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Counteractive Effects of Norepinephrine and Amphetamine on Ouabain-Induced Amnesia

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GIBBS, M. E. AND K. T. NG. *Counteractive effects of norepinephrine and amphetamine on ouabain-induced amnesia.* PHARMAC. BIOCHEM. BEHAV. 6(5) 533–537, 1977. – Ouabain, administered 5 min prior to learning induces amnesia for a single-trial passive avoidance task in day-old chickens by inhibiting memory formation during the labile sodium-pump dependent phase. Amphetamine and norepinephrine (NE) successfully counteract ouabain-induced amnesia when administered immediately after learning. The actions of these drugs on ouabain-induced amnesia parallel that of diphenylhydantoin (DPH), and within similar time constraints. It is concluded that amphetamine (through release of NE) and norepinephrine exert their effects through stimulation of Na^+/K^+ ATP'ase activity. This conclusion is supported by the observation that these drugs do not overcome potassium chloride (KCl) inhibition of memory formation in the short-term phase prior to formation of labile sodium-pump dependent memory, and the fact that the noradrenergic blockers, propranolol and piperoxane do not alter the couteractive influence of DPH on cycloheximide (CXM) inhibition of the protein synthesis dependent long term memory phase which follows the labile phase.

Amphetamine Norepinephrine Ouabain-induced amnesia Labile memory
Na^+/K^+ ATP'ase activity Chickens Diphenylhydantoin

CYCLOHEXIMIDE-INDUCED amnesia for single trial passive avoidance learning in day-old chickens has been successfully counteracted by amphetamine [5], norepinephrine (NE) and noradrenergic agonists [6] and also by diphenylhydantoin (DPH) an antiepileptic drug which stimulates Na^+/K^+ ATP'ase activity [7]. The action of amphetamine appears to be due to norepinephrine release in this situation [6].

It has been suggested that the formation of permanent memory in this learning situation occurs in three sequentially and functionally dependent phases [8]. These phases are a short-term phase (STM) lasting 10 min, followed by a labile phase lasting 30 min, and finally the long term phase (LTM). The formation of memory in the three phases is inhibited respectively by 1 or 2 mM KCl; ouabain, which inhibits the sodium pump (Na^+/K^+ ATP' ASE activity), and cycloheximide (CXM) a protein synthesis inhibitor.

Amphetamine, NE and DPH have been postulated to overcome CXM-induced amnesia by their actions on the labile memory trace, prolonging this phase of memory storage until protein synthessis recovers from CXM inhibition [7,9]. Biochemical evidence has been obtained showing that these drugs do stimulate Na^+/K^+ ATP'ase activity in chicken forebrain homogenate [7,9].

DPH, a Na^+/K^+ ATP'ase stimulant, probably directly antagonizes the inhibitory action of ouabain. In behavioural experiments, DPH is effective in overcoming ouabain-induced amnesia when administered prior to complete formation of labile memory (i.e. within 10 min of learning) [7]. The action of DPH at the particular dose used in these experiments (0.1 ml of 10^{-4}M, administered subcutaneously) does not seem to be due to norepinephrine release as α and β noradrenergic antagonists do not prevent its counteracting CXM-induced amnesia or its facilitation of memory under normal conditions. The basis of action of DPH, therefore, involves direct stimulation of Na^+/K^+ ATP'ase activity, rather than by release of norepinephrine.

Therefore, if the mechanism of action of amphetamine (through release of NE) and norepinephrine in overcoming CXM-induced amnesia is via their modulation of Na^+/K^+ ATP'ase activity and labile memory formation, then they should be able to antagonize the inhibition of memory by ouabain within the same time constraints as DPH. To be consistent with the hypothesis these drugs should not alleviate the inhibition of STM by 1 mM KCl.

METHOD

Procedure

The procedure as reported by Gibbs [5] was followed. Day-old white-Leghorn black-Australorp cockerels were trained to peck at a 4 mm diameter chromed bead, dipped in water, presented for 10 sec. On a single aversive learning

trial, a similar bead dipped in the aversant, methyl anthranilate, was also presented for 10 sec. The data from chickens failing to peck during this trial were discarded at the end of the experiment. A different group of 20 chicks were used for each treatment-retention interval condition. Retention, measured as percentage of chicks avoiding the lure, was tested using a chromed bead identical to the training bead but without the aversant, presented for 10 sec.

Drugs and Injections

All drugs were made up in sterile NaCl 0.15 mM (0.9%). Ouabain (Sigma; 0.4 mg/chick), potassium chloride (KCl, 1.0 mM, 1.5 μg/chick), or saline (NaCl, 0.9%) was injected intracranially 5 min before learning. The bilateral site of each 10 μl freehand injection was the centre of the forebrain at a depth of 3.0 mm.

D-amphetamine sulphate (1.0 mg/kg) or l-norepinephrine bitartarate (50 μg/kg) in 0.1 ml volume was injected under the skin on the ventral side of the rib cage. The doses of amphetamine and norepinephrine were based on the optimum doses for alleviating CXM-induced amnesia reported by Gibbs [6]. The drugs were administered at various times from 5 sec to 60 min after learning.

Retention was tested at various times between 5 and 180 min after learning or between 20 and 170 min after administration of amphetamine.

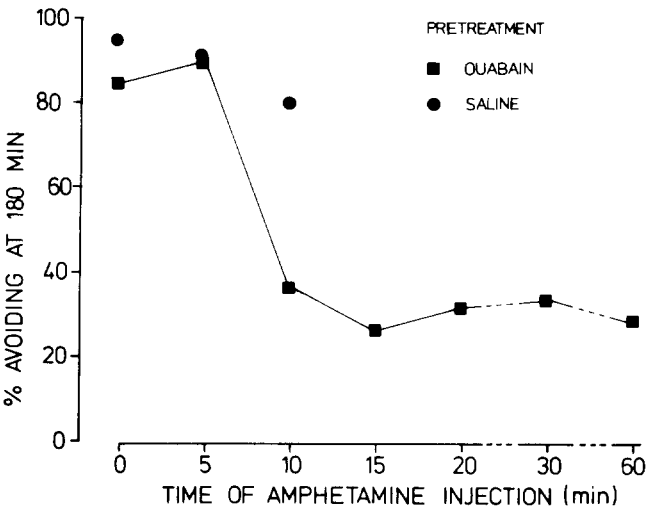

FIG. 1. Chickens were pretreated 5 min before learning with ouabain or saline. 1.0 mg/kg amphetamine in 0.1 ml was administered subcutaneously at various times between 5 sec and 60 min after learning. Retention was measured 180 min after learning. Amphetamine effectively overcame ouabain-induced amnesia only when injected up to 5 min after learning. Using Rodger's [14] planned contrasts on proportions, differences between saline and ouabain pretreated groups were not significant with amphetamine administered at 5 sec and 5 min after learning ($p>0.05$) but significant with amphetamine administered at 10 min after learning ($p<0.05$).

RESULTS

Time of Injection of Amphetamine or Norepinephrine

Amphetamine was administered 5 sec, 5, 10, 15, 20, 30 or 60 min after learning to chicks pretreated with ouabain 5 min before learning. Retention was tested at 180 min after learning.

Amphetamine was effective in overcoming ouabain-induced amnesia (80% – 90% avoidance) when administered up to 5 min after learning. From 10 min on, the drug had no effect (Fig. 1). NE administered 5 sec after learning also overcame amnesia measured at 180 min, but when administered 10 min after learning resulted in only 15% avoidance. These results contrasted markedly with those from the administration of amphetamine and NE to CXM-pretreated chicks. At 10 and 30 min, both drugs overcame CXM-induced amnesia measured at 180 min after learning [6]. Finally, amphetamine resulted in increased percentage of retention (95%, 90% and 80% avoidance) at 180 min in saline pretreated chicks when given 5 sec, 5 and 10 min after learning (respectively) (Fig. 1), the saline only level being approximately 70%.

Time Course of Retention Following Amphetamine or Norepinephrine Administration 5 Sec After Learning

Amphetamine or nonrepinephrine were administered at 5 sec after learning to chicks pretreated with ouabain (5 min before learning). Retention was tested at 10, 20, 30, 60, 90, 120 or 180 min after learning. Control animals were given saline alone with no ouabain-pretreatment, or no post-learning treatment following ouabain-pretreatment. The results are summarized in Fig. 2.

Ouabain-induced amnesia, normally evident at testing 20, 30 and 180 min after learning, was overcome by both amphetamine and norepinephrine. The retention levels at these times and at 180 min were also somewhat higher than those obtained from saline treated controls. These results were confirmed with amphetamine administered 5 min after learning and retention tested at 180 min.

Between 60 and 120 min after learning, however, administration of amphetamine and norepinephrine resulted in marked decreases in the percentage of ouabain-pretreated chicks avoiding, the level at 90 min being similar to that obtained with ouabain alone. A similar effect was reported for CXM-pretreated animals [5]. With amphetamine given 10 min after learning to saline and CXM-pretreated chicks, retention levels of 46% and 45% respectively were obtained at 60 min. The same treatment with norepinephrine yielded retention levels of 40% and 42% respectively. To determine whether this effect was due to memory loss or to general performance inhibition, amphetamine alone was administered 5 min before learning and retention measured at 10, 30, 60, 90, 120 or 180 min after learning. The dip in performance between 60 to 120 min was confirmed (Fig. 3)

Furthermore, amphetamine alone was administered 10 min or 60 min after learning, and retention tested at 20, 50, 80, 110 and 170 min after administration of the drug. A marked decrease in retention levels occurred between 50 and 80 min after administration of the drug (Fig. 4.). It is clear that the effect is due to a general performance deficit induced by the drug and related to the time of administration of the drug rather than to time of learning. The procedure was repeated with norepinephrine (Table 1), the results are consistent with those obtained with amphetamine.

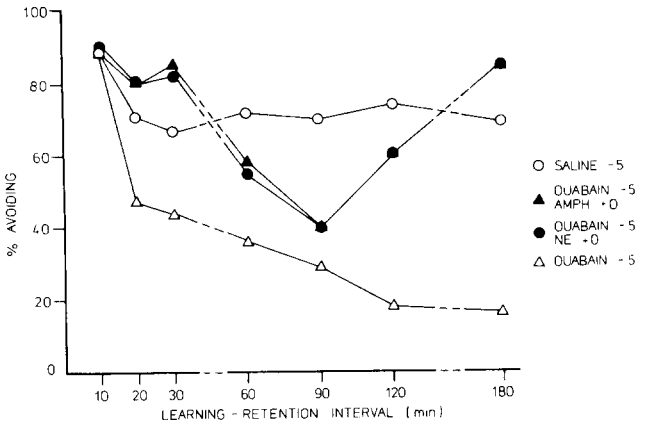

FIG. 2. Chickens with ouabain 5 min before learning were administered 1.0 mg/kg amphetamine or 50 μg/kg norepinephrine 5 sec after learning. Control animals received either saline or ouabain pretreatment with no post-learning treatment. Retention was tested at various times between 10 and 180 min after learning. No significant differences in proportion avoiding were found at any learning-retention interval between amphetamine and norepinephrine treated groups (all $p>0.05$). Significant differences are present between amphetamine or norepinephrine treated groups and ouabain-only controls at 20, 30, 120, and 180 min learning-retention intervals ($p<0.05$). A significant difference between amphetamine or norepinephrine treated groups and saline-only groups was obtained only at the 90 min learning-retention interval ($p<0.05$). Amphetamine or norepinephrine successfully counteracts ouabain-induced amnesia during the labile phase. They also produce a performance deficit between 60 min and 120 min after learning with complete recovery by 180 min.

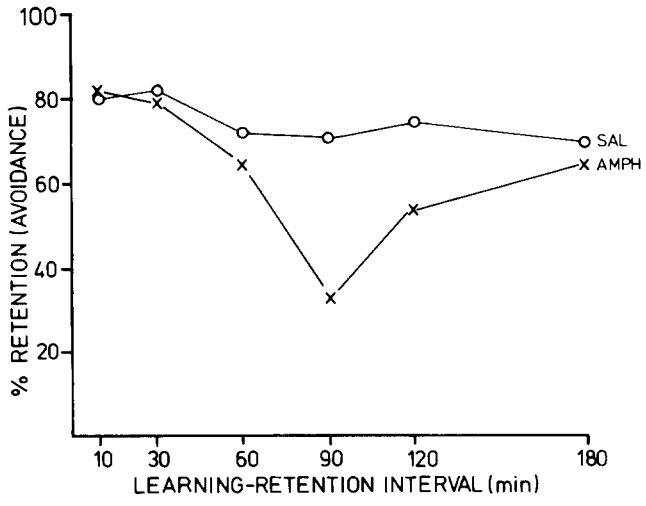

FIG. 3. 1.0 mg/kg amphetamine or 0.9% saline was administered 5 min before learning and retention tested at various times between 10 and 180 min after learning. A significant difference ($p<0.05$) in proportion of chickens avoiding the lure is found between saline and amphetamine groups at the 90 min training-test interval. The recovery at 180 min suggests that the difference at 90 min is a performance difference.

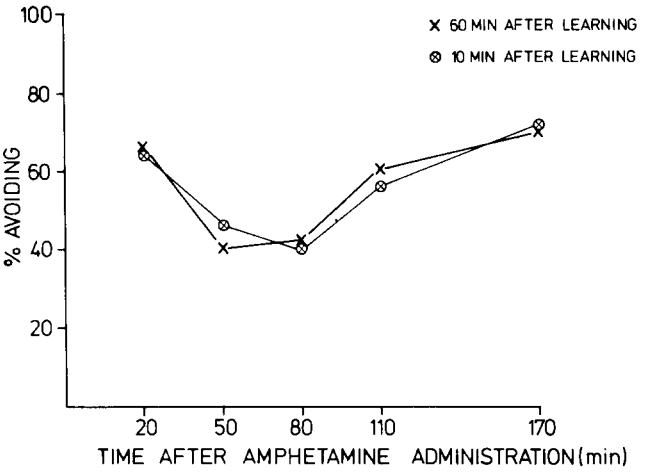

FIG. 4. 1.0 mg/kg amphetamine was administered 10 or 60 min after learning and retention was tested at various times between 20 and 170 min after administration of the drug. Although no difference between any pair of proportions is significant at the 0.05 level, it is clear for both times of administration that the performance trends are consistent with those observed in Figs. 2 and 3.

TABLE 1

GENERAL PERFORMANCE INHIBITION DUE TO NOREPINEPHRINE

Time of NE (50μg/kg) Administration after Learning (min)	Time of Testing after NE administration (min)	% Avoiding
60	20	70.0
	50	40.0
	80	40.0
	110	60.0
	170	70.0
10	50	40.0
	80	45.0

Effect of Amphetamine on KCl-Induced Amnesia

KCl was administered 5 min before learning and amphetamine 5 sec after learning. Retention was tested at 5, 10, 15, 20, 25, 30, 60 and 180 min after learning. Amphetamine did not overcome KCl-induced amnesia (Fig. 5).

DISCUSSION

The results from these experiments provide further confirmatory evidence for a distinction between short-term and labile memory. Amphetamine overcomes ouabain-induced but not KCl-induced amnesia. The results are also consistent with the argument that the labile phase, unlike the short-term phase, of memory formation is dependent on Na^+/K^+ ATP'ase activity, which is inhibited by ouabain.

Both amphetamine and NE administration result in increased extraneuronal norepinephrine, the former indicrectly through stimulated NE release [2,18]. As norepinephrine has been shown to increase Na^+/K^+ ATP'ase activity [9,10,17] the increased extraneuronal nor-

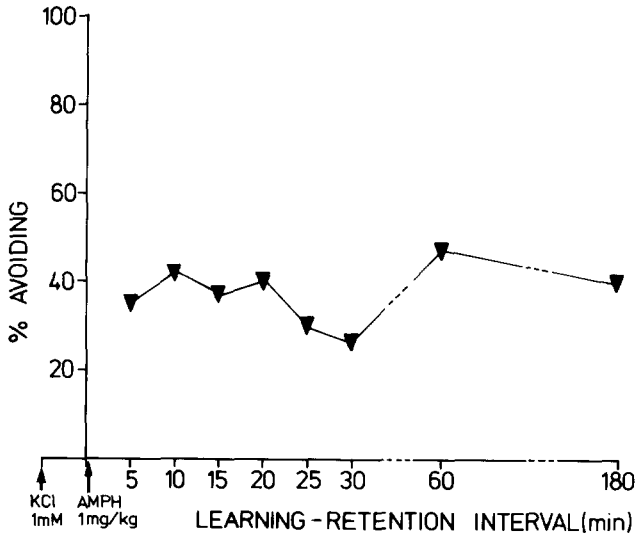

FIG. 5. 1mM KCl was administered intracranially to chickens 5 min before learning followed by 1.0 mg/kg amphetamine subcutaneously at 5 sec after learning. Amphetamine does not counteract KCl-induced amnesia.

epinephrine from administration of amphetamine and norepinephrine should increase the activity of this enzyme. Increased Na^+/K^+ ATP'ase activity is an action these drugs have in common with the effect of 10^{-4} M DPH [7] thus providing an explanation for the action of these drugs in overcoming ouabain inhibition of labile memory formation.

Since DPH (10^{-4} M) facilitates accumulation of norepinephrine in brain slices [1] and decreases NE release [12] while amphetamine inhibits neuronal NE uptake and accumulation [2, 4, 13, 15] and increases its release, it seems unlikely that this action of amphetamine on NE reuptake is involved in the effect of amphetamine on ouabain-induced amnesia. This interpretation rests somewhat on the possible involvement of Na^+/K^+ ATP'ase activity in NE reuptake. It has been postulated that Na^+/K^+ ATP'ase activity has a direct role in the reuptake of biogenic amines [10,16] and ouabain has been shown to block NE reuptake [3]. However, there is some doubt on this point as Cahill and Medzihradsky [4] found that although amphetamine inhibited NE reuptake, it did not inhibit Na^+/K^+ ATP'ase activity in synaptosomes. In addition the possibility that NE release is the substantive biochemical basis for labile memory is not consistent with the effects of α and β noradrenergic blockers. As can be seen from Table 2, the presence of noradrenergic blockers (piperoxane or propranolol) does not overcome the counteractive effect DPH on CXM-induced amnesia with retention measured at 3 hr after learning. The normal retention rate with CXM under these conditions is approximately 20%. The α and β noradrenergic blockers successfully overcame amphetamine counteraction of CXM-induced amnesia [6].

The observation that amphetamine (and NE) is noneffective in overcoming ouabain-induced amnesia when administered 10 min or later after learning suggests that labile memory is formed within the first 15 min after learning, while the short-term phase is still active. In the light of the fact that ouabain does not inhibit memory formation when administered 10 min after learning [11], these results also imply that Na^+/K^+ ATP'ase activity is involved in labile memory formation but not normally in its sugsequent maintenance. The present results lend weight to the postulate [7] that the phases of memory formation are sequentially and functionally dependent. Furthermore, the action of amphetamine (and NE) is immediate since ouabain-induced amnesia is overcome as early as 20 min after learning. The action is probably also long lasting since amphetamine (and NE) is effective in overcoming CXM-induced amnesia when administered as early as 5 sec and as late as 30 min after learning [5,6]. Thus while the labile phase of memory is still active, CXM-induced amnesia may be overcome by chemicals whose primary actions involve stimulation of sodium pump activity. As with short-term and labile memory, so long-term, protein synthesis dependent memory formation appears to be sequentially and functionally dependent on the preceding phase – labile memory.

TABLE 2

MODULATION BY NORADRENERGIC BLOCKERS OF DPH EFFECTS ON CXM-INDUCED AMNESIA

Pretreatment 5 min Before Learning	Treatment 5 min After Learning*	Retention at 3 hr
SAL	—	70.0%
SAL	SAL	63.2%
SAL	DPH	85.0%
SAL	DPH + PROPRANOLOL	85.0%
CXM	—	21.7%
CXM	SAL	21.1%
CXM	DPH	80.0%
CXM	DPH + PROPRANOLOL	85.0%
CXM	DPH + PIPEROXANE	78.9%

*DPH (10^{-4}M 2.7 μg/chick) ± propranolol (1.0 mg/chick) or piperoxane (1.0 mg/chick) were administered subcutaneously in 0.1 volumes.

Work in our laboratory has provided evidence suggesting that the amino acid substrate necessary for subsequent protein-synthesis associated with long-term memory formation is taken up in the first 10 min after learning via sodium pump action. While it may be argued that prolongation of sodium pump activity, or some associated event, maintains this substrate until such time as protein synthesis processes recover from CXM inhibition it does not explain why administration of amphetamine after 30 min following learning to CXM-pretreated animals produces decreasing counteractive effects consistent with a declining labile memory trace [6]. Nor can this be attributed to the performance decline between 60 and 120 min following learning observed in the experiments reported here, since the decline has been shown to be dependent on the time of administration of amphetamine rather than on time learning. A consistent interpretation is that the level of long-term memory formed is a function of the level of available labile memory.

The present results confirm the belief that ouabain-induced amnesia is produced by inhibition of Na^+/K^+

ATP'ase activity which is successfully counteracted directly by DPH and NE and indirectly by amphetamine through release of NE. Norepinephrine uptake does not appear to be implicated although the precise role of increased NE release per se and relative to the activity of the sodium pump is not known.

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