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ELECTROCONVULSIVE SHOCK AND BRAIN MUSCARINIC RECEPTORS: RELATIONSHIP TO ANTEROGRADE AMNESIA

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Summary

Rats were administered one electroconvulsive shock daily for 7 days (ECS x 7) and were killed 24 hours after the last treatment. Muscarinic cholinergic receptor number, as determined by [^{3}H] quinuclidinyl benzilate ([^{3}H]QNB) binding, was significantly reduced in the cerebral cortex. A parallel group of rats was trained on a passive avoidance task 24 hours following the last ECS and tested for retention of the original avoidance response 24 hours later; these animals exhibited a profound amnesia. Animals tested 1 hour following training were not amnestic, indicating that learning was unimpaired. Animals trained 7 days following ECS x 7 were not amnestic and [^{3}H] QNB binding changes were not demonstrable at this time. A single ECS which does not significantly affect cortical [^{3}H] QNB binding, did not induce amnesia in rats trained 24 hours after the treatment and tested 24 hours later. The parallel, cumulative nature of ECS-induced muscarinic receptor down-regulation and ECS-induced anterograde amnesia suggests a possible causative relationship.

Electroconvulsive shock (ECS) induces changes in brain cholinergic systems which are compatible with acute release of acetylcholine during the seizure. Ictal and post-ictal reductions in brain acetylcholine levels have been reported (1,2,3) as well as increases in acetylcholine esterase (3) and choline acetyltransferase activity (3). Repeated exposure of muscarinic cholinergic receptors (MCR) to endogenous or exogenous agonist is known to induce receptor subsensitivity as measured by [^{3}H] quinuclidinyl benzilate ([^{3}H] QNB) binding (4,5). Down-regulation of brain MCR has also been reported after repeated ECS administration (6,7).

Following 4 ECS daily over 4 days, Dashieff et al.(6) found a 19-25% reduction in [^{3}H] QNB binding in the rat hippocampus. Lerer et al. (7) reported a 13% and 15% down-regulation of MCR in rat hippocampus and cerebral cortex respectively after one ECS daily over 7 days. Lerer et al. (7) also found that concurrent ECS administration reversed the significant increase in cortical [^{3}H] QNB binding caused by chronic atropine administration. The changes reported by Dashieff et al. (6) and Lerer et al. (7) were, however, variable in magnitude and two other studies found ECS-induced reductions in [^{3}H] QNB binding to be non-significant statistically (8) or negligible (9). These studies reported data based on [^{3}H] QNB binding at a single ligand concentration. While our

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earlier work (7) and that of Dashieff et al. (6) had suggested that ECS-induced changes in specific binding were related to a reduction in receptor number, it was important to re-examine the effect of repeated ECS on cortical and hippocampal MCR on the basis of Scatchard analysis of individual binding data derived from a series of [^{3}H] QNB concentration points.

The functional significance of ECS-induced alterations in MCR sensitivity also remained to be determined. Repeated ECS administered to humans in the context of electroconvulsive therapy (ECT) is known to cause memory impairment characterized by both anterograde and retrograde amnesias (10). A functional alteration in cholinergic neurotransmission through subsensitive MCR might hypothetically underlie the ECS-induced memory deficits (11). This possibility was investigated by studying alterations in [^{3}H] QNB binding and memory function in parallel in groups of rats subjected to the identical chronic ECS regimen.

Materials and Methods

Animals: Male, albino rats (Sprague-Dawley) weighing 200-225 gm were used in all experiments. Rats were housed 3 per cage in a temperature controlled (24°C) environment with a regular 12 hr light-dark cycle (7 a.m. on/7 p.m. off). Food and water were continuously available.

ECS was administered via ear-clip electrodes using a Medcraft clinical ECT apparatus which supplies a constant, preset voltage output (settings used: 130 volts, 0.75 sec.). ECS was observed to regularly induce a tonic-clonic seizure lasting 20-25 sec. with full recovery within a few minutes. Control rats had electrodes applied with no current passed (sham ECS). Rats received either a single ECS (ECS x 1) or one ECS daily over 7 days (ECS x 7).

^{3}H-QNB Binding: Rats for [^{3}H] QNB binding studies were killed by decapitation 24 hours after the last ECS administration. Brains were rapidly removed and dissected over ice and tissues were stored at -70°C until assayed. Binding of [^{3}H] QNB was determined in accordance with the methods described by Wastek and Yamamura (12) with modifications. A Brinkmann Polytron (setting 7 for 15 s) was used to homogenize the samples in 50 volumes (w/v) of ice-cold 50 mM tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The homogenate was centrifuged at 30,000 g for 10 minutes. The supernatant was decanted and the resulting pellet was washed and centrifuged, as above, twice more. After the last centrifugation, the pellet was resuspended in 33.3 volumes (w/v) of ice cold 50 mM tris-HCl buffer as above. Protein was determined according to Lowry et al. (13) and averaged approximately 2 mg/ml. An aliquot of the final homogenate was taken and diluted with 50 mM Hepes buffer (pH 7.4) to a final protein concentration of 0.05 mg/ml for the QNB receptor binding assay.

Samples (500 µl) of the homogenate were incubated in duplicate at 37°C with 100 µl of [^{3}H] QNB at 10 [^{3}H] QNB concentrations between 2.5-300 pM final concentration (specific activity, 33.1 Ci/mmole from New England Nuclear) and with 100 µl of either 50 mM Hepes buffer or 5 µM final concentration of atropine sulfate. Total assay volume was adjusted to 2 ml with 50 mM Hepes buffer. After 60 minutes the incubate was diluted with 3 ml ice-cold Hepes buffer and filtered through Whatman GF/B glass filters using a modified Brandell cell harvester (Biomedical Research and Development Laboratory, Inc., Gaithersburg, MD). Filters were washed two more times with 3 ml of buffer and placed in glass scintillation vials with 10 ml Aquasol-2 (New England Nuclear) and counted for 10 minutes in a Beckman LS 6800 liquid scintillation counter. Counting efficiency was 45%. Specific binding was defined as that which was displaced by 5 µm atropine sulfate and represented approximately 87% of total binding. The number of receptor sites (Bmax) and the affinity constant (Kd) were determined from the Scatchard plot for each individual rat cerebral cortex and hippocampus.

Memory Testing:

Rats were trained on a passive avoidance task 24 hours after ECS x 1 or ECS x 7 and tested 1 hour or 24 hours after training. Training and testing were conducted in a two-compartment black plexiglass shuttle-box (76.2 x 34.3 x 50.8 cm high). The smaller (start) compartment of the shuttle-box was illuminated by five (7.5 watt D.C.) bulbs mounted along the top of the rear wall and separated from a larger dark compartment by a door which could be manually opened and closed from above. The floor was constructed of 0.6 cm diameter stainess-steel bars spaced 1.9 cm apart and connected to a Grayson-Stadler (Model 700) scrambled shock source. Each animal was initially placed in the lighted compartment with the door separating the two chambers closed and allowed 30 seconds to freely explore that side of the apparatus. The door was then raised and the animal was allowed to enter the dark compartment. Once the animal had completely crossed over to the dark side (all four paws) the door was lowered and a 3.0 sec, 0.5 mA inescapable shock was delivered via the grid floor. Immediately after the shock the animal was removed from the dark compartment and returned to his home cage. Any animal failing to cross from the lighted to the dark compartment within 300 sec was discarded from the experiment. The latency to cross from the lighted to the dark compartment was recorded for each animal. The same procedure was used for testing except that no shock was applied if and when the animal crossed over to the dark side. The initial latency to cross (i.e., step-through latency) from the lighted to the dark chamber was recorded. A test session lasted a maximum of 600 seconds. Any animals which failed to cross within 600 seconds were removed from the apparatus and assigned a maximum score of 600.

Statistical Analysis:

Differences between experimental and control groups were compared by Student's t-test (two-tailed) for [^{3}H]QNB binding studies and by Kruskal-Wallis one way ANOVA for memory testing.

Results

[^{3}H] QNB Binding: (Table 1) Rats killed 24 hours following the last of a series of one ECS daily over 7 days (ECS x 7) had a 10% reduction in MCR (Bmax) in the cerebral cortex compared to sham-treated animals. This difference was statistically significant ($p < .05$). Hippocampal MCR number (Bmax) was reduced by 5% and this finding was not significant. Affinity of [^{3}H] QNB for muscarinic cholinergic binding sites (Kd) was unaltered by ECS in both brain areas. An additional group of rats were decapitated one week after ECS x 7 (or Sham x 7) and [^{3}H] QNB binding in the cortex was assayed at 25 pM [^{3}H] QNB concentration. No difference in binding was observed (ECS 695 ± f.moles/mg protein, N = 11, vs. Sham 709 ± 13.15 f.moles/mg protein, N = 10).

Memory Testing: (Table 2) Rats trained 24 hours following a single ECS (ECS x 1) and tested 24 hours later, showed no evidence of memory impairment. However, rats trained 24 hours following a series of one ECS daily for 7 days (ECS x 7), exhibited a profound amnesia for the original avoidance habit when tested 24 hours following training ($p < .01$). Rats administered ECS x 7, trained 24 hours after the last ECS and tested 1 hour later, were not similarly amnestic indicating that learning was unimpaired. Rats trained one week after ECS x 7 (or Sham x 7) and tested 24 hours later showed no evidence of memory deficit. (ECS test latency 515 ± 60.44 sec, N = 6, vs. Sham 600 ± 0 sec, N = 6). There were no differences in training latency between any of the ECS and control groups.

TABLE I

Effect of ECS on [^{3}H] QNB Binding Parameters in Cerebral Cortex and Hippocampus.

	N	Bmax	Kd
		Fmoles/mg protein	pM
		$\bar{x}$ ± s.e.m.	$\bar{x}$ ± s.e.m.
Cerebral Cortex			
ECS	7	800 ± 26.5*	13.8 ± 1.54
Control	9	885 ± 25.6	15.2 ± 1.55
Hippocampus			
ECS	7	647 ± 47.0	17.4 ± 1.92
Control	9	704 ± 61.35	18.6 ± 1.70

Rats were decapitated 24 hours after the last of 7 daily ECS and [^{3}H] QNB binding in cerebral cortex and hippocampus was determined by individual Scatchard analysis for each ECS and control brain area. Differences were compared by Student's t-test.

* $p < .05$, ECS vs Control.

TABLE II

Effect of ECS on Delayed Recall of an Aversive Stimulus

	Train-Test Interval	N	Test Latency (sec)
			$\bar{x}$ ± s.e.m.
ECS x 1	24 hours	10	429 ± 82.81
Control		10	381 ± 85.36
ECS x 7	1 hour	10	510 ± 60.06
Control		10	448 ± 74.06
ECS x 7	24 hours	9	30 ± 7.03*
Control		10	484 ± 73.68

Rats were trained in a shuttlebox 24 hours after single (ECS x 1) or multiple ECS (ECS x 7) and tested 1 hour or 24 hours later.
Differences in step-through latency were compared by Kruskal-Wallis one way ANOVA.

* $p < .01$, ECS vs. Control ($H = 8.816$, df 1)

Discussion

The results of the [^{3}H] QNB binding studies confirm our previous report of a reduction in cortical MCR binding following repeated ECS administration (7). The effect is small in magnitude but is statistically significant and is due to a reduction in the number of muscarinic binding sites without alteration in the affinity of the ligand for the receptor. Although a trend towards reduction in hippocampal [^{3}H] QNB binding was present, this effect was not statistically significant. The discrepancy between this finding and previous reports (6,7) may be due to regional variations in the effect of ECS within the hippocampal formation. The more striking effect reported by Dashieff et al. (6) was observed in the dentate and hippocampal gyri whereas whole hippocampi were used in the present assays. Frequency of ECS administration may also be a factor; Dashieff et al. (6) administered 4 ECS daily over 4 days whereas the regimen used in the present study was one ECS daily for 7 days. Notwithstanding these possible explanations, the effect of repeated ECS on MCR in the hippocampus is not robust. It is noteworthy that the significant reductions in acetylcholine levels and increases in choline acetyltransferase activity reported by Longoni et al. (3) following ECS, were also localized to the cortex and not demonstrable in the hippocampus.

The results of the memory studies shed further light on the nature of the anterograde amnesia induced by ECS. Previous studies had shown that following a single ECS, the development of anterograde amnesia is dependent upon the interval between administration of the ECS and training; with intervals exceeding 30-60 minutes, amnesia was not induced (13,14). Longer ECS-training intervals may, however, be associated with deficits in long-term memory although immediate recall is unimpaired (15). In the present study the ECS-training interval was 24 hours. This is well beyond the maximal interval reported to be associated with long-term deficits following a single ECS (16). Deficits were not present in rats trained following ECS x 1 and tested 24 hours later but were profound in rats trained after ECS x 7 and tested 24 hours later. Rats trained 24 hours after ECS x 7 and tested 1 hour later were not amnestic. Repeated ECS thus cumulatively impairs either the stability of the memory trace or access to it over time while not impeding initial learning. This pattern of anterograde amnesia is strikingly similar to the deficits manifested by patients after a course of electroconvulsive therapy (ECT); in these patients recall is also intact 1 hour after training but impaired 24 hours later (10).

Considered in conjunction, the present findings indicate a parallel effect of repeated ECS on cortical [^{3}H] QNB binding and memory function. Both effects are cumulative in nature, are not present after ECS x 1 but are demonstrable after ECS x 7. One week following ECS x 7, recovery of memory function and normalization of cortical [^{3}H] QNB binding are demonstrable. Recent evidence strongly suggests an association between memory impairment and reduced cholinergic neurotransmission, in normal individuals and in patients with Alzheimer's disease (17). MCR subsensitivity may be functionally expressed as a net reduction in cholinergic neurotransmission and may, in this way, underlie ECS-induced anterograde amnesia. This hypothesis implies that chronic exposure of brain MCR to endogenous or exogenous agonist, which is known to down-regulate these receptors (4,5), should also induce anterograde amnesia. Loullis et al. (18) have in fact recently demonstrated that retention is impaired in rats trained on a passive avoidance task after chronic pretreatment with physostigmine. Loullis et al. (18) also observed enhanced retention following chronic administration of scopolamine which up-regulates brain MCR (5). Lerer et al. (7) found that concurrent atropine administration prevented ECS-induced MCR subsensitivity; the effect of concurrent anticholinergic (e.g., scopolamine) administration on ECS-induced anterograde amnesia remains to be investigated. A possible association between MCR subsensitivity and ECS-induced anterograde

amnesia may be relevant to the pathogenesis of memory deficits following ECT in humans.

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