Neuropharmacology 105 (2016) 318–328

Contents lists available at ScienceDirect

Neuropharmacology

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

Nicotinic acetylcholine receptor-mediated GABAergic inputs to cholinergic interneurons in the striosomes and the matrix compartments of the mouse striatum

Ritsuko Inoue [a], Takeo Suzuki [a, b, c], Kinya Nishimura [a, b], Masami Miura [a, *]

[a] Neurophysiology Research Group, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173-0015, Japan
[b] Department of Anesthegiology and Pain Management, Juntendo University School of Medicine, Tokyo 113-8421, Japan
[c] Department of Anesthesia, Tokyo Metropolitan Bokutoh Hospital, 4-23-15 Kotobashi, Sumida-ku, Tokyo 130-8575, Japan

ARTICLE INFO

Article history:
Received 12 September 2015
Received in revised form
30 December 2015
Accepted 20 January 2016
Available online 22 January 2016

Keywords:
Acetylcholine
Nicotinic
Cholinergic interneuron
Striosome
Matrix

ABSTRACT

The striatum consists of two neurochemically distinct compartments: the striosomes (or patches) and the extrastriosomal matrix. Although striatal neurons are strongly innervated by intrinsic cholinergic interneurons, acetylcholinesterase is expressed more abundantly in the matrix than in the striosomes. At present, little is known about the different cholinergic functions of the striatal compartments. In this study, we examined gamma-aminobutyric acidergic (GABAergic) inputs to cholinergic interneurons in both compartments. We found that nicotinic receptor-mediated GABAergic responses were evoked more frequently in the matrix than in the striosomes. Furthermore, a single action potential of cholinergic neurons induced nicotinic receptor-mediated GABAergic inputs to the cholinergic neurons themselves, suggesting mutual connections that shape the temporal firing pattern of cholinergic neurons. The nicotinic receptor-mediated GABAergic responses were attenuated by continuous application of acetylcholine or the acetylcholinesterase inhibitor eserine and were enhanced by desformylflustrabromine, a positive allosteric modulator of the α4β2 subunit containing a nicotinic receptor. These results suggest that the nicotinic impact on the GABAergic responses are not uniform despite the massive and continuous cholinergic innervation. It has been reported that differential activation of neurons in the striosomes and the matrix produce a repetitive behavior called stereotypy. Drugs acting on α4β2 nicotinic receptors might provide potential tools for moderating the imbalanced activities between the compartments.

1. Introduction

The striatum is developmentally, anatomically, and neurochemically divided into two distinct compartments: the striosomes (or patches) and extrastriosomal matrix (Eblen and Graybiel, 1995; Fujiyama et al., 2011; Gerfen, 1984; Graybiel et al., 1981; Graybiel and Ragsdale, 1978; Kawaguchi et al., 1989; Martin and Cork, 2014; Newman et al., 2015; Penny et al., 1988). Expression of acetylcholinesterase (AChE), a cholinergic marker in the brain, is most abundant in the matrix, and relatively low in the striosomes (Graybiel et al., 1986). Acetylcholine (ACh) is critically important for the striatal functions of motor control, reinforcement, and habit learning (Aosaki et al., 2010; Crittenden et al., 2014; Kaneko et al., 2000; Okada et al., 2014). Cholinergic interneurons that release ACh are few in number, but they prominently innervate throughout the striatum. Although the cholinergic interneurons are distributed throughout the striatum, they preferentially locate at the periphery of the striosomes in human, monkey, cat, and rodents (Bernacer et al., 2007; Jakab et al., 1996). Cholinergic dendritic arbors are dense in the matrix, because the striosomal cholinergic neurons extend their dendrites toward the matrix compartments

Abbreviations: ABT-418, 3-Methyl-5-[(2S)-1-methyl-2-pyrrolidinyl]isoxazole hydrochloride; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ACh, acetylcholine; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; GABA, γ-aminobutyric acid; dFBr, desformylflustrabromine hydrochloride; IPSC, inhibitory post-synaptic currents; mAChR, muscarinic acetylcholine receptor; nAChR, nicotinic acetylcholine receptor; PNU-120596, N-(5-Chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-urea.

* Corresponding author. 35-2, Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan.
E-mail addresses: inoritu@tmig.or.jp (R. Inoue), takeon@da2.so-net.ne.jp (T. Suzuki), juntendoski-50kinen@live.jp (K. Nishimura), mmiura@tmig.or.jp (M. Miura).

http://dx.doi.org/10.1016/j.neuropharm.2016.01.029
0028-3908/

(Crittenden et al., 2014; Graybiel et al., 1986). Furthermore, choline acetyltransferase (ChAT), a synthetic enzyme for ACh, is expressed in matrix cholinergic neurons later in development, suggesting the existence of subpopulations of cholinergic neurons (Van Vulpen and Van Der Kooy, 1996). Therefore, distinct cholinergic functions have been proposed for the striosomes and the matrix (Alcantara et al., 2001; Holt et al., 1996; Prensa et al., 1999).

We previously reported that endogenous striatal ACh and opioid substances have opposing effects on the activity of neural circuits only in the striosomes (Inoue et al., 2012). In the striosomes, activation of the μ-opioid receptor (MOR), expressed in striosomal medium-spiny (MS) projection neurons, reduces γ-aminobutyric acid type A ($GABA_A$) receptor-mediated currents. The suppressive effect of GABAergic inhibitory post-synaptic currents (IPSCs) is antagonized by the activation of M1 muscarinic acetylcholine receptors (mAChRs). In contrast to muscarinic drugs, the activation of nicotinic acetylcholine receptor (nAChR) lacks an apparent effect on MS projection neurons, although GABAergic interneurons respond to nicotinic stimulation (Luo et al., 2013). Considering that cholinergic interneurons receive inhibitory inputs from GABAergic interneurons (Gonzales et al., 2013), ACh released from cholinergic neurons might excite the nAChR-expressing GABAergic neurons, which then generate inhibitory inputs back to the cholinergic neurons themselves. Indeed, stimulation of cholinergic fibers evoked nAChR-mediated polysynaptic GABAergic currents in cholinergic neurons (Sullivan et al., 2008) and MS projection neurons (Miura et al., 2006). The mutual innervation between cholinergic and GABAergic neurons may provide a mechanism for shaping the temporal pattern of striatal activities, which contributes to the initiation and control of voluntary movements and habit learning (Aosaki et al., 1994; Brown et al., 2012; Cragg, 2006). These findings were likely obtained in the matrix, because of the small proportion of striosomes in the striatum. Here, we investigated whether these mutual circuits exist and work in the striosomes. We found that nAChR-mediated GABAergic inputs are more prominent in the matrix cholinergic neurons than in the striosomes, suggesting differing cholinergic modulation via nACh in the striosomes and the matrix compartments.

2. Materials and methods

2.1. Slice preparation

All experimental procedures were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology and were carried out in accordance with the Guidelines for Animal Experimentation of the Ministry of Education, Culture, Sports, Science, and Technology of Japan as well as the guidelines of the National Institutes of Health in the United States (Publications No. 8023, revised 1996). All efforts were made to minimize animal suffering and reduce the number of animals used. We used male and female TH-GFP mice (Matsushita et al., 2002) aged 15–28 days. Mice were anesthetized and decapitated. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 3 mM KCl, 1 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, and 10 mM glucose buffered to pH 7.4 with 26 mM $NaHCO_3$ and saturated with 95% O_2 and 5% CO_2. Coronal striatal slices of 300 μm in thickness were cut using a Pro 7 Linear Microslicer (Dosaka, Kyoto, Japan).

2.2. Electrophysiology

Striatal slices were placed on a recording chamber and continuously perfused with ACSF at a rate of 2.0–2.5 ml/min at 30 °C. Whole-cell patch-clamp recordings were collected by an EPC 10 Amplifier (Heka Elektronik, Lambrecht/Pfalz, Germany), with infrared differential contrast (IR-DIC) and fluorescence visualized using an Olympus BX50WI (Olympus, Tokyo, Japan) and two charge-coupled device cameras (Hamamatsu Photonics, Shizuoka, Japan; Dage-MTI, Michigan, IN, USA). Patch pipettes (4–6 MΩ) were fabricated from borosilicate glass capillaries (1.5 mm in outer diameter and 1.17 mm in inner diameter; Harvard Apparatus Ltd., Edenbridge, United Kingdom) on a PC-10 Puller (Narishige, Tokyo, Japan). For both current-clamp and voltage-clamp recordings, patch pipettes contained the following: 90 mM K-gluconate, 45 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 0.1 mM EGTA, 0.1 mM spermine, 4 mM Na_2-ATP, 0.3 mM Na-GTP, and 0.5% biocytin (brought to pH 7.3 with KOH; osmolarity: 280 mOsm; equilibrium potential of chloride (E_{Cl}): -24 mV). For some recordings, pipettes were filled with: 124 mM Cs-methanesulfonate, 11 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 4 mM Na_2-ATP, 0.3 mM GTP, 0.1 mM spermine, 5 mM QX-314, and 0.5% biocytin (brought to 280 mOsm and pH 7.3 with CsOH; E_{Cl}: -58 mV). Series resistance was compensated at 70%.

Striosomes were identified as bright areas with enhanced green fluorescent protein (EGFP) under a fluorescence microscope, as previously described (Inoue et al., 2012; Miura et al., 2007). We then switched to infrared-differential interference contrast visualization to find a cholinergic interneuron in one of the striosomes. The pipette was attached to the cell and suctioned after obtaining a stable seal of >1 GΩ to achieve the whole-cell configuration. Cholinergic neurons and fast-spiking (FS) interneurons in the striatum were identified according to their morphology using an IR-DIC video microscope. For the recording of FS neurons, we chose the cells having round and slightly larger cell bodies when compared to those of MS projection neurons. Their cell types were further identified by passing hyperpolarizing and depolarizing current pulses through them to determine their input resistances, spike widths, and firing patterns (Kawaguchi, 1993). In the experiments using Cs-based intracellular solution, we chose cells that had large elliptical somas with one or more thick dendrites. After electrical recordings, their morphology and the striosomes/matrix compartments were further confirmed by histochemical procedures (see below).

All recordings were taken from cells in the dorsal striatum in the presence of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptor antagonist; 25 μM D-(−)-2-amino-5-phosphonopentanoic acid (AP5), an N-methyl-D-aspartate receptor antagonist; and 1 μM atropine, a muscarinic receptor antagonist. Data were corrected for a junction potential of −10.2 mV and −9.7 mV for K-based and Cs-based solutions, respectively. If series resistance changed by 15% or more, the data were discarded. All drugs were purchased from Sigma (St Louis, MO, USA), except for desformylflustrabromine hydrochloride (dFBr) and AP5, which were purchased from Tocris Bioscience (Bristol, United Kingdom).

2.3. Histological procedures and morphological analysis

After recording, slices were fixed in phosphate-buffered 4% paraformaldehyde with 0.2% picric acid for 24 h at 4 °C. Slices were blocked with 10% normal goat serum and 0.3% Triton X-100 in phosphate-buffered saline (PBS) for 1 h and then incubated for 24 h with anti-μ-opioid receptor antibody (1:1000; Chemicon, Temecula, CA, USA) and Streptavidin Alexa Fluor 647 (1:500, Invitrogen, Carlsbad, CA, USA) at 4 °C. After the slices had been washed with PBS containing 0.1% normal goat serum, 1% bovine serum albumin, and 0.3% Triton X-100, they were incubated with Alexa Fluor 555-conjugated goat anti-rabbit IgG secondary antibody (1:500, Invitrogen). The slices were washed with PBS and mounted in