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Effects of Scopolamine on Extracellular Acetylcholine and Choline Levels and on Spontaneous Motor Activity in Freely Moving Rats Measured by Brain Dialysis

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TOIDE, K. *Effects of scopolamine on extracellular acetylcholine and choline levels and on spontaneous motor activity in freely moving rats measured by brain dialysis.* PHARMACOL BIOCHEM BEHAV **33**(1) 109–113, 1989.—The present study demonstrates the feasibility of measuring acetylcholine (ACh) and choline in perfusate samples collected by in vivo brain dialysis in the frontal cortex and hippocampus of freely moving rats in which spontaneous motor activity could be measured simultaneously. Systemically administered scopolamine increased the output of ACh about 10-fold and 20-fold in the frontal cortex and hippocampus, respectively. By contrast, scopolamine decreased the choline level in the extracellular fluid about 2-fold in both brain regions, possibly owing to enhanced choline uptake into the presynaptic nerve terminals. Scopolamine also increased spontaneous motor activity over the same time course as the changes in ACh and choline. These results indicate that the in vivo brain dialysis technique applied to freely moving rats may be useful in investigating ACh turnover and in studying the relation between cholinergic transmission and behavioral functions.

Acetylcholine release Choline uptake Brain dialysis Spontaneous motor activity Scopolamine Frontal cortex Hippocampus

NEUROTRANSMITTER levels in the extracellular fluid may reflect functional states of neurotransmitter systems. This concept has stimulated the development of techniques for measuring endogenous neuroactive compounds in the extracellular fluid. In vivo intracerebral dialysis has recently been reported to be useful in measuring extracellular fluid levels of monoamines, ACh and excitatory amino acids such as glutamate and aspartate (2–6, 18, 19, 24). This method involves perfusion through a tube containing a semipermeable membrane implanted in a discrete brain area. An important advantage of this technique is that spontaneous motor activity as well as other behavioral changes can be measured simultaneously.

It is now well established that a major neurochemical defect in senile dementia of the Alzheimer type is loss of presynaptic cholinergic indices in the cortex and hippocampus and such deficiency seems to be associated with the memory defects seen in aging and dementia (22). This suggests the importance of investigating ACh release in cortical regions as an index of cholinergic functions connected with behavioral change.

In the present study, in order to quantitate ACh and choline levels in extracellular fluid of the frontal cortex and hippocampus of rats, the brain dialysis technique was applied to freely moving animals that were directly connected to an electrochemical HPLC detector system. In addition, the changes in ACh and choline levels induced by the anticholinergic drug scopolamine, which affects cholinergic transmission, were assessed while simultaneously measuring the spontaneous motor activity of the perfused rats.

METHOD

Brain Dialysis and Surgery

Male Wistar rats (230–260 g) were anesthetized with pentobarbital sodium (40 mg/kg, IP) and placed in a stereotaxic frame. The skull was exposed, holes were drilled, and unilateral dialysis probes (Eicom, Kyoto) were implanted into the left frontal cortex (coordinates: A +3.0, L −2.0, V −4 mm) and left hippocampus (coordinates: A −7.5, L −4.5, V −7.0 mm), with distances reckoned from the bregma in accordance with the atlas of König and Klippel (10). A semipermeable regenerated cellulose tube (Gambro, Sweden) was glued along a U-shaped stainless steel guide and lengths of 2.0 mm (frontal cortex) and 3.0 mm

(hippocampus) from the top were kept free, exposing the dialysis surface. The device was fixed with dental cement and fastened with one screw onto the skull of the rat. To exclude the effects of anesthesia the rats were first allowed time to recover; perfusion experiments were carried out between 26 and 48 hr after surgery, during daylight hours. The rats were housed in plastic cages (40 × 30 × 28 cm) and were given water and food ad lib. The dialysis device was essentially the same as that described by Damsma (5), with only slight modifications. This dialysis device was connected to the perfusion pump (Eicom EP-50, Kyoto) and to the injection valve of the HPLC system by means of polyethylene tubing (length 40 cm, inner diameter 0.1 mm). The motor driven injection valve of an autoinjector (Eicom AS-10, Kyoto) was controlled by an adjustable electronic timer. The sample loop (100 μl) was held in the load position for 15 min and automatically switched to the injection position for 20 sec, after which the cycle was repeated. One stainless steel cannula was connected to the perfusion pump by a polyethylene tube, and the outlet of the other cannula was connected to the injection valve. In the present case, the internal standard, ethylhomocholine (EHC), delivered by the perfusion pump, was fed into the perfusate tube proximally to the injection valve. The dialysis tube was perfused at a constant rate of 2 μl/min with physiological saline at pH 6.3 containing 100 μM physostigmine sulfate. To estimate the variation between different dialysis devices, the efficiency for five probes was determined in vitro at 2 μl/min. The average recoveries for ACh and choline were 24.8 ± 1.3% and 29.6 ± 1.9% (n = 5, S.E.), respectively.

Assay of ACh and Choline

Measurements of ACh and choline were performed with some modifications of Damsma *et al.* (5). Briefly, a column (styrene polymer, Eicom AC-Gel, 6 × 150 mm) was used for separation. An enzymatic postcolumn reactor containing acetylcholinesterase (EC 3.1.1.7, type V-S, Sigma Chemicals) and choline oxidase (EC 1.1.3.17, Toyobo), covalently bound to aminopropyl bonded silicagel (Merck), converted ACh and choline to hydrogen peroxide, which was electrochemically (ECD, Bioanalytical Inc., USA) detected by a platinum electrode (Φ = 3 mm) at 450 mV. The column temperature was 33°C. The mobile phase, delivered by a dual piston pump (Bioanalytical Inc., USA) at 1.0 ml/min, was 0.1 M phosphate at pH 8.0, containing 0.6 mM tetramethylammonium chloride and 0.82 mM sodium 1-decanesulfonate.

Measurement of Spontaneous Motor Activity

The animals were placed in plastic cages (35 × 35 × 30 cm) and after perfusion for about thirty minutes, spontaneous motor activity was automatically measured by an Animex apparatus (Muromachi MK-110, Tokyo) every fifteen minutes, in parallel with brain dialysis.

Drugs and Chemicals

Physostigmine sulfate (Sigma) was added to the perfusion solution. Scopolamine sulfate (Sigma) was dissolved in saline and injected subcutaneously in doses of 1 ml/kg. Aqueous solutions were prepared from distilled water and all other chemicals were of analytical special grade.

Statistics

The calculated results were expressed as pmoles of ACh and choline per minute. The mean values of measurements performed at prescribed intervals after vehicle (control) and drug treatment were compared. Significant differences were assessed by Student's *t*-test with respect to ACh and choline levels, and by Wilcoxon 2-sample test with respect to spontaneous motor activity.

RESULTS

Chromatography

Figure 1 shows typical chromatograms of a standard sample containing 15 pmol of ACh, choline and EHC, and of brain perfusion samples. The EHC concentration in the brain samples was 30 pmol. The average values for ACh and choline were 0.102 ± 0.004 and 1.151 ± 0.014 (n = 9, pmol/min, S.E.), respectively, in the frontal cortex, and 0.114 ± 0.002 and 2.376 ± 0.042 (n = 9, pmol/min, S.E.), respectively, in the hippocampus. The detection limits of ACh and choline were 20 and 10 fmol/injection, respectively.

Effects of Scopolamine on In Vivo Dialysate Levels of ACh and Choline in Frontal Cortex and Hippocampus

Figure 2 (A and B) shows the temporal changes in ACh and choline levels in dialysates of the frontal cortex and hippocampus of freely moving rats before and after systemic administration of saline or of scopolamine (0.5 mg/kg, SC) which caused a marked increase of ACh and decrease of choline in both regions. The maximum level of ACh at 45 min after drug administration was approximately 10 times that of the control group in the frontal cortex and about 20 times that of the control group in the hippocampus. There was a gradual decrease to basal levels in both brain regions 120 min after drug administration. In contrast with these increases in ACh levels, choline levels dialysed in both brain regions were significantly decreased by scopolamine concomitantly with the ACh changes.

Effect of Scopolamine on Spontaneous Motor Activity in Perfused Rats

Figure 3 shows the effects upon spontaneous motor activity after SC administration of scopolamine in perfused rats.

Spontaneous motor activity increased significantly between 15 min and 120 min after drug administration, the strongest effect being manifested 30 min after drug administration.

DISCUSSION

Our results, as exemplified in Fig. 1, illustrate that ACh and choline peaks can be clearly identified in brain dialysis samples. It is also clear from our results that the anticholinergic drug scopolamine markedly increases the release of ACh in both the frontal cortex and hippocampus (Fig. 2 A and B). The magnitude of the increase in ACh release differs in the two brain regions, possibly owing to a difference of sensitivity to scopolamine. The effect of scopolamine on release of ACh may be principally attributed to a blocking of presynaptic muscarinic receptors, since evidence obtained in in vitro experiments has clearly established that anticholinergic drugs such as atropine and scopolamine block cholinergic action by inhibiting presynaptic muscarinic autoreceptors and thereby facilitate ACh release (8, 12, 13, 16).

Furthermore, the results presented here indicate that scopolamine induces decreases in choline levels inversely proportional to the concomitant changes of ACh levels in both brain regions. Numerous reports have indicated that anticholinergic drugs enhance the uptake of choline, probably by a feedback mechanism via a blocking action upon postsynaptic muscarinic receptors. The effects of anticholinergic agent upon ACh release and choline uptake have already been well established by in vitro experiments

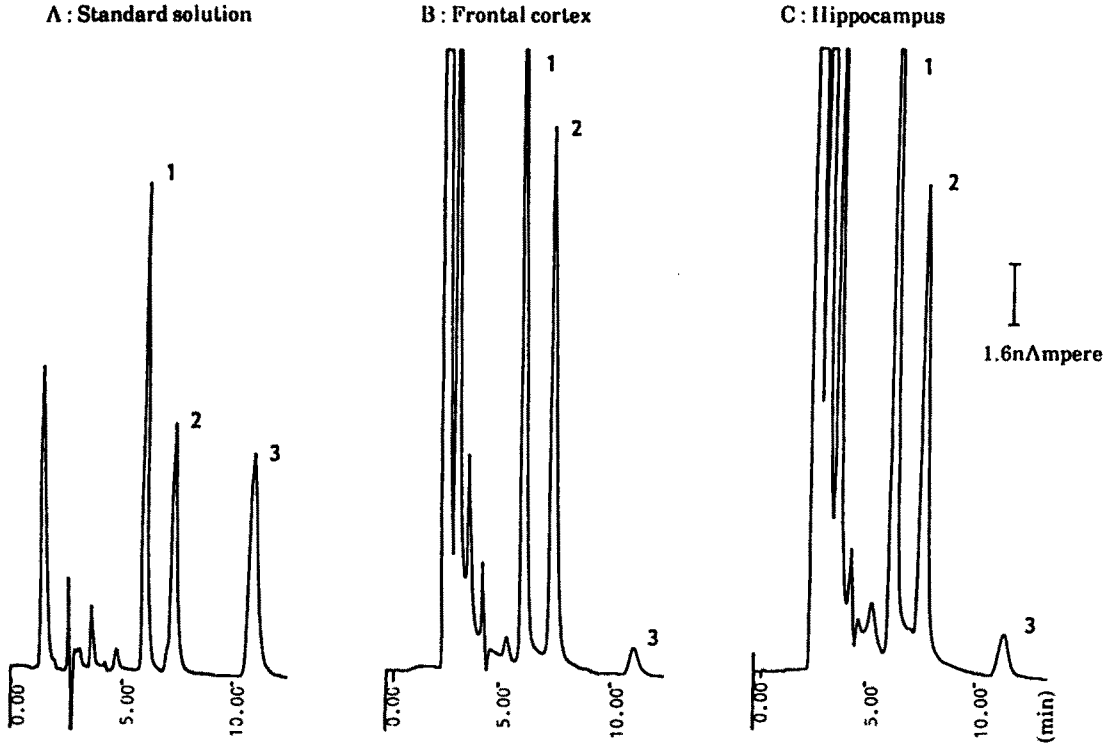

FIG. 1. Chromatograms of ACh, choline and EHC in the standard solution and in the dialysate from rat frontal cortex and hippocampus. (A) Standard solution containing 15 pmol of ACh, choline and EHC. (B and C) Thirty pmol of EHC and 30 μl of perfusate with physiological saline solution containing physostigmine. 1: Choline, 2: EHC, 3: ACh.

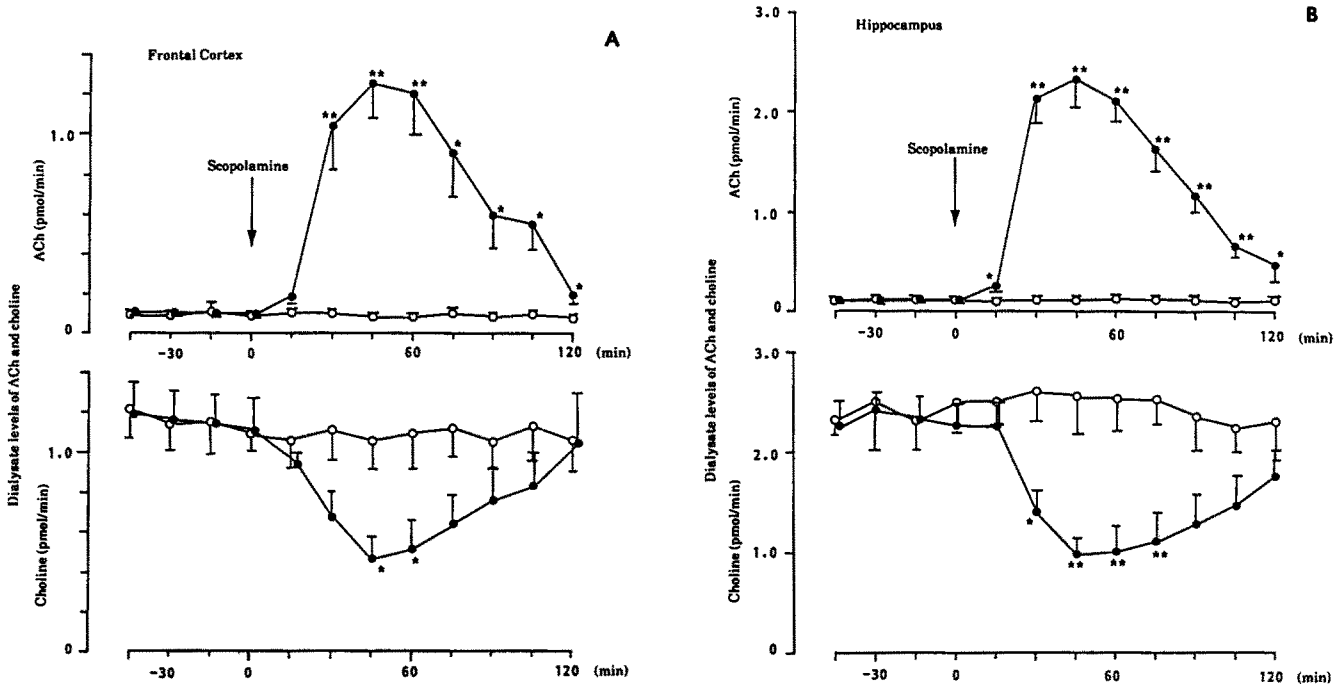

FIG. 2. Effect of scopolamine on the dialysate levels of ACh and choline from frontal cortex (A) and hippocampus (B) of freely moving rats. Saline (open circles) and 0.5 mg/kg scopolamine (closed circles) were injected subcutaneously. The data (mean and S.E. from 4 and 5 rats) represent the ACh and choline content for each 15-min fraction. *$p<0.05$, **$p<0.01$, Student's t-test.

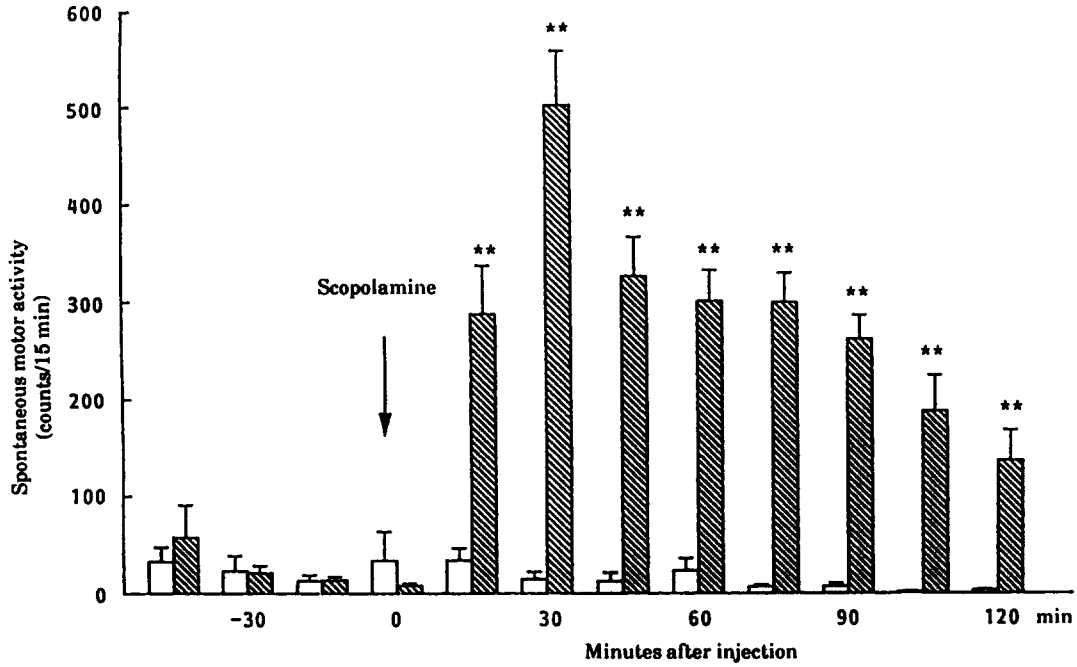

FIG. 3. Effects of scopolamine on spontaneous motor activity in perfused rats. Saline (open columns) and 0.5 mg/kg scopolamine (striped columns) were injected subcutaneously. Each column represents (mean and S.E. from 8 and 10 rats) the spontaneous motor activity for each 15 min. $^{**}p<0.01$, Wilcoxon 2-sample test.

using radioisotope techniques (7, 14, 15, 20, 23). The consistency of these in vitro data with our in vivo data validate the use of the dialysis technique to measure ACh turnover and indicate that, in vivo as in vitro, scopolamine may enhance ACh turnover by the inhibition of both pre- and postsynaptic muscarinic receptors.

Measurement of spontaneous motor activity was attempted concomitantly with the measurement of ACh and choline by brain dialysis in freely moving rats. Anticholinergic drugs such as scopolamine and atropine are generally believed to increase spontaneous motor activity (17). The results presented in Fig. 3 show that scopolamine induced a marked increase of spontaneous motor activity in the perfused animals and that this increase showed a temporal correlation with the changes in central ACh turnover. Previously, Aquilonius *et al.* (1) found some correlation between motor activity in rats and cortical ACh release as measured by the cup method.

Physostigmine is generally used to prevent the degradation of ACh by acetylcholinesterase, and therefore 100 μM of physostigmine was added to the perfusion solution in the present study. It is well known that systemic administration of physostigmine at high dosages induces side effects such as sedation, hypothermia, salivation, diarrhea, etc. (9,11). Under the conditions of the present experiments, however, rats exposed to physostigmine in the perfusion solution did not display such behavioral side effects but instead seemed perfectly normal in behaviors such as rearing, grooming, etc.

In conclusion, the present approach permits the direct detection of changes in ACh and choline levels in the extracellular space of discrete brain areas of experimental animals. The simultaneous detection of both ACh and choline is of particular importance, since it may be useful in assessing the cholinegic functions. Moreover, this technique permits observation of behavioral changes, e.g., spontaneous motor activity, concomitantly with the quantitative determination of ACh and choline. Hence, this approach is well suited for studying correlations between behavioral parameters and cholinergic transmission, especially ACh release.

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