Neuropharmacology 56 (2009) 975–983

Contents lists available at ScienceDirect

Neuropharmacology

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

Effects of nicotine on K^+ currents and nicotinic receptors in astrocytes of the hippocampal CA1 region

Miriam Hernández-Morales, Jesús García-Colunga*

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Boulevard Juriquilla 3001, Juriquilla, Querétaro 76230, Mexico

ARTICLE INFO

Article history:
Received 16 July 2008
Received in revised form
14 January 2009
Accepted 29 January 2009

Keywords:
Glial cells
Hippocampus
Nicotinic acetylcholine receptors
Potassium current
Astrocytes

ABSTRACT

Nicotine, the main addictive substance in tobacco, interacts with muscle and neuronal nicotinic acetylcholine receptors (nAChRs) that are also localized in astrocytes. We studied electrical effects elicited by nicotine in cultured astrocytes from the CA1 area of the rat hippocampus. Nicotine elicited different types of responses: sustained inward currents, decaying inward currents, and biphasic responses (an outward, followed by an inward current). Nicotine showed two opposite effects, an increase or a decrease of astrocyte membrane conductance, when voltage ramps were applied during sustained inward currents. The former was isolated by blocking K^+ currents with Cs^+ and was inhibited by mecamylamine. The latter was mimicked by tetraethylammonium ion, and was obtained in the presence of nAChR antagonists (mecamylamine, methyllycaconitine plus dihydro-β-erythroidine). Thus, these results indicate that nicotine activates nAChRs and directly inhibits K^+ currents in cultured astrocytes from the CA1 region of the rat hippocampus.

1. Introduction

Nicotine is the main substance responsible for tobacco dependence. Nicotine actions improve cognitive functions such as attention, learning, and memory and are also involved in certain pathologies, including Parkinson's and Alzheimer's diseases, schizophrenia, anxiety, and depression (Jensen et al., 2005; Le Houezec, 2003; Levin et al., 2006). All of these effects seem to be mediated by nicotine acting on nicotinic acetylcholine receptors (nAChRs) in several brain areas (Gotti et al., 2006; Mansvelder et al., 2006).

The nAChRs are ligand-gated cation channels endogenously activated by acetylcholine. They are pentameric proteins composed of distinct subunits (α2–α10 and β2–β4) and are widely distributed in the brain. Each nAChR subtype has different biophysical and pharmacological properties, depending on its subunit composition (Jensen et al., 2005). Activation of nAChRs allows the movement of Na^+, K^+, and Ca^{2+} across the cell membrane, resulting in a depolarizing response, whereas long exposure to the agonist desensitizes a fraction of nAChRs. The diverse functions and locations of nAChR subtypes underlie their modulating roles throughout the brain. Thus, presynaptic nAChRs modulate neurotransmitter release, postsynaptic nAChRs participate in fast excitatory transmission, and non-synaptic nAChRs interfere with many neurotransmitter systems by influencing neuronal excitability (Dani and Bertrand, 2007; Gotti et al., 2006).

Interestingly, nicotine also interacts at sites different from nAChRs, inhibiting voltage-gated K^+ channels (delayed-rectifying, inward-rectifying, and transient A-type channels) in ventricular myocytes, arterial smooth muscle cells, and heterologous expression systems. In these studies nicotine blocked K^+ currents, without activating nAChRs, by interacting directly with K^+ channels (Satoh, 2002; Tang et al., 1999; Wang et al., 1999a,b, 2000a,b).

On the other hand, nicotine produced membrane hyperpolarization, depolarization, or both effects in spinal cord astrocytes (Hösli et al., 1988). Furthermore, in cortical astrocytes, nicotine increased the intracellular Ca^{2+} concentration, and the increase was partially inhibited by the nAChR antagonists dihydro-β-erythroidine and methyllycaconitine (Oikawa et al., 2005). Although there are few studies on the effects of nicotine on astrocytes, they show different types of responses. For this reason, in the present work we studied two possible effects of nicotine in cultured astrocytes from the CA1 area of the rat hippocampus, activation of nAChRs and blockade of K^+ currents. Part of these results has been reported in abstract form (Hernández-Morales and García-Colunga, 2007).

2. Materials and methods

2.1. Astrocyte cultures

Every effort was made to minimize suffering and the number of animals used in all experiments. The animal experiments were approved by the ethical policies for

* Corresponding author. Tel./fax: +52 442 238 1063.
E-mail address: garciac@inb.unam.mx (J. García-Colunga).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.01.024

animal care and handling of the Universidad Nacional Autónoma de México. Astrocyte cultures were obtained as previously described (Montiel-Herrera et al., 2006; Reyes-Haro et al., 2005). Two newborn Wistar rats were decapitated and pithed. The brains were immediately removed and placed in a Petri dish containing cold control solution (see the section on Electrophysiological recordings). Brain slices were then obtained to dissect the hippocampus. Small pieces of the hippocampal CA1 area were cut using sharp needles. The pieces of tissue were placed in a 2-mL Eppendorf tube with control solution and passed several times through a Pasteur pipette to mechanically dissociate the cells. The suspension of cells was placed in a 35-mm Petri dish on a glass coverslip coated with 0.01% poly-L-ornithine. Then, we added 2 mL Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, 11 mg/mL sodium piruvate, 100 units/mL penicillin, and 100 μg/mL streptomycin. To select for astrocytes, after 24 h of culture, the Dulbecco's Modified Eagles Medium was replaced by serum-free neurobasal medium with 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and G5 supplement. The medium was changed every four days. All culture reagents were purchased from Gibco BRL (Carlsbad, CA, USA).

2.2. Immunocytochemical assay

After 4–7 days in culture, cells were rinsed twice with 0.1 M phosphate buffered saline (PBS, pH 7.4), fixed with 100% methanol for 10 min at −20 °C, rinsed three times with PBS, and kept for 1 h at room temperature in PBS containing 0.1% Triton X-100 plus 10% horse serum. Then, cell cultures were incubated overnight at 4 °C with a primary rabbit polyclonal antibody directed against glial fibrillary acidic protein (GFAP; Diasorin, Stillwater, MN, USA) diluted 1:10 in PBS. The cells were then washed three times with PBS and incubated for 1 h at room temperature with a secondary, fluorescein isothiocyanate (FITC)-conjugated goat antibody raised against rabbit IgG (Sigma, St. Louis MO, USA) at a dilution 1:250. Finally, coverslips were washed three times with PBS and mounted with glycerol. Cells were visualized with epifluorescence microscopy to determine the percent of immunoreactive cells. Images were digitalized with a monochromatic camera and edited with commercial software. In control experiments, cells were treated with the secondary antibody alone and did not fluoresce significantly.

2.3. Electrophysiological recordings

Membrane currents were recorded using the whole-cell voltage-clamp technique (Hamill et al., 1981). A fragment of coverslip, on which astrocytes had been cultured for 4–7 days, was placed in a recording chamber (~200 μL) on the stage of an inverted microscope (Olympus IX-70, Japan). The recording chamber was continuously superfused (4 mL/min) with control solution containing (in mM): 136 NaCl, 2.5 KCl, 2 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, and 10 D-glucose, pH adjusted to 7.4 with NaOH. Recording pipettes were made with borosilicate capillaries using a Sutter P-97 micropipette puller (Sutter Instruments, CA, USA). Pipettes were filled with a solution containing (in mM): 130 K-gluconate, 10 NaCl, 10 HEPES, 10 EGTA, 2 Mg-ATP, and 0.2 Na-GTP, pH adjusted to 7.2 with KOH, having resistance of 3–5 MΩ. In one set of experiments, pipettes were filled with a solution containing (in mM): 80 CsCl, 80 CsF, 10 EGTA, and 10 HEPES, pH adjusted to 7.2 with CsOH. All experiments were carried out at room temperature (20–23 °C). Transient capacitive and leak currents were electronically and digitally subtracted. The currents were recorded with an Axopatch 200B amplifier (Axon instruments, CA, USA), filtered at 5 kHz, and digitized at 100 Hz (for currents generated by nicotine), 3.3 kHz (for voltage pulses), or 0.5 kHz (for voltage ramps). The data were analyzed with pClamp 8.2 (Axon Instruments, CA, USA) and Microcal Origin 7 (OriginLab, MA, USA) software. The data are given as mean ± standard error. Comparisons of the mean values among groups were performed by the Student's *t*-test, where $P < 0.05$ was taken to indicate a statistically significant difference.

2.4. Drugs

The drugs used were 4-aminopyridine, mecamylamine hydrochloride (Tocris Ellisville, MO, USA), acetylcholine chloride, atropine, dihydro-β-erythroidine hydrobromide, (−)-nicotine hydrogen tartrate salt, methyllycaconitine citrate hydrate, and tetraethylammonium chloride (Sigma–Aldrich, St. Louis, MO, USA). The drugs were prepared as concentrated stocks in distilled water and stored frozen. On the day of use, drugs were diluted in control solution and applied in the bath superfusion fluid by gravity.

3. Results

Within a single study there are different and opposite effects induced by nicotine, either a hyperpolarization or a depolarization of astrocytes from the spinal cord, both mediated by activation of nAChRs (Hösli et al., 1988). To date there are few studies related to effects of nicotine on astrocytes (Hösli et al., 1988; Oikawa et al., 2005). Therefore, we explored the effects of nicotine on membrane currents in astrocytes from the CA1 area of the hippocampus.

Confluent cell cultures ($n = 5$) were immunolabeled with an antibody against glial fibrillary acidic protein (GFAP), and more than 99% of the cells were positive, indicating their astrocytic lineage (Fig. 1A and B). The cells were also identified as astrocytes according to morphological and electrophysiological criteria. They have an irregular cell body (from rounded to oval) with branching processes and, like hippocampal astrocytes *in situ* (Araque et al., 2002; Bushong et al., 2002), they present neither rapid Na^+ currents nor action potentials.

3.1. Nicotine induces different electrical responses in astrocytes

For recording purposes we used completely isolated astrocytes after 4–7 days in culture, i.e., with no neighboring cells. Of 180 astrocytes, 76 responded to the application of 1 mM nicotine. Interestingly, these cells showed different types of responses. Thus, nicotine generated a sustained inward current in 33 astrocytes with amplitude of 223 ± 32 pA (Fig. 1C, $n = 33$). In 11 astrocytes, nicotine induced an inward current that decayed in the continuous presence of nicotine, and the amplitude was highly variable, between 31 and 957 pA (Fig. 1D, $n = 11$). On the other hand, nicotine induced biphasic responses in 25 cells, an outward current (94 ± 25 pA) followed by an inward current (140 ± 22 pA) (Fig. 1E, $n = 25$). Finally, in a few astrocytes nicotine induced an outward current (data not shown), with amplitude between 41 and 1051 pA ($n = 7$). In all cases the current returned to its basal level after nicotine was removed. Hippocampal astrocytes responded to concentrations of nicotine as low as 1 μM; an example of a response to 10 μM nicotine is shown in Fig. 1D (inset; 56 ± 20 pA, $n = 8$).

It is important to note that no correlation was observed between the day of culture of the astrocytes (4–7 days) and the type of response induced by nicotine. Furthermore, the diverse responses induced by nicotine were observed in different astrocytes from the same cell culture. In addition, the different responses were independent of the nicotine concentration, and successive nicotine applications on one astrocyte evoked the same type of response (data not shown).

3.2. Opposite effects of nicotine on hippocampal astrocytes

Although nicotine elicited a variety of responses, including sustained and transient inward currents, outward currents, and biphasic currents, we focused on sustained inward currents, because these were the majority (43% of the responding astrocytes), and because of the possible relevance of such effect, in which a depolarization of an *in situ* astrocyte may lead to an increase of the intracellular Ca^{2+} concentration and then to the release of a transmitter that modulates neuronal activity (Jourdain et al., 2007; Parpura et al., 1994).

To examine nicotine effects on voltage-dependent currents, current–voltage (*I–V*) relationships were obtained by applying 1-s voltage ramps (from −120 to 80 mV) before and during the nicotine-induced, sustained inward current (Fig. 2A and B; vertical lines a–d). In 9 of 32 cells, the current generated by voltage ramp during the nicotine response showed an increase of the inward current and, to a lesser extent, an increase of the outward current, both as compared with the control (Fig. 2Ca and b). In this type of responses, the reversal potential of the *I–V* relationship during nicotine-induced current was displaced to positive voltages with respect to the control *I–V* relationship. In addition, the slope of the *I–V* relationship, which corresponds to the membrane conductance, increased at negative voltages compared with the control *I–V* relationship.