British Journal of Pharmacology (1998) 123, 920–926

Reversion of muscarinic autoreceptor agonist-induced acetylcholine decrease and learning impairment by dynorphin A (1–13), an endogenous κ-opioid receptor agonist

[1]Masayuki Hiramatsu, Hiroyasu Murasawa, Hiromasa Mori & Tsutomu Kameyama

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

1 We investigated whether carbachol, a muscarinic receptor agonist, induces learning and memory impairment, and if so, dynorphin A (1–13), an endogenous κ-opioid receptor agonist, ameliorates the impairment of learning and memory induced by carbachol, by use of a step-through type passive avoidance task.

2 Carbachol induced a dose-related dual response. Carbachol (1.66 pmol per rat) administered directly into the hippocampus significantly shortened the step-through latency, while lower (0.166 pmol per rat) and higher (16.6 pmol per rat) doses of carbachol did not induce learning or memory impairment.

3 Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) administered 5 min after carbachol injection significantly reversed carbachol-induced impairment of learning and memory.

4 Perfusion with carbachol (3×10^{-4} M) significantly decreased acetylcholine release in the hippocampus during perfusion as determined by *in vivo* brain microdialysis. This decrease in acetylcholine release was suppressed by co-perfusion with a low dose of atropine (10^{-7} M).

5 Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) immediately before carbachol perfusion completely blocked this decrease in extracellular acetylcholine concentration induced by carbachol.

6 These antagonistic effects of dynorphin A (1–13) were abolished by treatment with norbinaltorphimine (5.44 nmol per rat, i.c.v.), a selective κ-opioid receptor antagonist, 5 min before dynorphin A (1–13) treatment.

7 These results suggest that the neuropeptide dynorphin A (1–13) ameliorates the carbachol-induced impairment of learning and memory, accompanied by attenuation of the reductions in acetylcholine release which may be associated with dysfunction of presynaptic cholinergic neurones via κ-opioid receptors.

Keywords: Dynorphin A (1–13); κ-opioid receptor agonist; carbachol; muscarinic autoreceptor; learning and memory

Introduction

Memory deficit in presenile dementia and Alzheimer's disease have been proposed to be due to attenuated functional activity of central cholinergic neurones (Coyle *et al*., 1983). To clarify these functions, cholinergic innervation of the hippocampus and cortex has been studied extensively because of its involvement in cognitive processes such as learning and memory, arousal, and attention (Squire, 1992; Zola-Morgan & Squire, 1993). The principal therapeutic approach for Alzheimer's disease is the reinstatement of the level of postsynaptic cholinergic stimulation, either directly with muscarinic receptor agonists, or indirectly by elevating the levels of endogenous acetylcholine with acetylcholine esterase inhibitors (Rupniak, 1992). Although several cholinomimetic drugs have been tested clinically, only a few are currently available because of the rather limited clinical improvements (Davis *et al*., 1992). The clinical failure of cholinomimetics in the therapy of senile and presenile dementia may be related to their receptor non-specificity, the stimulation of both M_1 and M_2 receptors leading to functionally opposing effects.

Administration of cholinomimetic drugs affects cholinergic neurotransmission at different sites. In addition to stimulating postsynaptic receptors, cholinomimetic drugs also affect the release of endogenous acetylcholine by activating autoreceptors. For example, when muscarinic autoreceptors located on cholinergic nerve terminals were stimulated, acetylcholine release was decreased by inhibitory feedback regulation (Nordström & Bartfai, 1980; Marchi *et al*., 1981; Raiteri *et al*., 1984; Moor *et al*., 1995). Recent information has provided a useful and valuable new foundation for addressing currently unresolved questions related to muscarinic function. With respect to brain tissue, one such unresolved question of considerable interest is the characterization of muscarinic autoreceptor function. Quirion *et al*. (1989) described a selective and significant decrease in the number of high-affinity agonist binding sites, generally regarded as M_2, in the Alzheimer's disease brain tissue. They have also demonstrated that an M_2 muscarinic receptor antagonist, BIBN-99, facilitated acetylcholine release and cognitive performance in aged memory-impaired rats, and these findings may have implications for the treatment of degenerative disorders associated with impaired cholinergic function (Quirion *et al*., 1995).

We have recently found that administration of dynorphin A (1–13), an endogenous κ-opioid receptor agonist, completely blocked the decrease in extracellular acetylcholine concentration induced by galanin in the hippocampus of rats (Hiramatsu *et al*., 1996b). Galanin reduces cholinergic activity at presynaptic sites of the septo-hippocampal pathway and induces impairment of learning and memory (Fisone *et al*., 1987). Dynorphin A (1–13) significantly prevented galanin-induced impairment of the acquisition of learning and memory recall in rats (Hiramatsu *et al*., 1996b). However, the mechanism underlying this improvement of memory by dynorphin A (1–13) is still unknown.

[1] Author for correspondence.

A low dose of carbachol, a muscarinic receptor agonist, is supposed to act at presynaptic muscarinic receptors and modulates cholinergic activity (Sen & Bhattacharya, 1991; Moor *et al*., 1995). Presynaptically located muscarinic receptors are predominantly of the M_2 type and appear to reduce the synaptic release of acetylcholine (Sen & Bhattacharya, 1991). Moor *et al*. (1995) also found that local administration of carbachol in the hippocampus decreased the output of acetylcholine. The present study, therefore, was designed to test the hypothesis that dynorphin A (1–13) antagonizes both carbachol-induced learning and memory impairment and the decrease in cholinergic neurotransmission, via activation of κ-opioid receptors.

Methods

Animals

Male Sprague-Dawley rats (Japan SLC Inc., Japan), weighing between 250 and 350 g, were used. The animals were housed in a room with controlled lighting (12-h light/dark cycle, lights on; 08 h 00 min to 20 h 00 min) and temperature ($23 \pm 2^{\circ}$C) for at least 5 days before the experiments, and given free access to food and water. Experimental protocols concerning the use of laboratory animals were approved by the committee of Meijo University and followed the guidelines of the Japanese Pharmacological Society (Folia Pharmacol. Japon., 1992, 99: 35A) and the interministerial decree from May 25th, 1987 (the Ministry of Education).

Passive avoidance test

One group of rats was trained in a passive avoidance apparatus which consisted of two compartments, one light ($25 \times 15 \times 15$ cm high) and one dark, of the same size connected via a guillotine door. On day 1, each rat was placed in the light compartment and then allowed to enter the dark compartment. Rats that had latencies greater than 60 s were discarded as being outside the normal range (pre-acquisition trial). The acquisition trial was carried out 15 min after the pre-acquisition trial. Rats were placed in the light compartment and 30 s later the guillotine door was opened. Once the rat entered the dark compartment, the guillotine door was closed and an electric shock (0.5 mA for 3 s) was delivered to the animal via the floor. The animal was then put back into the home cage and the retention trial was carried out 24 h later. The rat was put in the light compartment and the time taken to enter the dark compartment was recorded (step-through latency). A maximum latency of 300 s was set.

Surgical procedure

Rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) administered intraperitoneally (i.p.). By use of coordinates from the stereotaxic atlas of Paxinos & Watson (1986), a guide cannula for the microdialysis probe was implanted unilaterally into the hippocampus, and that for drug injection was implanted into the lateral ventricle. The tips of the cannulae were positioned just above the hippocampus (A: -4.1, L: 2.0, V: 3.2 mm from the bregma) and the lateral ventricle (A: -1.0, L: 1.2, V: 4.5 mm from the bregma) of each rat. The animals were allowed to recover from the procedure for 3 to 7 days before the experiments. In the experiment, the dialysis probe (CMA/10, Bioanalytical Systems, Inc., Japan) was inserted through the guide cannula and a 3 mm length of dialysis membrane was then advanced into the hippocampus.

Sampling procedure

The other group of rats was used for microdialysis experiments. The dialysis probe was perfused with Ringer solution (composition in mM: NaCl 127.6, KCl 2.5, $CaCl_2$ 1.3, pH 6.4–6.8, containing 0.01 mM physostigmine) at the rate of 2 μl min^{-1}, connected to a microinfusion pump (Syringe Infusion Pump 22, Harvard Apparatus, MA) via a single-channel liquid swivel. The rats were placed in individual acrylic cages ($30 \times 30 \times 35$ cm high) and allowed to adapt for at least 60 min before the experiment was started. The dummy cannulae were replaced with dialysis probes and the perfusate was collected in small (250 μl) disposable microcentrifuge tubes secured to the middle of the tether. The total dead volume from the tip of the probe to the collection tube was usually 4 μl. About 3 h after the probe was inserted, samples (40 μl) were collected at 20 min intervals, and when at least three baseline samples were stable, the drugs were administered. Perfusate samples from the brain were taken up to 120 min after treatment with drugs or saline. The locations of dialysis probes were confirmed after the experiments.

Analysis of dialysates

Acetylcholine and choline in the dialysate were quantified by high-performance liquid chromatography (h.p.l.c.) by use of an immobilized enzyme column and an electrochemical detector (e.c.d.) (ECD-300, Eicom Corp., Japan). The mobile phase consisted of 0.1 M sodium phosphate buffer (pH 8.5) containing 200 mg l^{-1} 1-decanesulphonate sodium salt and 65 mg l^{-1} tetramethylammonium chloride (Fujimori & Yamamoto, 1987) was delivered by a pump (P-300, Eicom, Japan) at a flow rate of 0.6 ml min^{-1}. To protect the analytical column from impurities in the mobile phase and samples, a pre-column (Eicom) was placed between the pump and injector. Twenty-five microlitre aliquots of the perfusate samples were injected into the h.p.l.c. system and separated by a column of Eicompak AC-GEL (4.6×150 mm). The enzyme column containing acetylcholinesterase, and choline oxidase catalysed the formation of hydrogen peroxide from acetylcholine and choline. The resultant H_2O_2 was detected by e.c.d. with a platinum electrode at $+450$ mV. The average basal values of acetylcholine and choline (recorded in the presence of 0.01 mM physostigmine) were 0.22 ± 0.06 and 2.45 ± 0.46 pmol min^{-1} in the hippocampus. Although relatively high concentrations of physostigmine had to be used to improve sensitivity for acetylcholine detection, similar responses were observed when samples were collected over longer periods of time.

Drugs

The following drugs were used: sodium pentobarbitone, carbamylcholine chloride (carbachol) (Tokyo Chemical Industry Co., Ltd., Japan); dynorphin A (1–13) (Peptide Institute, Inc., Japan); nor-binaltorphimine dihydrochloride (n-BNI, Research Biochemicals, Inc., MA). Drugs were dissolved in isotonic saline solution (Otsuka Pharmaceuticals, Inc., Japan).

Dynorphin A (1–13) was administered i.c.v. 5 min after carbachol injection or at the same time as commencement of carbachol perfusion. Nor-binaltorphimine was administered i.c.v. 5 min before the dynorphin A (1–13) injection. Carbachol (3×10^{-5}–3×10^{-3} M) was perfused for 40 min and atropine (10^{-7} M) was co-perfused with carbachol for 40 min.

Data analysis

The behavioural data are expressed in terms of median and interquartile ranges. Significant differences were evaluated by the Mann–Whitney U-test for comparisons between two groups and Kruskal-Wallis non-parametric one-way analysis of variance followed by Bonferroni's test for multiple comparisons. Dialysis data are shown as means ± s.e.mean of the percentage of baseline level obtained from each rat before drug treatment. To compare the effects of drugs, data were analysed by two-way repeated measures analysis of variance followed by Bonferroni's test. The data for individual time points were analysed by one-way analysis of variance followed by Bonferroni's test. The total responses for each treatment, assessed as the area under the time response curves (AUC), were then calculated by the trapezoidal method. Statistical analysis of the behavioural data and AUC were carried out by use of the Kruskall-Wallis test followed by the Bonferroni's test for multiple comparison. $P<0.05$ was taken as the criterion for significance.

Results

Effects of dynorphin A (1–13) on the carbachol-induced learning and memory impairment

Carbachol (1.66 pmol per rat) injected into the hippocampus significantly shortened the step-through latency when administered 30 min before the acquisition trial (Figure 1b). Interestingly, carbachol induced a dose-related dual response. That is, lower (0.166 pmol per rat) and higher (16.6 pmol per rat) doses of carbachol did not induce learning or memory impairment. Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) administered 5 min after carbachol injection significantly and almost completely attenuated these impairments of learning and memory induced by carbachol in rats (Figure 2b). Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) itself administered 25 min before the acquisition trial, had no effect on learning and memory when administered alone (Figure 2).

Effects of carbachol on the extracellular acetylcholine levels

A significant treatment effect ($F_{3,140}=17.09$, $P<0.01$) for the acetylcholine levels was revealed by analysis of variance. Carbachol (3×10^{-5} and 3×10^{-4} M) significantly decreased the synaptic overflow of acetylcholine in the hippocampus by about 20 to 40% of the baseline levels from 20–40 min after injection (Figure 3a). This decrease elicited by carbachol lasted only for the period of perfusion, returning to baseline levels thereafter (Figure 3). On the other hand, carbachol (3×10^{-3} M) significantly increased the acetylcholine levels in the hippocampus, as revealed by two-way analysis of variance. Carbachol itself did not affect the choline level as compared with controls in the hippocampus (data not shown). Neither dynorphin A (1–13) (0.5 nmol per rat, i.c.v.), an endogenous κ-opioid receptor agonist, nor n-BNI (5.44 nmol per rat, i.c.v.), a selective κ-opioid receptor antagonist, affected the

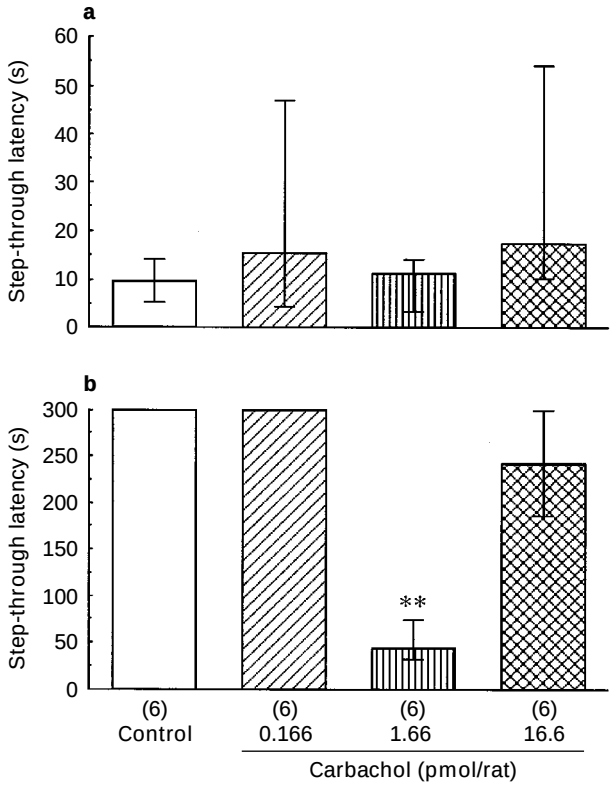

Figure 1 Effects of carbachol on normal rats in the step-through type passive avoidance test. Carbachol (0.166–16.6 pmol per rat) was injected into the hippocampus 30 min before the acquisition trial. The retention trial (b) was carried out 24 h after the acquisition trial (a). Values show the median (column), and first and third quartiles ranges (vertical lines). $^{**}P<0.01$ vs control (Bonferroni's test).

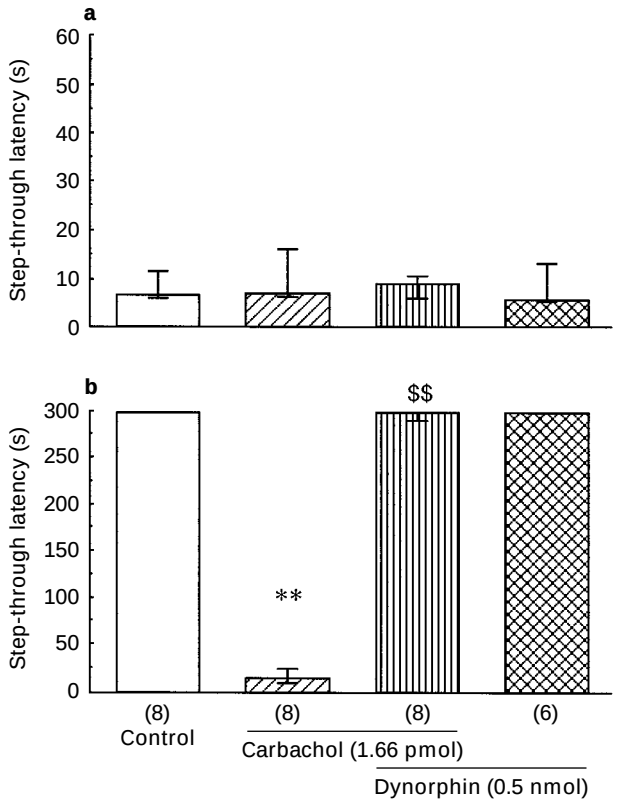

Figure 2 Effects of dynorphin A (1–13) administered before training on carbachol-induced learning impairment in passive avoidance test. Carbachol (1.66 pmol per rat) and dynorphin A (1–13) were injected into the hippocampus 30 min and i.c.v. 25 min before the acquisition trial, respectively. The retention trial (b) was carried out 24 h after the acquisition trial (a). Values show the median (column) and first and third quartiles ranges (vertical lines). $^{**}P<0.01$ vs control; $\$\$P<0.01$ vs carbachol alone (Bonferroni's test).

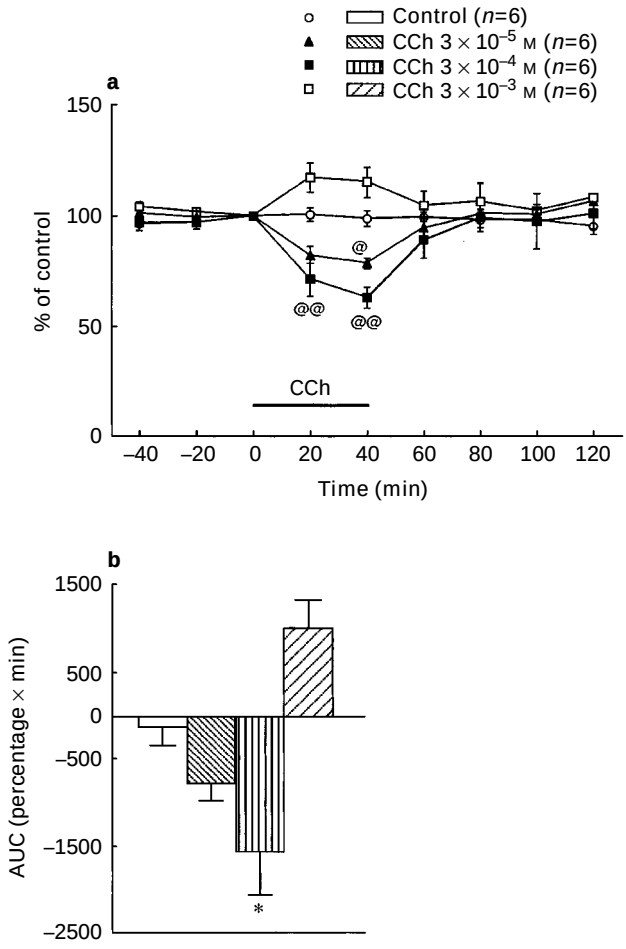

Figure 3 Effects of carbachol (CCh) on acetylcholine output (a) and area under the time response curve (AUC) (b) in the hippocampus. Carbachol or vehicle was perfused for 40 min. Values represent the means and vertical lines s.e.mean for 6 rats. (a): $P<0.01$ for control vs carbachol 3×10^{-5} M and control vs carbachol 3×10^{-3} M (two-way ANOVA followed by Bonferroni's test). @$P<0.05$, @@$P<0.01$ vs control (Bonferroni's test). (b): *$P<0.05$ vs control (Bonferroni's test).

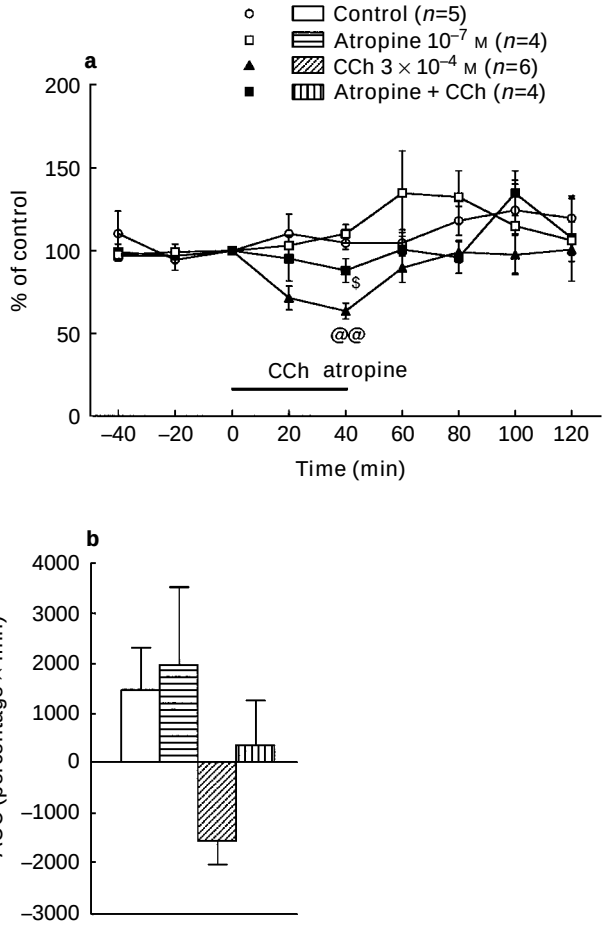

Figure 4 Effects of atropine on carbachol (CCh)-induced decrease in acetylcholine output (a) and area under the time response curve (AUC) (b) in the hippocampus. Atropine, carbachol, atropine (10^{-7} M) + carbachol (3×10^{-4} M), or vehicle was perfused for 40 min. Values represent the means and vertical lines s.e.mean for 4–6 rats. (a): $P<0.05$ for CCh vs atropine + CCh, $P<0.01$ for control vs CCh (two-way ANOVA followed by Bonferroni's test). @@$P<0.01$ vs control, \$$P<0.05$ vs CCh (Bonferroni's test).

extracellular acetylcholine levels in the hippocampus when administered alone (Hiramatsu *et al*., 1996b).

Effects of atropine on the carbachol-induced decrease in extracellular acetylcholine levels

It has been shown that a low dose of carbachol acts at presynaptic M_2 receptors and induces a decrease in acetylcholine release. To confirm this, the extracellular acetylcholine levels were measured after co-perfusion of a low dose of atropine with carbachol. The effects of treatment were significant in the hippocampus ($F_{3,105}=7.99$, $P<0.01$). Atropine (10^{-7} M) significantly antagonized the decrease in the extracellular acetylcholine level induced by carbachol in the hippocampus ($P<0.05$), although atropine itself did not modify the acetylcholine levels when administered alone (Figure 4). The total responses for each treatment assessed as the AUC also indicated that atropine tended to abolish the reduction in acetylcholine levels induced by carbachol (Figure 4b).

Effects of dynorphin A (1–13) on the carbachol-induced decrease in extracellular acetylcholine levels

In behavioural experiments dynorphin A (1–13) attenuated the carbachol-induced impairment of learning and memory in rats (Figure 2). To investigate the mechanism of this behavioural effect, the extracellular acetylcholine levels were measured after dynorphin A (1–13) had been administered just before carbachol perfusion. The effects of treatment were significant in the hippocampus ($F_{3,126}=20.70$, $P<0.01$). Dynorphin A (1–13) (0.5 nmol per rat) completely abolished the decrease in extracellular acetylcholine levels induced by carbachol in the hippocampus, although dynorphin A (1–13) did not modify the acetylcholine levels when administered alone (data not shown). This attenuation of the effect of carbachol by dynorphin A (1–13) was significantly antagonized by pretreatment with the selective κ-opioid receptor antagonist n-BNI ($P<0.01$) (Figure 5a). The decrease in acetylcholine levels were continued after the carbachol perfusion. The total responses for each treatment assessed as the AUC also indicated that dynorphin A (1–13) abolished the reduction in acetylcholine level, and this effect was antagonized by n-BNI (Figure 5b).

Discussion

Although investigations of learning and memory have focused primarily on cholinergic neurotransmission, findings of increased κ-opioid receptor density in the brain of

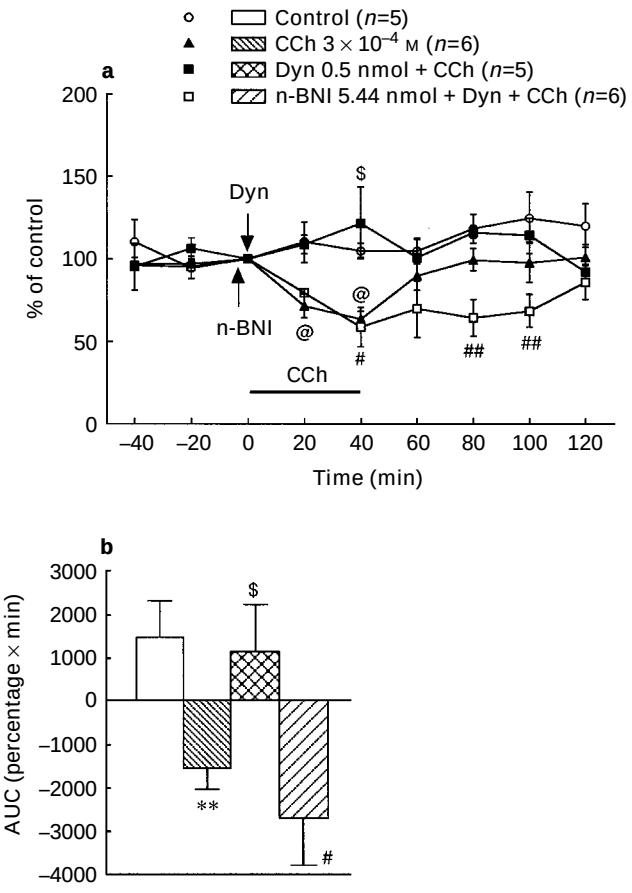

Figure 5 Effects of dynorphin A (1–13) (Dyn) on the carbachol-induced decrease in extracellular acetylcholine levels and its combination with nor-binaltorphimine (n-BNI) in the hippocampus. Carbachol (CCh, 3×10^{-4} M) was perfused for 40 min. Dyn (0.5 nmol per rat, i.c.v.) and n-BNI (5.44 nmol per rat, i.c.v.) were injected immediately and 5 min before carbachol perfusion, respectively. Values represent the means and vertical lines s.e.mean for 5–6 rats. (a): $P<0.01$ for control vs CCh, CCh vs Dyn + CCh, Dyn + CCh vs n-BNI + Dyn + CCh (two-way ANOVA followed by Bonferroni's test). @$P<0.05$ vs control, \$$P<0.05$ vs CCh, #$P<0.05$, ##$P<0.01$ vs Dyn + CCh (Bonferroni's test). (b): **$P<0.01$ vs control, \$$P<0.05$ vs CCh, #$P<0.05$ vs Dyn + CCh (Mann–Whitney U-test).

Alzheimer's patients (Hiller *et al*., 1987) and dynorphin A (1–8)-like immunoreactivity in the hippocampus of aged rats (Jiang *et al*., 1989) suggest that disruption of opioidergic neurotransmission also plays a role in the cognitive deficits associated with Alzheimer's disease and age. Our recent studies have indicated that selective κ-opioid receptor agonists, dynorphin A (1–13) and U-50,488H, improve scopolamine-, mecamylamine- and carbon monoxide-induced memory dysfunction, and the anti-amnesic effects of these drugs were blocked by administration of n-BNI (Hiramatsu *et al*., 1995; 1996a; 1998). Galanin, which decreased the amount of acetylcholine released in agreement with the findings of Fisone *et al*. (1987), significantly impaired the acquisition of learning and memory recall, and dynorphin A (1–13) improved the galanin-induced impairment of memory accompanied by blockade of the reduction of acetylcholine release in rats (Hiramatsu *et al*., 1996b). In normal mice, κ-opioid receptor agonists did not facilitate the acquisition of memory and n-BNI alone did not modify learning or memory (Hiramatsu *et al*., 1995; 1996a). Therefore, we proposed that the κ-opiodergic system in the brain may play an important role in modulating learning and memory when the cholinergic system has been impaired (Hiramatsu *et al*., 1988).

It has been proposed that galanin acts presynaptically on cholinergic neurones and reduces acetylcholine release (Fisone *et al*., 1987; 1991). In agreements with these previous studies, we found that galanin decreased acetylcholine release by acting at presynaptic cholinergic neurones in the hippocampus (Hiramatsu *et al*., 1996b). Interestingly, following preadministration of dynorphin A (1–13), galanin-induced learning and memory impairments and the decrease in acetylcholine release were almost completely abolished (Hiramatsu *et al*., 1996b). Behavioural impairments induced by galanin are believed to be exerted through interactions with the cholinergic forebrain neurones originating in the septal diagonal band nuclei and projecting to the hippocampus (Fisone *et al*., 1987; Givens *et al*., 1992). In the present study, carbachol at a dose of 1.66 pmol induced impairment of learning and memory, and decreased extracellular acetylcholine concentration in the hippocampus. A low dose of carbachol acts as a presynaptic M_2 receptor agonist and may reduce acetylcholine release (Sen & Bhattacharya, 1991; Moor *et al*., 1995), suggesting that carbachol acts at autoreceptors and reduces acetylcholine release, consequently impairing learning and memory. In this study, dynorphin A (1–13) was shown to ameliorate carbachol-induced learning impairment accompanied by abolishment of reductions in acetylcholine release. The effects of dynorphin A (1–13) were antagonized by n-BNI, a selective κ-opioid receptor antagonist. These results in rats are in good agreement with previous findings indicating that dynorphin A (1–13) improves galanin-induced impairment of memory processes in rats (Hiramatsu *et al*., 1996b). Taken together our findings indicate that dynorphin A (1–13) may act presynaptically on cholinergic neurones by activating κ-opioid receptors and prevent the effects of galanin and carbachol. However, it remains a possibility that administration of the drugs before acquisition could be affecting retention via several non-mnemonic processes.

Once postsynaptic acetylcholine receptors are depressed due to a decrease in acetylcholine release, dynorphinergic systems may be activated and act to normalize cholinergic neuronal transmission. Therefore, it is likely that cholinergic neurones in the hippocampus are regulated, at least in part, by κ-opioid receptors. Scopolamine blocks pre- and postsynaptic muscarinic receptors, and as a result, acetylcholine release is increased (Fisone *et al*., 1987; Billard *et al*., 1995). Dynorphin A (1–13) and U-50,488H improve scopolamine-induced learning impairment in mice (Itoh *et al*., 1993; Hiramatsu *et al*., 1996a). However, dynorphin A (1–13) did not affect the acetylcholine release evoked by scopolamine *in vivo* (Hiramatsu *et al*., 1998). Further investigations of these interactions are required to elucidate the mechanisms underlying the anti-amnesic actions of dynorphin A (1–13).

κ-Opioid receptor agonists such as U-50,488H and ethylketocyclazocine did not depress high-potassium- or electrically evoked acetylcholine release from rat hippocampal slices (Lapchak *et al*., 1989), frontal cortex (Heijna *et al*., 1990), or striatum (Mulder *et al*., 1991). Similarly, dynorphin A (1–13) did not depress the acetylcholine release from the rat hippocampus (Lapchak *et al*., 1989) or striatum (Mulder *et al*., 1984). However, raising the concentration of dynorphin A (1–13) reduced potassium-evoked [^{14}C]-acetylcholine release (Mulder *et al*., 1984). In the present study, dynorphin A (1–13) did not alter acetylcholine release from the hippocampus in normal rats. This was in accord with the results obtained by Lapchak *et al*. (1989) indicating that the effects of dynorphin A(1–13) and U-50,488H on acetylcholine release are confined

to evoked release; i.e. spontaneous acetylcholine release was not affected by either of these agents (Lapchak *et al*., 1989).

The modulation of the hippocampal cholinergic system implies a role for opiates in the processing and integration of newly acquired information and learning (Zager & Black, 1985). Studies by Izquierdo *et al*. (1980) and Rigter *et al*. (1980) have provided evidence suggesting that endorphins and enkephalins can cause impairment of memory storage, and the opiate-mediated inhibition of hippocampal acetylcholine might play a role in this phenomenon considering the known importance of cholinergic influence on cognitive function (Bartus *et al*., 1982). Our results indicate that dynorphin A (1–13) acts at κ-opioid receptors in the rat hippocampus modulating acetylcholine release when cholinergic neurotransmission is depressed.

Clinical improvement of memory deficits can be best brought about by selective M_1 receptor agonists or presynaptic M_2 receptor antagonists, both of which augment the functional activity of the cholinergic system (Quirion *et al*., 1995). Significant losses in both hippocampal and cortical choline-acetyltransferase (ChAT) activity (Coyle *et al*., 1983; Mullan & Crawford, 1993) and muscarinic M_2 receptors (Mash *et al*., 1985; Aubert *et al*., 1992) have been observed in Alzheimer's disease. However, in the case of advanced Alzheimer's disease, these two markers are still present often up to 40–45% of the normal levels (Mash *et al*., 1985; Aubert *et al*., 1992). Therefore, treatment strategies could take advantage of remaining cholinergic innervation by using appropriate pharmacological manipulations. The use of κ-opioid agonists could release the remaining feedback inhibition and facilitate acetylcholine release. Therefore, the development of κ-opioid receptor agonists may provide a strategy for the treatment of age-related memory impairment and for dementia, both of which are associated with degeneration of cholinergic neurones.

In conclusion, dynorphin A (1–13) did not affect cholinergic neurotransmission in normal rats. However, when the cholinergic neuronal systems were impaired by carbachol, for example, with reductions in acetylcholine release, dynorphin A (1–13) prevented this effect by activating κ-opioid receptors. Therefore, κ-opioid receptor agonists might be effective in the treatment of various forms of cognitive disturbances related to dysfunction of the presynaptic cholinergic system with beneficial effects on learning and memory. However, considerable research will still be necessary to fully understand the potential utility of κ-opioid receptor agonists in the treatment of cognitive dysfunction.

This study was supported in part by grants from the Kowa Life Science Foundation, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the Japan Smoking Research Foundation, the Science Research Promotion Fund from Japan Private School Promotion Foundation and INSERM/JSPS Joint Research Project, and by Grants-in-Aids for Scientific Research (No. 09672340) from the Ministry of Education, Science and Culture, Japan.

References

AUBERT, I., ARAUJO, D.M., CECYRE, D., ROBITAILLE, Y., GAUTHIER, S. & QUIRION, R. (1992). Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J. Neurochem.,* **58,** 529–541.

BARTUS, R.T., DEAN, R.L., BEER, B. & LIPPA, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science,* **217,** 408–417.

BILLARD, W., BINCH, III H., CROSBY, G. & MCQUADE, R.D. (1995). Identification of the primary muscarinic autoreceptor subtype in rat striatum as m2 through a correlation of in vivo microdialysis and in vitro receptor binding data. *J. Pharmacol. Exp. Ther.,* **273,** 273–279.

COYLE, J.T., DONALD, L.P. & DELONG, M.R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science,* **219,** 1184–1190.

DAVIS, K.L., THAI, L.J., GAMZU, E.R., DAVIS, C.S., WOOLSON, R.F., GRACON, S.I., DRACHMAN, D.A., SCHNEIDER, L.S., WHITEHOUSE, P.J., HOOVER, T.M., MORRIS, J.C., KAWAS, C.H., KNOPMAN, D.S., EARL, N.L., KUMAR, V., DOODY, R.S. & THE TACRINE COLLABORATIVE STUDY GROUP. (1992). A double-blind placebo-controlled multicenter study of tacrine for Alzheimer's disease. *N. Engl. J. Med.,* **327,** 1253–1259.

FISONE, G., BARTFAI, T., NILSSON, S. & HÖKFELT, T. (1991). Galanin inhibits the potassium-evoked release of acetylcholine and the muscarinic receptor-mediated stimulation of phosphoinositide turnover in slices of monkey hippocampus. *Brain Res.,* **568,** 279–284.

FISONE, G., WU, C.F., CONSOLO, S., NORDSTRÖM, O., BRYNNE, N., BARTFAI, T., MELANDER, T. & HOKFELT, T. (1987). Galanin inhibits acetylcholine release in the ventral hippocampus of the rat: Histochemical, autoradiographic, in vivo, and in vitro studies. *Proc. Natl. Acad. Sci. U.S.A.,* **84,** 7339–7343.

FUJIMORI, K. & YAMAMOTO, K. (1987). Determination of acetylcholine and choline in perchlorate extracts of brain tissue using liquid chromatography-electrochemistry with an immobilized-enzyme reactor. *J. Chromatogr.,* **414,** 167–173.

GIVENS, B.S., OLTON, D.S. & CRAWLEY, J.N. (1992). Galanin in the medial septal area impairs working memory. *Brain Res.,* **582,** 71–77.

HEIJNA, M.H., PADT, M., HOGENBOOM, F., PORTOGHESE, P.S., MULDER, A.H. & SCHOFFELMEER, A.N.M. (1990). Opioid receptor-mediated inhibition of dopamine and acetylcholine release from slices of rat nucleus accumbens, olfactory tubercle and frontal cortex. *Eur. J. Pharmacol.,* **181,** 267–278.

HILLER, J.M., ITZHAK, Y. & SIMON, E.J. (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.,* **406,** 17–23.

HIRAMATSU, M., HYODO, T. & KAMEYAMA, T. (1996a). U-50,488H, a selective κ-opioid receptor agonist, improves carbon monixide-induced delayed amnesia in mice. *Eur. J. Pharmacol.,* **315,** 119–125.

HIRAMATSU, M., MORI, H., MURASAWA, H. & KAMEYAMA, T. (1996b). 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.* (in press).

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

ITOH, J., UKAI, M. & KAMEYAMA, T. (1993). Dynorphin A-(1–13) markedly improves scopolamine-induced impairment of spontaneous alternation performance in mice. *Eur. J. Pharmacol.,* **236,** 341–345.

IZQUIERDO, I., PAIVA, A.C.M. & ELISABETSKY, E. (1980). Post training intraperitoneal administration of leu-enkephalin and β-endorphin causes retrograde amnesia for two different tasks in rats. *Behav. Neural Biol.,* **28,** 246–250.

JIANG, H.-K., OWYANG, V., HONG, J.-S. & GALLAGHER, M. (1989). Elevated dynorphin in the hippocampal formation of aged rats: Relation to cognitive impairment on a spatial learning task. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 2948–2951.

LAPCHAK, P.A., ARAUJO, D.M. & COLLIER, B. (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,** 313–325.

MARCHI, M., PAUDICE, P. & RAITERI, M. (1981). Autoregulation of acetylcholine release in isolated hippocampal nerve endings. *Eur. J. Pharmacol.,* **73,** 75–79.

MASH, D.C., FLYNN, D.D. & POTTER, L.T. (1985). Loss of M2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science,* **228,** 1115–1117.

MOOR, E., DE BOER, P., AUTH, F. & WESTERINK, B.H.C. (1995). Characterization of muscarinic autoreceptors in the septo-hippocampal system of the rat: a microdialysis study. *Eur. J. Pharmacol.,* **294,** 155–161.

MULDER, A.H., BURGER, D.M., WARDEH, G., HOGENBOON, F. & FRANKHUYZEN, A.L. (1991). Pharmacological profile of various κ-agonists at κ-, μ- and δ-opioid receptors mediating presynaptic inhibition of neurotransmitter release in the rat brain. *Br. J. Pharmacol.,* **102,** 518–522.

MULDER, A.H., WARDEH, G., HOGENBOON, F. & FRANKHUYZEN, A.L. (1984). κ- and δ-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. *Nature,* **308,** 278–280.

MULLAN, M. & CRAWFORD, F. (1993). Genetic and molecular advances in Alzheimer's disease. *Trends Neurosci.,* **16,** 398–403.

NORDSTRÖM, O. & BARTFAI, T. (1980). Muscarinic autoreceptor regulates acetylcholine release in the rat hippocampus: In vitro evidence. *Acta Physiol. Scand.,* **108,** 347–353.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain Stereotaxic Coordinates*. New York: Academic Press.

QUIRION, R., AUBERT, I., LAPCHAK, P.A., SCHAUM, R.P., TEOLIS, S., GAUTHIER, S. & ARAUJO, D.M. (1989). Muscarinic receptor subtypes in human neurodegenerative disorders: Focus on Alzheimer's disease. *Trends Pharmacol. Sci.,* **10,** 80–84.

QUIRION, R., WILSON, A., ROWE, W., AUBERT, I., RICHARD, J., DOODS, H., PARENT, A., WHITE, N. & MEANEY, M.J. (1995). Facilitation of acetylcholine release and cognitive performance by an M2-muscarinic receptor antagonist in aged memory-impaired rats. *J. Neurosci.,* **15,** 1455–1462.

RAITERI, M., LEARDI, R. & MARCHI, M. (1984). Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. *J. Pharmacol. Exp. Ther.,* **228,** 209–214.

RIGTER, H., HANNAN, T.J., MESSING, R.B., MARTINEZ, J.L. Jr., VASQUEZ, B.J., JENSEN, R.A., VELIQUETTE, J. & MCGAUGH, J.L. (1980). Enkephalins interfere with acquisition of an active avoidance response. *Life Sci.,* **26,** 337–345.

RUPNIAK, N.M.J. (1992). Profile of cholinomimetic drugs in primates: status of screens for potential Alzheimer therapies. *Drug Dev. Res.,* **27,** 77–88.

SEN, A.P. & BHATTACHARYA, S.K. (1991). Effect of selective muscarinic receptor agonists and antagonists on active-avoidance learning acquisition in rats. *Ind. J. Exp. Biol.,* **29,** 136–139.

SQUIRE, L.R. (1992). Memory and the hIppocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol. Rev.,* **99,** 195–231.

ZAGER, E.L. & BLACK, P.M. (1985). Neuropeptides in human memory and learning processes. *Neurosurgery,* **17,** 355–369.

ZOLA-MORGAN, S. & SQUIRE, L.R. (1993). Neuroanatomy of memory. *Annu. Rev. Neurosci.,* **16,** 547–563.

(Received June 30, 1997
Revised November 6, 1997
Accepted November 12, 1997)