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Amnesia Attenuation Specificity: Propranolol Reverses Norepinephrine but not Cycloheximide-Induced Amnesia[1]

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ELLIS, M. E., R. F. BERMAN AND R. P. KESNER. *Amnesia attenuation specificity: Propranolol reverses norepinephrine but not cycloheximide-induced amnesia.* PHARMACOL BIOCHEM BEHAV 19(5) 733–736, 1983.—Post-trial injections of norepinephrine (NE) or cycloheximide (CHX) into the amygdala produces a long-term retention deficit (amnesia) for a 1-trial footshock experience in rats. Concomitant post-trial injections of the adrenergic antagonist, propranolol, prevents NE-, but not CHX-induced amnesia. These results indicate separate mechanisms of action for amnesia produced by intracranial CHX and NE injections.

Amygdala Amnesia Norepinephrine Cycloheximide Propranolol Differential attentuation Memory mechanisms Aversive information processing

RETROGRADE amnesia can be produced by a variety of experimental means, including electroshock, localized electrical brain stimulation, various pharmacological treatments, and inhibition of cerebral protein synthesis. Many of these procedures produce global and multiple changes in neural function, raising the possibility that a single effect common to several amnestic treatments may underlie the memory impairment. This point has taken on added importance by the recent reports that peripherally administered adrenergic antagonists (e.g., phenoxybenzamine, phentolamine, propranolol) can apparently reduce, or in some cases reverse, several examples of experimental amnesia. Based on these data, Gold and Sternberg [11] have suggested that noradrenergic perturbation may represent such a single, common mechanism underlying several amnesias.

In our own work [2,13], a small amount of the antibiotic cycloheximide (CHX) injected bilaterally into the amygdala of rats produces a time-dependent, dose-dependent passive avoidance deficit. Autoradiographic techniques localized the effect to the region of the amygdala. We now report that the beta-adrenergic antagonist, propranolol (PROP), does not reverse amnesia produced by amygdaloid CHX injections, while it does reverse amnesia produced by similar amygdaloid injections of norepinephrine (NE). These results substantiate the importance of the NE system in memory processes, but indicate that some amnesias (e.g., amnesia following intracranial CHX injection into the amygdala) appear to be independent of NE disturbance.

METHOD

Procedure

Subjects. Sixty-five adult male Long Evans rats with initial weights ranging from 300 to 325 g were individually housed in wire cages under a 12 hr photoperiod (onset 0800 hr). All animals had ad lib access to food and water. Fifty-two rats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg, IP) and given atropine sulfate (0.1 mg, IP) as a prophylactic just prior to surgery. Each animal was stereotaxically implanted bilaterally with chronic stainless steel cannulas aimed for the amygdala region (coordinates relative to bregma with head level: posterior 2.0 mm, lateral 3.5 mm, vertical 8.5 mm). Cannulas were fixed to the skull with two stainless steel screws and dental acrylic cement. Following surgery, procaine penicillin-G + dihydrostreptomicin (0.1 cc, IM, Combiotic, Pfizer, New York, NY), and oxytetracycline HCl with Polymixin-B sulfate (topical antiseptic, Terramycin with Polymixin-B sulfate, Pfizer, New York, NY), were administered to retard infection. Rat weights were monitored daily beginning with the second day post-surgery. Implanted animals were allowed to recover for at least 10 days or until preoperative weight had been exceeded before being used experimentally. The remaining 13 rats served as non-operated controls.

Passive Avoidance Training

The passive avoidance training apparatus was a

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rectangular, red, Plexiglas box (30×60×40 cm) divided into two equal compartments (neutral-side and goal-side) by a manually operated sliding door. A metal watering tube protruded through a hole in the far wall of the goal-side. The floor of the apparatus consisted of brass grates which could be electrified for footshock. Latencies to enter the goal-side, begin drinking, and consume the first 10 licks were recorded with digital relay circuitry.

Rats were given a single training trial in the passive avoidance apparatus per day. Training was initiated by restricting water access to 15 min/day in rats' home cages for two days. On the next two days, rats were given 10 minutes access to water in the goal-side of the apparatus plus 5 minutes in the home cages. Rats were then conditioned on subsequent days to run as soon as the sliding door was opened from the neutral-side to the goal-side. Rats were allowed 5 minutes to drink in the apparatus; then they were returned to their home cages where, after several minutes delay, they had an additional 10 minutes of access to water. Water consumption was monitored by weighing the water bottles before and after the home cage drinking period. Training was continued until each rat ran from neutral- to goal-side and began drinking in less than 10 sec over two consecutive days. The rats typically required 5–7 days of training to reach this criterion. The day after reaching the 10 sec criterion, implanted rats were randomly assigned to one of six groups. All rats including the non-operated controls were then given a single passive avoidance training trial. The procedure consisted of placing the rat in the neutral-side, allowing it to enter the goal-side and drink from the tube for 60 sec, and then giving it a 3 sec, 3 milliamperes inescapable footshock.

Non-operated control rats (NOC, n=13) were removed from the apparatus approximately 15 sec after footshock, handled for about 3 minutes, and returned to their home cages. Rats in the other six groups were removed from the apparatus 15 sec following the shock and given intracranial injections.

Intracranial Drug Injections

The intracranial cannulas were hand-tooled from 23 and 30 ga stainless steel, thin-walled hypodermic tubing (Temper Needle, Superior Co., Wapakaneta, OH), in 5–14 mm lengths according to need. The cannulas were double-walled with a 23 ga outer guide cannula and a 30 ga obturator cut flush with the distal, implanted end of the guide cannula.

All intracranial injections were made through a 30 ga stainless steel injector fashioned to the exact 14 mm length of the 23 ga guide cannula. The injector was connected with approximately 35 cm of polyethylene tubing to a 10 μl microsyringe (Hamilton Co., Reno, NV). Bilateral amygdaloid injections were made in volumes of 1 μl/cannula at the rate of 1 μl/min. Rats were permitted freedom of movement about their home cages within the length of the polyethylene tubing during the injection period. Rats were restrained only to manipulate the injectors or the obturators. Special buffered saline (SBS) [4] was the vehicle in which norepinephrine (NE), propranolol and CHX were dissolved for intracranial administration. Different groups of animals received either SBS (n=7), 1 μg norepinephrine (NE; n=8), 10 μg cycloheximide (CHX; n=12), 1.5 μg propranolol (PROP; n=9), 10 μg cycloheximide immediately followed by 1.5 μg propranolol (CHX+PROP; n=6), and 1 μg norepinephrine immediately followed by 1.5 μg propranolol (NE+PROP; n=10). Drug dosages used (i.e., 10 μg CHX, 1 μg NE) were chosen from earlier work that established the minimum effective dose of either CHX (10 μg) or NE (1 μg) that resulted in shock avoidance deficits following posttrial bilateral amygdaloid injection [2,5]. Similarly, a maximum dose of 1.5 μg propranolol (PROP) was chosen because higher doses (i.e., 1.7 μg or higher) in themselves produce retention disruption [2].

Non-operated and injected rats were allowed at least 14 additional minutes access to water following the footshock-injection period. The animals were allowed to drink until pre-shock consumption levels had been exceeded to compensate for the loss of four minutes drinking time in the passive avoidance apparatus.

Retention Tests

Retention of the footshock experience was tested for the seven groups at 24 hr following the shock-avoidance training trial. Each animal was individually placed in the neutral side of the apparatus and the sliding door was opened. The latency to enter the goal-side, the latency to enter and initiate drinking, and the latency to enter and consume the first 10 licks were recorded. Latency scores were used as the main index of retention. Long latencies were interpreted as good retention of the aversive footshock experience, and relatively shorter latencies were interpreted as evidence of poor retention.

Water consumption was monitored following the retention test during the 10 minute home cage drinking period. All subjects were then placed on ad lib chow and water until sacrifice and perfusion in preparation for histological procedures.

Histology

Following completion of the experiment the implanted rats were anesthetized with 1 ml of sodium pentobarbital, heparinized, and perfused percardially with 10% formalin in isotonic saline. Brains were excised, stored in 10% formalin/isotonic saline for 10 days, frozen and cut at 50 micron sections through the cannula tracks and stained with cresyl violet. Cannula placements within the amygdala were confirmed by reference to the König and Klippel atlas [14]. All animals had cannula placements bilaterally within the region of the amygdala. The placements were very similar to that reported in Ellis and Kesner [5] and Berman, Kesner, and Partlow [2].

RESULTS

Lick latencies were log transformed prior to analysis by a one-way ANOVA and Newman-Keuls tests. Tenth-lick latencies provided the most consistent data, and results using this measure are shown in Fig. 1. As evident in the figure, non-operated (NOC), SBS-injected (saline) and propranolol (PROP) injected rats showed good retention of the shock avoidance training as evidenced by relatively long latencies to enter the goal-box and complete 10 licks compared to preshock latencies (i.e., <10 sec). In contrast, CHX and NE injected rats showed impaired retention performance replicating previous reports for this task [2,5]. Most notable in the figure is the apparent reversal by propranolol of NE-induced retention deficits, while CHX effects on retention were unaffected. Statistical analysis indicated that there was an overall significant treatment effect, $F(6,58)=9.98$, $p<0.01$. Further Newman-Keuls tests revealed that the latencies scores for groups NE, CHX and CHX+PROP were significantly lower ($p<0.05$) than those of

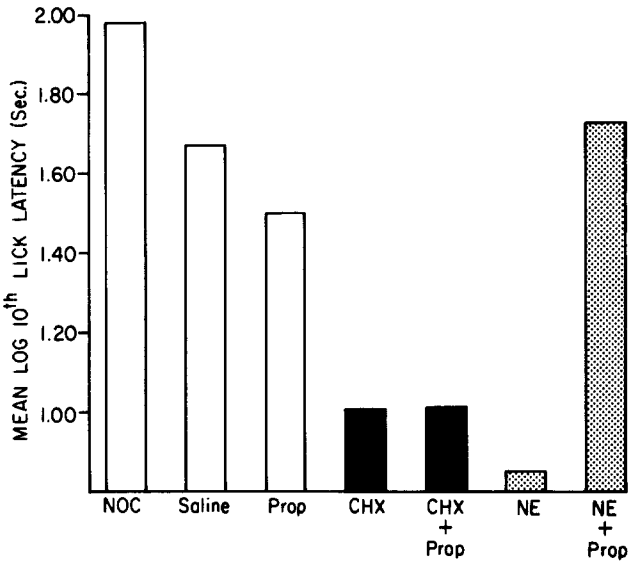

FIG. 1. Mean 10th lick latency (log sec) 24 hr after footshock as a function of posttraining intracranial injections of specific pharmacological agents into the amygdala. The following groups of animals were used: NOC (non-operated control), saline (special buffered saline control), PROP (1.5 μg propranolol), CHX (10 μg cycloheximide), CHX+PROP (10 μg cycloheximide followed by 1.5 μg propranolol), NE (1 μg norepinephrine), and NE+PROP (1 μg norepinephrine followed by 1.5 μg propranolol).

groups NOC, saline, PROP and NE+PROP. Hence, NE-induced amnesia was significantly reduced by PROP while CHX-induced amnesia was not.

DISCUSSION

The present results demonstrate that posttrial amygdaloid (central) injections of norepinephrine (NE) or cycloheximide (CHX) impair shock avoidance performance in rats tested 24 hr after training. Concurrent amygdaloid injections of propranolol (PROP), a beta-adrenergic antagonist, reverses the impairment produced by NE, but not that produced by CHX. We interpret these findings as evidence for fundamentally different mechanisms underlying NE- and CHX-induced amnesias. Only a single dose of each agent was tested. However, the levels of CHX, NE, and PROP used were chosen to maximize the likelihood of demonstrating an amelioration of amnesia by PROP. Also, since PROP at higher doses produces amnesia [5], it is unlikely that higher doses of PROP would have been effective in blocking CHX-induced amnesia. Finally, the dose of PROP used was effective in reversing NE-induced amnesia.

Earlier work implicating the role of the amygdala in aversive information processing suggested that PROP-induced deficits in a simple step-through task could be reversed with NE [10]. Todd and Kesner [20], using the same 1-trial passive avoidance task as that used in the present study, reported amnesia in rats following amygdaloid injections of physostigmine. Combined injection of physostigmine and atropine blocked the amnesia. Similarly, Gallagher and Kapp [9] reported that posttrial levorphanol injections into the amygdala produced passive avoidance impairment. Again, combined amygdaloid injection of levorphanol with naloxone blocked the amnesia. Viewed together, these data clearly point to a critical involvement of amygdaloid activity in memory processes associated with shock avoidance conditioning in rats. They also indicate that amnesia in rats can be produced by perturbation of any one of the several neuroregulatory systems identified in the region of the amygdala [1] and further suggest an interaction of transmitter systems in memory formation. They do not clearly point to a single common neurobiological mechanism underlying experimental amnesia. Such a common mechanism has been proposed by Gold and Sternberg [11].

The amnesia found in the present study following amygdaloid injection of NE can be easily interpreted by the Gold and Sternberg [11] hypothesis as the result of a local (i.e., amygdaloid) increase in NE. The reversal of amnesia by propranolol indicates that adrenergic antagonists such as propranolol may under certain circumstances attenuate amnesia.

However, it is still unclear how amnesia following CHX injection into the amygdala can be explained. First, in the present study concurrent propranolol injection with CHX did not affect the observed amnesia. Second, there is little, if any, direct evidence for an increase in central NE release produced by CHX. In the single study addressing this issue, Freedman, Judge and Quartermain [8] report that *in vitro* K^+-stimulated release of NE carried out in hypothalamic slices is reduced from 9% above baseline to approximately 4% above baseline by CHX. The significance of this small *in vitro* reduction of K^+-stimulated release of NE by CHX is unclear. It is noteworthy that a decrease, not an increase, was observed. Third, even peripheral injections of relatively large amounts of CHX do not markedly alter basal NE levels [3] and direct injection of CHX into the brainstem proximal to noradrenergic perikarya does not result in amnesia for shock avoidance, while a similar amygdaloid injection does produce amnesia [7]. Fourth, while tyrosine hydroxylase activity is decreased by peripheral CHX injection, and central tyrosine levels increase as a consequence [6,12], the effects of CHX on tyrosine metabolism have been dissociated from the amnesia produced by CHX [7,15].

In view of the evidence, the conclusion that centrally administered CHX produces amnesia by interfering with NE synthesis or release does not appear to be warranted. Until more information concerning the mechanism of memory formation is available, the precise mechanism of action of CHX on memory will likely remain unknown. However, the most consistent hypothesis concerning the action of CHX is still that it produces amnesia via its major pharmacological activity, i.e., inhibition of protein synthesis [7].

In summary, the present study provides additional support for the importance of the amygdala in memory of shock avoidance conditioning. The results also indicate that central NE may play a role in normal memory processes and in certain types of experimental amnesia. However, they do not support the hypothesis that CHX produces amnesia via action on NE synthesis and release, and therefore fail to support the Gold and Sternberg [11] hypothesis of a common mechanism underlying experimental amnesia.

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