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Neurobiology of Aging 5806 (2002) 1–3

NEUROBIOLOGY OF AGING

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Microdialysis without acetylcholinesterase inhibition reveals an age-related attenuation in stimulated cortical acetylcholine release

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Received 4 October 2002; received in revised form 21 November 2002; accepted 5 December 2002

Abstract

Aging-related differences in the ability of cortical cholinergic inputs to respond to local depolarization was assessed in young (3–6 months) and old (26–33 months) awake rats using in vivo microdialysis in the absence of an inhibitor of acetylcholinesterase. Rats were perfused, using a within-subjects, repeated session design, with vehicle (aCSF) or K^+ (25, 50, 100 mM). Perfusion of K^+ resulted in a dose-dependent increase in cortical ACh efflux with comparable efflux seen between the two ages following 25 mM (50%) and 50 mM (100%) K^+. In contrast, aged rats exhibited a marked attenuation (330%) in ACh efflux relative to young adult rats (650%). These data reveal aging-related decreases in the responsiveness of cortical cholinergic afferents, tested under physiologically relevant conditions, to local depolarization and may provide a neuronal mechanism contributing to the cognitive deficits reported in normal aging- and age-related pathological conditions.

Keywords: Acetylcholine; Prefrontal cortex; Microdialysis; Aging; Potassium; Rat

1. Introduction

Several morphological alterations occur in the cholinergic forebrain with normal aging in the rat; however, the consequences of these age-related changes for normal presynaptic function of cortical cholinergic afferents remain unclear [12]. In vivo microdialysis and subsequent measurement of ACh efflux permits a dynamic measure of the functional integrity of cortical cholinergic afferents during aging in the rat. Previous microdialysis studies focusing on potential age-related alterations in cortical ACh release have used acetylcholinesterase (AChE) inhibitors in the perfusion medium, and did not reveal differences in basal cortical ACh efflux between young and aged rats [6,8,9]. However, a reduced potency of local depolarization following perfusion of elevated potassium (K^+) to stimulate cortical ACh efflux in aged rats has been observed [1,9]. Although useful in the detection of basal levels of ACh, the use of AChE inhibitors in the perfusion medium is potentially problematic for the interpretation of drug effects and for the study of alterations in ACh release associated with aging and other pathological states. Local perfusion of AChE inhibitors tonically alters cholinergic receptor activity by increasing extracellular levels of ACh [15] and this may affect the excitability of cortical choniergic inputs [7]. In addition, AChE inhibitors produce changes in local concentrations of other neurotransmitters [5,10]. The utilization of recent developments in the methodology associated with the electrochemical detection of ACh now permits reliable detection of basal and stimulated ACh release in the absence of an AChE inhibitor [4,14], thus, permitting more sensitive and physiologically relevant study of potential alterations in cortical ACh release associated with aging. The purpose of the present experiment was, therefore, to re-evaluate findings from prior studies characterizing potential age-related alterations in the regulation of basal cortical ACh efflux and to further characterize the age- and dose-related effects of local K^+ perfusion on cortical ACh efflux without the use of an AChE inhibitor in the perfusion medium.

2. Methods

Fischer-344/Brown Norway (NIA) hybrid rats aged 3–6 and 26–33 months served as subjects. Rats were handled and habituated to the testing environment for 4 days prior

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0197-4580/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
doi:10.1016/S0197-4580(02)00226-9

to surgery. Under isoflurane anesthesia (2–3% inhalant via Kopf stereotaxic adapter), animals were implanted with microdialysis guide cannulae (Sci Pro; 15 mm) into the medial prefrontal cortex (mPFC) using the following stereotaxic coordinates relative to bregma [11]: AP, +2.7 mm; ML, ±0.8 mm; DV, –1.0 from dura. Following the last microdialysis session, guide cannulae and microdialysis probe placements were verified histologically using standard perfusion and light microscopy techniques. Microdialysis sessions were conducted every other day for a total of four sessions per animal. This repeated perfusion method has been validated for a variety of transmitters in several brain regions [2]. During each session, animals were placed in a circular Plexiglas testing chamber for 30 min, after which a concentric dialysis probe (SciPro: 3.0 mm membrane, O.D. 0.24 mm) was inserted into the guide cannula and continuously perfused (1.25 μl/min) with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl, 155.0; $NaHCO_3$, 27.5; KCl, 2.4; Na_2SO_4, 0.5; KH_2PO_4, 0.5; $CaCl_2$, 1.1; $MgCl_2$, 0.8; glucose, 1.0; pH 7.0. For elevated K^+ manipulations, the perfusion medium was changed to aCSF containing a final K^+ concentration of 100 mM. Session order (VEH, 25, 50 mM K^+) was counterbalanced across the first three sessions, and 100 mM K^+ was always used on session four for all subjects. Three hours following insertion of probes, samples were collected every 15 min during baseline conditions, during perfusion of K^+, and following return to normal aCSF. Quantification of ACh in mPFC dialysates was achieved using high-performance liquid chromatography with electrochemical detection (9 μl per injection). Briefly, a pre-column enzyme reactor (ESA, Inc.) was used to oxidize choline and reduce H_2O_2 in samples prior to separation of ACh and choline by a C-18 carbon polymer column (ESA, Inc.) using a sodium phosphate mobile phase. Post-column hydrolysis of ACh was achieved using an enzyme reactor containing covalently-bound acetylcholinesterase and choline oxidase. Subsequent electrochemical detection of H_2O_2 was achieved using a peroxidase-wired glassy carbon electrode [3]. The limit of detection under these conditions was 5.0 fmol on column per 12 μl injection.

3. Results

Mean (±S.E.M.) ACh efflux during baseline conditions did not differ between young (14.0 ± 1.0 fmol, $n = 6$) and aged (15.0 ± 0.5 fmol, $n = 6$) rats. Stable and comparable levels of cortical ACh efflux were observed irrespective of age over repeated microdialysis sessions. A mixed factor ANOVA revealed no main effect of age, session, and no interaction between the two (all Ps > 0.05). To account for variations in probe recovery, all data were subsequently expressed as mean percent change from baseline.

Fig. 1 illustrates the dose- and age-related effects of K^+ perfusion on cortical ACh efflux across collection intervals. Consistent and comparable levels of cortical ACh were observed in both young and aged rats throughout the entire VEH (aCSF) session. Concentration-dependent ($F(3, 30) = 25.38$, $P < 0.05$) and time-dependent ($F(11, 110) = 20.36$, $P < 0.05$) increases in cortical ACh efflux were observed

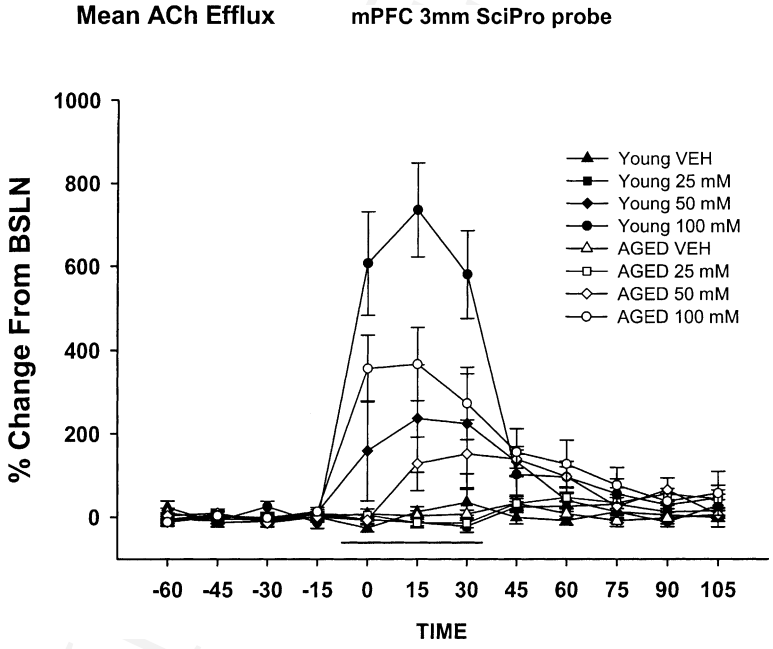

Fig. 1. Mean (±S.E.M.) percent change from baseline ACh efflux in young and aged rats during baseline collections and following perfusion of aCSF containing VEH, 25, 50, or 100 mM K^+ (see inset) through the microdialysis probe. Time along the abscissa refer to the endpoint of the 15 min collection interval. The first K^+ perfusion occurred at 15 min and was removed prior to the 60 min collection.

in both young and aged animals following K^+ perfusion. There was no main effect of age ($P > 0.05$) Relevant to the hypothesis being tested, there was a significant three-way interaction among age, concentration, and time ($F(33, 330) = 2.28$, $P < 0.05$). While the response to the low (25 mM) and middle (50 mM) concentrations of K^+ was comparable between the two ages, cortical ACh efflux following the high concentration (100 mM) in aged subjects (~330% increase) was significantly reduced relative to that seen in young subjects (~650% increase, $t(10) = 2.24$, $P < 0.05$).

4. Discussion

Studies of the regulation of cortical ACh release have typically employed microdialysis techniques that utilize AChE inhibitors in the perfusion medium to improve detection limits. As a consequence of AChE inhibition, persistent elevations in extracellular levels of ACh have been found to influence cholinergic transmission via pre- and post-synaptic muscarinic/nicotinic receptors. These effects may alter the excitability of the basal forebrain cortical cholinergic system (BFCS) via autoreceptors and long-loop feedback pathways [7] as well as alter the regulation of other neurotransmitter systems in cortex and interact with other pharmacological manipulations [4].

Consistent with previous reports [8,9], basal cortical ACh efflux was found to be comparable in young and aged subjects. The data from the present study indicate that without cholinesterase inhibition, and therefore, under more physiologically relevant conditions, no apparent age-related differences in basal dialysate levels of cortical ACh were observed. Neuronal depolarization by perfusion of K^+ through the microdialysis probe produced robust dose-related elevations in cortical ACh efflux. There was, however, an attenuation in K^+-stimulated efflux following the highest concentration (100 mM) in aged animals. These findings replicate prior findings from this laboratory [9] and substantiate an age-related alteration in the normal regulation of ACh release from BFCS projections. The underlying mechanisms of this reduced potency of K^+ to stimulate cortical ACh in aged animals remain to be determined. Although there are indications that these findings are likely not due to limitations in ACh synthesis and release from cortical cholinergic terminals [9], further studies without the use of AChE inhibitors are required to determine the exact nature of this age-related effect. In general, these data serve to further illustrate the utility of in vivo microdialysis for the study of cortical cholinergic transmission in the intact nervous system as well in the cognitive disturbances that accompany aging and a variety of neuropsychiatric disorders [13].

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