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Improvement by dynorphin A (1-13) of galanin-induced impairment of memory accompanied by blockade of reductions in acetylcholine release in rats

[1]M. Hiramatsu, H. Mori, H. Murasawa & T. Kameyama

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

1 Human galanin (0.32 nmol per rat, i.c.v.), an endogenous neuropeptide, administered 30 min before acquisition or retention trials, significantly impaired the acquisition of learning and recall of memory in a step-through type passive avoidance performance.

2 The role of dynorphin A (1-13) in learning and memory is controversial. Dynorphin A (1-13) (0.5 nmol per rat, i.c.v.) administered 5 min before galanin injection, completely antagonized these impairments.

3 Galanin significantly decreased acetylcholine release in the hippocampus 40 to 120 min after injection as determined by *in vivo* brain microdialysis. This peptide also decreased acetylcholine release, albeit to a lesser extent, from the frontal cortex.

4 Dynorphin A (1-13) (0.5 nmol per rat, i.c.v.) 5 min before galanin injection, completely blocked the decrease in extracellular acetylcholine concentration induced by galanin.

5 These antagonistic effects of dynorphin A (1-13) were abolished by treatment with nor-binaltorphimine (5.44 nmol per rat, i.c.v.), a selective κ-opioid receptor antagonist, 5 min before dynorphin A (1-13).

6 Dynorphin A (1-13) (0.5 nmol) itself had no effect on learning and memory and on the acetylcholine concentration in the hippocampus or the frontal cortex in normal rats.

7 These results suggest that the neuropeptide dynorphin A (1-13) ameliorates the galanin-induced impairment of learning and memory accompanied by abolition of reductions in acetylcholine release via κ-opioid receptors.

Keywords: Dynorphin A (1-13); galanin; kappa opioid receptor; acetylcholine; hippocampus; learning and memory; *in vivo* microdialysis

Introduction

Reports of increased κ-opioid receptor density in the brain of 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 may play a role in the cognitive deficits associated with Alzheimer's disease and aging. Of particular interest was the observation that an endogenous κ-opioid agonist, dynorphin A (1-13), improves scopolamine-induced impairment of spontaneous alternation performance in mice (Itoh *et al.*, 1993) and carbon monoxide-induced delayed amnesia in mice (Kameyama *et al.*, 1994a; Hiramatsu *et al.*, 1995). However, whether dynorphins improve the memory process is still controversial. For example, post-training administration of dynorphin A (1-13) has no effect on inhibitory avoidance or shuttle avoidance responses (Izquierdo *et al.*, 1985) and impairs retention of inhibitory avoidance but not of Y-maze discrimination (Introini-Collison *et al.*, 1987). Colombo *et al.* (1992) reported that dynorphin A (1-13) impaired memory in a dose-dependent manner. However, injection of U-50,488, a selective κ-opioid receptor agonist showed a biphasic effect on memory; low doses tended to enhance, albeit not significantly, while high doses significantly impaired memory in two day old chicks. Therefore, the role of κ-opioid receptors in memory formation may depend biphasically on the dosage of agonist used.

Also, there is evidence that high concentrations of dynorphin decrease [^{14}C]-acetylcholine release (Mulder *et al.*, 1984). On the other hand, the activation of κ-opioid receptors by dynorphin had no effect on high potassium or glutamate-evoked acetylcholine release in rat striatal slices (Arenas *et al.*, 1990), and electrical stimulation or high potassium concentration-evoked release of acetylcholine output in brain slices (Lapchak *et al.*, 1989; Heijna *et al.*, 1990). Furthermore, recent results from our laboratory indicate that low doses of dynorphin have no effect on acetylcholine release in normal rats as measured by microdialysis (Mori *et al.*, 1995).

Galanin was originally identified as a neuropeptide of 29 amino acids in extracts of porcine intestine (Tatemoto *et al.*, 1983). Galanin-like immunoreactivity is widely distributed throughout the central nervous system (Rökaeus *et al.*, 1984; Skofitsch & Jacobowitz, 1985; Melander *et al.*, 1986a) and in peripheral tissues (Melander *et al.*, 1985a; Rökaeus, 1987). Specific binding sites for galanin have been demonstrated in discrete areas of the brain (Skofitsch *et al.*, 1986); autoradiographic studies revealed a high density of galanin binding sites in the hippocampus (Fisone *et al.*, 1987) which appear to be presynaptic since they are reduced by lesioning. With regard to its physiological roles in the central nervous system, galanin has been shown to modulate cholinergic activity (Melander *et al.*, 1986b; Mastropaolo *et al.*, 1988; Wenk & Rökaeus, 1988; Fisone *et al.*, 1991; Aspley & Fone, 1993). This peptide is co-localized with acetylcholine in neurones of the basal forebrain-cortical and the septo-hippocampal pathways (Melander *et al.*, 1985b; Senut *et al.*, 1989), and may have an important role in memory processes (Crawley & Wenk, 1989). Galanin reduces the evoked release of acetylcholine *in vitro* in slice preparations and *in vivo* as measured by microdialysis (Fisone *et al.*, 1987). Behaviourally, galanin inhibits acquisition of place dis-

[1] Author for correspondence.

crimination in the Morris swim maze (Sundstrom *et al.*, 1988), impairs the ability of acetylcholine to improve spatial memory in rats with ibotenic acid lesions of the basal forebrain cholinergic system (Mastropaolo *et al.*, 1988), impairs working memory in the T-maze paradigm (Givens *et al.*, 1992) and impairs delayed nonmatching-to-sample performance in rats (Robinson & Crawley, 1993).

The presence of galanin within cholinergic neurones suggested its potential importance in the pathology of Alzheimer's disease (Crawley & Wenk, 1989). The profound degeneration of basal forebrain cholinergic neurones in Alzheimer's disease is reflected in deficits in cholinergic activity markers (Johnston *et al.*, 1979). Gabriel *et al.* (1994) reported that human galanin-like immunoreactivity was increased in the postmortem cerebral cortex of patients with Alzheimer's disease. As galanin appears to act as an inhibitory modulator of cholinergic neurotransmission, the blockade of galanin activity may be useful in the treatment of Alzheimer's disease (Consolo *et al.*, 1994). We reported recently that dynorphin A (1-13) attenuates galanin-induced impairment of memory in mice (Kameyama *et al.*, 1994b). The present study was designed to test the hypothesis that dynorphin antagonizes both galanin-induced learning 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 ± 2°C) for at least 5 days before the experiments, and given free access to food and water.

Surgical procedure

Rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) administered intraperitonally (i.p.). Using coordinates from the stereotaxic atlas of Paxinos & Watson (1986), guide cannulae for microdialysis probes were implanted unilaterally into the hippocampus and the frontal cortex, and a guide cannula for drug injection was implanted unilaterally 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), the frontal cortex (A: +1.5, L: 2.0, V: 2.5 mm from the bregma, at a 25° angle against a cross-section), 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 prior to 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 and the frontal cortex.

Passive avoidance test

One group of rats was trained in a passive avoidance apparatus which consisted of two compartments, one light ($25 \times 15 \times 15 \times$ 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 entry latencies greater than 60 s were discarded as being out with 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 animals 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.

Sampling procedure

A separate 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 0.1 mM physostigmine) at the rate of 2 μl min^{-1}, connected to a microinfusion pump (Syringe Infusion Pump 22, Harvard Apparatus, MA, U.S.A.) 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 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 readings from 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.) using an immobilized enzyme column and an electrochemical detector (e.c.d.) (ECD-300, Eicom, Japan). The mobile phase consisted of 0.1 M sodium phosphate buffer (ph 8.5) containing 200 mg l^{-1} 1-octane sulphonic acid sodium salt and 65 mg l^{-1} tetramethylammonium chloride (Fujimori & Yamamoto, 1987) was delivered by a pump (TriRotor V, Japan Spectroscopic Co., Ltd., Japan) at a flow rate of 1.0 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 μl aliquots of the perfusate samples were injected into the h.p.l.c. system and separated by a column of Eicompak AC-GEL (6.0×150 mm). The enzyme column containing acetylcholinesterase, and choline oxidase catalyzed 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 0.1 mM physostigmine) were 0.22 ± 0.06 and 2.45 ± 0.46 pmol min^{-1} in the hippocampus and 0.26 ± 0.03 and 1.41 ± 0.40 pmol min^{-1} in the frontal cortex, respectively. Although relatively high concentrations of physostigmine had to be used to improve sensitivity for acetylcholine detection, similar responses to galanin were observed when samples were collected over longer time periods.

Drugs

The following drugs were used: sodium pentobarbitone (Tokyo Chemical Industry Co., Ltd., Japan); dynorphin A (1-13), human galanin (Peptide Institute, Inc., Japan); nor-binaltorphimine (nBNI) (Research Biochemicals, Inc., MA, U.S.A.). Drugs were dissolved in isotonic saline solution (Otsuka Pharmaceuticals, Inc., Japan).

Data analysis

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 Scheffe's test. The data for individual time points were analysed by one-way analysis of variance followed by Scheffe'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 using the Kruskall-Wallis test followed by the Bonferroni test for multiple comparison. $P<0.05$ was taken as the criterion for significance.

Results

Effects of dynorphin A (1–13) on galanin-induced learning impairment

Galanin (0.32 nmol per rat, i.c.v.) significantly impaired the acquisition of learning when administered 30 min before the acquisition trial (Figure 1a). Galanin also impaired the recall of memory when administered 30 min before the retention trial (Figure 1b). Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) significantly and almost completely attenuated these impairments of learning and memory induced by galanin in rats (Figure 1a,b). Dynorphin A (1–13) (0.5 nmol per rat, i.c.v.) itself administered 30 min before acquisition or retention trials, had no effect on learning and memory when administered alone (Figure 1a,b).

Effects of galanin, dynorphin A (1–13), and nBNI on the extracellular acetylcholine and choline levels

A significant treatment effect ($F_{3,144}=21.92$, $P<0.01$) for the acetylcholine levels was revealed by analysis of variance. Galanin (0.32 nmol per rat, i.c.v.) significantly decreased the overflow of acetylcholine in the hippocampus ($P<0.01$) by about 30% of the baseline levels from 40–120 min after injection (Figure 2a). This decrease elicited by galanin lasted for at least 120 min, returning to baseline levels thereafter (data not shown). There was also a significant treatment effect in the frontal cortex ($F_{3,144}=7.924$, $P<0.01$). At the same dose, galanin also decreased the acetylcholine levels transiently in the frontal cortex ($P<0.01$) at the second 20 min sampling period. The decreased extracellular acetylcholine level in the frontal cortex returned to baseline level by 80 min (Figure 2b). Galanin itself did not affect the choline level as compared with controls in the hippocampus and frontal cortex (data not shown). Neither dynorphin A (1–13) (0.5 nmol per rat, i.c.v.), and endogenous κ-opioid receptor agonist, nor nBNI (5.44 nmol per rat, i.c.v.), a selective κ-opioid receptor antagonist, affected the extracellular acetylcholine levels in the hippocampus or frontal cortex when administered alone (Figure 2a,b).

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

In behavioural experiments, dynorphin A (1–13) attenuated galanin-induced impairment of memory in mice (Kameyama *et al.*, 1994b) and in rats (Figure 1a,b). To investigate a possible

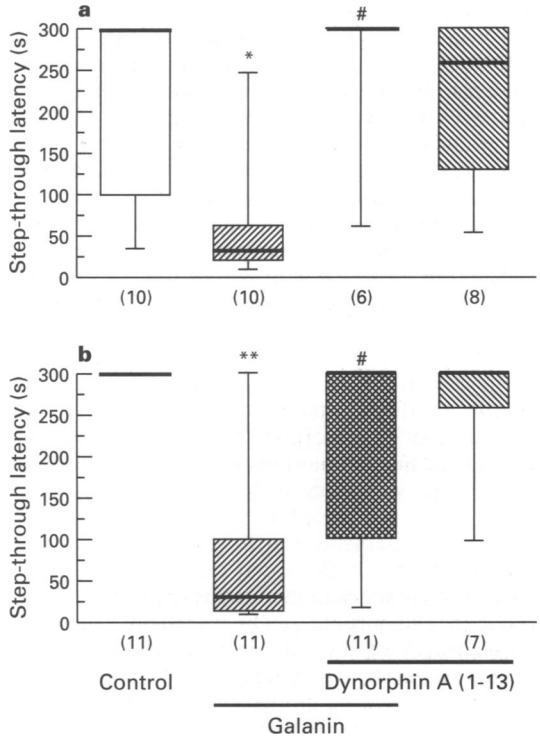

Figure 1 Effects of dynorphin A (1-13) on galanin-induced impairment of learning and memory in the step-through type passive avoidance test. Dynorphin A (1-13) (0.5 nmol per rat, i.c.v.) and galanin (0.32 nmol per rat, i.c.v.) were injected into the lateral ventricles 35 and 30 min before acquisition (a) or retention trials (b), respectively (as indicated by the bars). The retention trial was carried out 24 h after acquisition trial. Each value shows that median (horizontal bar), first and third quartiles (vertical column) and 10th and 90th percentiles (vertical lines). $^{*}P<0.05$, $^{**}P<0.01$ vs. control, $^{\#}P<0.05$ vs. galanin alone (Bonferroni's test).

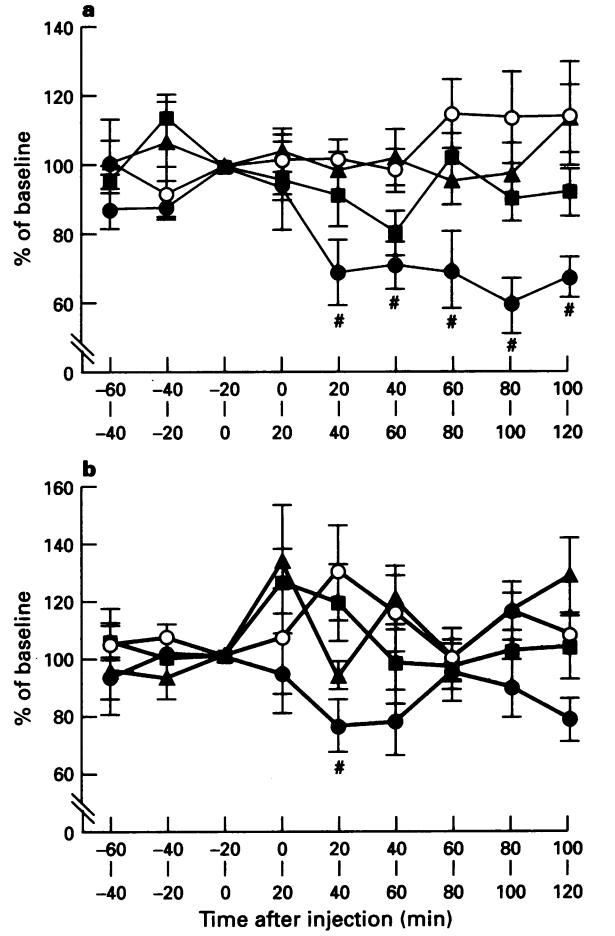

Figure 2 Effects of galanin (Gal), dynorphin A (1-13) (Dyn) or norbinaltorphimine (nBNI) on acetylcholine output from the hippocampus (a) and frontal cortex (b). Gal (●, 0.32 nmol per rat, i.c.v.), Dyn (■, 0.5 nmol per rat, i.c.v.), nBNI (▲, 5.44 nmol per rat i.c.v.) or vehicle (○) were injected at 0 min. Values represent the means ± s.e.mean for 5 rats. (a): $P<0.01$ for [control] vs. [Gal], [Gal] vs. [Dyn] and [Gal] vs. [nBNI], (b): $P<0.05$ for [Gal] vs. [Dyn], $P<0.01$ for [control] vs. [Gal] and [Gal] vs. [nBNI] (two-way ANOVA followed by Scheffe's test). $^{\#}P<0.05$ vs. control (Scheffe's test).

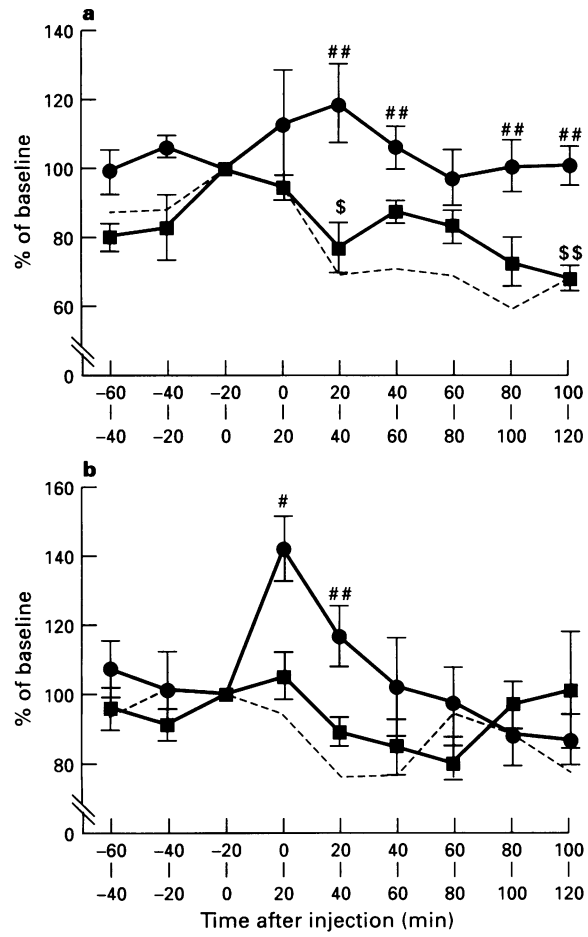

Figure 3 Effects of dynorphin A (1-13) (Dyn) on the galanin-induced decrease in extracellular acetylcholine levels in the hippocampus (a) and the frontal cortex (b). Gal (0.32 nmol per rat, i.c.v.) was injected at 0 min. Dyn (0.5 nmol per rat, i.c.v.) and nBNI (5.44 nmol per rat, i.c.v.) were injected 5 and 10 min before Gal, respectively. Gal alone (- - - -); Gal plus Dyn (●); Gal plus Dyn plus nBNI (■). Values represent the means ± s.e.mean for 5 rats. (a):, $P<0.01$ for [Gal] vs. [Gal + Dyn], [Gal + Dyn] vs. [Gal + Dyn + nBNI] (b): $P<0.05$ for [Gal + Dyn] vs. [Gal + Dyn + nBNI], $P<0.01$ for [Gal] vs. [Gal + Dyn] (two-way ANOVA followed by Scheffe's test). $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. Gal alone $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. Gal + Dyn (Scheffe's test).

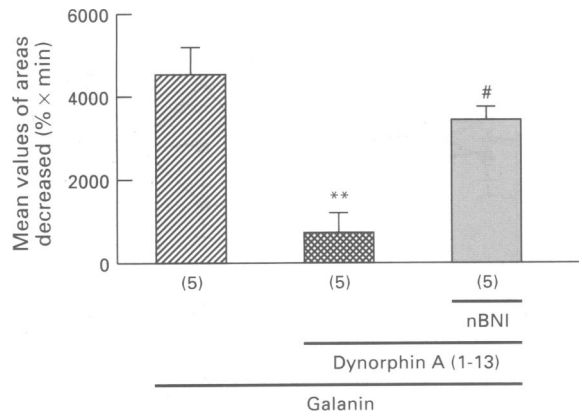

Figure 4 Area under the time response curve (AUC) after injection of galanin, dynorphin A (1-13), nBNI and their combinations in extracellular acetylcholine levels in the hippocampus. Gal (0.32 nmol per rat, i.c.v.) was injected at 0 min. Dyn (0.5 nmol per rat, i.c.v.) and nBNI (5.44 nmol per rat, i.c.v.) were injected 5 and 10 min before Gal, respectively. Mean values of AUC of acetylcholine concentration decreased from 100% in the hippocampus are shown as means ± s.e.mean $^{**}P<0.01$ vs. galanin alone, $^{\#}P<0.05$ vs. dynorphin + galanin (Bonferroni's test).

mechanism of this behavioural effect, the extracellular acetylcholine levels were measured after dynorphin A (1 – 13) was administered before galanine injection. The effects of treatment were significant in the hippocampus ($F_{2,108}=5.121$, $P<0.01$). Dynorphin A (1 – 13) (0.5 nmol per rat) completely abolished the decrease in extracellular acetylcholine levels induced by galanin in the hippocampus ($P<0.01$), although dynorphin A (1 – 13) did not modify the acetylcholine levels when administered alone (Figure 3a). This abolition of the galanin effect by dynorphin A (1 – 13) was significantly antagonized by pretreatment with the selective κ-opioid receptor antagonist, nBNI ($P<0.01$) (Figure 3a). The total responses for each treatment assessed as the AUC also indicated that dynorphin A (1 – 13) abolished the reduction in acetylcholine levels, and this effect was antagonized by nBNI (Figure 4). Similar effects of dynorphin A (1 – 13) and nBNI were observed in the frontal cortex ($P<0.01$ and $P<0.05$, respectively) (Figure 3b).

Discussion

This study demonstrated that dynorphin A (1 – 13) reverses learning and memory impairment accompanied by abolition of reductions in acetylcholine release induced by galanin. These results in rats are in close agreement with previous reports indicating that dynorphin A (1 – 13) improves scopolamine-induced impairment of memory processes in mice (Itoh *et al.*, 1993) and carbon monoxide-induced delayed amnesia in mice (Kameyama *et al.*, 1994a, Hiramatsu *et al.*, 1995). In these amnesia models, it has been suggested that cholinergic neurotransmission is disrupted, since some nootropics such as nefiracetam and NIK-247, which may facilitate cholinergic neuronal system (Sarter, 1991), have ameliorative effects (Yoshida *et al.*, 1992, Hiramatsu *et al.*, 1992; 1994). Dynorphin A (1 – 13) itself did not modify memory processes in normal mice (Itoh *et al.*, 1993; Kameyama *et al.*, 1994b; Hiramatsu *et al.*, 1995) or in rats (Figure 1). Therefore, we hypothesized that dynorphin A (1 – 13) improves learning and memory processes only when cholinergic neurotransmission is disrupted.

Several reports have indicated that galanin acts as an inhibitory modulator of acetycholine function in the hippocampus. Galanin reduces the *in vivo* and *in vitro* evoked release of acetylcholine (Fisone *et al.*, 1987; Consolo *et al.*, 1991) and inhibits slow cholinergic e.p.s.p.s. in CA1 pyramidal neurones elicited by endogenous acetylcholine release (Dutar *et al.*, 1989). Therefore, it has been proposed that galanin acts presynaptically on cholinergic neurones and reduces acetylcholine release. In agreement with these previous reports, our results indicated that galanin decreased acetylcholine release acting at presynaptic cholinergic neurones in the hippocampus and to a lesser extent in the frontal cortex. Interestingly, following preadministration of dynorphin A (1 – 13), galanin-induced learning and memory impairments and the decrease in acetylcholine release were almost completely abolished. 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 (Givens *et al.*, 1992). Recently, we showed that dynorphin A (1 – 13) attenuated galanin-induced impairment of memory in mice (Kameyama *et al.*, 1994b). These effects by dynorphin A (1 – 13) were antagonized by nBNI, a selective κ-opioid receptor antagonist. Taken together, our findings indicate that dynorphin A (1 – 13) may act presynaptically on cholinergic neurones relative to κ-opioid receptors and prevent the effects of galanin.

Recently, Consolo *et al.* (1994) reported that galanin is released from the neuronal compartment in the hippocampus in an impulse flow-dependent manner. The low stimulation frequency of the neurones containing acetylcholine and galanin selectively releases acetylcholine, while at higher stimulation frequencies galanin is also released. Galanin administered exogenously acts at presynaptic cholinergic neurones and reduces acetylcholine release. When postsynaptic acetylcholine

receptors are depressed due to decrease in acetylcholine release, dynorphinergic systems may be activated and act to normalize the 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 presynaptic muscarinic receptors, and as a result, acetylcholine release is increased (Fisone *et al.*, 1987). Dynorphin A (1–13) improves scopolamine-induced learning impairment in mice (Itoh *et al.*,1993). However, dynorphin A (1–13) did not affect the acetylcholine release evoked by scopolamine *in vivo* (data not shown). Further investigation of these interactions is 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), and 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 or frontal cortex in normal rats. This was supported by the results reported by Lapchak *et al.* (1989) indicating that the effects of dynorphin A (1–13) and U-50,488H on acetylcholine release were confined to evoked release; i.e. spontaneous acetylcholine release was not affected by either of these agents (Lapchak *et al.*, 1989).

Mulder *et al.* (1984) found that lower doses of dynorphin A (1–13) inhibited both the spontaneous efflux of tritium and the potassium-induced release of [^{3}H]-dopamine from rat striatal slices, whereas the release of [^{14}C]-acetylcholine was not significantly affected. Similar effects were also reported with administration of the selective κ-agonist, U 50,488H, at concentrations below 1 μM (Schoffelmeer *et al.*, 1988). Previously, we reported that the dose of dynorphin A (1–13) tested in this study did not alter dopamine release in the striatum, whereas higher doses of this peptide (2.5 and 5 nmol per rat, i.c.v.) exhibited an inhibitory effect as measured by microdialysis (Mori *et al.*, 1993). Therefore, even very low doses, dynorphin A (1–13) may be capable of modulating neuronal functions. Furthermore, a selective κ-opioid receptor antagonist, nBNI, did not alter acetycholine release in the hippocampus or frontal cortex. This suggests that endogenous κ-agonists such as dynorphin A (1–13) may not modulate tonic acetylcholine release from cholinergic neurones in the hippocampus and frontal cortex.

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 influences on cognitive function (Bartus *et al.*, 1982). Our results indicated that dynorphin A (1–13) acts at κ-receptors in the rat hippocampus and/or frontal cortex modulating acetylcholine release when cholinergic neurotransmission is depressed.

In conclusion, dynorphin A (1–13) did not affect cholinergic neurotransmission in normal rats. However, when cholinergic neuronal systems were impaired by galanin, for example, with reductions in acetylcholine release, dynorphin A (1–13) prevented this effect by activating κ-opioid receptors. It has been reported that the basal forebrain may be hyper-innervated by galanin-containing fibres in Alzheimer's disease (Chan-Palay, 1988; Beal *et al.*, 1990). 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.

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