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Short communication

Effects of estrogen on potassium-stimulated acetylcholine release in the hippocampus and overlying cortex of adult rats

Robert B. Gibbs [a,*], Ahmad Hashash [b], David A. Johnson [b]

[a] *Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, 1004 Salk Hall, Pittsburgh, PA 15261, USA*
[b] *Department of Pharmacology-Toxicology, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282-1504, USA*

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Abstract

In vivo microdialysis techniques were used to examine the effects of estrogen on potassium-stimulated acetylcholine release in the hippocampus and overlying cortex of adult, ovariectomized rats. Estrogen treatment resulted in a significant increase in the percent change in acetylcholine release induced by potassium relative to controls, particularly after prolonged (90 min) exposure to high potassium. The data suggest that estrogen may help to maintain cholinergic function under conditions where cholinergic afferents to the hippocampal formation and cortex are challenged or impaired.

Keywords: In vivo microdialysis; Choline acetyltransferase; Basal forebrain; Alzheimer's disease; Aging

Cholinergic neurons located in the medial septum, the diagonal band of Broca, and the nucleus basalis magnocellularis, are the main source of cholinergic innervation to the hippocampal formation and cortex and play an important role in learning and memory processes. Numerous studies suggest that decreases in hippocampal and cortical cholinergic activity contribute to cognitive decline associated with aging and Alzheimer's disease (see [6] for review). Significant decreases in cholinergic parameters, as well as in the number of cholinergic neurons located in the MS and NBM, have consistently been detected in the brains of Alzheimer's patients [2,3,10,23]. In addition, manipulations which disrupt basal forebrain cholinergic function produce significant learning and memory deficits in animals and in humans which are similar in many ways to those associated with aging and disease (see [4] for review). Conversely, agents which increase cholinergic function have been shown to reduce cognitive deficits associated with injury, aging, and disease in animals and humans [1,12,13,16,17,27], suggesting that agents which help to maintain basal forebrain cholinergic function may likewise help to prevent or reduce the development of age-and disease-related cognitive decline.

Recent studies have shown that the gonadal steroid, estrogen, can have significant positive effects on basal forebrain cholinergic neurons. Specifically, estrogen replacement has been shown to increase the expression of choline acetyltransferase (ChAT) mRNA and protein in the MS and NBM [7–9], and to increase ChAT activity [15,25] as well as high affinity choline uptake [18,25] in the MS, hippocampus, and cortex of ovariectomized rats. Short-term estrogen treatment has also been shown to counteract the impairment of T-maze performance induced by scopolamine [5] in female rats, and to enhance spatial memory performance in young and aged male rats [14]. Packard et al. [21] also recently showed that intrahippocampal injections of estradiol can enhance spatial memory, as well as prevent scopolamine-induced memory impairment, in males. These data are consistent with recent reports showing beneficial effects of estrogen on cognitive function in women with Alzheimer's disease [11,19,20], as well as a decreased risk of developing Alzheimer's disease in women with prior estrogen use [22,26]. Collectively, the data are consistent with the idea that estrogen can help to reduce or delay the development of Alzheimer's-related dementia via effects on basal forebrain cholinergic neurons and the

* Corresponding author. Fax: +1 (412) 624-1850; E-mail: gibbsr@vms.cis.pitt.edu

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maintenance of hippocampal and cortical cholinergic function. However, direct evidence showing that estrogen administration can significantly affect acetylcholine (ACh) release in the hippocampal formation and cortex has not yet been reported.

In the present study, in vivo microdialysis techniques were used to examine the effects of estrogen replacement on basal and potassium-stimulated acetylcholine release in the hippocampus and overlying cortex of adult Sprague–Dawley rats. Ovariectomized animals were purchased and housed for at least 2 weeks prior to use with food and water available ad libitum. Animals received a 3 mm silastic capsule containing either 17β-estradiol (E) crystals ($n = 7$) or nothing ($n = 7$), implanted subcutaneously in the neck region. The estrogen-containing capsules produce serum levels of estradiol of approximately 50 pg/ml.

Microdialysis was performed 10–11 days following capsule implantation. This time-point was chosen based on previous studies showing increases in ChAT and ChAT activity following 7–10 days of estrogen treatment [8,15]. Animals were anesthetized with urethane (1.5 g/kg in normal saline) and a 3 mm loop-type microdialysis probe (ESA cat #70-0402) was slowly lowered into the right hippocampal formation and overlying cortex (−3.4 mm from bregma, 1.18 mm lateral, −3.4 mm ventral; see Fig. 1). Probes were perfused at a rate of 1 μl/min with artificial cerebrospinal fluid (ACSF; 144.3 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.7 mM NA_2HPO_4, 0.5 mM NaH_2PO_4) containing 1 μM neostigmine bromide. The acetylcholinesterase inhibitor was added to obtain detectable quantities of ACh. Samples were collected continuously every 30 min for a period of 2 h in order to provide sufficient time for basal acetylcholine release to stabilize. Two additional samples (B1 and B2) were then collected as a measure of basal ACh release. Probes were then perfused with ACSF containing 60 mM potassium and samples were collected every 30 min for an additional 90 min. Animals were then perfused with paraformaldehyde, the brains were removed and sectioned to verify placement of the probes. Immediately before each experiment, probes were dialyzed against solutions of ACh (0.05–1.0 μM in ACSF) to estimate the in vitro efficiency of the probes.

Fig. 1. Photomicrograph illustrating the tract * produced by the microdialysis probe. Scale bar = 150 μm.

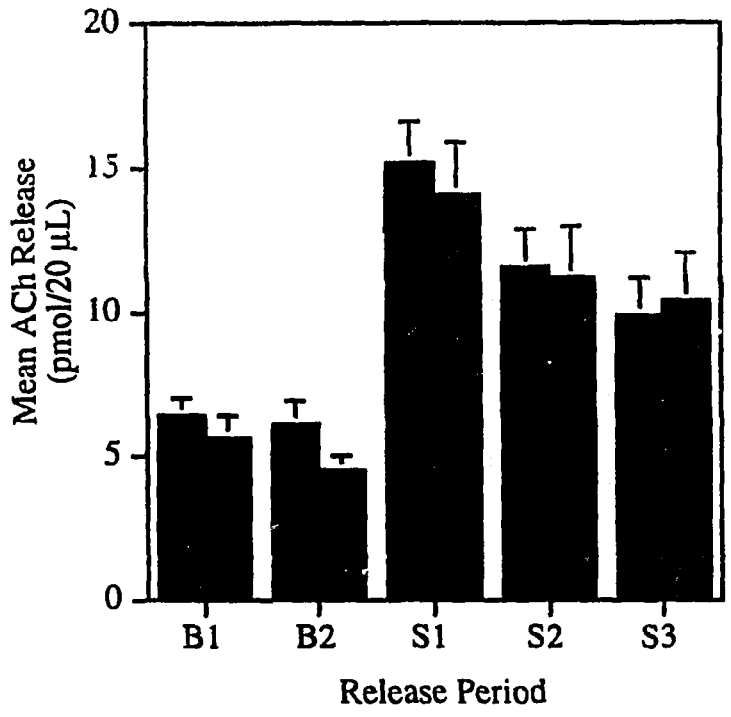

Fig. 2. Mean ACh release ± S.E.M. during each release period for estrogen-treated animals (shaded bar) and controls (black bar). Note the decline in release during the period of stimulated release (S1–S3) in both estrogen-treated and control animals.

Samples were analyzed using HPLC, enzymatic conversion, and electrochemical detection as previously described [24]. ACh values were compared with a 1-pmol standard solution (20 μl of 0.05 μM ACh in ACSF). Values were then corrected for probe recovery and expressed as picomoles per 20 μl sample. Samples collected 30, 60, and 90 min after switching to high potassium were designated S1, S2, and S3 and were used as a measure of potassium-stimulated release. Effects of treatment on basal release, stimulated release, and the percent change in release (calculated for each animal as the percent change relative to the average of B1 and B2), were analyzed using a one-way analysis of variance with repeated measures. Univariate F-tests were used to compare treatment effects at each time-point during the period of stimulated release. All statistics were performed using Systat v5.2 for Macintosh.

A significant decrease in the average stimulated release over time was detected in both estrogen-treated and control animals ($F = 24.1$, $P < 0.0001$; Fig. 2); however, no significant effects of estrogen on the average basal, average stimulated, total basal, or total stimulated ACh release were detected (Table 1). In contrast, there was a significant effect of treatment on the percent change in the mean and total potassium-stimulated release relative to baseline ($F = 5.14$, $P = 0.04$) with a greater increase in ACh release observed in E-treated animals relative to controls (Table 1). This was particularly true for the samples obtained 90 min after initiating potassium-stimulated release (S3) at which time the average increase in ACh release was 105.6% ± 14.3 in E-treated animals vs. 59.1% ± 11.7 in non-E-treated controls ($P = 0.027$; Fig. 3). The percent change in ACh release was also greater in E-treated ani-

Table 1
Baseline and potassium-stimulated acetylcholine release

	OVX	E-treated
Average basal	6.2 ± 0.6	5.0 ± 0.7
Average stimulated	12.2 ± 1.3	11.9 ± 1.7
Average % change	97.5 ± 12.6	137.8 ± 12.6 *
Total basal	12.4 ± 1.1	10.0 ± 1.4
Total stimulated	36.6 ± 3.9	35.6 ± 5.0
Average % change	196.2 ± 18.9	256.7 ± 18.9 *

Values indicate average and total ACh release ± S.E.M. during the 60 min baseline period and the 90 min stimulated period, and the average percent change in mean and total ACh release relative to baseline. $n = 7$/group. * $P = 0.04$ relative to ovariectomized controls.

mals than in non-E-treated controls in samples collected 30 and 60 min after initiating potassium-stimulated release (186.4% ± 24.3 vs. 146.8% ± 17.3 at S1; 121.3% ± 13.0 vs. 86.5% ± 13.5 at S2); however, these differences were not statistically significant.

These data demonstrate that short-term continuous estrogen replacement can significantly affect potassium-stimulated acetylcholine release in the adult rat hippocampal formation and overlying cortex. In particular, the data suggest that estrogen replacement may enable cholinergic afferents to sustain higher levels of acetylcholine release over time. This finding is consistent with the recent studies cited above which have demonstrated significant increases in cholinergic parameters, as well as significant enhancements in learning and memory function, following short-term estrogen replacement. The fact that no significant effects on average or total ACh release were detected may be due to the substantial inter-animal variation in absolute levels of ACh release (coefficient of variation ranged from 23.5 to 37.8%). Calculating the percent change in release for each animal eliminates one source of inter-animal variability and, therefore, provides a more sensitive measure of changes in potassium-stimulated release.

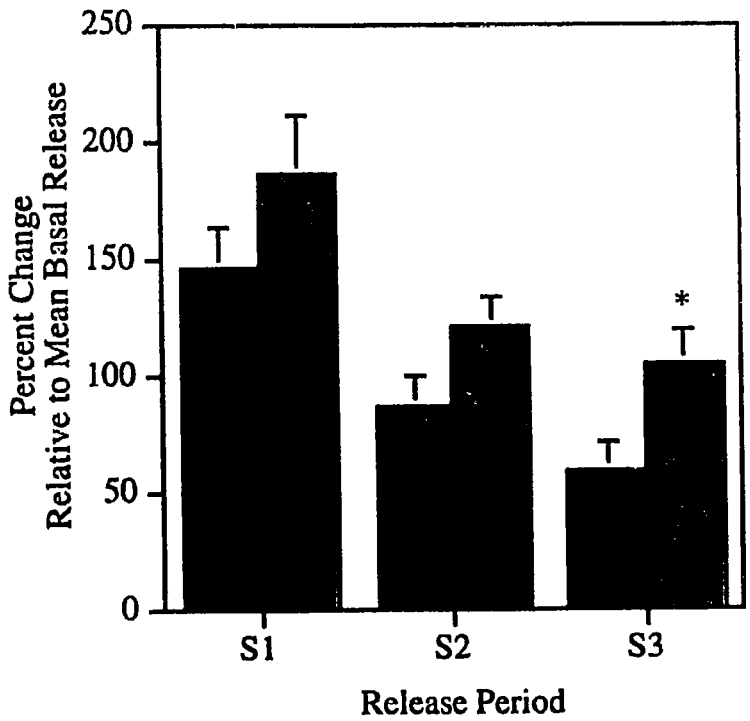

Fig. 3. Mean ACh release ± S.E.M. induced by potassium during each release period. Percent change relative to baseline was calculated for each animal and then used to calculate the group average. Note that the percent change in release is greater in E-treated animals (shaded bar) than in controls (black bar) during all three release periods and is statistically significant at S3 (* $P < 0.05$ relative to non-E-treated controls).

Whether estrogen treatment results in a greater increase in ACh release immediately upon activation or maintains a given level of ACh release for a longer period of time is not entirely clear. Following the initiation of potassium-stimulated release, levels of ACh decreased over time suggesting either a depletion of ACh from the cholinergic terminals or a decrease in the responsiveness of the terminals to elevated levels of potassium. In the present study, the average percent change in release induced by potassium was higher in E-treated vs. control animals at all three time-points (30, 60, and 90 min), however, the difference between E-treated and control animals was statistically significant only at the 90 min (S3) time-point. This suggests that in E-treated animals, the rate at which ACh release decreases over time during exposure to high potassium is reduced relative to the rate of decrease in non-E-treated controls. This is consistent with the idea that estrogen treatment reduces the rate at which ACh is depleted from cholinergic terminals during prolonged periods of activation. Previous studies have demonstrated that short-term estrogen treatment produces increases in ChAT mRNA and protein within cholinergic neurons in the MS and NBM [7–9], as well as increases in ChAT activity [15,25] and high affinity choline uptake [18,25] in the hippocampus and cortex. Increases in ChAT, ChAT activity, and high-affinity choline uptake could all contribute to increased synthesis of ACh, and thereby reduce the rate at which ACh stores are depleted during periods of prolonged activation. Consequently, one effect of estrogen may be to help maintain a threshold level of cholinergic function under conditions where the cholinergic neurons are severely challenged or impaired. This hypothesis is consistent with the ability for estrogen to overcome age- and scopolamine-related memory deficits, and provides a mechanism by which estrogen replacement may help to reduce or delay cognitive deficits associated with aging and Alzheimer's disease in women. Additional studies will need to be performed to more directly assess the effects of estrogen on ACh release over time.

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