



CHOLINE ENHANCES SCOPOLAMINE-INDUCED ACETYLCHOLINE RELEASE IN DORSAL HIPPOCAMPUS OF CONSCIOUS, FREELY-MOVING RATS

Darrell A. Jackson, Udo Kischka and Richard J. Wurtman

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology
E25-604, Cambridge, Massachusetts 02139, USA

(Received in final form October 13, 1994)

Summary

We examined the effects of exogenous choline (30, 60, 120 mg/kg, i.p.) on basal and scopolamine-evoked acetylcholine (ACh) release in awake animals, using *in vivo* microdialysis. After collection of 3-4 baseline dialysate samples (15 min each), rats received either saline or choline chloride and 4 additional samples were collected. All animals then received scopolamine hydrochloride (0.5 mg/kg, i.p.) and 6 additional samples were collected. Basal ACh release in animals receiving choline did not differ from that in rats given saline, nor from ACh release prior to choline administration. Scopolamine alone increased average ACh levels in dialysates from 1.22 ± 0.54 to 11.18 ± 3.07 pmol/15 min (mean \pm SD; $p = 0.001$); administration of 60 mg/kg or 120 mg/kg of choline chloride significantly enhanced maximal scopolamine responses by about 55%. These results suggest that supplemental choline enhances evoked ACh release in hippocampus of freely-moving rats.

Key Words: choline, acetylcholine, microdialysis, scopolamine, hippocampus

Brain acetylcholine (ACh) levels are maintained within relatively narrow limits, indicating that the rate of ACh synthesis is adequate for sustaining the neurotransmitter's release under normal conditions. However, when ACh release is sustained, its levels can decrease significantly as shown in whole brain (1,2) and brain slices (3,4), indicating that synthesis is then unable to keep up with demand. Some (3-8) but not all investigators (9-13) have observed that choline supplementation can increase brain ACh concentrations *in vivo*, and can prevent the depletion of ACh tissue levels otherwise observed in brain slices subjected to prolonged stimulation. Moreover, preincubation of hippocampal slices with choline can significantly increase the release of ACh evoked by incubation with potassium (14) or with aminopyridines (15).

We now report that choline, given peripherally, causes a dose-dependent increase in scopolamine-evoked ACh release in hippocampus of awake, freely-moving rats.

Methods

ANIMALS: Male Sprague-Dawley rats (250-300 g) obtained from Charles River Laboratories (Wilmington, MA), were housed in groups of three and kept in a 12:12 hour light/dark cycle.

Water and food were provided ad libitum.

SURGERY AND DIALYSIS: Rats were anesthetized with equithesin (chlornembutol 0.3 ml/100 g, i.p.) and placed in a Kopf stereotaxic small animal frame. Concentric 3 mm microdialysis probes with a molecular weight cutoff of 5000-6000 g were chronically implanted in the ventrolateral aspect of the right hippocampus. Implantation coordinates used with reference to bregma and dura were: P = 5.8 mm, L = 5.5 mm and V = 6.0 mm (16). Probes were permanently implanted using dental cement and jeweler screws anchored to the skull. Prior to probe implantation, a polyethylene tube (0.51 mm internal diameter and 1.56 mm outer diameter) was implanted chronically within the peritoneal cavity and allowed to exit the body at the top of the head, along with microdialysis tubing. Animals were allowed to recover from anesthesia and surgery for 24-36 h. Approximately 3 h prior to collecting dialysate samples for basal choline and acetylcholine determinations, probes were perfused with artificial cerebrospinal fluid (containing, in mM: NaCl, 121; NaHCO₃, 25; KCl, 3.5 CaCl₂, 1.2; MgCl₂, 1.2; NaH₂PO₄, 1; Neostigmine, 10 μM: bubbled with 95% O₂ and 5% CO₂ for 15 min, pH 7.4) at a rate of 2 μl/min. During the experiment, animals were allowed to have free access to water and food and were exposed to ambient air. All experiments were carried out between 11:00 am and 6:00 pm. Dialysates were collected in fractions representing 15 min intervals. After collection of 4 basal extracellular samples, rats with chronically implanted hippocampal microdialysis probes received choline chloride (60 mg/kg, i.p.). Four additional dialysate samples were collected with animals then receiving scopolamine hydrochloride (0.5 mg/kg, i.p.) and sample collection continuing for an additional 90 min.

Analysis

Dialysate ACh and choline concentrations were determined by HPLC with an enzyme reactor containing acetylcholinesterase and choline oxidase, coupled to an electrochemical detector for hydrogen peroxide (Bioanalytical Systems Inc., West Lafayette, IN), as described by Potter et al. (17). At the end of the experiment, the animals were sacrificed and their brains removed to verify probe placement and to assess the condition of tissue surrounding the implantation site. The percentage recovery of each microdialysis probe measured for ACh and choline were determined to be 24% and 26%, respectively. Choline chloride was obtained from the Sigma Chemical Company (St. Louis, MO).

STATISTICS: Significance of difference between the saline control and choline groups was determined by two-way analysis of variance (ANOVA) and Tukey test. Statistical tests were performed with the aid of SYSTAT version 5 software (Systat Inc., Evanston, IL) on a Macintosh IICI personal computer.

Results

Stability in baseline acetylcholine and choline levels was achieved prior to either saline or choline supplementation. Each sample represented 15 min collection time (n=4, saline; n=8, choline); basal choline levels were determined to be 13.47 ± 2.2 for saline and 12.01 ± 1.28 for choline. Systemic administration of choline chloride (60 mg/kg, i.p.) elevated choline levels in hippocampal dialysates maximally (almost 3-fold) 15 min post-injection (Fig. 1). Two-way ANOVA followed by a Tukey test was used to determine the statistical significance of difference between the two groups. Extracellular choline levels were significantly elevated 15 min following choline administration compared with those of saline-treated control rats (P = 0.004). No other statistically significant differences were found. These elevated levels returned to baseline values (i.e., within 36% of pre-injection choline levels) by 60 min. Systemic injection

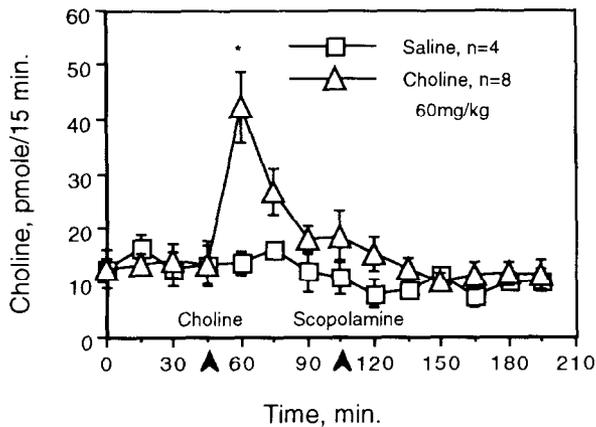


FIG. 1

Effect of choline or administration (60 mg/kg i.p.) on extracellular hippocampal choline levels. The results are expressed as means \pm SEM. * $P = 0.004$ Tukey HSD multiple comparisons matrix of pairwise comparison probabilities; Systat statistical program.

of the muscarinic antagonist scopolamine decreased extracellular choline concentrations in choline-pretreated rats, and in rats that had received saline (Fig. 1). Basal acetylcholine levels ranged from 0.41 to 1.87 pmol/15 min for choline groups and 0.71 to 1.96 pmol/15 min for saline control. Pretreatment of rats with 30, 60 or 120 mg/kg, i.p., choline chloride resulted in no significant differences in basal ACh levels compared with those in rats receiving equal volumes of saline (Fig. 2).

Administration of 30 mg/kg, i.p., of choline chloride 1 h prior to scopolamine, i.p., did not potentiate scopolamine-evoked ACh release (i.e., compared with that in rats receiving saline prior to the scopolamine; Fig. 2). However, administration of 60 or 120 mg/kg choline chloride did significantly ($P < 0.002$ or $P < 0.009$; two-way ANOVA) enhance scopolamine-evoked hippocampal ACh release; maximal release, observed 30 min after scopolamine injection, was about 50% greater than in animals not receiving choline chloride.

Discussion

These data demonstrate that choline administration can potentiate scopolamine-evoked ACh release in hippocampus of awake, freely-moving rats. Consistent with previous findings (18,19), this treatment also elevated dialysate choline levels within 15 min of injection and, by 30 min, these levels had begun to return to preinjection concentrations. Providing supplemental choline chloride did not appear to affect basal ACh release, as discussed previously (3,14). A potential problem associated with using microdialysis techniques to measure ACh release is the necessity of including a cholinesterase inhibitor in the dialysate medium in order to measure basal ACh levels. These drugs can depress ACh release in slice preparations (20) as well as in brain in vivo (21), perhaps via enhanced activation of muscarinic autoreceptors by the increased synaptic levels of ACh. In the present study, the failure of choline chloride supplementation to enhance basal hippocampal release may have been an artifact arising from the inclusion of the cholinesterase inhibitor in the perfusion medium.

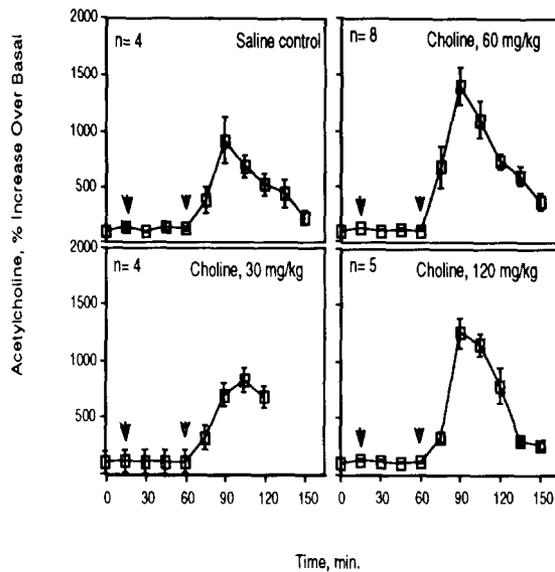


FIG. 2

Dose-dependent effect of choline pretreatment on scopolamine-evoked ACh release within rat hippocampus. The solid arrows indicate when saline or choline was administered and the clear arrows indicate when rats received scopolamine (0.5 mg/kg, i.p.). Percent values were derived from the last basal ACh value preceding choline or saline administration. Values are expressed as means \pm SEM.

In accordance with previous findings (22), peripheral administration of the non-selective muscarinic antagonist, scopolamine, caused a pronounced enhancement of hippocampal ACh release. Extracellular ACh levels were maximally elevated 30 min following scopolamine administration, gradually returning to near-basal levels by 120 min. In the present study, pretreatment with choline produced a dose-dependent enhancement of scopolamine-evoked ACh release. It was previously observed that atropine-evoked release of ACh in the striatum is enhanced by pretreatment with choline (13) and it is well established that presynaptic muscarinic autoreceptors modulate ACh release (23,24).

In conclusion, ACh release in the awake rat's hippocampus, when muscarinic autoreceptors are blocked, is affected by the availability of choline.

Acknowledgments

These studies were supported in part by a grant (MH-28783) from the National Institute of Mental Health. Dr. Jackson is also the recipient of support from Training Grant (T32MH15761).

References

1. B.HOLMSTEDT, Ann. N.Y. Acad. Sci. 144 433-458 (1967).
2. B.A. TROMMER, D.E. SCHMIDT and L. WECKER, J. Neurochem. 39 1704-1709 (1982).
3. WECKER, L., J. Neurochem. 51 497-504 (1988).
4. ULUS, I.H., WURTMAN, R.J., MAURON, C. and BLUSZTAJN, J.K., Brain Res. 484 217-227 (1989).
5. COHEN, E.L. and WURTMAN, R.J., Science 191 561-562 (1976).
6. HAUBRICH, D.R., WANG, P.F.L., CLODY, D.E. and WEDEKING, P.W., Life Sci. 17 975-980 (1975).
7. HIRSCH, M.J. and WURTMAN, R.J., Science 202 223-225 (1978).
8. WECKER, L., CAWLEY, G. and Rothermel, S., J. Neurochem. 52 568-575 (1989).
9. FLENTGE, F. and VAN DEN BERG, C.J., J. Neurochem. 32 1331-1333 (1979).
10. JOPE, R.S., J. Pharmacol. Exp. Ther. 220 322-328 (1982).
11. SHERMAN, K.A., ZIGMOND, M.J. and HANIN, I., Neuropharmacology 20 921-924 (1981).
12. BRUNELLO, N., CHENEY, D.L. and COSTA, E., J. Neurochem. 38 1160-1163 (1982).
13. WESTERINK, B.H.C. and DE BOER, P., Neurosci. Lett. 116 297-301 (1990).
14. WECKER, L., J. Neurochem. 57 1119-1127 (1991).
15. BUYUKUYSAL, R.L., Holmes, T.C. and Wurtman, R.J., Brain Res. 541 1-6 (1991).
16. PAXINOS, G. and WATSON, C., The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, Sydney (1986).
17. POTTER, P.E., MEEK, J.L. and NEFF, N.H., J. Neurochem. 41 188-194 (1983).
18. WECKER, L. and SCHMIDT, D.E., Brain Res. 184 234-238 (1980).
19. KLEIN, J., KOPPEN, A. and LOFFELHOLZ, K., J. Neurochem. 55 1231-1236 (1990).
20. BOURDOIS, P.S. and MITCHELL, J.F., Br. J. Pharmacol. 52 509-517 (1974).
21. DE BOER, P., WESTERINK, B.H.C. and HORN, A.S., Neurosci. Lett. 116 357-360 (1990).
22. TOIDE, K. and ARIMA, T., Eur. J. Pharmacol. 173 133-141 (1989).
23. NORDSTROM, O. and BARTFAI, T., Acta Physiol. Scand. 108 347-353 (1980).
24. MEYER, E.M. and OTERO, D.H., J. Neurosci. 5 1202 (1985).