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Acetylcholine release in the hippocampus: effects of cholinergic and GABAergic compounds in the medial septal area

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The medial septal area (MSA) contains cholinergic and GABAergic neurons that send projections to the hippocampus. These neurons have both cholinergic and GABAergic receptors. This study was designed to determine the effects of intraseptal infusions of cholinergic and GABAergic drugs, which alter mnemonic processes, on hippocampal acetylcholine (ACh) release. Hippocampal ACh release was assessed using in vivo microdialysis and HPLC-EC. Oxotremorine and scopolamine produced a dose-dependent decrease in hippocampal ACh release. Muscimol decreased hippocampal ACh release at both high and low doses, although not in a dose-dependent manner. The effects of scopolamine and muscimol are consistent with a role of ACh in mnemonic processing.

The major cholinergic innervation of the hippocampus arises from cells in the medial septal area (MSA), which includes the medial septal nucleus and the diagonal band [12,13,22,23]. The MSA contains at least two populations of neurons, cholinergic and GABAergic [3], which send projections to the hippocampus via the fimbria-fornix [4,12,17]. Approximately 30% of these projections are cholinergic and the remaining 70% are GABAergic [17]. The cholinergic neurons project to the hippocampal pyramidal cells, the dentate granule cells and the inhibitory interneurons [23,24,26]. The GABAergic neurons project primarily to the inhibitory interneurons [1,11,16]. Thus, the MSA is anatomically situated to modify information processing in the hippocampus.

Cholinergic and GABAergic neurons in the MSA have both cholinergic and GABAergic receptors, as well as receptors for other neurotransmitters [3,21,27]. Neurons in the septal area are regulated by GABAergic afferents possibly from the lateral septum [20,21] and a cholinergic input from axon collaterals or from the brainstem cholinergic system [3,21,28]. The electrophysiological activity of MSA neurons was modulated by iontophoretic application of cholinergic and GABAergic compounds [18,19]. Cholinergic agonists and GABAergic antagonists increased the activity of MSA neurons, whereas cholinergic antagonists and GABAergic agonists decreased the activity of these neurons. Thus, intraseptal infusions of cholinergic or GABAergic compounds can alter the activity of MSA neurons.

Memory was altered by intraseptal infusions of cholinergic and GABAergic compounds [4–6,14]. Oxotremorine improved the choice accuracy of aged rats in a recent memory task on a T-maze [25]. Scopolamine and muscimol decreased the choice accuracy of young rats in a variety of tasks: recent memory in T-maze and radial arm maze, place discrimination in a Morris water maze, and visual discrimination [4,5,10,14]. These results demonstrate that direct manipulations of the cholinergic and GABAergic neurons in the MSA can alter mnemonic processes.

The cholinergic hypothesis of learning and memory states that the cholinergic system is crucial for the acquisition and retrieval of memories [2]. The cholinergic MSA neurons play an important role in the cholinergic hypothesis because they project to the hippocampus, an area important for memory processing. Based on this hypothesis, drugs that increase hippocampal ACh release should improve mnemonic processes. By contrast, drugs that decrease hippocampal ACh release should impair mnemonic processes. In the present experiment, drugs and doses that effectively altered performance in mnemonic tasks were infused into the MSA. The release of ACh in the hippocampus and overlying cortex was examined.

Nine female Fischer 344 rats, approximately 3 months

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of age, were anesthetized with a mixture of 33% O_2, 66% N_2O_2 and 1–2% ethrane (Anaquest) and implanted with a microdialysis and drug infusion guide cannulae. The microdialysis guide cannula was placed above the dura, 4.0 mm posterior and 2.5 mm lateral to bregma. The guide cannula for drug infusion was placed 0.7 mm anterior and 1.5 mm lateral to bregma and 4.6 mm ventral to dura, at a 15° angle with the tip toward the midline. The guide cannula for drug infusion was a 10 mm long, 26 gauge stainless steel tubing. Both cannulae were fixed to the skull with dental acrylic (Bioanalytical Systems).

Experiments were carried out 24 h after cannulae implantation. A microdialysis probe (Bioanalytical System, CMA/10 dialyzing length = 3 mm) was placed in the hippocampus and overlying cortex and continuously perfused with physiological Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl) containing 100 μM neostigmine bromide, an acetylcholinesterase inhibitor. Microdialysate samples were collected by perfusing the Ringer's solution into the brain at a rate of 5 μl/min. Each sample period was 5 min. Six or seven baseline dialysate samples were collected prior to drug infusion and twelve samples were collected after drug infusion. During the entire session, the rat was able to move freely in a testing chamber (22 cm × 23 cm × 21 cm) constructed of clear Plexiglas.

Each rat was randomly assigned to one of the 3 drug groups: (1) scopolamine (SCOP, 15 and 30 μg), (2) oxotremorine, (OXO, 0.5 and 2 μg), and (3) muscimol (MUS, 15 and 30 ng). Each group had 3 rats. Each rat received a total of 3 infusions of one drug in the following order: high dose of the drug, low dose of the drug and saline. All compounds were obtained from Sigma Chemical (St. Louis, MO, USA) and were dissolved in 0.9% sterile saline solution. The drug injector (12 mm long, 26 gauge stainless steel tube) was inserted into the guide cannula, extending 2 mm from the tip of the guide cannula. Each drug was delivered (Sage Instruments syringe pump, Model 341B) at a rate of 0.1 μl/min for 5 min. During the drug infusion, the rat was free to move about the chamber. The injector was left in place for an additional minute and then removed. Three days were allowed between microinfusions.

Following the infusion experiments, a lethal dose of choral hydrate was given and the rats were transcardially perfused with a 10% formalin solution. The brain was removed and cut into 50 μm coronal sections. Sections containing the tracks from the drug infusion and microdialysis probe were mounted and Nissl stained using Neutral red. This histopathological evaluation was done to verify anatomically the placement of the microdialysis probe and drug infusion cannula, and to examine cells in the vicinity of the infusion for gliosis. The microdialysates were analyzed for their ACh content using HPLC with electrochemical detection [7,8,15]. The detection limit of the ACh assay was 2.5 fmol.

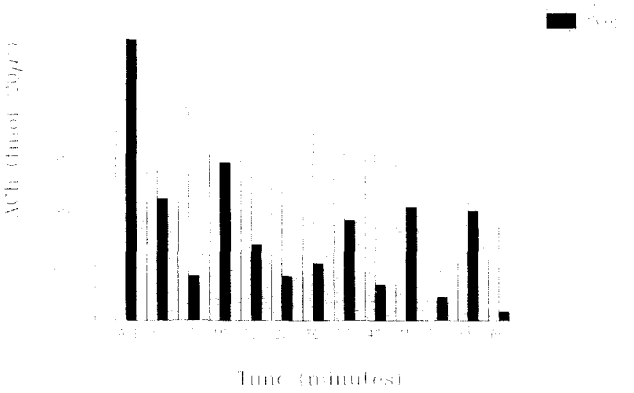

Fig. 1. Scopolamine (30 μg) decreased hippocampal ACh levels as compared to saline. The amount of ACh in each 5 min microdialysate sample collected from the same rat in two separate sessions are illustrated. Values are calculated using an external standard. BASE equals the average ACh level prior to infusion.

For each session, baseline release was determined using the mean ACh level of the 4–5 samples immediately prior to infusion. Amounts of ACh in each of the twelve dialysate samples collected during and after drug infusion were expressed relative to baseline. The mean change for each 5 min sample was calculated across all rats in the same drug condition. Multiple planned comparisons were made between saline and low dose infusions and between saline and high dose infusions within each drug group. The overall session mean was also computed using all 12 sample means. A negative value indicated a decrease of ACh release from baseline and a positive value indicated an increase of ACh release from baseline.

Histological evaluation of the tissue revealed correct placement of the drug infusion cannula and microdialysis probe in all of the rats for which data is reported. A glial reaction was noted around the injection site in the MSA and in the area of the microdialysis probe in the hippocampus. A lesion approximately the diameter of the probe and drug injector was also noted.

Intraseptal infusions of saline altered hippocampal ACh release in the hippocampus in 5 of the 9 rats. The saline and SCOP (30 μg) sessions for one rat are depicted in Fig. 1. The overall mean percentage change of ACh release following saline was 22%, 23%, and 9% for SCOP, OXO, and MUS, respectively (Fig. 2b).

Both scopolamine, a cholinergic antagonist, and oxotremorine, a cholinergic agonist, decreased hippocampal ACh in a dose-dependent manner (Fig. 2). The low dose of SCOP (15 μg) and OXO (0.5 μg) did not produce ef-

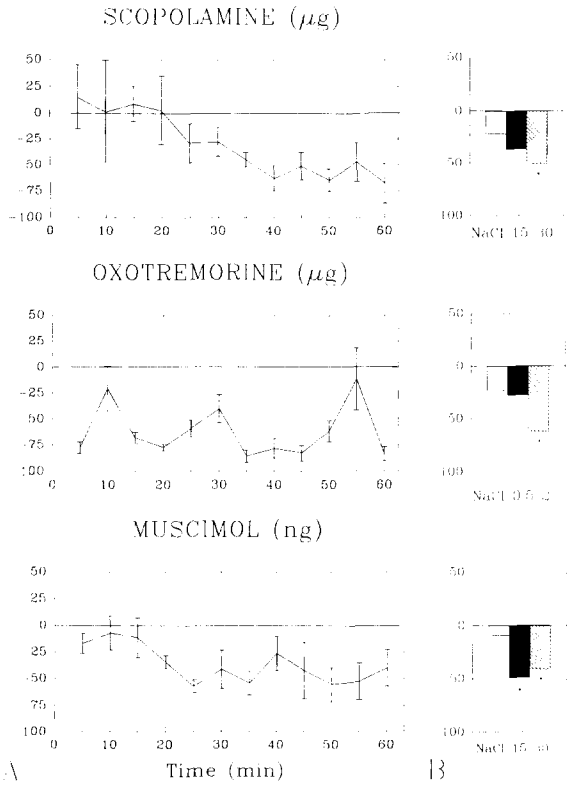

Fig. 2. Intraseptal infusions of SCOP, OXO, and MUS decreased hippocampal ACh release. Drug effects are expressed as the mean percentage change from baseline. Time response curves for the high dose of each compound are illustrated in Fig. 2a. Each point represents the mean value of samples from all rats in the drug group ± S.E.M. The overall session mean for each of the drug conditions (saline, low and high dose) is shown in Fig. 2b. $*P < 0.001$, compared to saline.

fects that were significantly different from saline. The high dose of SCOP (30 μg) and OXO (2 μg) decreased hippocampal ACh levels as compared to the saline control ($F_{1,22} = 10.449$, $P = 0.004$; $F_{1,22} = 20.743$, $P = 0.000$, respectively). Both doses of muscimol, 15 and 30 ng, significantly decreased ACh levels as compared to saline ($F_{1,22} = 11.594$, $P = 0.003$; $F_{1,22} = 8.595$, $P = 0.008$, respectively).

The effects of scopolamine and muscimol on ACh release are consistent with the action of these compounds on the cholinergic neurons in the MSA and impairments in memory. Cholinergic antagonists and GABAergic agonists decrease the activity of MSA cholinergic neurons [18,19]. Working memory was impaired following intraseptal infusions of scopolamine and muscimol [5,14]. In the present study, intraseptal infusion of SCOP (15 or 30 μg) and MUS (15 or 30 ng) decreased ACh release in the hippocampus. Thus, these results are consistent with the idea that drugs that decrease the activity of MSA neurons also decrease hippocampal ACh release and impair memory.

Oxotremorine decreased ACh release in a dose-dependent manner. OXO (2 μg) decreased ACh release below that of the saline controls, whereas OXO (0.5 μg) had little effect. Both doses of oxotremorine improved memory of aged rats in a T-maze alternation tasks (Markowska et al., 1991). If drugs that improve memory do so by increasing ACh release, then oxotremorine should have increased ACh release in the hippocampus following both doses. One possible explanation for this inconsistency is the age of the rat. The present study was performed in young animals and the behavioral data was obtained in aged animals. Intraseptal infusion of carbachol, another cholinergic agonist, increased ACh in young rats [9], but has not been shown to improve behavior in young rats (Givens, unpublished observation).

Combined data from microinfusions and microdialysis can provide information regarding the interaction between the cholinergic and GABAergic neurons of the septohippocampal system. This information may help to elucidate the neural mechanisms by which the MSA can modulate behavioral performance. By providing neuroanatomical specificity, intracranial microinfusions are a powerful way to pharmacologically manipulate small populations of neurons. The results of the present study are consistent with a number of predictions of cholinergic function based on electrophysiological and behavioral studies. Given the complexity of the septohippocampal system, additional studies are necessary to determine the precise nature of the interactions between the cholinergic and GABAergic systems.

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