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Research report

Low voltage-activated calcium and fast tetrodotoxin-resistant sodium currents define subtypes of cholinergic and noncholinergic neurons in rat basal forebrain

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Abstract

Neurons of the basal forebrain (BF) possess unique combinations of voltage-gated membrane currents. Here, we describe subtypes of rat basal forebrain neurons based on patch-clamp analysis of low-voltage activated (LVA) calcium and tetrodotoxin-resistant (TTX-R) sodium currents combined with single-cell RT-PCR analysis. Neurons were identified by mRNA expression of choline acetyltransferase (ChAT+, cholinergic) and glutamate decarboxylase (GAD67, GABAergic). Four cell types were encountered: ChAT+, GAD+, ChAT+/GAD+ and ChAT−/GAD− cells. Both ChAT+ and ChAT+/GAD+ cells (71/75) displayed LVA currents and most (34/39) expressed mRNA for LVA Ca^{2+} channel subunits. $Ca_v3.2$ was detected in 31/34 cholinergic neurons and $Ca_v3.1$ was expressed in 6/34 cells. Three cells expressed both subunits. No single neurons showed $Ca_v3.3$ mRNA expression, although BF tissue expression was observed. In young rats (2–4 mo), ChAT+/GAD+ cells displayed larger LVA current densities compared to ChAT+ neurons, while these latter neurons displayed an age-related increase in current densities. Most (29/38) noncholinergic neurons (GAD+ and ChAT−/GAD−) possessed fast TTX-R sodium currents resembling those mediated by Na^+ channel subunit $Na_v1.5$. This subunit was expressed predominately in noncholinergic neurons. No cholinergic cells (0/75) displayed fast TTX-R currents. The TTX-R currents were faster and larger in GAD+ neurons compared to ChAT−/GAD− neurons. The properties of ChAT+/GAD+ neurons resemble those of ChAT+ neurons, rather than of GAD+ neurons. These results suggest novel features of subtypes of cholinergic and noncholinergic neurons within the BF that may provide new insights for understanding normal BF function.
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Theme: Excitable membranes and synaptic transmission
Topic: Calcium channel structure, function and expression

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1. Introduction

Basal forebrain cholinergic neurons project to the hippocampus and neocortex and modulate cortical plasticity, attention, and cognition [14,48,52]. Some of these neurons are lost in age-related neurodegenerative diseases, such as Alzheimer's disease [43,58]. The basis of selective vulnerability of cholinergic cells is unknown, but distinctive properties of cholinergic subtypes could be responsible for cell loss and other age-related neurological dysfunction. There are also diverse populations of noncholinergic BF neurons, including GABAergic, glutamatergic, and peptidergic [20,35]. However, the description of these cells, particularly noncholinergic, nonGABAergic cells, is limited [9].

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Classically, BF cholinergic neurons were identified by electrophysiology and immunohistochemical labeling of cholinergic markers [17,18,29,36]. Recently, single-cell RT-PCR has been used to detect the expression of cholinergic markers [27,45,47,60,62]. This technique has permitted the identification of neurons that express mRNA for both the cholinergic marker ChAT and the GABAergic marker GAD [22,55]. In addition to its utility for cell identification, single-cell RT-PCR has been coupled with patch-clamp electrophysiology to correlate voltage- and ligand-gated channel function with gene expression (reviews, Refs. [3,11,39,54]).

Because mRNA expression patterns in cholinergic and noncholinergic BF neurons revealed several potential subtypes [22], we sought correlations between mRNA expression and electrophysiological properties that could help to define these subtypes. We hypothesized that subtypes of cholinergic neurons might be identified by properties of low voltage-activated (LVA) calcium currents, including LVA channel α_1 subunit mRNA expression. Cholinergic BF neurons demonstrate prominent LVA currents [1,19,29,59] and age-related plasticity [41]. LVA currents are particularly important for neuronal function in that they mediate a host of actions, including intrinsic membrane oscillations, burst firing patterns, seizure activity, synaptic plasticity, and calcium influx [25,44]. Three members of the gene family encoding LVA channel α_1 subunits, $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}) and $Ca_v3.3$ (α_{1I}), are present within the basal forebrain [56] and could be differentially expressed in cholinergic neurons. We therefore undertook this investigation using single-cell mRNA analysis of LVA Ca^{2+} channel subunits combined with patch-clamp analysis of LVA currents.

Fortuitously, we identified a tetrodotoxin-resistant (TTX-R) fast sodium current not previously reported in basal forebrain neurons. This current resembles a "heart-like" TTX-R current described in the medial entorhinal cortex [57] and proposed to be carried by Na^+ channels expressing the $Na_v1.5$ α subunit [23]. Other TTX-R currents with distinct properties have been described in dorsal root ganglion neurons [50] and associated with nociceptive transmission and $Na_v1.8$ + 1.9 Na^+ channel α subunits [10,15,64]. In the basal forebrain, the fast TTX-R Na^+ current is recorded in noncholinergic neurons only and has different properties associated with the expression or non-expression of GAD67 mRNA. These noncholinergic neurons also show $Na_v1.5$ mRNA expression.

2. Materials and methods

2.1. Experimental animals

Male Fischer 344 rats were obtained from Harlan (Indianapolis IN, NIA breeding colony). Data were compared from animals in two age groups, 2–4 months (young) and 24–27 months (aged). Animals had food and water available ad libitum and were maintained on a 12-h light/dark cycle. Handling and care of the animals was in accordance with policies of Texas A&M University System and the NIH.

2.2. Acutely dissociated neurons

Individual medial septum/nucleus of the diagonal band (MS/nDB) neurons were obtained using methods described previously [19,42]. Briefly, isoflurane (Abbott Labs, N. Chicago IL) was used to anesthetize rats prior to decapitation. Coronal brain slices (440 μm) were cut and microdissected to isolate the MS/nDB region and the tissue was enzymatically treated (trypsin ~0.7 mg/ml; Sigma Type XI). Cells were triturated through a series of glass pipettes and dispersed onto glass coverslips in a recording chamber. Individual cells were viewed using an inverted microscope (Axiovert 100 or 35, Zeiss, Oberkochen, Germany). Dissociated neurons were perfused at a rate of ~2 ml/min with a physiological solution containing (mM): 140 NaCl, 3 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 33 D-glucose, 10 HEPES, (pH 7.4 with NaOH, osmolarity 310–330 mOsm). Drugs and salts were obtained from Sigma, St. Louis MO, except as noted.

2.3. Electrophysiology

Whole-cell patch-clamp recording of voltage-gated barium currents was used to study the Ca^{2+} channel function. Sterilized patch pipettes (KG-33, 1.5 mm OD, Garner Glass, Claremont CA) were pulled by a Brown and Flaming puller (Sutter Instr., Novato, CA) and lightly polished. Cell capacitance (pF) was neutralized by the potentiometer on the patch amplifier and this value was used as an indicator of cell size. The bath recording solution contained (mM): 0.0005 tetrodotoxin (TTX, Calbiochem, La Jolla, CA), 132 NaCl, 2 $BaCl_2$, 2 $MgCl_2$, 33 D-glucose, 10 HEPES, 10 TEA chloride (tetraethyl ammonium chloride) pH = 7.4, osmolarity 315–325 mOsm. A few experiments were conducted using this solution with 2 mM Ca^{2+} as the charge carrier. The Na^+-free recording solution contained (mM); 120 choline chloride, 2 $BaCl_2$, 2 $MgCl_2$, 44 D-glucose, 10 HEPES, 10 TEA chloride and TTX 0.5 μM. The pipette solution contained; 120 mM CsCl or 120 mM Cs-acetate and 15 mM CsCl, 20 or 10 mM TEA chloride, 10 mM EGTA (ethyleneglycol-bis (β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), 2 mM $MgCl_2$, 100 μM GTP (guanosine 5′-triphosphate), 10 or 20 mM HEPES, 4 mM ATP (adenosine-triphosphate, pH 7.2, osmolarity ~290 mOsm). The CsCl pipette solution was made with nuclease-free water (Promega, Madison, WI) and was used for all cells identified by single-cell RT-PCR analysis. The Cs-acetate pipette solution was used for the experiments (Fig. 3) in which there was no RT-PCR identification. Liquid junction potentials were corrected with the pipette offset prior to recording. LVA barium currents were generated with

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