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# Effects of nocistatin on nociceptin-induced impairment of learning and memory in mice

Masayuki Hiramatsu *, Kaori Inoue

*Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Tenpaku-ku, Nagoya 468-8503, Japan*

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**Abstract**

We investigated the effects of nociceptin/orphanin FQ and nocistatin on learning and memory function as measured in a step-down type passive avoidance task and spontaneous alternation of Y-maze with mice. Nociceptin (0.5–5.0 nmol/mouse, i.c.v.) 30 min before the training session or Y-maze test, dose dependently shortened the step-down latency and impaired spontaneous alternation, while there was no significant effect of nocistatin (0.5–5.0 nmol/mouse). Interestingly, nocistatin (5.0 nmol) significantly improved the nociceptin (5.0 nmol)-induced impairment of learning and memory without changing motor activity or response to electric shocks. These results suggest that nocistatin, a new biologically active peptide now found to also counteract the impairment of learning and memory induced by nociceptin, plays an important role in the regulation of learning and memory process in the central nervous system. 

*Keywords:* Nocistatin; Nociceptin; Orphanin FQ; Dynorphin A; Passive avoidance; Learning; Memory

## 1. Introduction

Nociceptin, also known as orphanin FQ, is an endogenous ligand for the orphan opioid receptor-like, receptor 1 (ORL1), and resembles to some extent the endogenous opioid peptide dynorphin A-(1–17) (Meunier et al., 1995; Meunier, 1997). When administered intracerebroventricularly (i.c.v.) to mice, nociceptin induces hyperalgesia and a decrease in motor activities (Reinscheid et al., 1995) or stimulates locomotor and exploratory behaviors (Florin et al., 1996). On the other hand, nocistatin, which was recently isolated from the same precursor as nociceptin, blocks nociceptin-induced allodynia and hyperalgesia, and attenuates pain evoked by prostaglandin $E_2$ (Okuda-Ashitaka et al., 1998).

It is known that opioid peptides acting on opioid receptors can modulate hippocampal synaptic functions. Although ORL1 receptors which display close homology with classical opioid receptors are abundant in the hippocampus, little is known regarding their role in synaptic function. Recently, Sandin et al. (1997) showed that nociceptin microinjected into the hippocampus impairs spatial learning in rats, and also Manabe et al. (1998) showed that long-term potentiation and memory were facilitated in mice lacking nociceptin receptors. Further, Yu et al. (1997) suggested that nociceptin could function as an inhibitory modulator regulating synaptic transmission and synaptic plasticity in the hippocampus. These findings suggest that activation of ORL1 receptors may play an important role in the synaptic plasticity involved in learning and memory.

In the hippocampus, dynorphins may function as neurotransmitters or modulators to regulate the excitability of neurons (Jiang et al., 1989; Wagner et al., 1993; Xie and Lewis, 1995), which has been suggested to play a role in a process associated with learning and memory (Gallagher et al., 1983). We previously reported that dynorphin A-(1–13), a κ-opioid receptor agonist, improved impairments of learning and memory in mice and rats by κ-opioid receptor-mediated and/or non-opioid mechanisms (Hiramatsu et al., 1995, 1996, 1998a,b). Since the effects of nociceptin on hippocampal long-term potentiation are similar to that reported for dynorphin A (Wagner et al., 1993; Drake et al., 1994), some common mechanisms underlying learning and memory function may exist. Here, we investigated whether these orphan neuropeptides modulate learning and

* Corresponding author. Tel.: +81-52-832-1781; Fax: +81-52-834-8780; E-mail: mhiramt@meijo-u.ac.jp

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memory functions, using for the purpose a step-down type passive avoidance task and spontaneous alternation of Y-maze in mice.

## 2. Materials and methods

### 2.1. Animals

Seven-week-old male ddY mice (Japan SLC, Japan) were kept in a controlled environment (23 ± 1°C, 50 ± 5% humidity), with a 12-h light/12-h dark cycle (light on 0800h–2000h) and given food and tap water ad libitum. 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 of May 25th, 1987 (the Ministry of Education).

### 2.2. Drugs

Nociceptin and nocistatin (Peptide Inst., Osaka, Japan) were dissolved in 0.9% saline. Both peptides were administered 30 min before the training session for the passive avoidance task and the Y-maze session. They were injected into the lateral ventricle (i.c.v.) of the mouse brain according to the method of Haley and McCormick (1957) in a volume of 5 μl/mouse under brief ether anesthesia.

### 2.3. Step-down type passive avoidance task

A step-down type of passive avoidance task, as described previously (Hiramatsu et al., 1995), was used with some modifications. The apparatus consisted of a transparent acrylic rectangular cage (30 × 30 × 40 cm high) with a grid floor with a wooden platform (4 × 4 × 4 cm) in the center, set in a semi-soundproof wooden outer box (35 × 35 × 90 cm high). Illumination was provided by a 15-W illumination lamp above the apparatus. An electric current (1 Hz, 500 ms, 80 V DC) was delivered to the grid floor from an insulated stimulator (SEN-3201, Nihon Koden, Japan).

Each mouse was placed on the wooden platform. When the mouse stepped down from the platform onto the grid floor, an electric shock was delivered for 15 s. The retention test was carried out 24 h after the training session in a manner similar to the training except that no electric shock was delivered to the grid floor. Each mouse was placed on the platform and step-down latency was recorded. An upper cut-off time of 300 s was set.

### 2.4. Responses to electric shock

The responses to electric shock during the training session were recorded. The following scores were given based on the responses to electric shock: 3 = jumping, 2 = vocalization, 1 = flinching, 0 = no response. Shock sensitivity is shown as the total score, which was the sum of each score for 15 s.

### 2.5. Spontaneous alternation behavior

Immediate working memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze (Hiramatsu et al., 1997). Each mouse, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into

Table 1
Effects of nociceptin (A) and nocistatin (B) on step-down latency and sensitivity to electric shocks during the training period in mice

| Treatments | Doses (nmol/mouse) | Step-down latency (s), median (range) | Shock sensitivity, median (range) | *N* |
|---|---|---|---|---|
| *(A)* | | | | |
| Control | 0 | 224.0 (166.5–300.0) | 36.5 (35.0–38.0) | 12 |
| Nociceptin | 0.5 | 166.5 (86.5–244.0) | 37.0 (35.0–38.5) | 12 |
| Nociceptin | 1.5 | 60.5 (41.0–143.5) | 37.0 (34.5–38.0) | 12 |
| Nociceptin | 5.0 | 26.0 (19.0–47.0)[a] | 36.0 (33.5–39.5) | 12 |
| *(B)* | | | | |
| Control | 0 | 204.5 (127.0–300.0) | 37.5 (33.0–40.0) | 14 |
| Nocistatin | 0.5 | 182.5 (100.5–271.0) | 35.5 (34.0–37.5) | 12 |
| Nocistatin | 1.5 | 172.5 (117.5–261.5) | 35.5 (34.0–37.5) | 12 |
| Nocistatin | 5.0 | 158.0 (114.5–270.0) | 36.0 (34.8–37.0) | 13 |

Mice were treated with nociceptin and nocistatin (0.5–5.0 nmol/mouse, i.c.v.) 30 min before the training session.
The following scores were given based on the response to each electric shock (1 Hz, 500 ms, 80 V, DC).
Shock sensitivity is shown as the total score which was the sum of each score for 15 s as follows: 3 = jumping, 2 = vocalization, 1 = flinching, 0 = no response.
Values are shown as the median and range (first and third quartiles).
*N* shows the number of mice used.
Significance levels: [a] $P < 0.01$ vs. control (Bonferroni's test).

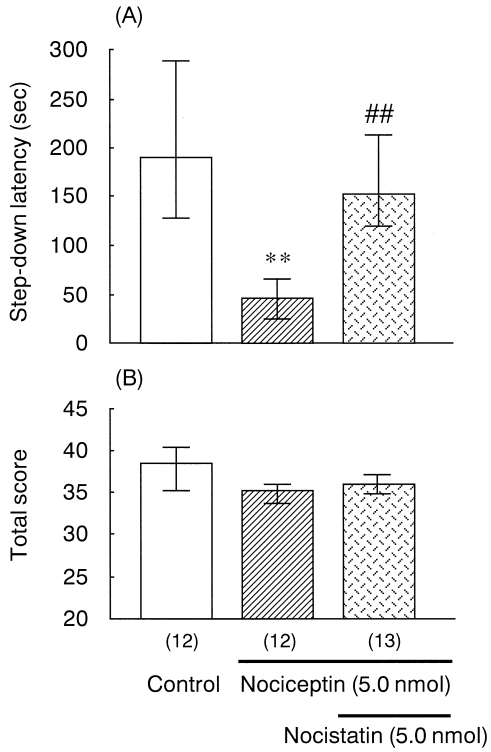


Fig. 1. Effects of nocistatin on nociceptin-induced impairment of learning and memory in the passive avoidance task (A) and sensitivity to electric shocks during the training period (B). Mice were treated intracerebroventricularly with nociceptin and nocistatin (5.0 nmol/mouse) 30 min before the training session. The following scores were given based on the response to each electric shock (1 Hz, 500 ms, 80 V, DC). Shock sensitivity is shown as the total score which was the sum of each score for 15 s as follows: 3 = jumping, 2 = vocalization, 1 = flinching, 0 = no response. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels; $**P < 0.01$ vs. control, $\#\#P < 0.01$ vs. nociceptin alone (Bonferroni's test).

the three arms, on overlapping triplet sets. The effect was calculated as percentage alternation according to the following formula: percentage alternation = {(number of alternation)/(total number of arm entries − 2)} × 100%.

### 2.6. Data analysis

The behavioral data are expressed in terms of median and interquartile percentile ranges. The significance of differences was evaluated using the Kruskal–Wallis nonparametric one-way analysis of variance followed by Bonferroni's test for multiple comparisons. The criterion for significance was $P < 0.05$ in all statistical evaluations.

## 3. Results

Administration of nociceptin (0.5–5.0 nmol/mouse, i.c.v.) 30 min before the training session significantly shortened the step-down latencies in the retention test in a dose-dependent manner (Table 1). On the other hand, nocistatin had no significant effect on the step-down latencies in the retention test (Table 1). Neither nociceptin nor nocistatin induced significant changes in the response to electric shocks at the same dose range as used in the passive avoidance test (Table 1).

Interestingly, nocistatin (5.0 nmol/mouse) 30 min before the training session significantly reversed the shortening of step-down latency induced by nociceptin (5.0 nmol/mouse) in the retention test without affecting the response to electric shocks (Fig. 1).

Administration of nociceptin (0.5–5.0 nmol/mouse) 30 min before the test session in the Y-maze dose dependently decreased the percent alternation, and the effects of nociceptin at doses of 1.5 and 5.0 nmol were significant (Table 2). Nociceptin did not change the total number of arm entries. On the other hand, neither percent alternation nor total number of total arm entries 30 min after nocistatin (0.5–5.0 nmol/mouse) were affected (Table 2).

Table 2
Effects of nociceptin (A) and nocistatin (B) on normal mice in the Y-maze test

| Treatments | Doses (nmol/mouse) | Percent alternation, median (range) | Total arm entries, median (range) | *N* |
|---|---|---|---|---|
| *(A)* | | | | |
| Control | 0 | 79.3 (70.0–84.8) | 26.0 (22.0–27.8) | 15 |
| Nociceptin | 0.5 | 69.6 (64.3–79.7) | 25.0 (22.0–31.8) | 15 |
| Nociceptin | 1.5 | 62.5 (60.3–74.8)[a] | 27.0 (22.3–32.0) | 15 |
| Nociceptin | 5.0 | 63.0 (58.1–69.2)[b] | 19.0 (18.3–25.8) | 15 |
| *(B)* | | | | |
| Control | 0 | 76.2 (64.7–83.5) | 26.0 (23.3–27.8) | 7 |
| Nocistatin | 0.5 | 78.9 (68.4–87.0) | 24.0 (20.5–34.8) | 9 |
| Nocistatin | 1.5 | 76.0 (67.7–79.7) | 30.0 (26.3–34.0) | 9 |
| Nocistatin | 5.0 | 81.0 (78.7–87.6) | 31.5 (25.5–34.5) | 8 |

Mice were treated intracerebroventricularly with nociceptin or nocistatin (0.5, 1.5 and 5.0 nmol/mouse) 30 min before the test session.
Values are shown as the median and range (first and third quartiles).
*N* shows the number of mice used.
Significance levels: [a]$P < 0.05$, [b]$P < 0.01$ vs. control (Bonferroni's test).

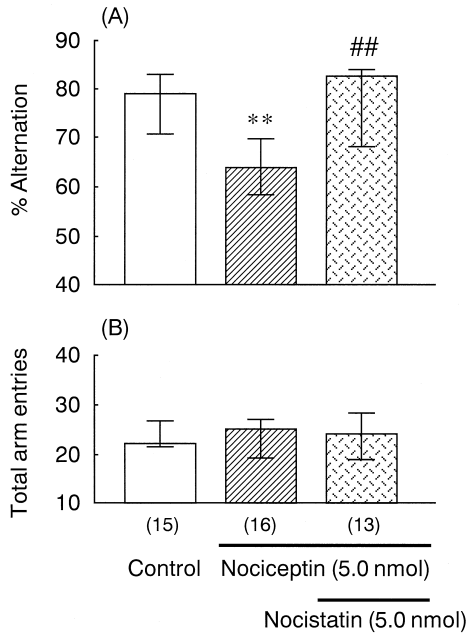


Fig. 2. Effects of nocistatin on nociceptin-induced impairment of spontaneous alternation (A) and total arm entries (B) in the Y-maze test. Mice were treated intracerebroventricularly with nociceptin and nocistatin (5.0 nmol/mouse) 30 min before the test session. Data are shown as median (vertical column) and first and third quartiles (vertical line). The number of mice used is shown in parentheses. Significance levels; $**P < 0.01$ vs. control, $\#\#P < 0.01$ vs. nociceptin alone (Bonferroni's test).

In agreement with the result of the passive avoidance test, nocistatin (5.0 nmol/mouse) 30 min before the test session significantly reversed the impairment of spontaneous alternation induced by nociceptin (5.0 nmol/mouse) without affecting the total number of arm entries (Fig. 2).

## 4. Discussion

Although investigations of learning and memory have focused primarily on cholinergic neurotransmission, peptidergic neurotransmission may also play a role in cognitive deficits associated with Alzheimer's disease and age (Hiller et al., 1987; Jiang et al., 1989). Nociceptin has some structural similarity to the endogenous opioid peptide, especially dynorphin A. However, it has been reported that nociceptin shows different characteristics in pharmacological profiles and does not bind to classical opioid receptors with high affinity (Meunier et al., 1995; Reinscheid et al., 1995; Mathis et al., 1997). Our previous reports showed that dynorphin A-(1–13) improved memory dysfunction in several animal models of amnesia (Hiramatsu et al., 1995, 1996, 1997, 1998a). On the other hand, Sandin et al. (1997) demonstrated that nociceptin impaired memory function. Furthermore, ORL1 receptor knockout mice showed greater learning ability and better memory than did control mice (Manabe et al., 1998). In accordance with these findings, our present results also demonstrated that nociceptin impaired learning and memory function. Together, these findings make it likely that nociceptin and dynorphin A modulate learning and memory function through different neuronal mechanisms.

An antisense oligodeoxynucleotide targeting the first coding exon of an opioid clone (KOR-3) potently blocks nociceptin-induced hyperalgesia without interfering with nociceptin-induced analgesia, while antisense probes based on the second and third coding exons prevent only analgesia (Mathis et al., 1997). The authors suggested that there are heterogeneous, functionally active nociceptin receptors in mouse brain. Although nocistatin does not bind to the ORL1 receptor (Okuda-Ashitaka et al., 1998), it blocks nociceptin-induced allodynia and hyperalgesia (Okuda-Ashitaka et al., 1998). Interestingly, our present results also showed that nocistatin reversed the nociceptin-induced impairment of learning and memory and the impairment of spontaneous alternation (Figs. 1 and 2). It has been reported that nocistatin binds to its own binding sites in the membranes prepared from mouse brain with high affinity (Okuda-Ashitaka et al., 1998). Together, these receptors may have opposite roles in pharmacological effects induced by nociceptin and nocistatin, and these two peptides may play opposite roles in the central nervous system (CNS). The significance of these observations is not yet clear, but they do suggest that additional studies are needed to more fully define these potential nociceptin systems.

Nociceptin is known to have many effects on the CNS, including alterations of spontaneous activity, antinociception and aversive motivation (Meunier et al., 1995; Reinscheid et al., 1995; Florin et al., 1996). Therefore, pretraining administration of nociceptin and nocistatin may alter locomotor activity, pain sensitivity to electric shocks and/or motivation, and these effects may alter the behavioral test conditions in a non-specific manner. Evaluation of the pain response (flinching, vocalization and jumping) to electric shocks showed that the drug tested in avoidance studies had no significant effect on pain sensitivity as compared to its effect in the control group. Thus, nociceptin-induced learning and memory impairments and their improvement by nocistatin do not result from antinociceptive and motor effects.

It has been reported that dynorphin A-(1–13) exerts so-called 'non-opioid effects' (Faden, 1992). We have recently shown that a des-Tyr$^1$-dynorphin analog, dynorphin A-(2–13) improved scopolamine-induced learning and/or memory impairment (Hiramatsu et al., 1998b). Further studies examining the possibility of interaction with κ-opioidergic and nociceptin-mediated systems will be necessary to explore the possibility of counterbalance mechanisms between these peptide activities involved in the physiological functioning of the learning and memory network.

In conclusion, the present results suggest that nociceptin can function as an inhibitory modulator regulating synaptic

transmission and nocistatin can counteract this modulating effect, suggesting that these endogenous peptides may play an important role in the synaptic plasticity involved in learning and memory. Although the functional significance of these findings remains to be elucidated, our data have implications for further clarification of the role of the neuropeptide system in the CNS and in memory function.

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