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BRIEF COMMUNICATION

Effect of Cobra Neurotoxin on Retention of a Brightness Discrimination in Rats[1]

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MOSS, D. E. AND J. B. ROGERS. *Effect of cobra neurotoxin on retention of a brightness discrimination in rats.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1147–1148, 1975. – In view of some recent evidence that blockade of nicotinic acetylcholine receptors might interfere with memory recall, the possibility that intracranial injections of purified cobra neurotoxin, an irreversible nicotinic receptor blocker, would produce long-lasting amnesia was explored with a brightness discrimination habit in rats. The results indicate that even the highest tolerable dose of neurotoxin had no detectable effect on memory recall.

Neurotoxin Nicotinic receptors Memory

A large body of evidence has been accumulating which suggests that cholinergic neurotransmission is necessary for the recall of memory. In particular, anticholinesterases and drugs that block muscarinic acetylcholine receptors have been shown to influence retention of a brightness discrimination habit in rats by facilitating and disrupting recall [2]. Recent evidence demonstrating that puromycin, a powerful amnestic agent with long-lasting effect [5], blocks the response to acetylcholine in neuromuscular transmission in a manner that is only partly reversible [9], suggests that long-term blocking of nicotinic receptors might be a cause of long-lasting amnesia. However, choice of a nicotinic receptor blocker to test this hypothesis requires special attention to confounding side effects of the drug. The use of gallamine (Flaxedil), d-tubocurarine, hexamethonium, and other similar well known nicotinic blocking agents would be wholly unsuitable for this purpose because they are virtually all potent inhibitors of acetylcholinesterase [3,6]. The effect of these commonly known nicotinic blockers would be confounded with acetylcholinesterase inhibition and, therefore, muscarinic stimulation or blocking effects. Alternatively, snake venom neurotoxin has no effect on acetylcholinesterase activity [4]. While it should be noted that Dr. Paul Boquet could not demonstrate the labelling of cholinergic receptors in mouse brain with tritiated neurotoxin (personal communication), it is a specific irreversible nicotinic receptor blocker [7] reported to bind nicotinic receptors from mammalian central nervous system tissue [1]. Therefore, the purpose of the present investigation was to determine if intracranial injections of purified cobra neurotoxin would produce amnesia for a brightnes discrimination habit in rats similar to that found in earlier experiments [2].

METHOD

Animals and Surgery

One week prior to the beginning of training, 51 male Sprague Dawley rats weighing approximately 350 g were implanted with chronic guide cannulae so subsequent intracranial injections could be made without anesthetic. The bilateral cannulae were placed at bregma –3.6 mm and 5.0 mm lateral to the midsagittal suture with the upper incisor bar 5.0 mm above the interaural line so injections 8.0 mm below dura were in the area of the ventral hippocampus and entorhinal cortex; an injection site which produces reliable and complete puromycin-induced amnesia in rats (unpublished data). The cannulae were 5.0 mm lengths of 22 ga steel tubing which were placed so they extended 1.0 mm below dura. The intracranial injections were made with an injection cannula which was curved so the tip was 1.5 mm off center when it was placed through a guide cannula. Injections were made with the tip directed rostrally, laterally, and caudally through each guide cannula

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to insure wide distribution of the neurotoxin. Each injection contained from 1 to 5 μl volume depending upon the dose of neurotoxin.

Procedure

The animals were trained and retention tested by relearning in a Y maze identical to that employed by Wiener and Deutsch [8]. The grid floor was connected to a shock source which delivered 1.75 mA foot shock. Some animals were taught to enter the lighted alley, others were taught to enter the darkened alley. Training was continued with a 20 sec intertrial interval until the animal made 11 correct responses out of 12 trials. Rats taking fewer than 15 or more than 70 trials were eliminated from the experiment. Retention testing was conducted in exactly the same manner as the training during the period 3 to 6 days after training when muscarinic receptor blockers interfere with memory recall [2].

Group 1 (n = 8) was used to determine if injection of toxin would produce a lasting decrement in recall, therefore, 30 μg of toxin were injected immediately after training and retention testing was conducted 6 days later. Group 2 (n = 6) was injected with 45 μg of toxin 3 days after training and retention tested 24 hr later to parallel earlier experiments [2]. Group 3 (n = 12) was used to determine if toxin administered over several days would have a greater effect than one single dose, therefore, 20 μg of toxin were administered immediately after training and 10 μg were administered 24 and 48 hr thereafter. Retention testing for Group 3 followed the last injection by 48 hr to allow the animals to recover from the injections.

The cobra neurotoxin used in these experiments was purified α-toxin from *Naja nigricollis* venom and was the generous gift of Docteur Paul Boquet of the Institut of Pasteur, Paris. The crystalline toxin was dissolved in 0.1 M phosphate (Na) buffer, pH 7.0, and stored at 4° protected from light. The concentration of toxin was 5 μg/μl and dose was varied by changing the volume of the injection.

An estimate of toxin distribution achieved by the multiple intracranial injections used in these experiments was determined by injecting 0.1 percent malachite green in isotonic saline using the same techniques and procedures used with the toxin injections. Twenty four hr after the stain was injected, the brains were removed, frozen, and sectioned and the distribution of the stain was recorded.

RESULTS AND DISCUSSION

The multiple intracranial injection procedure used resulted in the distribution of malachite green throughout the hippocampus and entorhinal cortex from approximately bregma −1.0 to −5.4 mm with the heaviest staining from bregma −2.8 to −4.4 mm. Stain was also observed to spread to the lateral ventricles and other surrounding tissue suggesting that the toxin was widely distributed throughout the brain. The LD_{50} for this cobra neurotoxin injected intracranially in rats under these experimental conditions was determined to be approximately 55 μg in 18 rats given 30, 60, or 80 μg. There was 100 percent survival at 30 μg, 34 percent survival at 60 μg, and 100 percent death at 80 μg. All animals not surviving died between 4 and 24 hr after injection, apparently from respiratory failure.

The rats required an average of 48 trials in training (36 trials before the criterion trials) and all animals, regardless of toxin injections administered in Groups 1, 2, and 3, showed excellent retention equivalent to unoperated controls in spite of the fact that rats retention tested in Group 3 all showed extreme muscular weakness after the first few trials in the retention test. They had to literally drag themselves through the maze, suggesting that there was a substantial amount of toxin outside the brain which interfered with voluntary motor function, presumably by blocking neuromuscular transmission. These results suggest strongly that memory recall does not depend on nicotinic receptors affected by cobra neurotoxin. It is not possible, however, to completely rule out nicotinic cholinergic functions in memory insofar as there may be nicotinic receptors in the central nervous system that are not blocked by cobra neurotoxin.

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