

## PROCEEDING

# Postnatal development of inhibitory synaptic transmission to superior salivatory neurons in rats

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**Abstract :** The primary parasympathetic center of the submandibular and sublingual salivary glands is the superior salivatory (SS) nucleus, and its neurons receive excitatory (glutamatergic) and inhibitory (GABAergic and glycinergic) synaptic transmissions in rats. In the present study, we focused on the postnatal development of inhibitory transmission to SS neurons. Gramicidin-perforated whole-cell patch-clamp recordings were performed in rat brainstem slices on postnatal day 2 (P2)-P14. Developmental changes in the intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>in</sub>) were examined based on the reversal potentials of total inhibitory postsynaptic currents (GABAergic plus glycinergic), which were evoked by electrical stimulation near the recording neuron. The [Cl<sup>-</sup>]<sub>in</sub> in the P8-P14 group was significantly lower than in the P2-P7 group. The effect of GABA application at the resting potentials changed from depolarization to hyperpolarization around P8, suggesting that SS neurons acquired mature inhibitory systems around P8. The period at which GABA responses change from excitatory to inhibitory in SS neurons was discussed compared with those of the forebrain, brainstem, and spinal neurons. *J. Med. Invest.* 56 Suppl. : 270-272, December, 2009

**Keywords :** superior salivatory neurons, development, GABAergic, glycinergic, gramicidin-perforated patch clamp

## INTRODUCTION

The superior salivatory (SS) nucleus is the primary parasympathetic center of the submandibular and sublingual glands. We previously revealed the excitatory (glutamatergic) and inhibitory (GABAergic and glycinergic) synaptic inputs to SS neurons in neonatal rat brainstem slices [1]. Postnatal development of the central nervous system is generally observed in ligand-gated channels such as glutamate,

GABA<sub>A</sub>, and glycine receptors. Especially, GABA<sub>A</sub> and glycine receptor-mediated responses show a marked change. That is, the activation of GABA<sub>A</sub> and glycine receptors causes hyperpolarization in mature neurons, but depolarization in immature neurons [2, 3]. Thus, such an electrophysiological characteristic of the inhibitory synaptic transmission is one of the indications for the development of SS neurons.

In the present study, we focused on the development of inhibitory synaptic transmission to SS neurons innervating the salivary glands. Many SS neurons showed depolarization on GABA application during the first week of postnatal life, and, thereafter, showed hyperpolarization. These results suggest that SS neurons acquire functional inhibitory

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synaptic transmission around P8.

**METHODS**

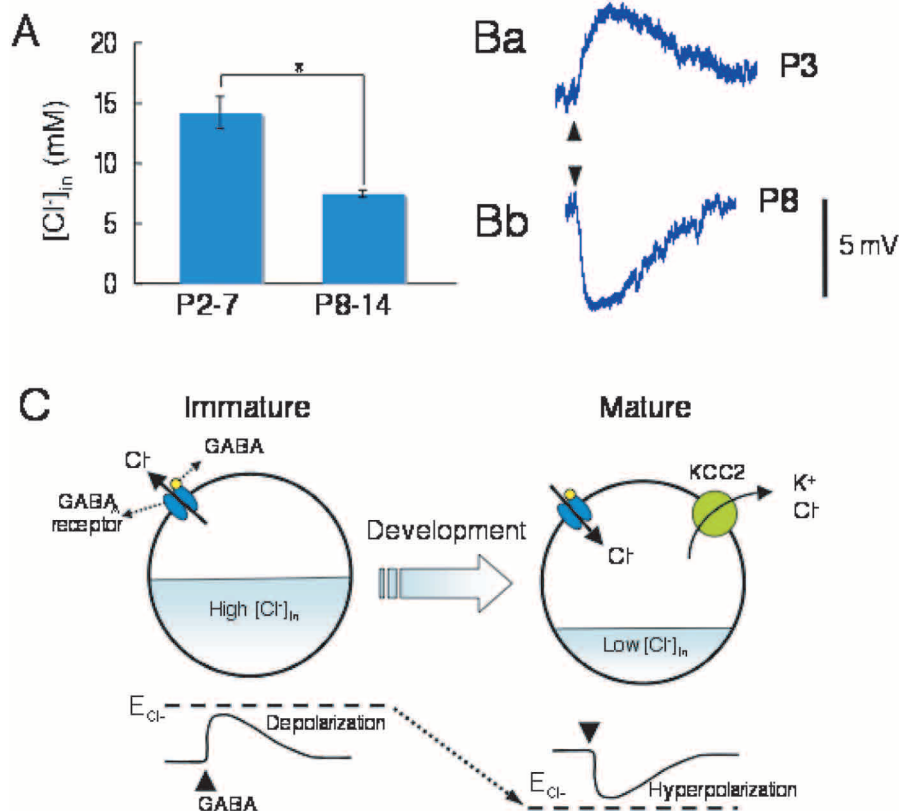
Wistar rats at P1 to P14 were used in the present study. SS neurons innervating the submandibular salivary glands were labeled by the retrograde axonal transport of a fluorescent dye, Texas Red, injected into the chorda-lingual nerve. Gramicidin-perforated patch-clamp recordings were applied to the labeled SS neurons. Neural excitability on GABA<sub>A</sub> receptor activation is affected by the intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>in</sub>). To examine the [Cl<sup>-</sup>]<sub>in</sub> of SS neurons, inhibitory postsynaptic currents were evoked by electrical stimulation near the SS neuron used for recording at various potentials. The [Cl<sup>-</sup>]<sub>in</sub> was calculated by the reversal potential (V<sub>rev</sub>). GABA (1 mM, 50-100 ms) was applied *via* pressure injection near the SS neuron used for recording when it was at its resting potential.

**RESULTS AND DISCUSSION**

*Developmental change of [Cl<sup>-</sup>]<sub>in</sub> in SS neurons*

The V<sub>rev</sub> in SS neurons tended to have a more negative potential with advancing postnatal age. The developmental decrease of V<sub>rev</sub> is attributed to the decrease of the [Cl<sup>-</sup>]<sub>in</sub>. The [Cl<sup>-</sup>]<sub>in</sub> in P2-P7 and P8-P16 groups was 18.3 ± 3.0 (n=27) and 7.8 ± 0.6 mV (n=14), respectively (P < 0.01, Fig. 1A). This difference could lead to variations in the responses on the activation of GABA<sub>A</sub> receptors. GABA application induced the depolarization of SS neurons at P2-P7 (n=10/12). In contrast, after P8, GABA caused hyperpolarization (n=7/9) (Fig. 1B, C).

The period at which GABA action switches from depolarization to hyperpolarization may be different depending on the central regions. In the forebrain, brainstem, and spinal neurons, the switching period of the forebrain is the slowest (around P21). The switching period tends to be earlier in the order of the spinal cord [4], brainstem [5], and forebrain



**Fig. 1** Effect of GABA on the membrane potential of developing SS neurons with an intact [Cl<sup>-</sup>]<sub>in</sub>. A, [Cl<sup>-</sup>]<sub>in</sub> calculated from V<sub>rev</sub> in P2-P7 and P8-P14 groups. The [Cl<sup>-</sup>]<sub>in</sub> of P8-P14 is significantly lower than that of P2-P7 (p < 0.05). B, example of perforated patch-clamp recordings showing depolarization (Ba, P3 neuron) and hyperpolarization (Bb, P8 neuron) in response to GABA application (arrowhead) at resting potentials. C, GABA action and Cl<sup>-</sup> homeostasis. Binding of GABA and GABA<sub>A</sub> receptors induces the opening of Cl<sup>-</sup> channels. In immature neurons, the efflux of Cl<sup>-</sup> causes the depolarization (excitation) of the neuron. In contrast, in a mature neuron, an influx of Cl<sup>-</sup> causes hyperpolarization (inhibition). Generally, the developmental decrease in the [Cl<sup>-</sup>]<sub>in</sub> is attributed to the expression of KCC2 with development. KCC2 extrudes Cl<sup>-</sup> out of the cell.

neurons [6]. The switching period in the SS neurons (P8) is similar to that in the brainstem neurons. Since the brainstem and spinal cord neurons participate in essential functions such as reflexes in daily life, the synaptic functions of the brainstem and spinal cord may mature relatively earlier than those of the forebrain.

*Physiological significance of GABA excitation in SS neurons*

GABA and glycine during the early postnatal period might serve as trophic factors [2, 3] to influence synapse maturation in developing SS neurons. The GABAergic excitatory action induced  $\text{Ca}^{2+}$  entry into neurons *via* NMDA receptors and voltage-dependent  $\text{Ca}^{2+}$  channels. This  $\text{Ca}^{2+}$  influx is thought to be important in the regulation of various transcription factors which are involved in synapse development. The GABA-induced excitation may have a functional significance in immature SS neurons.

In the first postnatal week, SS neurons receive exclusively excitatory inputs due to the excitatory action of inhibitory inputs in addition to innate glutamatergic excitatory inputs. These excessive excitatory inputs are necessary for the production of saliva by immature salivary glands to moisten the oral cavity.

In conclusion, SS neuron responses to GABA switch from a depolarizing to hyperpolarizing action after P8. This suggests that the inhibitory synaptic transmission in SS neurons is functional until 3 weeks postnatal, at which time feeding behavior begins in rats.

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