ELSEVIER

Brain Research Bulletin 65 (2005) 11–16

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Effects of the metabotropic glutamate receptor agonist, ACPD, on the extracellular concentrations of GABA and acetylcholine in the prefrontal cortex of the rat during the normal process of aging

Gregorio Segovia*, Francisco Mora

Department of Physiology, Faculty of Medicine, Universidad Complutense, Ciudad Universitaria, s/n, 28040 Madrid, Spain

Received 4 May 2004; received in revised form 1 October 2004; accepted 8 October 2004
Available online 15 December 2004

Abstract

The aim of the present study was to investigate the effects of activation of metabotropic glutamate receptors (mGluR) on the extracellular concentrations of GABA and acetylcholine in the prefrontal cortex of freely moving rats of different groups of age. Perfusion, through the microdialysis probe, of the agonist of mGluR, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 100, 500 and 1000 μM), in the prefrontal cortex of young rats produced a dose-related increase of the dialysate concentrations of GABA. The effects of perfusion of ACPD on the concentrations of GABA were attenuated in middle-aged rats. In the prefrontal cortex of aged rats, perfusion of ACPD produced no changes in dialysate concentrations of GABA at any of the doses used. Conversely, perfusion of ACPD (100, 500 and 1000 μM) in the prefrontal cortex of young, middle-aged and aged rats did not modify the dialysate concentrations of acetylcholine. Basal concentrations of acetylcholine in the prefrontal cortex of middle-aged and aged rats were significantly lower than those in young rats. In contrast, basal dialysate concentrations of GABA were not significantly different in young, middle-aged and aged rats. These results suggest that the interaction GABA–glutamate in the prefrontal cortex, mediated by mGluRs, changes with age.

Keywords: Microdialysis; Neurotransmitter interaction

1. Introduction

The prefrontal cortex, due to its role in cognition, emotion and motivation, is a relevant area for understanding the normal process of aging [16]. The prefrontal cortex contains intrinsic glutamatergic pyramidal neurons and GABAergic interneurons that are reciprocally interconnected [11,41]. Glutamatergic terminals arising from other cortical areas and the thalamus and GABAergic terminals arising from the ventrotegmental area have been shown to synapse on neurons in the prefrontal cortex [6,29,41]. Also, the prefrontal cortex receives an important projection from cholinergic neurons located in the basal forebrain that synapse onto the glutamatergic pyramidal neurons [11,42]. Therefore, an imbalance between the neurotransmitters glutamate–GABA–acetylcholine in the prefrontal cortex could be associated with the cognitive deficits that occur during aging [5,19,44].

Several neurobiological changes have been described in the prefrontal cortex of the rat during the normal process of aging [9,15,31,34]. For instance, the activity of cholinergic neurons projecting to the prefrontal cortex seems to be altered during normal aging [4,12,26]. Also, changes in the cortical glutamatergic system, such as an atrophy of the dendritic tree or a reduction in the number and function of ionotropic glutamate receptors, has been described [18,25,28,34]. However, few studies have been devoted to investigate the effects of aging on the interaction of neurotransmitters in this area of the brain [18,30,31].

Based on pharmacological and cloning studies, eight metabotropic glutamate receptors (mGluR) with splice vari-

* Corresponding author. Tel.: +34 91 394 14 37; fax: +34 91 394 16 28.
E-mail address: segoviag@med.ucm.es (G. Segovia).

0361-9230/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2004.10.010

ants have been identified and classified into three groups based on sequence homology, pharmacology and transduction mechanisms [7,10]. Specific age-dependent changes in the expression and binding of these receptors have been described in the frontal cortex of the rat [23,37]. Based on this, the aim of the present study was to investigate the possible changes during aging of the effects of the activation of mGluR on the release of GABA and acetylcholine in the prefrontal cortex of the awake rat. For that the agonist of mGluR, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), was perfused (through the microdialysis probe) in the prefrontal cortex of freely moving rats of different groups of age. ACPD is a non-selective agonist active at all groups of mGluR although with lower affinity for group III receptors [7,10]. Therefore, perfusion of ACPD through the microdialysis probe at the doses used should produce the simultaneous stimulation of all mGluR in the prefrontal cortex.

2. Materials and methods

2.1. Animals and surgery

Young (2–4 months), middle-aged (9–14 months) and aged (22–24 months) male Wistar rats were housed in individual wire mesh cages, provided with food and water ad libitum, and maintained in a temperature-controlled room under a light/dark cycle (lights on/off at 8:00 p.m./8:00 a.m.). All in vivo experiments were conducted during the dark period of the light/dark cycle and followed the guidelines of the International Council for Laboratory Animal Science (ICLAS).

Under Equithesin (2 ml/kg i.p.) anaesthesia rats were stereotaxically implanted with bilateral guide cannulas to accommodate microdialysis probes in the medial prefrontal cortex of the rats [35]: 3.6 mm rostral and 0.9 mm lateral from Bregma and 4.5 mm ventral from dura mater [22].

2.2. Microdialysis

Three to four days after surgery, microdialysis experiments were performed on the freely moving rat as previously described [32,35]. The probes (dialysis membrane of 4 mm in length) were perfused with artificial CSF (composition in mM: NaCl, 137; $CaCl_2$, 1.2; KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 0.5; Na_2HPO_4, 2; glucose, 3; pH 7.3), containing neostigmine 1 μM, at a flow rate of 2 μl/min. After basal concentrations of GABA and acetylcholine were established (3 h of perfusion), 20-min samples were collected and immediately stored at −80 °C until analysed. The first three samples were used as control. After the experiments, the animals were perfused intracardially with 0.9% saline followed by 10% formalin and the brain was removed for verification of the placement of the microdialysis probe.

2.3. GABA and acetylcholine analysis

The GABA content of samples was analysed by reverse-phase HPLC and fluorometric detection, with precolumn *o*-phthalaldehyde derivatisation, according to a method described previously [32,35]. The acetylcholine content of samples was analyzed by HPLC and electrochemical detection [20].

2.4. Chemicals

(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylicacid (ACPD) was purchased from Tocris Cookson Ltd. (Bristol, UK). ACPD was dissolved in CSF from stock solutions (stored at −80 °C) before infusion through the microdialysis probe. Doses of ACPD used (100–1000 μM) are in the range of those described in the literature for in vivo studies [7,30].

2.5. Statistical analysis

One-way ANOVA followed by Dunnett's *t*-test was performed to compare basal dialysate concentration (average of the three sample values) of GABA and acetylcholine in the different groups of age studied. Pearson's coefficient and independence test were used for the study of correlations between basal extracellular concentrations of neurotransmitters and age of the animals. For the study of the effects of aging on the actions of ACPD, a three-way ANOVA (age × dose × time) with repeated measures design was used to perform planned comparisons [40]. For three-way ANOVA analysis, absolute microdialysis data were normalised by subtracting basal concentration (average of the three sample values) to each post-basal sample.

3. Results

3.1. Effects of aging on basal concentrations of GABA and acetylcholine in the prefrontal cortex

Basal dialysate concentrations of GABA were not significantly different in the prefrontal cortex of young, middle-aged and aged rats (Fig. 1). In contrast, basal concentrations of acetylcholine in middle-aged and aged rats were significantly lower than in young rats ($F_{2,42} = 6.22$; $p < 0.01$) (Fig. 1). Moreover, a significant correlation between the basal acetylcholine concentration and age was obtained ($r = -0.46$; $n = 45$; $p < 0.001$).

3.2. Effects of aging on the changes of dialysate GABA and acetylcholine induced by ACPD in the prefrontal cortex

Perfusion of the metabotropic agonist ACPD (100, 500 and 1000 μM) in the prefrontal cortex of young rats increased the dialysate concentrations of GABA: at the dose of