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Differences in developmental changes in GABAergic response between bushy and stellate cells in the rat anteroventral cochlear nucleus

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ABSTRACT

Many mammalian central nervous system neuron responses mediated by $GABA_A$ receptors undergo a developmental transition from excitation to inhibition, but little is known about the time of this switch in specific cell types in the developing anteroventral cochlear nucleus (AVCN). In the present study, bushy and stellate cells, two major cell types in the AVCN, were identified according to their morphology and electrophysiology. The equilibrium potential of GABA-evoked currents (E_{GABA}) was examined using the gramicidin-perforated patch-clamp technique. We found that the action of GABA in bushy and stellate cells switched from predominantly depolarizing to predominantly hyperpolarizing with respect to their resting membrane potential (V_{rest}) at different postnatal ages. Such a switch in the GABA response of bushy cells occurred before the first postnatal week, whereas that in stellate cells happened at the end of the second postnatal week. Furthermore, we discovered that bushy cells had a more depolarized V_{rest} than did stellate cells before the second postnatal week; however, the E_{GABA} of bushy and stellate cells was not significantly different. Thus, the discrepancy in the timing of the developmental shift from depolarizing to hyperpolarizing GABA responses between bushy and stellate cells may be due to the difference in their V_{rest}, but not due to E_{GABA} itself. These results suggest that GABAergic inhibition functions earlier in bushy than in stellate cells. In contrast, the longer excitatory action of GABA on stellate cells possibly renders them more vulnerable than bushy cells to excitotoxic substances during early development.

1. Introduction

Auditory nerve fibers carry electrical signals from the hair cells to the cochlear nuclear complex, which is the first synaptic station along the central auditory pathway that processes peripheral auditory signals. The anteroventral cochlear nucleus (AVCN) is one of the subdivisions of the cochlear nuclear complex. Two types of projection neurons, bushy and stellate cells, are intermingled in the AVCN. They receive direct input from the auditory nerve fibers, and their axons form distinct ascending pathways and project to nuclei in the auditory brainstem. All bushy cells project to the ipsilateral lateral superior olive (LSO) and contralateral ventral lateral lemniscus. Some bushy cells also project to both the ipsilateral and contralateral medial superior olivary nucleus (MSO) and the contralateral medial nucleus of the trapezoid body (MNTB) (Glendenning et al., 1981; Schofield and Cant, 1997; Cant and Benson, 2003). Stellate cells project to the contralateral inferior colliculus through the MNTB (Doucet and Ryugo, 1997), and some directly project to the contralateral cochlear nucleus (Arnott et al., 2004). Bushy cells have distinct biophysical membrane properties that result in little temporal summation of synaptic inputs. They are well suited for conveying and processing precise temporal information. In contrast, stellate cells sum inputs in time and are suitable for integrating auditory signals coming at different times (Cameron et al., 1983; Oertel, 1983).

Identifying bushy and stellate cells is unambiguous using morphology and electrophysiology. Bushy cells typically have one or two primary dendrites. Two dendritic processes usually originate from the opposite poles of the cell body and proceed away from each other (Paolini and Clark, 1998; Pocsai et al., 2007). The dendrites of bushy cells characteristically end in a spray of thinner and shorter braches. The stellate cell, also known as a multipolar cell, has a cell body with three or more primary dendrites (Wu and Oertel, 1984). These dendrites are slender and sparsely branched. As the morphology alone provides limited information for cell identification, electrophysiology can also be applied to assist in classifying AVCN neurons. Bushy and stellate cells respond differently to a current injection (Oertel, 1983; Wu and Oertel, 1984; Schwarz and Puil, 1997; Fujino and Oertel, 2001). Bushy cells fire

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a single or a few action potentials at the onset of a suprathreshold depolarizing current step, whereas stellate cells fire multiple action potentials sustained throughout the period of current injection.

Gamma-aminobutyric acid (GABA) and glycine are two major inhibitory neurotransmitters in the adult central nervous system (CNS), including the AVCN (Juiz et al., 1996; Lim et al., 2000). Activation of $GABA_A$ and glycine receptors may result in an influx of Cl^- and, therefore, hyperpolarization of the neuron. However, in some immature neurons, activation of $GABA_A$ and glycine receptors leads to depolarization and subsequent Ca^{2+} influx (Flint et al., 1998). Furthermore, this effect acts as a trophic signal for facilitating synapse formation and promoting morphological differentiation in developing neurons (Flint et al., 1998; Kirsch and Betz, 1998; Kullmann et al., 2002). Many studies have demonstrated a shift from mainly depolarizing to hyperpolarizing action of GABA at the early postnatal period in developing CNS neurons (Zhang et al., 1990; Balakrishnan et al., 2003; Milenkovic et al., 2007). The purpose of the present study was to investigate the switch time of the response mediated by $GABA_A$ receptors in developing bushy and stellate cells and to determine whether the switch time is cell-type specific. We also examined the resting potential of the two cell types to understand the cause for the different switch times of GABA-induced responses.

2. Materials and methods

The experimental protocol was approved by the ethics review committee for animal experimentation of Shanghai Jiao Tong University. All animal procedures used in this study followed local and international guidelines on the ethical use of animals, and all efforts were made to minimize the possible pain and discomfort of animals during the experimental procedures.

2.1. Preparation of slices and acute dissociation of AVCN neurons

Sprague-Dawley (SD) rats aged 5–15 post-natal days (P5–P15) (either gender) were used for the electrophysiology experiment. Animals were decapitated under deep anesthesia with sodium pentobarbitone (55 mg/kg, i.p.), and the brain was rapidly removed from the skull and immersed in ice-cold incubation solution. Coronal slices (~300-μm thick) containing the AVCN were cut using a vibratome (VT-1000s, Leica). The brain slices were incubated in the same solution oxygenated with 95% O_2–5% CO_2 at room temperature for 20–30 min before being transferred to a Petri dish filled with standard solution. The AVCN region was located using a dissecting microscope (XTL-2400; SOIEC, Shanghai, China). To obtain mechanically dissociated neurons, a fire-polished glass pipette was placed on the surface of the AVCN region and gently oscillated for 2–4 min at 50 Hz with amplitude of 0.1–0.2 mm. The dissociated AVCN neurons that adhered to the bottom of the Petri dish were ready for electrophysiological recordings about 20 min after the dissociation.

2.2. Solutions

The incubation solution for slices contained the following (in mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 24 $NaHCO_3$, and 10 glucose. The standard solution contained (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and had a pH of 7.4 after adjustment with Tris-base. The recording pipette solution for the perforated patch recording contained (in mM): 150 KCl and 10 HEPES (adjusted to pH 7.2 with Tris-base). Gramicidin was first dissolved in methanol to a concentration of 10 mg/ml and then diluted to a final concentration of 50–80 μg/ml in the pipette solution immediately before use and was only used for a maximum of 2 h.

2.3. Drugs

The drugs used in the present study included GABA, bicuculline (a $GABA_A$-receptor antagonist), 3-amino-propyl (diethoxymethyl)-phosphinic acid (CGP 35348, a $GABA_B$-receptor antagonist), and gramicidin. All chemicals were purchased from Sigma (St. Louis, MO, USA). GABA was dissolved in distilled water to make a stock solution. Bicuculline was dissolved in dimethyl sulfoxide to prepare a stock solution. All stock solutions were stored in single-use aliquots at −20 °C. Aliquots were diluted to the final solution prior to application and then applied to isolated neurons using a Y-tube system that completely exchanged the external solution with the drug solution within 10–20 ms (Wakamori et al., 1993).

2.4. Electrophysiological recordings and data analysis

Membrane currents and voltages were continuously measured with a patch-clamp amplifier (EPC 10; HEKA, Lambrecht/Pfalz, Germany). All data were low-pass-filtered at 1–3 kHz, digitized at sample rates of 3–10 kHz, and acquired by Pulse 6.0 software (HEKA). Cells in the dish were visualized using a microscope equipped with differential interference contrast optics (TE-2000U; Nikon, Tokyo, Japan). Patch electrodes were made from borosilicate capillary glass (outer diameter: 1.5 mm; inner diameter: 0.86 mm) using a vertical pipette puller (P-9; Narishige, Tokyo, Japan). The resistance of the electrode was 4–7 MΩ. The series resistance was compensated by 75–80%. Gramicidin-perforated patch recording was started only after the series resistance was stabilized below 50 MΩ (about 25 min after making the GΩ seal). The liquid junction potential was compensated. All experiments were performed at room temperature (22–26 °C).

In all experiments, the resting potential (V_{rest}) of a neuron was examined by holding the neuron in current-clamp mode at the zero current level after a perforated patch was attained. GABA-induced currents (I_{GABA}) were recorded by pressure application of GABA to neurons for 2 s at different holding potentials ranging from −80 mV to 0 mV (in 20-mV steps). The interval between voltage steps was at least 1 min (Ehrlich et al., 1999). E_{GABA} was measured when I_{GABA} was obtained at voltage levels from −80 mV to 0 mV. A current–voltage (I–V) curve was made by plotting the peak amplitude of I_{GABA} against the clamped voltage. All data are expressed as mean ± SEM, and comparisons between groups were made using the Student's paired t-test. $P < 0.05$ was considered significant.

3. Results

3.1. Cell identification

Data were obtained from perforated patch-clamp recordings from 67 bushy cells and 74 stellate cells. The identification of bushy and stellate cells in the AVCN was based on their morphology and electrophysiology. Bushy cells had a round or oval cell body and one or two processes orientated in opposite directions relative to the soma (Fig. 1A). Stellate cells typically had a cell body with a multipolar shape and three or more processes radiating from the cell body (Fig. 1B). The intrinsic membrane properties of these two cell types were also different. Bushy cells responded to a depolarizing current injection with only one action potential at the beginning of the current pulse (Fig. 1C), whereas stellate cells fired sustained spikes throughout the current pulse (Fig. 1D), and the number of spikes increased with current strength.

3.2. I_{GABA} in the recorded neurons

Gramicidin-perforated patch-clamp recording was used to record I_{GABA} mediated by the $GABA_A$ receptor without disrupting the normal intracellular Cl^- concentration ($[Cl^-]_i$). As GABA can activate both $GABA_A$ and $GABA_B$ receptors, and metabotropic $GABA_B$ receptors are present in the AVCN (Lim et al., 2000), the selective $GABA_B$-receptor antagonist, CGP 35348, was added to the external solution to block currents that might be induced by activating the $GABA_B$ receptors. Under the voltage-clamp mode, a pressure application of 100 μM GABA elicited inward currents in both bushy and stellate cells at a holding potential of −80 mV (Fig. 2A and B). The currents were completely inhibited by adding 10 μM bicuculline, confirming that the inward currents produced by GABA were mediated by $GABA_A$ receptors.

To investigate E_{GABA}, the membrane potential of bushy and stellate cells at P7 was clamped at various levels between −80 mV and 0 mV (Fig. 2C and D). As the membrane potential became more positive, the direction of I_{GABA} gradually reversed from inward to outward. To measure E_{GABA} more accurately, the peak amplitude of I_{GABA} was plotted against the voltage steps, and I–V relationship fitted with a linear regression were obtained (Fig. 2E and F). E_{GABA} was determined as the voltage with which the I–V curve crosses the zero current. Average E_{GABA} was −62.8 mV in P7 bushy cells ($n = 14$), which was more hyperpolarized than their V_{rest} (V_{rest} = −54.5 mV as indicated by the dotted line in Fig. 2E). Average E_{GABA} was −58.9 mV