Neurochemistry International 100 (2016) 30–34

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Nicotinic receptor subtypes differentially modulate glutamate release in the dorsal medial striatum

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form
8 August 2016
Accepted 23 August 2016
Available online 26 August 2016

ABSTRACT

The dorsal medial striatum is a crucial part of the neural network that subserves dynamic, goal-directed behaviors. Functional output of this nucleus is shaped, in part, by the influence of glutamatergic inputs. Striatal cholinergic systems have the capacity to modulate these excitatory inputs through presynaptic nicotinic acetylcholine receptors (nAChRs); however, the individual contribution of the two major nicotinic receptor subtypes, α4β2 and α7, to such modulation is not well characterized. In the present experiments, glutamate biosensors were used to monitor nAChR-dependent glutamate release with high temporal precision in the dorsal medial striatum of rats. Both α4β2 and α7 nAChRs were found to potently modulate glutamate release; however the two receptor subtypes do so in strikingly different ways. α7 nAChRs appear to enhance release from glutamatergic terminals. In contrast, α4β2 nAChRs act as a brake on glutamate release via an interaction with local dopaminergic inputs and D2 receptors. Combined, the present data reveal the capacity of local striatal cholinergic signaling to dynamically modulate excitatory inputs through nAChRs.

1. Introduction

The dorsal medial striatum (DMS) is a critical node in the circuitry that affords flexible and motivated action (Balleine and O'Doherty, 2010; DiFeliceantonio et al., 2012; Liljeholm et al., 2011; Richard et al., 2013; Yin et al., 2005a,b). Identifying the neural mechanisms that gate inputs to the DMS is of considerable scientific interest and potential therapeutic value. Anatomical and behavioral evidence suggest that DMS function is heavily influenced by glutamatergic inputs arising from the prefrontal cortex and thalamus, as well as more restricted projections from structures such as the basolateral amygdala (Guo et al., 2015; Mailly et al., 2013; Yin et al., 2005a; 2005b).

Glutamatergic projections to the DMS are likely subject to a high degree of modulation in the terminal field by both dopaminergic input and by local interneuron populations (Lovinger, 2010). Cholinergic interneurons are a major source of acetylcholine in the DMS, and have the capacity to directly modulate striatal input via pre-synaptic nAChRs (Cepeda et al., 2001; Jones et al., 2001; Livingstone and Wonnacott, 2009; Wang et al., 2014a; 2014b). Where presynaptic nAChR modulation of dopaminergic inputs to the DMS has been extensively characterized, direct modulation of glutamatergic inputs by nAChRs is less well understood. This disparity is due in part to a lack of studies directly measuring glutamate release with high temporal and spatial precision in the DMS. In the present experiments, modulation of second-to-second glutamate release in the DMS by the two major nAChR subtypes in the CNS (heteromeric α4β2s and homomeric α7s) was examined using enzyme-selective biosensors. The combined results demonstrate that α4β2 and α7 nAChRs have dissociable influences on glutamate release, giving local cholinergic neurotransmission the capacity to dynamically sculpt excitatory input to the DMS.

2. Methods

2.1. Subjects

Subjects were adult male Long-Evans hooded rats (n = 20), between 3 and 6 months old. All animals were handled and cared for in accordance to the Guide for the Care and Use of Laboratory Animals. All procedures were performed with the approval of the Pfizer Institutional Animal Care and Use Committee.

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http://dx.doi.org/10.1016/j.neuint.2016.08.009
0197-0186/

2.2. Drugs and chemicals

Nicotine, eticlopride (Etic), L-glutamic acid, bovine serum albumin, Nafion™, and glutaraldehyde were obtained from Sigma (St. Louis, MO). Glutamate oxidase (GO; EC 1.4.3.11) was purchased from AMSbio (formerly Seikagaku; Cambridge, MA). MLA and DHβE were purchased from Tocris Biosciences (Bristol, UK).

2.3. Glutamate biosensors

Teflon coated, platinum-iridium wire (Pt/Ir; 203.2 μm coated, 127 μm uncoated diameter; A-M Systems, Carlsborg, WA) was spun together using a Neuralynx (Bozeman, MO) tetrode spinner. A hot-air gun was used to warm the Teflon and bind the two strands. The bounded Pt/Ir wire was then cut in to individual ~1 cm long bundles. The Teflon coating was removed from the bottom ~5 mm of both wires, and these two wires were then separated using forceps. Gold pins were soldered to each wire and used to connect to a potentiostat (EDAQ model EA362, Dual Picostat). The other end of the bundle was left unaltered, leaving two adjacent 127 μm diameter Pt/Ir discs, separated by ~50 μm of Teflon coating, to be used as biosensors.

Electrodes were cleaned and then coated with Nafion™, baked at 170 °C for 4 min, to repel anionic interferents from the Pt/Ir sites. The bound wire assembly was threaded through a guide cannula then insulated and sealed in place with epoxy (Loctite Hysol E−90). One of the two Pt/Ir discs was coated with glutamate oxidase (GO) cross-linked with a bovine serum-albumin (BSA)-glutaraldehyde mixture (1% GO, 1% BSA and 0.125% glutaraldehyde) under a microscope using a 25 gauge, 1-μL Hamilton syringe (Hamilton, Reno, NV, USA). The second disc was coated with the BSA-glutaraldehyde solution alone and served to record background activity. Enzyme-coated microelectrodes were then allowed to cure for 48−72 h in a desiccator.

2.4. Calibration and preparation of glutamate biosensors

Prior to use *in vivo*, each biosensor was calibrated *in vitro* to confirm sensitivity and selectivity for glutamate over other common electroactive species in the brain. Calibrations were performed using fixed-potential amperometry with a voltage of 0.7 versus an Ag/AgCl reference electrode in a beaker containing 0.05 M PBS solution, constantly stirred at 37 °C. Data were acquired at a rate of 10 Hz. After allowing 20 min for stabilization of background currents, ascorbic acid (AA; 20 mM), glutamate (20 mM), and dopamine (DA; 2 mM) were added to the calibration beaker such that the final concentrations of the solutions were 250 μM AA, 20, 30, and 35 μM glutamate and 2 μM DA. The slope (sensitivity), linearity (R^2), and selectivity ratio over AA, were calculated for each individual recording site. The electrodes used in the present experiments were characterized by sensitivity for detecting glutamate: 15.19 ± 1.71 pA/μM, selectivity for glutamate:AA of 140.88 ± 22.29, and a highly linear response to increasing glutamate concentrations (20−35 μM): R^2: 0.99 ± 0.00.

After calibration, electrodes were equipped with a 33 gauge infusion stylet threaded through a 27 gauge guide cannula (Plastics One, Wallingford, CT). The infusion stylet extended 2 mm below the bottom of the guide cannula, and was positioned in sculpting putty such that is rested in between the 2 working electrodes of the biosensor, ~50−100 μm away from the recording surfaces. Once positioned, the guide cannula/infusion stylet was fixed into place with dental wax.

2.5. Surgery and electrode implantation

Animals were anesthetized with urethane (1.5 g/kg). When the pedal reflex could no longer be elicited, animals were placed into a stereotaxic frame (Kopf Instruments, Tujunga, CA). The skin above the skull was removed and three craniotomies were made: 1) One craniotomy was centered over the DMS to accommodate the glutamate biosensor (AP: +1.8, ML: ± 1.8, DV: −3.5−4.5). 2) Another craniotomy was made in the opposite hemisphere to accommodate the Ag/AgCl reference electrode. A final craniotomy was made in the same hemisphere as the glutamate biosensor where a stainless steel auxillary electrode was implanted. Dura was carefully peeled back above the DMS, and the glutamate biosensor/infusion cannula assembly was then immobilized and slowly lowered into the DMS with a micromanipulator (Narishige model MO-10, Tokyo).

2.6. Experimental protocol

To explore the capacity of nAChRs to modulate glutamate release in the DMS, we adapted a protocol used to study nAChR modulation of neurotransmitter release in the rodent cortex (Parikh et al., 2008, 2010). A single concentration and volume of nicotine (4 nmol in 200 nL total), selected based on previous data showing that such concentrations produce robust increases in cortical glutamate release (Parikh et al., 2008, 2010), was infused into the recording region over the course of 5 s via the infusion stylet connected to a 2 μL Hamilton syringe via polyethylene tubing. The capacity of local nicotine infusions to evoke changes in extracellular glutamate concentrations was characterized at baseline through a series of 2 local infusions, spaced 10 min apart. After these 2 infusions, the α4β2 nAChR antagonist DHβE (1.6 nmol in 500 nL), the α7 nAChR antagonist MLA (800 pmol in 500 nL), the D2 antagonist eticlopride (Etic; 1.33 nmol in 500 nL), or aCSF vehicle (500 nL) was then infused into the recording region. After allowing 10 min for current signals to stabilize, the capacity of nicotine (4 nmol) to evoke glutamate release was again characterized.

2.7. Glutamate signal processing

For the *in vivo* experiments, data were acquired at a rate of 1 Hz. Given the possibility that oxidation of dopamine could contribute to measured signals, current recordings from each platinum site were normalized by dividing the raw current value at each time point by the change in current following the addition of DA observed on that site during calibration. The normalized currents were then "self-referenced" by subtracting the currents recorded at the control sites form those recorded on glutamate oxidase-coated sites (Parikh et al., 2004), yielding currents that reflect the oxidation of glutamate at the electrode surface. The peak amplitude of current change after local drug infusion was calculated as the difference between the average self-referenced current value over the 10 s preceding the infusion and the largest current deflection over the 100 s immediately following the infusion. For the quantification of the effects of local drug administration on the amplitudes of evoked glutamate release, the peak amplitudes from the pre-antagonist (or vehicle) nicotine infusions were compared to post-antagonist (or vehicle) infusions and expressed as a percentage of pre-antagonist (or vehicle) release. These percent of baseline values were averaged for each post-antagonist infusion, yielding 1 post-infusion value per animal and condition for statistical comparison.

2.8. Statistical analysis

The changes in glutamate signal amplitudes following each drug