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Role of Cholinergic and GABAergic Neuronal Systems in Cycloheximide-Induced Amnesia in Mice

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NABESHIMA, T., Y. NODA, K. ITOH AND T. KAMEYAMA. *Role of cholinergic and GABAergic neuronal systems in cycloheximide-induced amnesia in mice.* PHARMACOL BIOCHEM BEHAV 31(2) 405–409, 1988.— The role of cholinergic and GABAergic neuronal systems on the cycloheximide (CXM)-induced amnesia was investigated using the step-down-type passive avoidance task in mice. CXM (7.5–120 mg/kg, SC) given just after the training caused amnesia (indicated by short latency to step down from the platform on the grid floor) in the retention test conducted 24 hr later in a dose-dependent fashion. In the CXM (60 mg/kg)-treated mice, a choline esterase inhibitor, physostigmine (PHY; 0.125 and 0.25 mg/kg, IP), or GABA agonists, muscimol (1 and 2 mg/kg, IP) and baclofen (6 and 12 mg/kg, IP), given just after training markedly prolonged step down latency (SDL), indicating reversal of amnesia. The antiamnesic action of PHY (0.125 mg/kg) was almost completely antagonized by a central acetylcholine antagonist, scopolamine (3 mg/kg, SC), but not by a peripheral acetylcholine antagonist, butylscopolamine (3 mg/kg, SC). Furthermore, the antiamnesic action of muscimol (2 mg/kg) was reversed by GABA antagonists, picrotoxin (0.5 mg/kg, SC) and bicuculline (0.5 mg/kg, SC), while the effect of baclofen (12 mg/kg) was reversed by picrotoxin (0.5 mg/kg), but not by bicuculline (0.5 mg/kg). These results suggest that the dysfunction of cholinergic and GABAergic neuronal systems play an important role in the CXM-induced memory impairment on the passive avoidance task.

Cycloheximide Amnesia Memory Cholinergic neuronal system GABAergic neuronal system Passive avoidance Mice

IT has been shown that administration of protein synthesis inhibitors such as cycloheximide (CXM) and puromycin, shortly before or immediately after trainings, impairs the development of long-term memory in a variety of species and for a variety of tasks (1, 4, 32), although acquisition and short-term memory are normal (8,31). These findings have been taken as support for the idea that one of the required steps in long-term memory is brain protein synthesis at or near the time of training, and that protein synthesis inhibitors induce amnesia by inhibiting the synthesis of proteins specifically required for long-term memory.

It is also known that certain types of drug treatment reverse the amnesia produced by many of protein synthesis inhibitors; general stimulants, adrenergic agonists, and certain hormones have been shown to have these effects (13, 15, 24, 29). Furthermore, there are several lines of evidence that GABA may have a role in the regulation of brain protein synthesis (27,33), and that cholinergic neuronal system is involved in the formation of memory (21, 25, 30).

A variety of other drugs has also been shown to affect memory processes. We previously have reported that facilitation and blockade of central cholinergic transmission enhances and impairs memory respectively in mice using passive avoidance task (19), and that the GABAergic neuronal dysfunction produces impairment in the memory retention of passive avoidance and rapidly learned conditioned suppression tasks (22). These results suggest that the cholinergic and GABAergic neuronal systems play an important role in the memory consolidation in mice.

In the present study, to clarify an involvement of the cholinergic and GABAergic neuronal systems in the protein synthesis inhibitor, CXM-induced amnesia, the effect of cholinomimetic and GABA agonists on CXM-induced amnesia were investigated using passive avoidance task in mice.

METHOD

Subjects

Male mice of the ddY strain (Shizuoka Laboratory Animal Center) weighing 30–35 g were used as subjects. They were housed in stainless-steel cages, were given food and tap water ad lib and were kept in a regulated environment (23±1°C, 50±5% humidity), with a 12-hr-light/12-hr-dark cycle (8 a.m. to 8 p.m.).

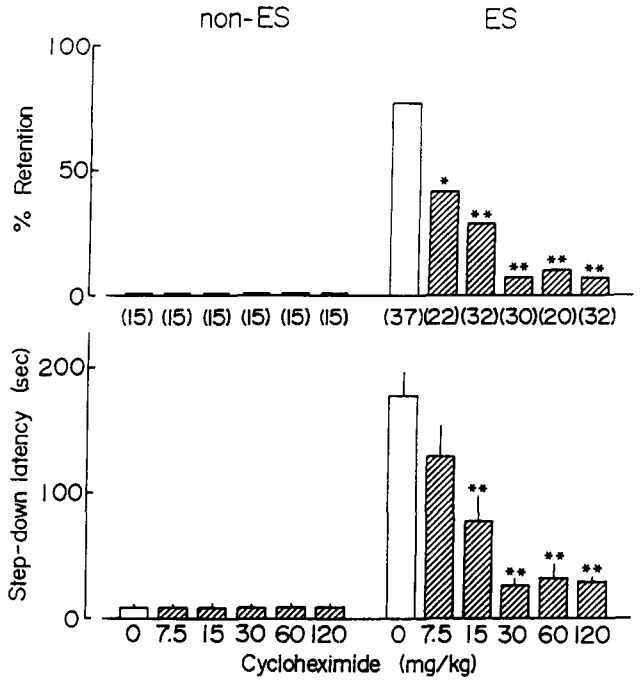

FIG. 1. Effect of cycloheximide on the retention of passive avoidance in mice. Number in the parentheses shows the number of animals used. $*p<0.05$, $**p<0.01$ vs. vehicle-treated ES group.

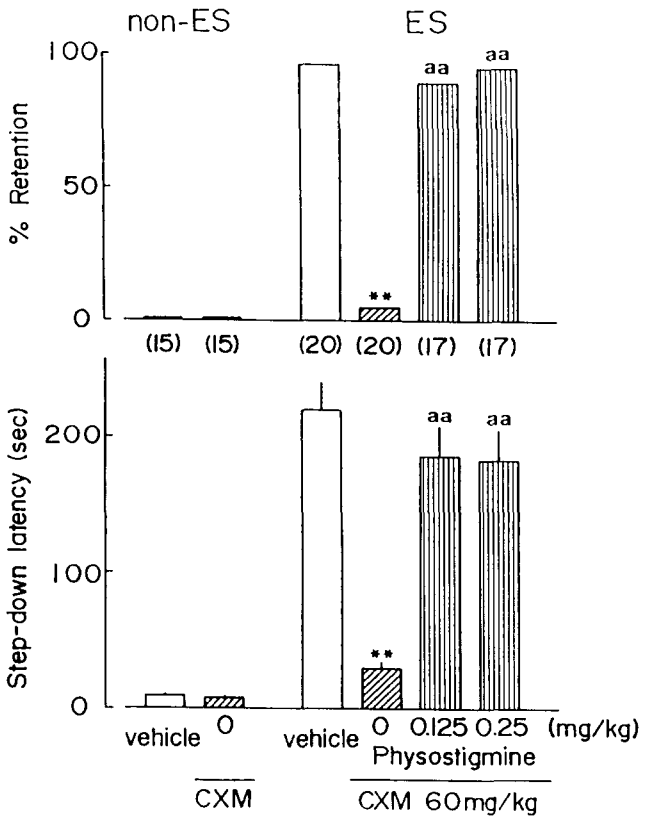

FIG. 2. Effect of physostigmine (PHY) on cycloheximide (CXM)-induced decrease of the retention of passive avoidance in mice. Number in the parentheses shows the number of animals used. $**p<0.01$ vs. vehicle-treated ES group; $^{aa}p<0.01$ vs. CXM-treated ES group.

Drugs

Cycloheximide (CXM; Sigma), physostigmine sulphate (PHY; Merck), scopolamine hydrobromide (SCOP; Katayama), butylscopolamine (BSCOP; Maruko), muscimol (Sigma), baclofen (Ciba-Geigy), were dissolved in a 0.9% saline solution. Picrotoxin (Tokyo Chem. Co.) was dissolved in a minimal amount of 0.1 N HCl to expedite solubility prior to the addition of water (final pH of the solution=5.0). Bicuculline (Sigma) was dissolved in citrate buffer pH 4 (prepared by mixing 50 ml of 0.1 M citrate buffer pH 2.2 with 6 ml of 1 N NaOH) with the aid of sonication (28).

Test Procedure

The experiment was carried out as previously described using a transparent acrylic rectangular cage (30×30×40 cm high) with a grid floor and semi sound-proofed wooden outer box (35×35×90 cm high) with a 15-W illumination lamp. In the center of the grid floor, a wooden platform (4×4×4 cm) was set up (19).

Each mouse was placed on this wooden platform, and when the mouse stepped down from the platform and placed all its paws on the grid floor, an intermittent electric shock (ES; 1 Hz, 500 msec, 60 V DC) was delivered continuously for 15 sec in the training. The step-down latency (SDL) was measured, and the animals in the range of criteria (SDL 3–15 sec) were used for retention test. Twenty-four hr after the training, each mouse was again placed on the platform, and the SDL was recorded. The animal showing over 60 sec of SDL was regarded as reaching the criterion of memory retention. Percent of retention = 100 × (the number of animals clearing the criterion)/(the number of animals tested) (19).

Drug Treatment

Mice were treated with drugs just after the training to avoid a direct acute effect on the behavior in the training and retention test (18). Muscimol, baclofen, PHY, SCOP, BSCOP, picrotoxin and bicuculline were used at the doses which did not affect the memory process by themselves.

Statistics

Statistical differences in the SDL between the drug-treated and control groups were analyzed using the two-tailed Mann-Whitney U-test. The two-tailed Fisher's exact probability test was used for statistical analysis of difference in % of retention between drug-treated and control groups.

RESULTS

Effect of Cycloheximide on the Retention of Passive Avoidance Task in Mice

To develop an amnesia model using present method, mice were given CXM at different doses just after the training (Fig. 1). In the non-ES groups, the SDLs and % of retention of drug-treated groups did not indicate a significant difference compared with vehicle-treated group. It appears that the posttraining-treatment of CXM did not affect the step-down ability of mice in the retention test. In the vehicle-treated ES group, the SDLs and % of retention were significantly longer and higher, respectively, in the retention test

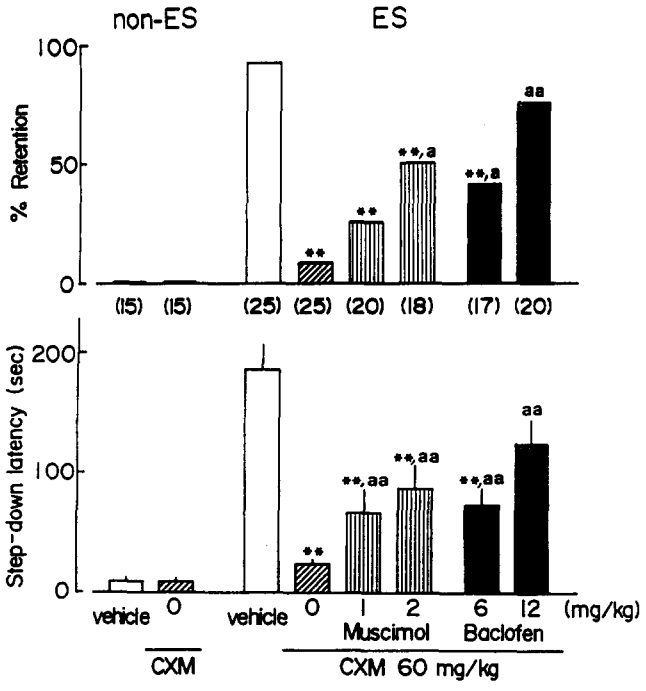

FIG. 3. Effect of muscimol and baclofen on cycloheximide (CXM)-induced decrease of the retention of passive avoidance in mice. Number in the parentheses shows the number of animals used. $^{**}p<0.01$ vs. vehicle-treated ES group; $^{a}p<0.05$; $^{aa}p<0.01$ vs. CXM-treated ES group.

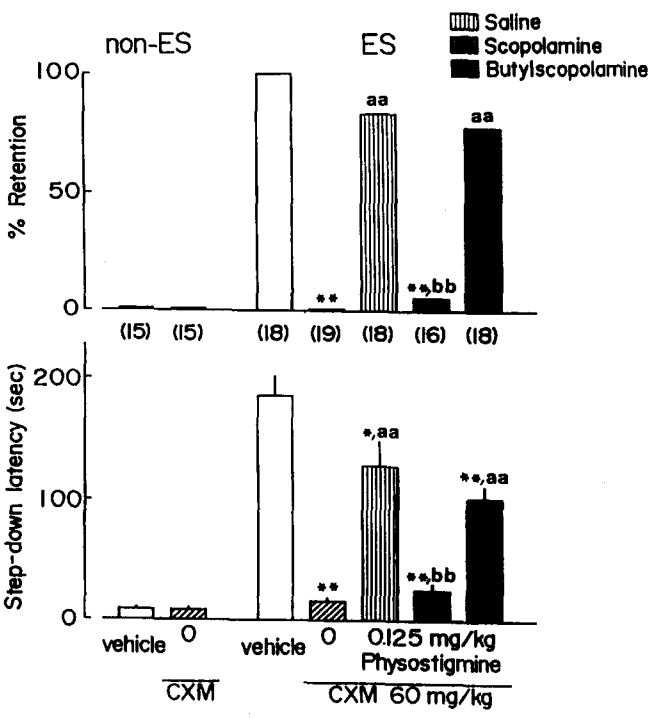

FIG. 4. Effect of scopolamine and butylscopolamine on physostigmine-induced recovery from impairment of memory by cycloheximide (CXM). Number in the parentheses shows the number of animals used. $^{*}p<0.05$, $^{**}p<0.01$ vs. vehicle-treated ES group; $^{aa}p<0.01$ vs. CXM-treated ES group; $^{bb}p<0.01$ vs. CXM + physostigmine-treated ES group.

than those in the non-ES groups. The administration of CXM (7.5–120 mg/kg, SC) just after the training shortened the SDL and decreased % of retention in a dose-dependent fashion.

Effect of Cholinomimetic and GABA Agonists on the Cycloheximide-Induced Amnesia in Mice

We investigated whether a choline esterase inhibitor, PHY, or GABA agonists, muscimol and baclofen, affect the CXM-induced amnesia on the passive avoidance task in mice.

As shown in Figs. 2 and 3, in the vehicle-treated ES group, the SDL was significantly prolonged and % of retention was increased in the retention test compared with the vehicle-treated non-ES group. The prolonged SDL was shortened and % of retention was decreased by CXM (60 mg/kg, SC). The amnesic action of CXM indicated by the shortened SDL and the decreased % of retention was attenuated by the administration of PHY (0.125 and 0.25 mg/kg, IP), muscimol (1 and 2 mg/kg, IP) and baclofen (6 and 12 mg/kg, IP).

Effect of Acetylcholine or GABA Antagonists on the Cholinomimetic- and GABA Agonist-Induced Recovery From Impairment of Memory by Cycloheximide

We investigated to confirm whether cholinomimetic- and GABA agonist-induced recovery from impairment of memory by CXM was developed through the central cholinergic and GABAergic neuronal systems, respectively.

Cholinergic neuronal system. As shown in Fig. 4, PHY (0.125 mg/kg) significantly decreased the amnesic effect of CXM, with the results shown in Fig. 2. PHY-induced recovery from memory impairment by CXM (antiamnesic action) was significantly antagonized by combination with a central acetylcholine antagonist, scopolamine (SCOP, 3 mg/kg, SC), but not with a peripheral acetylcholine antagonist, butylscopolamine (BSCOP, 3 mg/kg, SC). It appears that the PHY-induced improvement of amnesia may be mediated by a central cholinergic system.

GABAergic neuronal system. As shown in Table 1, muscimol (2 mg/kg) and baclofen (12 mg/kg) significantly decreased the amnesic effect of CXM, with the result shown in Fig. 3. The antiamnesic action of muscimol was significantly antagonized by combination with GABA antagonists, picrotoxin (0.5 mg/kg, SC) and bicuculline (0.5 mg/kg, SC). Moreover, the action of baclofen was significantly antagonized by picrotoxin (0.5 mg/kg) but not by bicuculline (0.5 mg/kg) It appears that the GABA agonist-induced improvement of amnesia may be mediated by a central GABAergic neuronal system.

DISCUSSION

Over the past several years many investigators have reported that pretraining or posttraining administration of the protein synthesis inhibitor, CXM, induces amnesia of the training experience when the animal is tested at some later time. This amnesia has usually been interpreted as resulting from an inhibition of synthesis of cerebral proteins during the critical posttraining consolidation period which prevents the manufacture of proteins necessary for long-term memory (2, 3, 11, 12). We tried to develop a CXM-induced amnesia model using the present method. Treatment with CXM just

TABLE 1

EFFECT OF PICROTOXIN AND BICUCULLINE ON MUSCIMOL- AND BACLOFEN-INDUCED RECOVERY FROM IMPAIRMENT OF MEMORY BY CYCLOHEXIMIDE (CXM)

Treatment	Dose (mg/kg)	N	Step-Down Latency (sec)
Vehicle	—	15	133.6 ± 23.4
CXM	60	15	58.9 ± 12.7†
CXM + Muscimol	60 + 2.0	15	119.5 ± 19.6‡
CXM + Muscimol + Picrotoxin	60 + 2.0 + 0.5	15	64.0 ± 20.1*§
CXM + Muscimol + Bicuculline	60 + 2.0 + 0.5	15	55.8 ± 19.4†¶
CXM + Baclofen	60 + 12.0	15	123.0 ± 18.7‡
CXM + Baclofen + Picrotoxin	60 + 12.0 + 0.5	15	62.4 ± 19.8†#
CXM + Baclofen + Bicuculline	60 + 12.0 + 0.5	15	70.6 ± 18.8

CXM (SC), GABA agonists (IP) and GABA antagonists (SC) were administered just after the training. N: number of animals.

*$p<0.05$, †$p<0.01$ vs. vehicle-treated group; ‡$p<0.01$ vs. CXM-treated group; §$p<0.05$, ¶$p<0.01$ vs. (CXM + muscimol)-treated group; #$p<0.01$ vs. (CXM + baclofen)-treated group.

after the training shortened the SDL in the retention test in a dose-dependent fashion. Significant amnesic action (indicated by the shortening of SDL and the decreased % of retention) was obtained by the treatment with CXM (15–120 mg/kg). This result suggests that CXM seems to disrupt selectively the memory consolidation process. CXM may inhibit the protein synthesis related to the cholinergic and GABAergic neuronal systems which play an important role in the memory process, because acetylcholine and GABA agonists recovered impairment of memory induced by CXM. Some biochemical experiments are required to identify what protein in both neuronal systems is responsible for the memory process.

Numerous reports have indicated that learning and memory can be modified by GABA-related agents which affect the central nervous system. We previously have reported that the GABAergic neuronal dysfunction induced by GABA antagonists produces impairment in the memory retention of passive avoidance and rapidly learned conditioned suppression tasks (22). This finding has suggested that the GABAergic neuronal system in the brain is involved in the learning and memory processes, and via its ability as an inhibitory transmitter GABA might have an effect on the memory "consolidation" processes. On the contrary, a variety of reports have suggested that a blocker of GABA neurotransmission facilitates the "acquisition" of learning tasks. From these reports, there is a possibility that GABA agonists would cause impairment of memory. If the centrally active GABA agonists could interfere with the "consolidation" of memory regarding passive avoidance task, animals which were injected with GABA agonist and CXM would show a shortened SDL compared with the CXM-treated group. In the present study, however, GABA agonists, muscimol and baclofen, antagonized CXM-induced amnesia on the passive avoidance task in mice. As described in theDrug Treatment section, drugs were used at the doses which did not affect the memory process by themselves. It has been suggested that a decrease in the levels of excitement is required for the instigation of the memory "fixation" process, although an increase in the levels of excitement is required for the "acquisition" of learning. Our result may support this theory since GABA agonists, which had an inhibitory effect, improved CXM-induced impairment of memory, while GABA antagonists which had a stimulating effect impaired learning and memory when administered just after the training. Therefore, it seems more reasonable to assume that GABA agonist-induced improvement of memory is caused on the "consolidation" process. Furthermore, our findings are in agreement with the results of the experiments with injection of GABA-level increasing agent (14) and GABA (ICV) (17), and of clinical reports of GABA-induced memory enhancement (9,26). On the other hand, there are several lines of evidence that GABA may have a role in the regulation of brain protein synthesis. In a word, the decrease of protein synthesis of brain was induced by an inhibition of GABA synthesis (27). Thus, the involvement of a GABAergic neuronal system in the amnesic action induced by protein synthesis inhibitors was also considered. Furthermore, it has suggested that GABA neuronal system is involved in the formation and storage of long-term memory (17,20). Taken together, there is a possibility that the GABAergic neuronal system may affect specifically the process of learning and memory by changing the mechanisms of protein synthesis in the brain. These results suggest that GABAergic neuronal system may play the important role in the long-term memory process.

Moreover, muscimol-induced improvement of memory was antagonized by GABA antagonists, picrotoxin and bicuculline, while effect of baclofen was also antagonized by picrotoxin, but not by bicuculline. The reason for the inconsistency of this result may be due to the difference of GABA receptor sites of baclofen and bicuculline. In fact, the presence of a novel receptor for the neurotransmitter GABA in mammalian brain slice has been described (5, 6, 16). Baclofen is stereospecifically active at the $GABA_b$ site whereas it is devoid of activity at the classical $GABA_a$ site (bicuculline-sensitive site) (5, 16, 23). In addition, a central acetylcholine antagonist, SCOP, antagonized GABA agonist-induced recovery from impairment of memory by CXM on the passive avoidance task in mice, but not a peripheral acetylcholine antagonist, BSCOP (data not shown). It suggests that GABA agonist may affect the memory process through the cholinergic functions in part. Furthermore, GABA agonist-induced improvement of memory was produced by a centrally acting GABA neuronal system. However, under the present experimental conditions, there is a possibility that the other mechanisms not described above may exist which influence the effect of GABAergic neuronal system on the memory process. On this point further investigation is required.

Moreover, the dosage of CXM (60 mg/kg) used in this present experiment seems to be rather lower than previous reports (7, 10, 18). This task seems to be useful to examine a role of CXM-induced amnesia in learning and memory in animals, since a high dose of CXM inhibits protein synthesis not only in the central nervous system, but also in the peripheral nervous system. However, there is a possibility that CXM-induced amnesia is not produced by a direct inhibition of protein synthesis, because the amnesia was observed even at the low dose (15 mg/kg). It has been suggested recently that the amnesia following CXM administration is not due to inhibition of protein synthesis itself but rather to impairment of acetylcholine neurotransmission. A transient increase in

muscarinic receptor population of about 21% has been shown to occur in the forebrain of chicks 30 min after a one-trial passive avoidance learning task (25). The training-induced elevation in muscarinic receptor density was blocked by CXM administered intracranially 5 min prior to the training. These results suggest that a change in quantity of muscarinic receptors may also be part of the process of memory acquisition and storage, and that the changes in receptors may not be produced by inhibition of protein synthesis, since the change is so rapid. There is a possibility that CXM affects the muscarinic receptors directly and produces an amnesia. This hypothesis has been proved by following present experimental data: PHY itself, which fails to affect protein synthesis, attenuates the amnesic effect of protein synthesis inhibitors, while CXM-induced amnesia is also caused by the combination of PHY in the presence with the central acetylcholine antagonist, SCOP, but not with the peripheral acetylcholine antagonist, BSCOP. Consequently, it is suggested that the dysfunction of central cholinergic neuronal system relates to the amnesia-inducing effects of protein synthesis inhibitors.

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