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Inhibitory effects of beta-amyloid on the nicotinic receptors which stimulate glutamate release in rat hippocampus: the glial contribution

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ABSTRACT

We investigated on the neuronal nicotinic acetylcholine receptor subtypes involved in the cholinergic control of *in vivo* hippocampal glutamate (GLU), aspartate (ASP) and inhibitory γ-aminobutyric acid (GABA) overflow. We also investigated on the possible contribution of nicotinic acetylcholine receptors subtypes present on astrocytes in the regulation of the three neurotransmitter amino acids overflow using hippocampal gliosomes and on the effects of beta-amyloid (Aβ) 1–40 on the nicotinic control of amino acid neurotransmitter release. Nicotine was able to enhance the *in vivo* overflow of the three amino acids being more potent in stimulating GLU overflow. The α7 selective agonist PHA543613 induced an overflow very similar to that of nicotine. The α4β2 selective agonist 5IA85380 was significantly less potent in inducing GLU overflow while the overflow of ASP and GABA were almost inconsistent. Aβ1–40 inhibited the neurotransmitter overflow stimulated by PHA543613 but not the one evoked by 5IA85380. In hippocampal gliosomes nicotine elicited selectively GLU overflow which was also evoked by 5IA85380 and by the α7 selective agonist choline. Nicotine- and choline-induced glutamate overflow in gliosomes was inhibited by Aα1–40. In conclusion nicotine administration *in vivo* elicits hippocampal GLU release mostly through α7 nicotinic acetylcholine receptors likely present both on neurons and astrocytes. Aβ inhibitory effect on the nicotinic-control of GLU release seems to depend primarily to the inhibition of α7 nicotinic acetylcholine receptors functional responses.

1. Introduction

It is well established that the cholinergic system modulates the function of glutamatergic system in several brain areas mainly through the activation of neuronal nicotinic acetylcholine receptor subtypes. These receptors are associated to permeability to Ca^{2+} thereby facilitating events such regulation of second messenger cascades (Khiroug et al., 2003), cell survival (Mechawar et al., 2004) and apoptosis (Berger et al., 1998) as well as the direct stimulation glutamate (GLU) release (Toth, 1996; Fedele et al., 1998; Marchi et al., 2002). Moreover, specific intracellular mechanisms have been also shown to be involved in the nicotinic modulation of GLU release (Lambe et al., 2003; Dickinson et al., 2007; Zappettini et al., 2010). This modulatory effect may also be relevant given that, in the hippocampus, glutamatergic and cholinergic systems have undoubtedly a fundamental role in the mechanisms of learning and memory (Picciotto et al., 1995; Levin and Simon, 1998; Giacobini, 2003; Errico et al., 2011; Parri et al., 2011) and both systems undergo age- and pathology-associated changes such as attention and memory (Woodruff-Pak and Gould, 2002; Mattson, 2008).

Recent studies *in vivo* and *in vitro* have shown that beta-amyloid (Aβ) was able to modulate the function of neuronal nicotinic acetylcholine receptor subtypes (Dougherty et al., 2003; Liu and Wu, 2006; Puzzo et al., 2008; Tong et al., 2011; Lilja et al., 2011; Ni et al., 2013). Moreover, non-neurotoxic Aβ 1–40 concentrations were able to modulate the nicotine-evoked release of both excitatory GLU and aspartate (ASP) and inhibitory γ-aminobutyric acid (GABA) and glycine (Mura et al., 2010, 2012; Zappettini et al., 2012). It is important to recall that GLU is essential to memory formation so its function might be pivotal to Alzheimer disease (AD) progression (Revett et al., 2013).

The relevance of the activation of different neuronal nicotinic acetylcholine receptor subtypes which stimulate *in vivo* the release of GLU has been so far poorly investigated. Interestingly, nicotinic acetylcholine receptors are present in several non-neuronal cells

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including astrocytes (Sharma and Vijayaraghavan, 2001; Lim and Kim, 2003; Patti et al., 2007) and therefore part of the GLU which is released *in vivo* might result also from these cells (Santello and Volterra, 2009; Santello et al., 2011). In the present study we have first comparatively investigated, *in vivo*, the stimulatory effects of two selective α4β2 and α7 neuronal nicotinic acetylcholine receptor agonists on the overflow of endogenous GLU, GABA and ASP and the modulatory effects of the Aβ peptide 1–40 on the function of these neuronal nicotinic acetylcholine receptor subtypes. We have then studied the effects of selective neuronal nicotinic acetylcholine receptors agonists and Aβ peptide on amino acid release using hippocampal gliosomes an *in vitro* preparation originating from adult astrocytes (Stigliani et al., 2006; Patti et al., 2007; Milanese et al., 2010; Matos et al., 2012a,b).

From our results we can conclude that the administration *in vivo* of nicotine and of two selective α4β2 and α7 nicotinic acetylcholine receptor agonists elicits a significant release of GLU, ASP and GABA in the rat hippocampus. The inhibitory effect of Aβ on the modulation of the three amino acid release *in vivo* seems to depend primarily on the interaction with the α7 nicotinic acetylcholine receptors. Moreover, both α7 and α4β2 nicotinic acetylcholine receptors are present on rat hippocampal gliosomes and modulate the release of GLU but not that of ASP and GABA and Aβ is able to inhibit only the α7 but not the α4β2 nicotinic acetylcholine receptor subtype.

2. Materials and methods

2.1. Animals and brain tissue preparation

Adult male Wistar rats (200–250 g, Harlan, Udine) were used for both *in vivo* experiments and as brain tissue source for *in vitro* experiments. Animals were housed at constant temperature (22 ± 1 °C) and relative humidity (50%) under a regular light–dark schedule (light 7 a.m. to 7 p.m.). The *in vitro* experimental procedures were approved by the Ethical Committee of the Pharmacology and Toxicology Section, Department of Pharmacy, in accordance with the European legislation (European Communities Council Directive of 24 November 1986, 86/609/EEC) and were approved by Italian legislation on animal experimentation (Decreto Ministeriale number 124/2003-A). The *in vivo* protocol was approved by Ethical Committee of Pavia's University (session of October 11th 2011, minutes 3/2011) according to international regulations for the care and treatment of laboratory animals, to the Italian Act (D.L. n. 116, GU, suppl. 40, 18 February, 1992) and to EEC Council Directive (86/609, OJ L 358, 1, 12 December, 1987). All efforts were made to minimize animal suffering and to use the minimal number of animals necessary to produce reliable results

2.2. In vivo experiments

2.2.1. Microdialysis probe implantation

Rats were anesthetized with Equithesin 3 ml/kg (pentobarbital 9.7 g, chloral hydrate 42.5 g, $MgSO_4$ 21.3 g for 1 l, 10% ethanol, 40% propylene glycol v/v) administered intraperitoneally and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The skin was shaved, disinfected, and cut with a sterile scalpel to expose the skull. A hole was drilled to allow the implantation of the probe into the brain parenchyma. The probe was implanted in the hippocampus (CA1/CA2 regions; AP −5.8 mm, ML ± 5.0 mm from bregma and DV −8.0 mm from dura) according to Paxinos and Watson atlas (1986), and secured to the skull with one stainless steel screw and dental cement. All *in vivo* experiments were performed using microdialysis probes, made in our laboratory according to the original method described by Di Chiara (1990) (EmophanBellco Artificial OR-internal diameter 200 μm, cut-off 40 kDa; Bellco, Mirandola, Modena, Italy), with a nominal active length of 5 mm. Finally, the skin was sutured, and the rats were allowed to recover from anesthesia for at least 24 h before the neurotransmitter release study. Previous immunohistochemical analysis has shown that the administration of Aβ1–40 through the dialysis probe at the concentrations tested *in vivo* (1 μM and 10 μM) allowed the delivery of the peptide within the hippocampus. Despite the fact that we do not know the exact amounts of Aβ reaching the tissue, there was a visible positive correlation between the concentration administered and the signal of Aβ immunoreactivity in the tissue. Moreover, immunohistochemical analysis shows that no evident signs of apoptosis were observed within the area of amyloid diffusion as shown by Hoechst staining (Mura et al., 2012).

2.2.2. Microdialysis samples collection

Microdialysis experiments were performed on conscious freely moving rats. On the day of the experiments (24 h after the surgical procedure), the probe was perfused with artificial CSF containing 145 mM NaCl, 3.0 mM KCl, 1.26 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.4 mM Na_2HPO_4, buffered at pH 7.2–7.4 and filtered through a Millipore 0.2 μm pore membrane. In all experiments, the microdialysis membrane was allowed to stabilize for 1 h at the flow rate of 4 μl/min, without collecting samples. At the end of the stabilization period, three samples were collected to evaluate baseline release of GLU, ASP and GABA and then the specific treatment started. All treatments were administered by manually switching syringes and tubing connections to allow drugs diluted in artificial CSF to flow through the probes. Tubing switches were performed taking care to maintain constant flow rates and collection volumes. Both basal and treatment samples were collected every 20 min in 100 μl Eppendorf tubes. The flow rate of 4 μl/min was maintained using a 1000 μl syringe (Hamilton) and a microinjection pump (CMA/100, CMA/Microdialysis AB). *In vitro* recovery of the probe for GLU was 15.21 ± 0.42 (Mura et al., 2012). Each rat was used for only one microdialysis session. At the end of each experiment, animals were sacrificed by guillotine, rat brains were removed and the position of the microdialysis probe was verified by histological procedures, slicing the tissues by a cryostat microtome (LEICA CM 1510). Only data from rats in which probe tracks were exactly located in the target area were used for statistical analysis.

2.3. In vitro experiments

2.3.1. Experiments of release

Rats were killed by decapitation and the hippocampus rapidly removed at 0–4 °C. Purified gliosomes were prepared essentially as described by Nakamura et al. (1993) with minor modifications (Stigliani et al., 2006). The tissue was homogenized in 10 volumes of 0.32 M sucrose, buffered to pH 7.4 with Tris (final concentration 0.01 M) using a glass Teflon tissue grinder (clearance 0.24 mm). The homogenate was centrifuged at 1000*g* for 5 min, to remove nuclei and cellular debris, and the supernatant was gently stratified on a discontinuous Percoll gradient (2%, 6%, 10% and 20% (v/v) in Tris-buffered sucrose) and centrifuged at 33,500*g* for 5 min. The layer between 2% and 6% Percoll (gliosomal fraction) was collected, washed by centrifugation and resuspended in physiological medium containing 125 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 1 mM NaH_2PO_4, 22 mM $NaHCO_3$ and 10 mM glucose, pH 7.2–7.4 (aeration with 95% O_2 and 5% CO_2). Gliosomes were incubated at 37 °C for 15 min and at the end of the incubation period, identical portions of the gliosomal suspension was layered on microporous filters at the bottom of parallel superfusion