A Non-α7 Nicotinic Acetylcholine Receptor Modulates Excitatory Input to Hippocampal CA1 Interneurons

MANICKAVASAGOM ALKONDON1 AND EDSON X. ALBUQUERQUE1,2

1Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201; and 2Departamento de Farmacologia Básica e Clínica, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21944, Brazil

Received 22 August 2001; accepted in final form 6 November 2001

Alkondon, Manickavasagom and Edson X. Albuquerque. A non-α7 nicotinic acetylcholine receptor modulates excitatory input to hippocampal CA1 interneurons. J Neurophysiol 87: 1651–1654, 2002; 10.1152/jn.00708.2001. The nonnicotinic acetylcholine receptor (nAChR), particularly the α7 subtype, has received profound attention for its role in modifying excitatory postsynaptic currents (EPSCs) in hippocampal pyramidal neurons as well as in neurons from other brain regions. Here, we tested the possibility that an nAChR could affect EPSCs in the interneurons of rat hippocampal slices. Using whole-cell patch-clamp technique on CA1 stratum radiatum interneurons and U-tube application of agents, we show that nicotinic agonists enhance EPSC frequency in interneurons. Among the agents tested, cytisine and mecamylamine were the most effective agonist and antagonist, respectively, suggesting a role for α3β4-containing nAChRs in the modulation of interneuron EPSCs. Ligands selective for the α7 nAChR had very little or no effect on interneuron EPSCs. Low concentrations of nicotine also enhanced EPSC frequency, implicating the involvement of non-α7 nAChRs in controlling interneuron excitability in smokers. We conclude that nAChR-dependent EPSC modulation in the hippocampus is both subtype- and neuron-specific and that a non-α7 nAChR, presumably α3β4, controls glutamate transmission to CA1 interneurons.

INTRODUCTION

In several brain regions, the strength of glutamate-dependent neurotransmission can be enhanced via nicotinic acetylcholine receptors (nAChRs), primarily through the highly Ca2+-permeable α7 subtype (Albuquerque et al. 1997; Alkondon et al. 1996; Aramakis and Metherate 1998; Gil et al. 1997; Gray et al. 1996; Ji et al. 2001; McGehee et al. 1995); however, in certain brain areas, non-α7 nAChRs affect glutamate transmission (Gil et al. 1997; Guo et al. 1998; Vidal and Changeux 1993). Nicotine-induced long-term potentiation has been attributed to its action on α7 nAChRs present on glutamate terminals (Fujii et al. 2000; Mansvelder and McGehee 2000). In the rat hippocampus, pyramidal neuron excitatory postsynaptic current (EPSC) is enhanced by α7 nAChR activation (Gray et al. 1996; but see Vogt and Regehr 2001); however, it is not known whether interneuron EPSCs are regulated by α7 or any other nAChRs. Because glutamate EPSCs remain the major excitatory stimulus to the interneurons (McBain et al. 1999), we tested the effects of activation of nAChRs on interneuron EPSCs in the hippocampus and attempted to determine pharmacologically the nature of the nAChR subtype involved.

METHODS

Whole-cell patch-clamp experiments were performed on CA1 stratum radiatum (SR) interneurons of rat hippocampal slices obtained from the brain of 15- to 24-day-old Sprague-Dawley rats. All recording conditions, solutions, and analyses were as previously described (Alkondon et al. 2000). The slices were superfused with artificial cerebrospinal fluid (ACSF) at 2 ml/min in the presence of atropine (1 μM) to block the muscarinic receptors. All recordings were performed at room temperature (20–22°C). Agonists were applied via a U-tube, and antagonists were applied via bath superfusion (Alkondon et al. 2000). Data are expressed as the mean ± SE.

RESULTS

In the absence of any antagonists, both EPSCs and inhibitory postsynaptic currents (IPSCs) were recorded from CA1 SR interneurons at −68 mV (Fig. 1A). The EPSCs could be blocked by 6-cyano-7-nitroquinolinone-2,3-dione (CNQX, 10 μM), whereas the IPSCs were sensitive to bicuculline (5 or 10 μM). Glutamate EPSCs were short in duration (rise time = 1.08 ± 0.06 ms; τdecay = 3.8 ± 0.34 ms; n = 9), and GABAergic IPSCs longer (rise time = 2.83 ± 0.20 ms; τdecay = 14.6 ± 1.29 ms; n = 9). Following this initial characterization, bicuculline (5 or 10 μM) was routinely included in the ACSF to block IPSCs that allowed the study of EPSCs in isolation.

U-tube application of a short pulse of acetylcholine (ACh; 100 μM) increased the frequency of EPSCs in 72 of 104 CA1 SR interneurons (Fig. 1B). CNQX (10 μM) abolished both spontaneous and ACh-triggered EPSCs (Fig. 1B). Although ACh increased significantly (P < 0.001 by Student’s paired t-test) the frequency of EPSCs (n = 72 neurons) from a control value of 0.09 ± 0.011 to 0.76 ± 0.074 Hz, neither the rise time (0.99 ± 0.05 ms in control to 1.07 ± 0.05 ms in ACh) nor the τdecay (2.83 ± 0.15 ms in control to 2.70 ± 0.14 ms in ACh) of the EPSCs was changed by the agonist. In 54 of 72 neurons, ACh also significantly enhanced the mean peak amplitude of EPSCs (P < 0.001) from a value of 17.2 ± 0.9 to 25.0 ± 1.5 pA (Fig. 1B). In the other 18 neurons, the ACh-induced in-
crease in the frequency of EPSCs was not accompanied by a change in the peak amplitude of the currents. ACh-induced changes were also evident in the cumulative plots of the inter-event interval and the amplitude of the events (Fig. 1C).

To investigate which nAChR subtype might be involved in the modulation of glutamate transmission to the interneurons, different nicotinic agonists were compared with respect to their effectiveness to increase the frequency of EPSCs. It has been shown that a preferential activation of certain nAChR subtypes occurs with choline (α7), dimethyl phenyl piperazinium (DMPP; α3β2), and cytisine (α3β4) (Albuquerque et al. 1997; Luetje and Patrick 1991; Quick et al. 1999), and therefore, a rather reliable correlation can be made between sensitivity to agonist and nAChR subtype. U-tube application of nicotine (100 μM; 6–12 s) triggered a short burst of EPSCs, including some with large amplitudes that were rarely observed under control (Fig. 2A). In neurons (n = 6) in which ACh (100 μM) elicited both nicotinic inward current and enhanced frequency of EPSCs, choline (1 mM; 12 s) induced a slight increase in the EPSC frequency (1 of 6 cells; Fig. 2B) or primarily the nicotinic current only (Fig. 2C). A higher concentration of choline (10 mM; n = 12) evoked large-amplitude nicotinic currents, but did not increase the frequency of EPSCs. Comparison of the agonists in the same neurons, as shown in Fig. 2D, indicated that cytisine was the most effective agonist for inducing EPSCs. An analysis of the frequency changes in various neurons indicated a statistically significant increase in the number of EPSCs (Fig. 2E) by four agonists with an order of effectiveness as follows: cytisine > nicotine > DMPP > ACh. Bath application of low concentrations of nicotine (250 and 500 nM) also enhanced EPSC frequency (Fig. 2F).

Nicotinic antagonists are also useful in distinguishing between various nAChR subtypes (Albuquerque et al. 1997). Methyllycaconitine (MLA; 10 nM) and α-bungarotoxin...
channel blocker, reduced significantly ACh-mediated increase in the EPSC frequency, but did not affect the nicotinic current (Fig. 3, E and F). In the presence of TTX (n = 6), ACh did not trigger any large-amplitude EPSCs.

**DISCUSSION**

The main finding of this study is that a non-α7 nAChR, probably α3β4, which can be activated by low concentrations of nicotine, regulates glutamate synaptic transmission to CA1 interneurons. The strong agonist response to cytisine, a potent inhibitory response to mecamylamine, and a poor inhibitory response to DHβE of EPSCs recorded from CA1 SR interneurons, suggest the involvement of α3β4-containing nAChRs and negate the involvement of α4β2-containing nAChRs. The present results, however, cannot exclude the participation of other potential combinations of subunits (e.g., α3β2β4) that are yet to be confirmed. In addition, we deduce based on a weak choline response and a MLA-insensitive ACh response that an α7 nAChR-dependent EPSC modulation is negligible in the CA1 region of the SR interneurons that contrasts with the previous reports of the presence of an α7 nAChR-dependent EPSC modulation in CA1 pyramidal neurons (Gray et al. 1996; Ji et al. 2001). Thus we propose the existence of a neuron-specific control of excitatory inputs by distinct subtypes of nAChRs in the hippocampus, i.e., an α7 regulating the function of pyramidal neurons and a non-α7, presumably α3β4, regulating interneurons (see Fig. 4). Such a dichotomy can add to the flexibility the brain ought to have to achieve fine tuning of several functions.

The nAChR subtypes that modulate EPSCs differ in their location and the mechanism by which they affect the transmitter release. For instance, α7 nAChRs in many systems are

We thank Dr. E.F.R. Pereira for the valuable suggestions on the manuscript. We acknowledge the technical assistance of M. Zelle, B. Marrow, and B. Alkondon.

This study was supported by National Institutes of Health Grants NS-25296, NS-41671, and ES-05730.

REFERENCES