PROCEEDING

Immunohistochemical study on the distribution and origin of GABAergic nerve terminals in the superior salivatory nucleus

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Abstract : The superior salivatory nucleus (SSN) is the primary parasympathetic center controlling submandibular salivatory secretion. Our previous electrophysiological study revealed that many SSN neurons receive GABAergic and glycinergic synaptic inputs. In the present study, we examined the distribution of GABAergic and glycinergic nerve terminals, $GABA_A$ receptors in the SSN, and the origin of GABAergic nerve terminals innervating the SSN. Glutamic acid decarboxylase (GAD) and glycine transporter 2 (GLYT2) were used as markers of GABAergic and glycinergic nerve terminals, respectively. GAD- and GLYT2-positive nerve terminals and $GABA_A$ receptors were examined immunohistochemically in SSN neurons labeled by the retrograde axonal transport of FastBlue (FB) injected into the chorda-lingual nerve. The SSN neurons abundantly contained GAD-positive nerve terminals and $GABA_A$ receptors, suggesting that SSN neurons undergo strong GABAergic inhibition. The origin of GABAergic terminals was examined in neurons labeled by the retrograde transport of FluoroGold (FG) injected into the SSN. GAD was used as a marker of GABAergic neurons. Numerous FG-labeled neurons were found in the forebrain and brainstem. However, in FG-labeled neurons, GAD-positive neurons were occasionally observed in the reticular formation of the brainstem. These findings suggest that SSN neurons mainly receive GABAergic projections from the reticular formation. J. Med. Invest. 56 Suppl. : 264-266, December, 2009

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INTRODUCTION

The superior salivatory nucleus (SSN), the primary center for salivary secretion, is located in the lateral reticular formation of the medulla oblongata in rats (1-3). Based on neuroanatomical studies (4-7) using methods involving the retrograde transsynaptic transport of a virus and retrograde axonal transport of horseradish peroxidase (HRP), it has been suggested that the nucleus innervating the SSN involves the brainstem and forebrain ; the brainstem includes the parabrachial nucleus, sensory trigeminal nucleus, nucleus of the solitary tract, and its surrounding reticular formation ; the

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forebrain includes the insular cortex, central nucleus of the amygdala, bed nucleus of stria terminals, and paraventricular nucleus of the hypothalamus. It is well-known that stimulation of the cerebral cortex (8) and hypothalamus (9) evokes salivary secretion. These neuroanatomical and *in vivo* studies suggest that salivary secretion is controlled not only by the brainstem, but also by the forebrain.

Meanwhile, our electrophysiological studies showed that most SSN neurons innervating the salivary glands and tongue receive excitatory glutamatergic inputs, and inhibitory GABAergic and glycinergic inputs. However, it is unclear how the numerous SSN neurons are controlled by both the forebrain and brainstem with respect to inhibitory salivary control.

In this study, we aimed to examine the distribution of GABAergic and glycinergic nerve terminals in the SSN, and the origin of GABAergic terminals innervating the SSN.

MATERIALS AND METHODS

1. Immunohistochemical staining of GABAergic and glycinergic nerve terminals and $GABA_A$ receptors in the SSN

SSN neurons innervating the submandibular salivary glands were stained by the retrograde transport of a fluorescent tracer, Fast Blue (FB). Male Wistar rats (290-310 g) were anesthetized with sodium pentobarbital and urethane (*i.p.*, 525 and 50 mg/kg, respectively), and then FB (2%, 2 μl) was injected into the left chorda-lingual nerve. After a survival period of 2 days, the rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Glutamic acid decarboxylase (GAD) and glycine transporter 2 (GLYT2), were investigated as markers of glycinergic and GABAergic nerve terminals, respectively. These markers were detected immunohistochemically in coronal brainstem sections (5 μm).

2. Immunohistochemical staining of GABAergic neurons innervating SSN neurons

The distribution of neurons innervating SSN neurons was examined based on the retrograde transport of a fluorescent tracer, FluoroGold (FG). For the injection of FG, male Wistar rats (290-310 g) were anesthetized with sodium pentobarbital and urethane (*i.p.* 525 and 50 mg/kg, respectively) and mounted on a stereotaxic apparatus. FG (2%, 0.05 μl) was injected into the SSN (coordinates : 10.6 mm caudal to the bregma, 2.2 mm lateral to the midline, 7.3 vertical to the cerebellar surface) using a Hamilton microsyringe with a glass micropipette (30-40 μm tip diameter). The injection was performed on the left side. After a survival period of 2 days, the rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). As a marker of GABAergic neurons, glutamic acid decarboxylase (GAD) was used. GAD was examined immunohistochemically in coronal brain sections (5 μm). These sections were observed using a fluorescence microscope. Series of sections obtained from FG-injection site (750 μm) were not used for the analysis.

RESULTS AND DISCUSSION

1. Distribution of GABAergic and glycinergic nerve terminals and $GABA_A$ receptors in the SSN

Almost all SSN neurons were positive for GAD and $GABA_A$ receptors. The number of GLYT2-positive neurons was lower. This suggests that SSN neurons innervating the salivary glands received strong GABAergic inputs.

2. Origin of GABAergic terminals in SSN neurons

Numerous neurons labeled with FG were observed in the forebrain and brainstem. In the forebrain, labeled cells were seen mainly in the bed nucleus of the stria terminalis (BST), central nucleus of the amygdala (CeA), and lateral hypothalamic area (LH). These labeled cells were distributed on the ipsilateral side of the FG injection site. The insular cortex (IC) was found on the contralateral side. In the brainstem, labeled cells were observed in the parabrachial nucleus (PBN), reticular formation (RF), sensory trigeminal nucleus (STN), and nucleus of the solitary tract (NST). These cells were distributed mainly on the ipsilateral side of the injection site. However, in the RF and NST, many labeled cells were present on the contralateral side of the injection site, also.

GAD-positive neurons were very sparse in the forebrain and brainstem, and mainly observed in the RF bilaterally. These results suggest that neurons projecting directly to the SSN neurons are distributed in various nuclei, and GABAergic neurons are less abundant in the nucleus.

CONCLUSION

1. SSN neurons innervating the submandibular salivary glands contained abundant $GABA_A$ receptors and GAD-positive nerve terminals.

2. SSN neurons received numerous direct innervations from the forebrain and brainstem.

3. A direct GABAergic projection from the lateral hypothalamus and central nucleus of the amygdala was rare, and that from the lateral reticular formation of the brainstem occurred occasionally.

4. Many GABAergic neurons directly projecting to SSN neurons may be located in close proximity to the SSN neurons.

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