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# The Effect of Memory Blocking Antibiotics and their Analogs on Acetylcholinesterase[1]

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SPRINGER, A. D., J. SCHACHT AND B. W. AGRANOFF. *The effect of memory blocking antibiotics and their analogs on acetylcholinesterase.* PHARMAC. BIOCHEM. BEHAV. 5(1) 1–3, 1976. – The ability of antibiotics to inhibit acetylcholinesterase was measured in homogenates of goldfish brain. Puromycin aminonucleoside was the most potent inhibitor followed by puromycin, cycloheximide and acetoxycycloheximide. Puromycin effectively impaired retention of active-avoidance learning in goldfish when injected either immediately before or after training, while puromycin aminonucleoside did not regardless of injection time. These results suggest that the known amnestic effects of puromycin, cycloheximide and acetoxycycloheximide are not a consequence of interference with acetylcholinesterase.

Acetylcholinesterase Memory Puromycin Puromycin aminonucleoside Cycloheximide Amnesia Acetoxycylcoheximide Goldfish

THE use of antibiotics that block protein synthesis to study memory formation is a well known approach, but it remains uncertain whether the established actions of these drugs or some side effects mediate the retention deficit. For example, it has been postulated that memory consolidation in the goldfish is disrupted by an inhibition of protein synthesis produced by puromycin [3]. Other inhibitors of protein synthesis such as cycloheximide [2] and acetoxycycloheximide [3], similarly block memory formation. Puromycin has also been reported to interfere with cholinergic neuromuscular transmission [10], depress postganglionic responses of the superior cervical ganglion of the rat [9] and to inhibit acetylcholinesterase (AChE) *in vitro* [6,7]. Since memory disruption has been reported to result from interference with cholinergic mechanisms in rat brain by other agents [4], these data have been interpreted to suggest that puromycin may impair memory fixation not via inhibition of protein synthesis but rather via interference with the cholinergic system at the level of AChE [6,7].

If interference with AChE were the basic mechanism of action of puromycin, then one should expect similar amnestic agents to share this property, while structural derivatives of these antibiotics that do not interfere with memory formation should not. We report here the effect on AChE of 3 agents, known to be amnestic in the goldfish, puromycin, cycloheximide and acetoxycycloheximide, as well as the ineffective analog of puromycin, puromycin aminonucleoside [3].

## METHOD

### *Biochemical Assay*

Common goldfish (*Carassius auratus*) weighing 8–10 g were obtained from Ozark Fisheries (Stoutland, Mo.). For AChE assays, brains (approximately 80–100 mg) were removed following spinal section, collected and homogenized in 0.32 M sucrose. Cellular debris and nuclei were removed by centrifugation at 1000 g for 10 min. The supernatant fraction was used in the determination of acetylcholinesterase by the procedure of Ellman, *et al.* [5]. Assays were performed at room temperature and the reaction was started by the addition of the enzyme preparation. Acetylthiocholine and puromycin were purchased from Sigma Co., St. Louis, Mo., puromycin aminonucleoside and cycloheximide from Nutritional Biochemical Co., Cleveland, Ohio.

### *Behavioral Assay*

One hundred-eleven goldfish were housed in 750-liter tanks for approximately 1–2 weeks after arrival and were then placed in separate 1.5-liter tanks for 1–2 days prior to Day 1 of the experiment. They were maintained at 20° ± 1°C in continuous light and were not fed.

Active-avoidance training was performed in an automated aquatic shuttlebox which differed from a previously described box [2] in that it contained a 6 mm thick, black Plexiglas partition that completely divided the box into 2 compartments. A 4 × 3 cm hole was centered in the barrier

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dividing the 2 compartments. The bottom of the hole was 3 cm from the floor of the tank and the water level in the tank was 5 cm.

On Day 1 fish were acclimated in individual shuttleboxes in the dark for 5 min prior to the onset of 15 training trials. Each trial lasted 1 min and began with 15 sec of light presented on the side of the box occupied by the fish, followed by 20 sec of light paired with shock (3.5 v, 60 HZ, *rms*, 100 msec duration, 2.5 sec interpulse interval). An escape response was recorded by means of photodetectors when a fish crossed the barrier in response to shock and an avoidance response was recorded when a fish crossed the barrier prior to shock onset. Trials were intitiated every 60 sec, so that escape or avoidance responses terminated the trial (light and shock off) and initiated an intertrial interval of at least 25 sec of darkness.

The fish were divided into 5 groups. Two groups received an intracranial injection of 130 μg of puromycin dihydrochloride in 10 μl saline ($n = 19$) or an equimolar amount of puromycin aminonucleoside (70 μg in 10 μl saline; $n = 23$) immediately prior to placement in the apparatus. Two groups received an injection of puromycin ($n = 22$) or puromycin aminonucleoside ($n = 22$) immediately following training. A fifth group did not receive any drug treatment ($n = 25$). The 4 initial groups were given 15 retraining trials on Day 4 while the fifth group was retrained on Day 8. Previous experiments indicated that a 3 or 7 day train–retrain interval results in comparable retention in control fish.

## RESULTS

The effects of the antibiotics on AChE activity are summarized in Fig. 1. The drugs were studied at 3 different concentrations and representative curves are presented. For acetoxycycloheximide, inhibition was determined at only one concentration because of scarcity of the substance. All of the antibiotics examined clearly inhibit AChE. Analysis of the kinetic data suggest noncompetitive inhibition, but does not rule out some degree of mixed inhibition. Inhibitor constants calculated from the data in Fig. 1 indicate that puromycin aminonucleoside is the most potent inhibitor, while acetoxycycloheximide is the weakest (Table 1).

A 2 × 2 ANOVA on mean training avoidances of the 4 drug treated groups (Table 2) found that the administration of drugs prior to training did not affect acquisition ($ps > 0.10$). Retraining avoidances for the puromycin and puromycin aminonucleoside groups were analyzed with a 2 × 2 ANOVA. The drug effect achieved significance ($F(1,82) = 6.92$, $p < 0.025$) indicating the amnestic effect of puromycin, while the effect of injection time and the interaction were not significant ($ps > 0.50$).

TABLE 1

INHIBITION OF AChE BY ANTIBIOTICS

| Drug | Ki |
|---|---|
| Puromycin aminonucleoside | $1.4 \times 10^{-4}$ |
| Puromycin | $3.7 \times 10^{-4}$ |
| Cycloheximide | $7.7 \times 10^{-3}$ |
| Acetoxycycloheximide | $15.3 \times 10^{-3}$ |
| None | Km = $2.7 \times 10^{-4}$ |

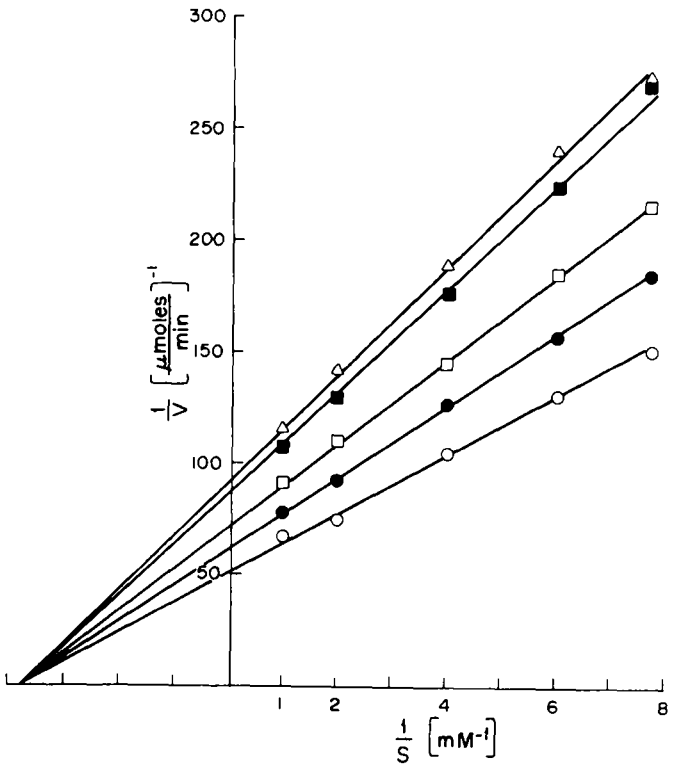


FIG. 1. Inhibition of AChE by antibiotics. AChE was assayed as described in Method. ○-○ no additions; ●-● $3 \times 10^{-4}$M acetoxycycloheximide; □-□ $3 \times 10^{-4}$M cycloheximide; ■-■ $1 \times 10^{-4}$M puromycin aminonucleoside; △-△ $3 \times 10^{-4}$M puromycin. Protein concentration: 80 μg/ml assay volume.

## DISCUSSION

All drugs examined inhibited AChE activity in preparations of goldfish brain. Compared to specific AChE inhibitors such as eserine or organophosphates [8], the concentrations of the antibiotics necessary to block AChE are several orders of magnitude higher. This result indicates that AChE is not a likely site for antibiotic action. Of particular significance is the ability of puromycin aminonucleoside to inhibit AChE to a greater degree than puromycin. Puromycin clearly impairs retention of training in goldfish while puromycin aminonucleoside has no effect on memory whether it is administered either before or after training. This result strongly suggests that the mechanism by which puromycin blocks memory fixation is unrelated to its effect on AChE. It should also be noted that 0.2 μg of acetoxycycloheximide [1] or 10 μg of cycloheximide effectively impair retention in goldfish [11]. Since the potency of the glutarimide antibiotics to inhibit AChE is in converse order, their ability to block memory also appears unrelated to their action on AChE.

The use of interventive drugs to study physiological mechanisms is never free of the problem that the drug may have side effects other than their known mechanism of action. It remains possible that the various macromolecular synthesis blocking agents affect memory formation by still other mechanisms. Whatever the ultimate mechanism of these agents, it appears evident that inhibition of AChE is not the means by which the various antibiotics impair memory fixation, and for the present, protein synthesis inhibition remains the most parsimonious explanation of their action.

TABLE 2

MEAN TRAINING AND RETRAINING AVOIDANCES*

| Time of injection | Training | | Retraining | |
|---|---|---|---|---|
| | Puromycin | Puromycin Aminonucleoside | Puromycin | Puromycin Aminonucleoside |
| Immediate pretrial | 1.53 (0.58) | 2.57 (0.50) | 2.16 (0.47) | 4.30 (0.91) |
| Immediate posttrial | 1.96 (0.39) | 1.73 (0.29) | 2.41 (0.62) | 4.09 (0.73) |
| No treatment | 2.20 (0.36) | | 4.40 (0.61) | |

*The mean number of avoidances (standard error in parentheses) are given for each 15 trials.

## REFERENCES


1. Agranoff, B. W. Recent studies on the stages of memory formation in the goldfish. In: *Molecular Approaches to Learning and Memory.* New York: Academic Press, 1970, pp. 35–39.
2. Agranoff, B. W. Effects of antibiotics on long-term memory formation in the goldfish. In: *Animal Memory.* New York: Academic Press, 1971, pp. 243–258.
3. Agranoff, B. W., R. Davis and J. J. Brink. Chemical studies on memory fixation in goldfish. *Brain Res.* **1**: 303–309, 1966.
4. Deutsch, J. A. The cholinergic synapse and the site of memory. *Science* **174**: 788–794, 1971.
5. Ellman, G. L., K. D. Courtney, V. Andres, Jr. and R. M. Featherstone. A new and rapid colormetric determination of acetylcholinesterase activity. *Biochem. Pharmac.* **7**: 88–95, 1961.
6. Moss, D. R., D. E. Moss and D. Fahrney. Puromycin as an inhibitor of acetylcholinesterase. *Biochem. biophys. Acta* **350**: 95–99, 1974.
7. Moss, D. E., D. R. Moss and D. Fahrney. Puromycin as an inhibitor of rat brain acetylcholinesterase. *Pharmac. Biochem. Behav.* **2**: 271–275, 1974.
8. O'Brien, R. D. Organophosphates and carbamates. In: *Metabolic Inhibitors.* New York: Academic Press, 1963, pp. 205–241.
9. Paggi, P. and G. Toschi. Inhibitors of protein synthesis involved in memory disruption: A study of their effects on sympathetic ganglion isolated *in vitro*. *J. Neurobiol.* **2**: 119–128, 1971.
10. Wulff, V. O. The effect of puromycin on neuromuscular transmission. *Pharmac. Biochem. Behav.* **1**: 177–182, 1973.