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Similar Effects of a Monoamine Oxidase Inhibitor and a Sympathomimetic Amine on Memory Formation

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GIBBS, M. E. AND K. T. NG. *Similar effects of a monoamine oxidase inhibitor and a sympathomimetic amine on memory formation.* PHARMAC. BIOCHEM. BEHAV. 11(3) 335–339, 1979.—Amnesia resulting from inhibition of cerebral protein synthesis by cycloheximide can be prevented by subcutaneous injection of the monoamine oxidase inhibitor pargyline (25 mg/kg) or the sympathomimetic amine metaraminol (3.0 mg/kg) administered up to 30 min following learning of a single trial passive avoidance task in day-old chickens. The injection has to be made during the life time of labile memory for the prevention of cycloheximide-induced amnesia. On the other hand, amnesia induced by the Na/K ATP'ase inhibitor ouabain can only be prevented if these two agents are administered up to 5 min after learning, i.e. during the life time of short-term memory. In addition, both agents produce a retrieval deficit 90 min after the injection, but only when memory is in long-term storage. These results are compared to those obtained with administration of norepinephrine, d-amphetamine and diphenylhydantoin.

Pargyline Metaraminol Ouabain Cycloheximide Day-old chickens
Single trial passive avoidance learning

AMNESIA for a single trial passive avoidance learning task in day-old chicks has been successfully induced by protein synthesis inhibitors such as cycloheximide (CXM) and anisomycin, by Na^+/K^+ ATP'ase inhibitors such as ouabain and ethacrynic acid [9, 14, 24], and by monosodium glutamate and low doses (1 to 2 mM) of potassium chloride [7,9]. The amnesic actions of these drugs are attributed to interference with different, but sequentially dependent, phases in the formation of memory: long-term protein synthesis dependent memory (LTM), labile sodium pump dependent memory, and short-term memory (STM), the latter postulated to be associated with a potassium conductance change induced hyperpolarization [7,9].

Both CXM and ouabain-induced amnesia can be counteracted by norepinephrine (NE) and amphetamine [4, 5, 10], as well as diphenylhydantoin (DPH), an antiepileptic drug [8]. DPH stimulates Na^+/K^+ ATP'ase activity and this appears to be the basis of its overcoming the inhibition produced by ouabain and CXM [8]. In the case of ouabain, DPH may directly counteract inhibition of Na^+/K^+ ATP'ase activity, while in the case of CXM it has been suggested that labile memory is prolonged or restimulated such that when protein synthesis, needed for LTM, starts to recover from CXM inhibition, consolidation can recommence. It has also been suggested that NE and amphetamine stimulate Na^+/K^+ ATP'ase activity, amphetamine acting through release of NE [5]. Biochemical evidence shows that both NE and amphetamine stimulate Na^+/K^+ ATP'ase activity in chicken forebrain homogenate [11]. There is evidence that the DPH effect is not due to NE release, since neither α nor β noradrenergic antagonists prevent NE's counteractive action on CXM-induced amnesia [9].

Monoamine oxidase inhibitors, including pargyline, pheniprazine, tranylcypromine and catron have been reported to overcome amnesia induced by protein synthesis inhibitors in mice [2, 18, 19, 20]. With the most potent inhibitor of monoamine oxidase activity, pargyline, the amnesic effects occurred in the presence of 90% or greater inhibition of MAO activity and significant increases in brain 5-hydroxytryptamine (5HT), dopamine (DA) and NE [21].

In this paper we report results of behavioral experiments on the time-dependent effects of pargyline on CXM and ouabain-induced amnesia for a single trial passive avoidance task in day-old chicks. These effects are compared with those of the sympathomimetic amine metaraminol, which has been suggested to increase NE levels, leading to end-product inhibition of NE synthesis. It was expected that both pargyline and metaraminol should overcome ouabain- and CXM-induced amnesia.

METHOD

Procedure

The procedure as reported by Gibbs [5] was adopted. Day-old white-Leghorn black Australorp cockerels were trained to peck at a 4 mm chromed bead dipped in water, and presented for 10 sec. Chicks failing to peck on this trial or to show characteristic disgust responses were eliminated from

subsequent data analysis. A different group of 20 chicks was used for each data point. Retention, defined as the proportion of chicks avoiding the bead, was measured using a similar chromed bead, presented dry for 10 sec.

Drugs and Injections

All drugs were made up in sterile NaCl (0.15M; 0.9%). Ouabain (Sigma, 0.4 mg/chick), cycloheximide (CXM, Upjohn; 20 mg/chick), or saline (NaCl; 0.9%) was administered intracranially in 10 μl volumes by freehand injection to the centre of each side of the forebrain, to a depth of 3 mm. Pargyline hydrochloride (Abbott Laboratories) or metaraminol bitartrate (Merck, Sharp and Dohme) in various doses was administered in 0.1 ml volumes by subcutaneous injection on the ventral side of the rib cage.

RESULTS

Dose Response Curves for Metaraminol or Pargyline

Chicks were treated with saline, ouabain or CXM 5 min before learning, followed by a subcutaneous injection of either saline, pargyline or metaraminol 5 min later. They were tested for retention 180 min after learning (Fig. 1).

Pargyline was administered in one of four doses: 5, 25, 50 or 75 mg/kg (Fig. 1a). Saline, ouabain and CXM treated control chickens received no pargyline injection. Twenty-five mg/kg of pargyline was optimum in preventing either ouabain- or CXM-induced amnesia, yielding very high levels of retention (90% and 95% respectively) compared to the 20% retention level shown by each of the drug control groups.

Metaraminol in one of four doses (0.5, 1.0, 2.0 or 3.0 mg/kg) was administered either 5 min or 10 min after learning (Fig. 1b, and 1c). Three mg/kg metaraminol was optimal in preventing ouabain-induced amnesia when administered 5 min after learning, with a 85% retention level compared to the 20% retention level with saline treatment. This dose had no effect on ouabain-induced amnesia when administered 10 min after learning. Three mg/kg metaraminol administered 5 min after learning was effective in preventing CXM-induced amnesia. Furthermore, the metaraminol effect on CXM-induced amnesia was present when administered 10 min after learning, in contrast to its lack of effect when administered at this time to ouabain-treated chicks. Twenty-five mg/kg pargyline and 3.0 mg/kg metaraminol were used in the subsequent experiments.

By using a successive discrimination avoidance task [6] it could be shown that the effects of pargyline and metaraminol were on retention rather than through inducement of general avoidance of pecking (Table 1). Chicks were trained to discriminate between a red and a blue bead by making the red bead aversive.

Time of Injection Effects of Pargyline and Metaraminol

Pargyline or metaraminol was administered between 5 and 60 min after learning to chicks pretreated with saline, ouabain or CXM 5 min before training (Fig. 2). Retention was tested 180 min after learning. Both pargyline (Fig. 2a) and metaraminol (Fig. 2b) were effective in preventing ouabain-induced amnesia when administered 5 min after learning, but not later. On the other hand, both drugs prevented CXM-induced amnesia when injected as late as, but not later than, 30 min after learning.

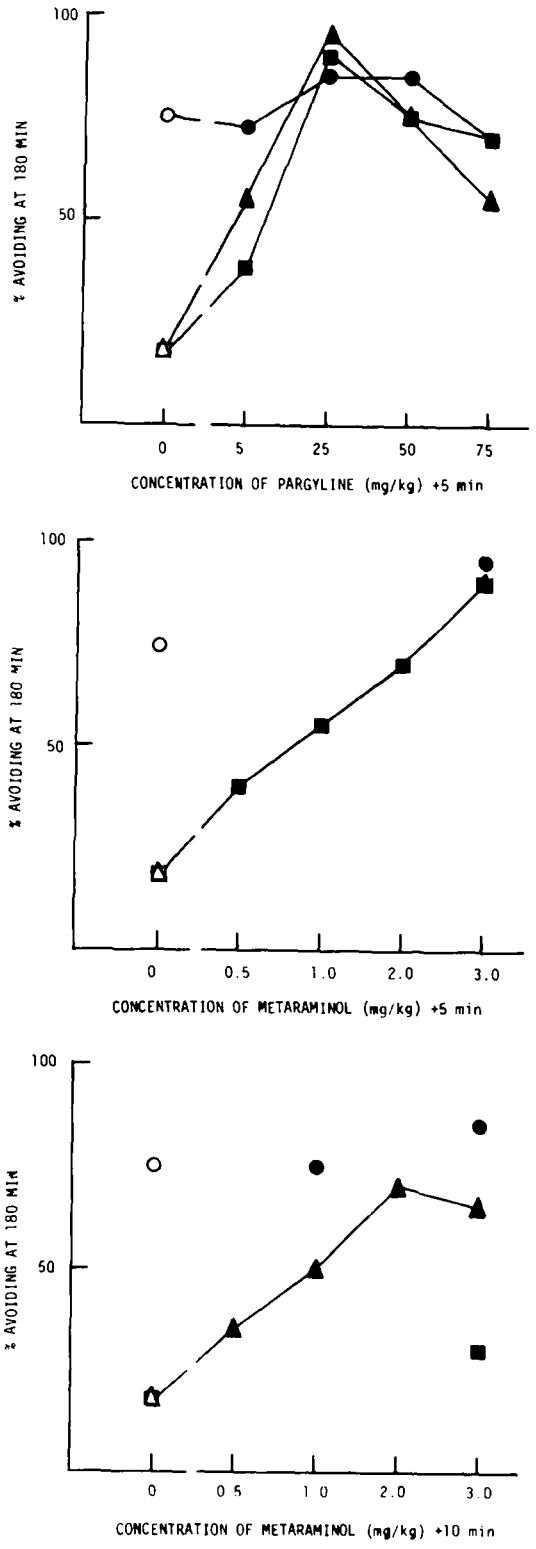

FIG. 1. Retention at 180 min in chicks given an intracranial injection of saline ●, ouabain ■, or CXM ▲ 5 min before learning and then given a subcutaneous injection of a range of concentrations of (a) pargyline administered 5 min after learning, (b) metaraminol administered 5 min after learning, or (c) metaraminol administered 10 min after learning. Controls were injected with subcutaneous saline ○ at the appropriate time after learning.

TABLE 1

EFFECTS ON RETENTION TESTING 180 MIN AFTER LEARNING OF PARGYLINE (25 MG/KG) OR METARAMINOL (3.0 MG/KG) ADMINISTERED 5 MIN AFTER DISCRIMINATION AVOIDANCE LEARNING

Treatment before learning	Treatment: Pargyline % avoidance of Red	Pargyline % avoidance of Blue	Metaraminol % avoidance of Red	Metaraminol % avoidance of Blue
Saline	90	0	85	0
CXM	95	15	80	15

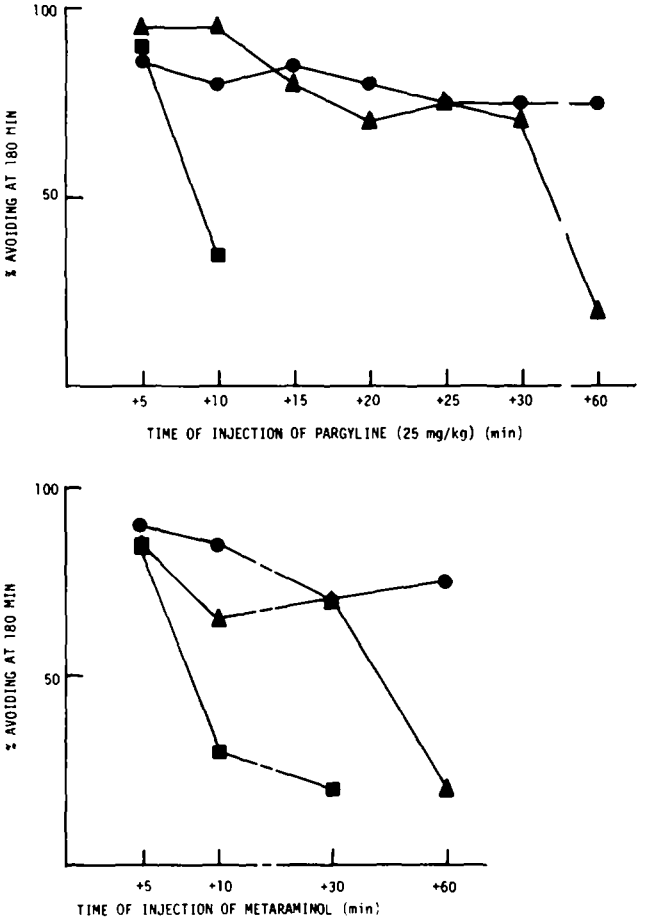

FIG. 2. Retention at 180 min after learning in chicks injected intracranially with saline ●, ouabain ■, or CXM ▲ before learning. Pargyline (25 mg/kg) or metaraminol (3.0 mg/kg) were injected subcutaneously at different times after learning.

Time Course of Retention Following Administration of Pargyline or Metaraminol

Pargyline, metaraminol or saline was administered 5 min after learning to chicks treated with saline, ouabain or CXM 5 min before learning. Retention was tested at 10, 30, 90 or 180 min after learning (Fig. 3). Both ouabain- and CXM-induced amnesias, normally evident by 15 and 60 min after

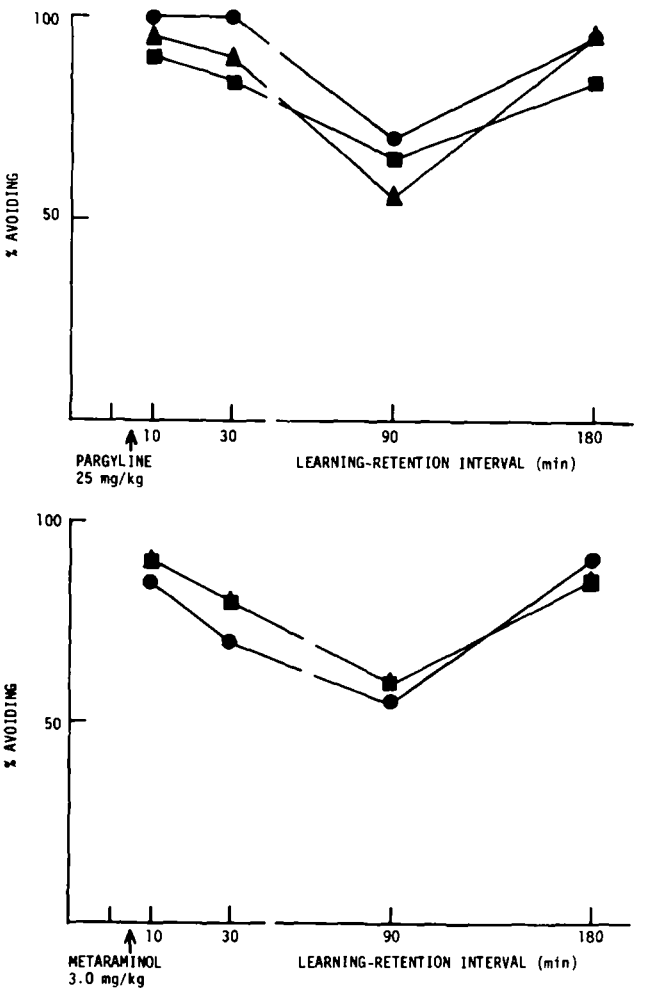

FIG. 3. Percent avoidance of chicks tested between 10 and 180 min after learning injected with saline ●, ouabain ■, or CXM ▲ before learning and given a subcutaneous injection of (a) pargyline or (b) metaraminol 5 min after learning.

learning respectively [9], were prevented by pargyline and metaraminol. Retention was high at 30 and 180 min after learning. However, there was a temporary retention deficit at 90 min after learning for both CXM- and ouabain-treated chicks administered either pargyline or metaraminol. A similar deficit was observed with both the saline-metaraminol and the saline-pargyline treated chicks. This phenomenon has also been reported with d-amphetamine and norepinephrine [4, 5, 9]. In addition, pargyline or metaraminol administered 10 or 60 min after learning and retention measured 60, 90 or 180 min *after administration of the drug* produced the same temporary decrease in retention levels 90 min *after drug administration* (Fig. 4). This strongly suggests that the deficit is due to performance deficit induced by the drugs and related to the time of administration of the drugs rather than to the time of learning. That the effect is not due to the injection *per se* is shown by the fact that chicks administered saline 60 min after learning the successive discrimination avoidance task yielded an avoidance level of 76.5 for the red

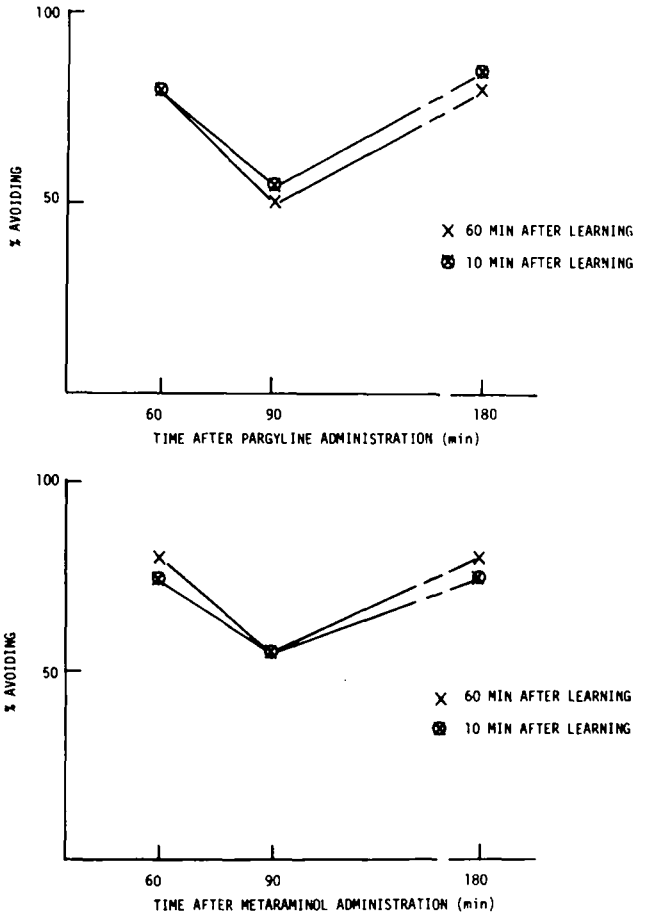

FIG. 4. Percent avoidance of chicks injected subcutaneously with (a) pargyline or (b) metaraminol 10 or 60 min after learning. Retention was tested at 60, 90 or 180 min after the subcutaneous injection.

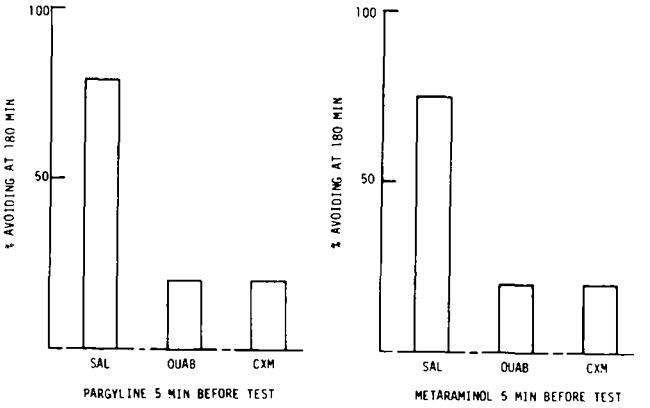

FIG. 5. Retention of chicks given an intracranial injection of saline, ouabain or CXM before learning, and a subcutaneous injection of (a) pargyline (25 mg/kg) or (b) metaraminol (3.0 mg/kg) 5 min before the test at 180 min.

bead and 17.6 for the blue bead when tested 90 min after drug administration.

Effect of Pargyline or Metaraminol Administered Immediately before Retention Test

When pargyline or metaraminol were administered 5 min before the 180 min retention test of the saline-, CXM-, or ouabain-treated chicks there was no difference in the level of retention when compared with chicks treated with saline, ouabain or CXM alone (Fig. 5; cf. Fig. 1).

DISCUSSION

Both ouabain- and CXM-induced amnesias are effectively prevented by the MAO inhibitor pargyline and the sympathomimetic agent metaraminol. The administration times that these two agents were effective in preventing amnesia differed for the two amnesic drugs. In the case of ouabain-induced amnesia, neither pargyline nor metaraminol was effective when administered later than 5 min after learning. With CXM-induced amnesia, however, both agents were successful in preventing amnesia when administered as late as 30 min after learning. The above findings match in almost every detail those reported for diphenylhydantoin, norepinephrine and amphetamine [4, 5, 8, 10]. The counteractive effects of both pargyline and metaraminol on ouabain- and CXM-induced amnesia may be explained in terms similar to those postulated for DPH, NE and amphetamine [9]; viz., through stimulation of Na^+/K^+ ATP'ase activity.

Increases in brain concentrations of endogenous monoamines following treatment with MAO inhibitors have been reported in the chicken [17]. It has been argued that inhibition of MAO prevents destruction, through oxidative deamination, of amines like tyramine, which are potent inhibitors of NE uptake and stimulators of NE release [23]. Similarly decreased synthesis of NE by metaraminol [12] has been attributed to release of NE from storage sites and subsequent feedback inhibition (see [15]).

The possibility arises, therefore, that the ability of pargyline and metaraminol to oppose the action of ouabain in producing amnesia may be attributed to increased NE levels which stimulate Na^+/K^+ ATP'ase activity and counteract ouabain inhibition of this activity [13]. While formation of ouabain-sensitive memory is dependent on Na^+/K^+ ATP'ase activity and is completed by 10 to 15 min after learning, maintenance of this memory is not since ouabain is ineffective in inhibiting memory when administered 10 min or later after learning [9]. Thus stimulation of ATP'ase activity, whether directly by DPH or NE, or indirectly through NE release by d-amphetamine, pargyline or metaraminol, should not be effective in overcoming ouabain-induced amnesia when injected 10 min or later after learning.

The basis for the effects of pargyline and metaraminol on CXM-induced amnesia may not be as clear-cut. Certainly, the results are not inconsistent with the hypothesis that CXM induces amnesia through inhibition of catecholamine synthesis [1, 3, 18, 19]. However, catecholamines are potent inhibitors of tyrosine hydroxylase [22] and drugs which increase brain levels of catecholamines may be expected to induce end-product feedback inhibition of catecholamine synthesis. This has been suggested as the basis for reduced turnover of endogenous brain NE during the inhibition of MAO [16]. As noted earlier decreased NE synthesis from both precursors: tyrosine and DOPA, following metaraminol

treatment has been attributed to NE release [12]. Other problems associated with the catecholamine hypothesis of CXM action have been detailed elsewhere [9]. The results for pargyline and metaraminol reported here may be interpreted in terms of the alternative hypothesis that these drugs prolong labile memory until protein synthesis recovers sufficiently from CXM inhibition to reinstitute LTM formation [8,9]. Since maintenance of labile memory appears to be independent of Na^+/K^- ATP'ase activity, pargyline and metaraminol may prolong this memory through continued reactivation of the trace by stimulation of ATP'ase activity. Thus the drugs effectively counteract CXM-induced amnesia only when administered up to 30 min following learning, i.e., prior to the decay of labile memory [9].

Pargyline and metaraminol yield a temporary retention deficit about 90 min after learning. Like NE and d-amphetamine (see [9,10]) the deficit is dependent on the time of administration of the drugs rather than on the time of learning. Thus administration of the drugs 60 min after learning, when protein synthesis dependent LTM is said to have been formed [9], produces a retention deficit 90 min later: recovery takes place after another 90 min. Moreover, the effect appears to be restricted to retrieval from the long term protein synthesis dependent phase. Metaraminol administered 60 min before learning of a passive avoidance task yielded retention levels of 90, 70, 75 and 75% when tested at 5, 20, 30 and 60 min following learning. These levels are comparable to those for saline treated chicks [9]. If the time parameters associated with the temporary retention deficits reported earlier were due to the time taken for the drugs to act, one might have expected a retention deficit around 30 min following learning when metaraminol was administered 60 min before learning. Thus the effect appears to be restricted to the LTM phase.

There is no immediately obvious explanation for these temporary deficits. No marked effects of this nature were observed with DPH [8,9]. The possibility that feedback inhibition of NE synthesis is involved merits a more detailed investigation, which is currently underway.

The experiments reported here suggest that the monoamine oxidase inhibitor pargyline as well as the sympathomimetic amine metaraminol are successful in preventing ouabain- and CXM-induced amnesia. The time of administration effects of these drugs as well as their time of action are consistent with the findings reported for norepinephrine, amphetamine and diphenylhydantoin. It is suggested therefore that the basis for their action is stimulation of Na^+/K^+ ATP'ase activity; in the case of ouabain-induced amnesia the drugs may prevent ouabain inhibition of Na^+/K^+ ATP'ase activity and in the case of CXM-induced amnesia the drugs may extend labile memory until some recovery from CXM-induced inhibition of protein synthesis has occurred.

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