effects of these κ-opioid receptor agonists were completely antagonized by pretreatment with nor-binaltorphimine (4 μg), a κ-opioid receptor antagonist. These results suggest that dynorphin A-(1–13) and U-50,488H improve the pirenzepine-induced dysfunction of working memory through an interaction with κ-opioid receptors, although the precise mechanism of interaction between κ-opioid and cholinergic systems is still unclear.

Because there is a report that the muscarinic $M_1$ receptor agonist McN-A-343 and the muscarinic $M_2$ receptor antagonists gallamine and AF-DX116 facilitate learning in an active avoidance paradigm in rats (Sen and Bhattacharya, 1991), it should be considered that pirenzepine impairs learning and memory by blocking mainly muscarinic $M_1$ receptors. Actually,

McN-A-343 has been shown to inhibit the pirenzepine-induced impairment of spontaneous alternation performance (Ukai et al., 1995a). Therefore, the improving effects of dynorphin A-(1–13) and U-50,488H on the pirenzepine-induced dysfunction of memory may result from the activation of cholinergic systems via muscarinic $M_1$ receptors.

Dynorphin A-(1–13) also improves the amnesia induced by scopolamine or basal forebrain lesion (Itoh et al., 1993a; Ukai et al., 1993; Ukai et al., 1995b). However, because $\kappa$-opioid receptor agonists inhibit the spontaneous release of acetylcholine from cholinergic neurons (Lapchak et al., 1989), dynorphin A-(1–13) or U-50,488H may postsynaptically activate cholinergic systems. Dynorphin A-(1–13) has been shown to decrease dopamine release in the striatum (Reid et al., 1988). In addition, haloperidol attenuates the scopolamine-induced impairment of working memory in a radial maze task (McGurk et al., 1988). Similarly, Itoh et al. (1993b) have reported that the dopamine $D_2$-selective receptor antagonist *S*(-)-sulpiride attenuates the scopolamine-induced impairment of spontaneous alternation performance. In contrast, the dopamine $D_2$ receptor agonist RU 24213 completely antagonizes the improving effects of dynorphin A-(1–13) on the scopolamine-induced impairment of spontaneous alternation performance (Itoh et al., 1993a). Therefore, the improving effects of dynorphin A-(1–13) or U-50,488H on the pirenzepine-induced impairment of spontaneous alternation performance may be based upon the inactivation of dopamine neurons through the mediation of $\kappa$-opioid receptors.

In conclusion, dynorphin A-(1–13) and U-50,488H improve the impairments of spatial memory resulting from the blockade of muscarinic $M_1$ receptors through the mediation of $\kappa$-opioid receptors.

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## References

Bymaster, F.P., I. Heath, J.C. Hendrix and H.E. Shannon, 1993, Comparative behavioral and neurochemical activities of cholinergic antagonists in rats, J. Pharmacol. Exp. Ther. 267, 16.

Caulfield, M.P.C., G.A. Higgins and D.W. Straughan, 1983, Central administration of the muscarinic receptor subtype-selective antagonist pirenzepine selectivity impairs passive avoidance learning in the mouse, J. Pharm. Pharmacol. 24, 131.

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.

Hagan, J.J., J.H.M. Jansen and C.L.E. Broekkamp, 1987, Blockade of spatial learning by the $M_1$ muscarinic antagonist pirenzepine, Psychopharmacology 93, 470.

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

Hiller, J.M., Y. Itzak and E.J. Simon, 1987, Selective changes in $\mu$, $\delta$ and $\kappa$ opioid receptor binding in certain limbic regions of the brain in Alzheimer's disease patients, Brain Res. 106, 17.

Hunter, A.J. and F.F. Roberts, 1988, The effect of pirenzepine on spatial learning in the Morris water maze, Pharmacol. Biochem. Behav. 30, 519.

Introini-Collison, I.B., 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 soponta-neous alternation performance in mice, Eur. J. Pharmacol. 236, 341.

Itoh, J., M. Ukai and T. Kameyama, 1993b, Dopaminergic involvement in the improving effects of dynorphin A-(1–13) on scopolamine-induced impairment of alternation performance, Eur. J. Pharmacol. 241, 99.

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

Lapchak, P.A., D.M. Araujo and B. Collier, 1989, Regulation of endogenous acetylcholine release from mammalian brain slices by opiate receptors: hippocampus, striatum and cerebral cortex of guinea pig and rat, Neuroscience 31, 313.

Levey, A.I., C.A. Kitt, W.F. Simonds, D.L. Price and M.R. Brann, 1991, Identification and localization of muscarinic acetylcholine receptor protein in brain with subtype-specific antibodies, J. Neurosci. 11, 3218.

McDaniel, K.W., W. Mundy and H.A. Tilson, 1990, Microinjection of dynorphin into the hippocampus impairs spatial learning in rats, Pharmacol. Biochem. Behav. 35, 429.

McGurk, S.R., E.D. Levin and L.L. Butcher, 1988, Cholinergic-dopaminergic interactions in radial-arm maze performance, Behav. Neural Biol. 49, 234.

Messer, W.S., Jr., G.J. Thomas and W. Hoss, 1987, Selectivity of pirenzepine in the central nervous system. II. Differential effects of pirenzepine and scopolamine on performance of a representational memory task, Brain Res. 407, 37.

Ofri, D., L. Fan, E.J. Simon and J.M. Hiller, 1992, Lesioning of the nucleus basalis of Meynert has differential effects on mu, delta and kappa opioid receptor binding in rat brain: a quantitative autoradiographic study, Brain Res. 581, 252.

Potter, L.T., D.D. Flynn, D.L. Hanchett, J. Kalinoski, Luber-Narod and D.C. Mash, 1984, Independent $M_1$ and $M_2$ receptors: ligands, autoradiography, and functions, Trends Pharmacol. Sci., Suppl. 5, 22.

Reid, M., M. Herrera-Marschitz, T. Hökfelt, L. Terenius and U. Ungerstedt, 1988, Differential modulation of striatal dopamine release by intranigral injection of $\gamma$-aminobutyric acid (GABA), dynorphin A and substance P, Eur. J. Pharmacol. 147, 411.

Sala, M., D. Braida, P. Calcaterra, M.P. Leone, F.A. Comotti, S. Gianola and E. Gori, 1991, Effect of centrally administered atropine and pirenzepine on radial arm maze performance in the rat, Eur. J. Pharmacol. 194, 45.

Sarter, M., G. Bodewitz, and D. Stephens, 1988, Attenuation of scopolamine-induced impairment of spontaneous alternation behaviour by antagonist but not inverse agonist and agonist $\beta$-carbolines, Psychopharmacology 94, 491.

Sen, A.P. and S.K. Bhattacharya, 1991, Effect of selective muscarinic

receptor agonists and antagonists on active-avoidance learning acquisition in rats, Indian J. Exp. Biol. 29, 136.

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

Ukai, M., N. Shinkai and T. Kameyama, 1995a, Cholinergic receptor agonists inhibit pirenzepine-induced dysfunction of spontaneous alternation performance in the mouse, Gen. Pharmacol. (in press).

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

Wall, S.J., R.P. Yasuda, F. Hory, S. Flagg, B.M. Martin, E.I. Ginns and B.B. Wolfe, 1991, Production of antisera selective for m1 muscarinic receptors using fusion proteins: distribution of m1 receptors in rat brain, Mol. Pharmacol. 39, 643.

Wallenstein, S., C.L. Zucker and J.L. Fleiss, 1980, Some statistical methods useful in circulation research, Circulation Res. 47, 1.