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The Antagonistic Effects of Naloxone on Cycloheximide and Anisomicin-Induced Amnesia

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KAMEYAMA, T., T. NABESHIMA AND T. KOZAWA. *The antagonistic effects of naloxone on cycloheximide and anisomycin-induced amnesia.* PHARMACOL BIOCHEM BEHAV **25**(3) 567–572, 1986.—The amnesia induced by cycloheximide (CXM) injected SC and by CXM or anisomycin injected ICV immediately after the training test was antagonized in combination with an opiate antagonist, naloxone (NLX). This antagonism occurred on both the passive avoidance- and escape-learning responses in a dose-dependent manner in mice. NLX alone (0.3–10.0 mg/kg) did not alter the performances of these tasks. Furthermore, the decrease in retention performance on shuttle avoidance in rats induced by CXM was also antagonized by NLX. Treatment with CXM and/or NLX did not affect spontaneous locomotor activity. The interaction of these drugs on the performance of the passive avoidance- and escape-learning and the shuttle avoidance tasks may be related to neurochemical memory processes. These results suggest that an opioid system may participate in the amnesic actions induced by protein synthesis inhibitors in these models.

Cycloheximide Anisomycin Amnesia Memory Naloxone Passive avoidance
Escape learning Shuttle avoidance

OVER the past twenty years, the effects of the protein synthesis inhibitors cycloheximide (CXM) and anisomycin (ANI) on the memory process have been investigated in experimental animals [1, 10, 13]. It is well known that both drugs produce retrograde amnesic effects, selectively on long-term memory, as reviewed by Barondes [3]. Furthermore, it is also known that the effect of protein synthesis inhibitors on memory is counteracted by catecholamine (CA), strychnine, amphetamine and picrotoxin which do not affect protein synthesis [14,32].

Recently the possibility of involvement of the opioids in the processes of learning and memory has been suggested [24]. In man, Reisberg *et al.* have shown an effect of the opioid antagonist, naloxone (NLX), on patients with senile dementia on a battery of cognitive tests, using a double-blind trial [33]. Since an absence of an effect was reported in the same year [5], there has been no agreement in this field as yet. In experimental animals, opiate agonists peripherally injected appear to inhibit acquisition of an aversively motivated task, either with classical conditioning [35], or with active avoidance [19,28]. Furthermore, β-endorphin and NLX have been found to have posttraining effects on memory, amnesic and facilitatory, respectively, in a very wide variety of tasks, including active and passive avoidance [2, 18, 19, 22, 23, 29], several types of habituation [20, 30, 34], alimentary conditioning [16], etc. NLX also blocks the stress-induced changes in retention, in a strain-dependent way, presumably by antagonizing the effects of stress-released endogenous opioids [8].

To clarify the involvement of an opioid system in protein synthesis inhibitor-induced amnesia, in the present study, the effects of NLX on CXM and ANI-induced amnesia were investigated using passive avoidance- and escape-learning in mice [27] in Experiment 1, and also shuttle avoidance in rats in Experiment 2.

EXPERIMENT 1: PASSIVE AVOIDANCE- AND ESCAPE-LEARNING RESPONSES IN MICE

METHOD

Subjects

Male mice of the ddY strain (Shizuoka Laboratory Animal Center, Japan) weighing 30–35 g were used as subjects. They were housed 10 per cage with ad lib access to water and food. The animals were kept in a regulated environment (23±1°C, 50±5% humidity), with a light-dark cycle: light off between 8 p.m. and 8 a.m. Each experimental group consisted of 20 mice.

Drugs

Naloxone hydrochloride (NLX; Endo Labs, Garden City, NY), cycloheximide (CXM; Sigma, St. Louis, MO) and anisomycin (ANI; Sigma, St. Louis, MO) were dissolved in physiological saline. CXM and NLX were injected subcutaneously and intraperitoneally, respectively (0.1 ml/10 g of body weight). Both CXM and ANI (5 μl/mouse) were injected intracerebroventricularly (ICV) using micro syringe (Hamilton, Reno, NV) and special injection needle (1/5, 3

mm, Natsume, Japan) by the method of Haley and McCormic [17].

Apparatus

The passive avoidance apparatus described in our previous paper [27] was used in this experiment. It consisted of a Plexiglas rectangular inner box (21×21×40 cm) and a grid floor (26 parallel steel rods, 3 mm in diameter, set 8 mm apart) with a wooden cubic platform (4×4×4 cm) set in the center, in a semi-soundproof wooden outer box (35×41×91 cm) with a 15 W tungsten lamp. The intermittent electric shocks (ES; 1 Hz, 0.5 sec, 60 V DC) were delivered through the grid floor by an isolated stimulator (Nihon Koden, Japan). The grid floor and platform were cleaned after the experiments with each group.

Procedures of Training and Retention Tests

Training test. Each mouse was placed on the platform gently and then, when the mouse stepped down from it and placed all its paws on the grid floor, the intermittent ES were delivered continually until the animal returned to the platform in order to escape from the ES [22,27]. The step-down latency (SDL) and escape latency (EL) were measured and the mice out of criterion ranges (SDL 3–15 sec and EL 15–60 sec) were not used for the following test. Each drug was injected after the training test as soon as possible.

Retention test. Twenty-four hr after the training test, each mouse was placed on the platform again, and the SDL was recorded. After the animal stepped down from, or stayed on the platform until cut-off time of measurement (300 sec), then it was placed in the corner of the grid floor with its tail toward the platform. ES were given again to the animal to record the EL of the retention test.

Experimental Procedures

Dose-dependent effects of NLX on the amnesia induced by subcutaneous injection of protein synthesis inhibitor. Mice were given CXM (150 mg/kg, SC) in combination with saline or NLX in doses of 0.3, 1, 3 and 10 mg/kg (IP) immediately after the training test. The retention test was performed 24 hr after the training test, and the antagonistic effects of NLX on the amnesia induced by CXM were determined.

Antagonistic effects of NLX on the amnesia induced by intracerebroventricular injection of a protein synthesis inhibitor. CXM or ANI (110 μg/mouse) was injected ICV [4] immediately after the training test and NLX was also given IP simultaneously. The retention test was performed 24 hr later, and the antagonistic effects of NLX on the drug-induced amnesia were determined.

Effects of NLX alone on the retention of passive avoidance- and escape-learning responses. The effects of NLX itself, administered immediately after training on the performance of the learning and memory were examined at the doses described above. The retention test was also carried out 24 hr after the training test.

Statistical Analysis

The data were analyzed by Kruskal-Wallis nonparametric one way analysis of variance and subsequently with 2-tailed Mann-Whitney U-test for individual groups. In all statistical evaluations, $p<0.05$ was used as the criterion for statistical significance.

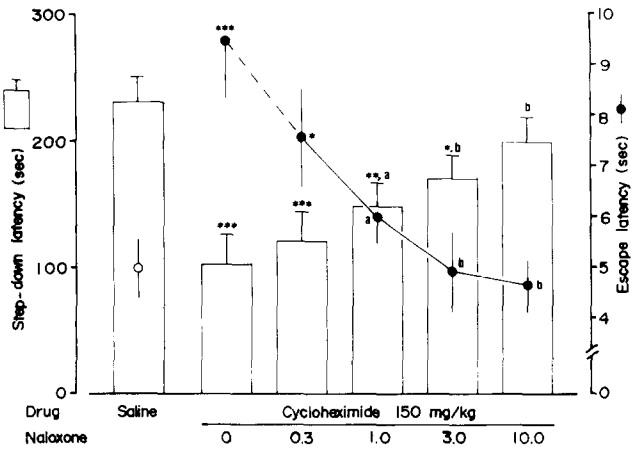

FIG. 1.The antagonistic effects of naloxone on the amnesic actions of cycloheximide on the retention of passive avoidance- and escape-learning responses in mice. Columns and circles show the step-down latency (SDL) and the escape latency (EL), respectively. Each group consisted of 20 mice. The scores represented mean±S.E. $*p<0.05$, $**p<0.01$ and $***p<0.0025$ vs. saline group. $^{a}p<0.05$ and $^{b}p<0.01$ vs. cycloheximide 150 mg/kg group.

RESULTS AND DISCUSSION

Dose-Dependent Antagonistic Effects of NLX on the Amnesia Induced by Subcutaneous Injection of a Protein Synthesis Inhibitor

We investigated whether the addition of NLX affects the CXM-induced amnesia. The means (±S.E.) of the SDL and EL in the training test were not significantly different among all the groups. They were distributed in the range of 7.22±0.73 to 7.71±0.67 sec and 30.6±2.3 to 31.9±2.5 sec, respectively. As shown in Fig. 1, the SDL and EL of the saline control group was significantly prolonged and shortened, respectively, in the retention test compared to those in the training test. The prolonged SDL was shortened, while the shortened EL was prolonged by CXM (150 mg/kg) given immediately after the training. The present results confirmed our previous report [27]. The amnesic actions of CXM, indicated by the shortening of SDL and the prolongation of EL, were attenuated by the simultaneous administration of NLX in a dose-dependent manner, and with significance among all CXM-treated groups: SDL, $F(4,95)=13.72$, $p<0.01$; EL, $F(4,95)=14.64$, $p<0.01$. On this occasion, more than 1 mg/kg of NLX had significant effects on both parameters in individual comparison. Furthermore, the group treated with NLX (10 mg/kg) recovered completely from CXM-induced amnesia. This result suggests the involvement of an opioid system in the amnesic effect of protein synthesis inhibitors.

Antagonistic Effects of NLX on the Amnesia Induced by Intracerebroventricular Injection of Protein Synthesis Inhibitors

The amnesic actions of protein synthesis inhibitors given by ICV injection and the effects of combination of NLX (IP) with them were investigated to confirm whether the amnesic effects of CXM injected peripherally developed through the central nervous system. In this experiment, the means (±S.E.) of the SDL and EL of all groups in the training test were not significantly different again: SDL, 7.33±0.46 to

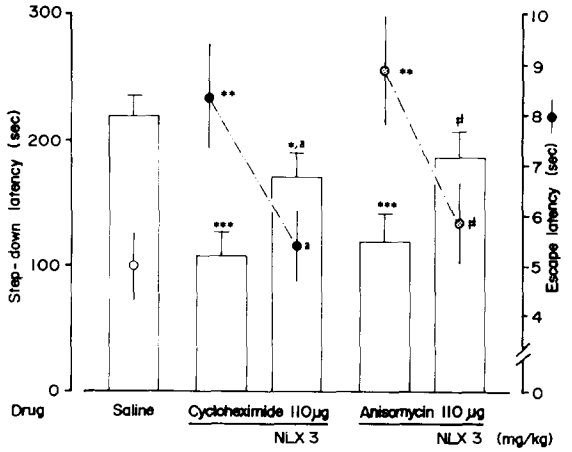

FIG. 2. Antagonistic effects of naloxone (NLX) on the amnesic actions induced by intracerebroventricular injections of protein synthesis inhibitors on the retention of passive avoidance- and escape-learning responses in mice. Columns and circles show the step-down latency (SDL) and the escape latency (EL), respectively. Each group consisted of 20 mice. The scores represented mean±S.E. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.0025$ vs. saline group. $^{a}p<0.05$ vs. cycloheximide (110 μg) group. $\#p<0.05$ vs. anisomycin (110 μg) group.

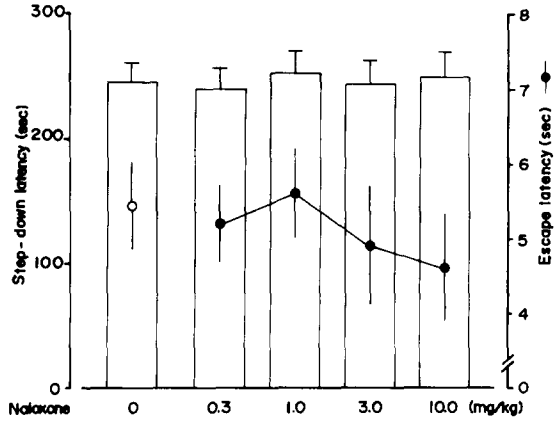

FIG. 3. Effects of naloxone alone on the retention of passive avoidance- and escape-learning responses in mice. Columns and circles show the step-down latency (SDL) and the escape latency (EL), respectively. Each group consisted of 20 mice. The scores represented mean±S.E.

7.49±0.67 sec; EL, 30.4±2.12 to 31.8±2.49 sec. As shown in Fig. 2, significant amnesia, indicated by shortening of the SDL and prolongation of the EL was induced both by CXM and also by ANI (each given separately in dosage of 110 μg ICV), in agreement with Barraco and Frank [4]. NLX (3 mg/kg) significantly decreased the amnesic effects of those protein synthesis inhibitors, with the results shown in Fig. 2. NLX antagonized not only CXM-induced but also ANI-induced amnesia. It appears that the amnesic action of protein synthesis inhibitors may be mediated by central opioid systems in part.

Effects of NLX Alone on Passive Avoidance- and Escape-Learning Responses

The SDL and EL of all groups on the training test were not significantly different: SDL, 7.40±0.61 to 7.76±0.51 sec; EL, 30.5±2.2 to 30.8±1.9. None of the SDLs or ELs in the retention test were altered significantly by administration of NLX (0.3–10 mg/kg) immediately after the training test (Fig. 3). The present result was quite different from that of previous reports which showed a memory-facilitating action of NLX [2, 6, 16, 19]. As reasons for this disagreement, the following may exist: first, the subject we used was the ddY strain of mice, while other authors used rats or other strains of mice. Second, the method we used is suitable for measuring both active and passive behavioral parameters, differing from the methods used in other reports, but it does not seem to be suitable for examining the memory-facilitating action, because the record of the SDL was cut off at a relatively short time (300 sec), since the SDL of control animals was very close to the cut off time of SDL.

EXPERIMENT 2

The amnesic action of a protein synthesis inhibitor was investigated by using a two-way shuttle avoidance response in rats, in order to confirm the results of the first experiment with mice.

METHOD

Subjects

Male rats of the Wistar strain (Shizuoka Laboratory Animal Center, Japan) weighing 250–300 g were used for this experiment. They were housed, 4 per wire mesh cage, and kept in a regulated environment (23±1°C, 50±5% humidity, light-dark cycle; light off between 8:00 and 20:00) with ad lib access to water and food.

Drugs

CXM and NLX were prepared in a manner similar to that in Experiment 1, and administered SC and IP to animals in a volume of 0.2 ml/100 g of body weight.

Apparatus

The study of shuttle avoidance was conducted in a rat shuttle box system (Lehigh Valley Electronics, Beltsville, ME) which was set in a soundproof room. This box (20×45×20 cm) was divided into two compartments by a 4.5 cm high hurdle which was electrified when the rats were trained to avoid a shock, because sometimes animals could avoid shocks by climbing onto the hurdle [25].

The spontaneous locomotor activity of the rats was measured by means of an Opto-Varimex (Columbus Instrument, Columbus, OH).

Procedures

Shuttle avoidance. The rats were initially habituated to the two-compartment conditioning apparatus, shuttle box for 15 min on the day before the training test. The training test session consisted of 30 trials, with an intertrial interval of 25 sec, during which time light (15 W, 28 V) and sound (10 KHz, 90 dB) signals were presented for 5 sec. If the animals did not go to the opposite chamber of the shuttle box during the presentation of the signals, a scrambled shock of 0.5 mA

TABLE 1

EFFECT OF NALOXONE (NLX) ON THE AMNESIC ACTION OF CYCLOHEXIMIDE (CXM) ON ACTIVE AVOIDANCE RESPONSE IN RATS (SHUTTLE-BOX)

Drugs (mg/kg)	n	Percent of avoidance	
		Training	Retention
Saline	25	27.1 ± 2.1*	75.6 ± 2.5
CXM 3	23	27.5 ± 2.1	55.4 ± 3.0‡
CXM 3 + NLX 2	11	28.2 ± 2.7	66.9 ± 3.7†§
CXM 3 + NLX 5	10	27.7 ± 2.8	70.7 ± 4.6¶
Saline + NLX 5	12	27.5 ± 2.5	72.2 ± 4.6

*Each value shows mean ± S.E.
†$p<0.05$ and ‡$p<0.0025$ vs. saline group.
§$p<0.05$ and ¶$p<0.01$ vs. CXM 3 mg/kg group.

(maximum 5 sec) was delivered through the cage floor [25,26]. Animals which performed 3–12 avoidances (10–40% of avoidance) in the training test were used for the retention test. Animals which met the specified criterion were divided randomly into 5 groups and treated with drugs immediately after the training test. The rats of groups I to V (inclusive) were given saline (SC), CXM (3 mg/kg SC), CXM + NLX (2 mg/kg IP), CXM + NLX (5 mg/kg IP) and saline + NLX (5 mg/kg IP), respectively. The retention test was carried out 7 days after the training test in the same manner as the method of Izquierdo *et al.* [19].

Spontaneous locomotor activity. Since the performance of active avoidance in the shuttle box is susceptible to changes in the activity of the animals, the effect of drugs on the spontaneous locomotor activity in rats was also investigated.

On the first day, the rats were habituated to the Plexiglas test cage (40×40×40 cm) surrounded with the Opto-Varimex for 15 min. Immediately after this exposure, the animals were divided randomly into 4 groups and treated with drugs. The rats of Groups I to IV (inclusive) were given saline (SC), CXM (3 mg/kg SC), CXM + NLX (5 mg/kg IP) and saline + NLX, respectively. The spontaneous locomotor activity of the rats was recorded on the 7th day after the treatment.

Statistical Analysis

The statistical analysis was carried out in the same way as described in Experiment 1.

RESULTS AND DISCUSSION

Shuttle Avoidance

As shown in Table 1, in the training test, the rats of each group equally avoided about 27.5% of 30 trials. In the retention test, the animals of the saline control group were able to avoid 75.6% of 30 trials; the group given CXM, however, showed a significantly lower level of avoidance (55.4%). These results suggest that the reduction of avoidance performance in the retention test ensued from the amnesic action of CXM. This hypothesis is supported by the report of Izquierdo [19].

On the other hand, NLX (2 and 5 mg/kg) administered immediately after the training decreased the amnesic effects of CXM significantly in a dose-dependent way, $F(2,41)=9.01$, $p<0.05$. In the CXM + NLX (5 mg/kg) group,

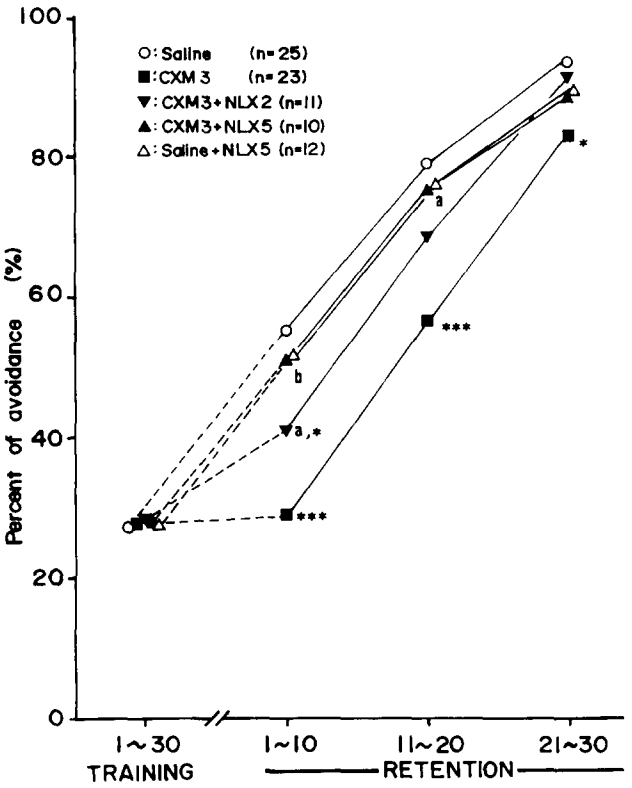

FIG. 4. Time course of the antagonistic effects of naloxone (NLX) on the amnesic action of cycloheximide (CXM) on the retention of active avoidance responses in rats (shuttle box). The numbers of abscissa show the numbers of trials in the training and retention tests. The symbols show the mean of the percent of avoidance. Number in the parentheses shows number of animals. *$p<0.05$ and ***$p<0.0025$ vs. saline group. [a]$p<0.05$ and [b]$p<0.01$ vs. CXM group.

TABLE 2

EFFECT OF CYCLOHEXIMIDE (CXM) AND/OR NALOXONE (NLX) ON THE LOCOMOTOR ACTIVITY IN RATS (OPTO-VARIMEX)

Drugs (mg/kg)	n	Activity counts
Saline	19	3936 ± 180*
CXM 3	8	3671 ± 412
CXM 3 ± NLX 5	8	3983 ± 348
Saline ± NLX 5	12	4039 ± 393

*Each value shows mean ± S.E. of activity counts for 15 min.

the % of avoidance reached almost the same level as that of the control group (70.7%). However, the administration of NLX (5 mg/kg) alone did not facilitate the avoidance performance of animals, in agreement with the results of Experiment 1. Since some previous authors have shown that post-training administration of NLX fails to produce a memory facilitating effect even at relatively high doses [6, 16, 19], our present results are consistent with previous findings.

Moreover, the avoidance performance in the retention test of 30 trials was divided into 3 blocks of 10 trials and analyzed in detail. In the first 10 trials of the retention test,

the differences of avoidance performance between the saline- and CXM-treated groups were considerable (26.5%), while there was only a small difference (10.6%) in the last 10 trials (Fig. 4). These results also indicated that the decrease of avoidance was caused by the amnesia-inducing action of CXM. If the avoidance reduction had resulted from a decrease of motor functions, the differences of avoidance performance between the control and CXM-treated groups would not be varied during the 3 blocks of 10 trials. The considerable difference on the first block especially, should be attributed to the disruptive action of protein synthesis inhibitors on the memory retrieval process as described by Miller and Springer [31].

Spontaneous Locomotor Activity

Neither CXM nor NLX significantly affected the spontaneous locomotor activity in rats 7 days after the treatment (Table 2). Furthermore, the number of the intertrial responses in the retention test of shuttle avoidance was not changed by CXM and NLX (data not shown). These results appear to prove that the effects of CXM and NLX are on the memory process in agreement with the results described above.

GENERAL DISCUSSION

CXM and ANI induced amnesia on the passive avoidance- and escape-learning responses in mice or on active avoidance in the shuttle box method in rats. NLX showed an anti-amnesic effect on passive and active avoidance responses. Furthermore, CXM-treated rats showed a remarkably low level of avoidance in the first 10 trials of the retention test, but they were able to avoid at nearly control level in the last 10 trials (Fig. 4). These results suggest that CXM seems to disrupt selectively the memory retrieval process [23]. CXM and NLX appear to act upon the process of the learning and memory, since these drugs failed to change the spontaneous locomotor activity which used to affect the avoidance performance.

Protein synthesis inhibitors are known to have an inhibitory action on CA biosynthesis, and CA release in the cerebrum [11, 12, 15]. These results suggest that they may play a role in the central CA neural systems. This hypothesis has been supported by experimental data showing that CA stimulation alone fails to affect protein synthesis, although it attenuates the amnesic effect of protein synthesis inhibitors [32], while an amnesia is also caused by the administration of the CA-depleting drugs [9]. Consequently, it is suggested that the CA systems may play many roles in the amnesia-inducing effects of protein synthesis inhibitors. Since the present results indicated the antagonistic actions of the opiate antagonist NLX against the different types of protein synthesis inhibitor-induced amnesia models, the involvement of an opioid system in the amnesic action induced by protein synthesis inhibitors should also be considered. Furthermore, it has been reported that the opiate agonist, pentazocine, decreases the protein synthesis in the mouse brain [36], and that the opiate antagonist, naltrexone, facilitates brain growth and increases the number of neural cells in infant rats [37]. Taken together, there is a possibility that the opioids may affect the process of learning and memory by changing the mechanisms of protein synthesis in the brain. However, as described above, the amnesic action of protein synthesis inhibitors are attenuated by drugs which themselves have no influence on protein synthesis [14]. Moreover, at doses that produce amnesic effect, enkephalins (Leu-, Met- and Des-Tyr-Met-) either increase or decrease protein synthesis in rat amygdala, caudate, hippocampus or the rest of brain [21]. The effects of opioids on memory are too fast to be attributed to affect on protein metabolism: they occur in a matter of minutes. Therefore, protein synthesis inhibitors may not affect the processes of memory via the function of protein synthesis but through neurohumoral systems. Probably the best explanation would seem to be that protein synthesis inhibitors may release opioids peptide and induce amnesia. In fact a number of memory-active substances (ACTH, epinephrine, etc.) release endogenous opioids [21] and their eventual amnesic effects can be thus explained.

Our previous results confirmed the data of Castellano *et al.* [7], that NLX antagonizes both morphine and immobilization stress-induced amnesia in passive avoidance response, but not that NLX alone facilitates the performance of the task in mice. It has been reported that NLX may affect the memory process through the adrenergic or the muscarinic cholinergic systems [2, 18, 22]. In these reports, NLX affects the memory at doses of less than 1 mg/kg and produces a U-shaped dose-response curve [2]. But, in the present results, NLX antagonized the CXM-induced amnesia dose-dependently and the maximum effect was obtained at 5 and 10 mg/kg in active and passive avoidance response, respectively. (These results may suggest that effect of opioids on memory process is not via the opiate μ-receptor.) Therefore, there remains a possibility that other mechanisms in addition to those described above may exist which influence the effect of NLX on the memory process. Further investigation is required to fully elucidate these important and fundamental physiologic processes.

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