PROCEEDING

Cevimeline enhances the excitability of rat superior salivatory neurons

Hirotaka Ueda[1,2], Yoshihiro Mitoh[1], Hiroyuki Ichikawa[3], Motoi Kobashi[1], Takashi Yamashiro[2], and Ryuji Matsuo[1]

[1]*Department of Oral Physiology,* [2]*Department of Orthodontics, Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama University, Okayama, Japan ; and* [3]*Division of Oral and Craniofacial Anatomy, Graduate School of Dentistry, Tohoku University, Sendai, Japan*

Abstract : Cevimeline, a therapeutic drug for xerostomia, is an agonist of muscarinic acetylcholine receptors (mAChRs), and directly stimulates the peripheral mAChRs of the salivary glands. Since cevimeline is distributed in the brain after its oral administration, it is possible that it affects the central nervous system. However, it is unknown how cevimeline affects the superior salivatory (SS) neurons, which control submandibular salivation. In the present study, we examined the effects of cevimeline on the SS neurons using the whole-cell patch-clamp technique in brain slices. In Wistar rats (6-10 days), the SS neurons were retrogradely labeled by Texas Red applied to the chorda-lingual nerve. Two days after injection, whole-cell recordings were obtained from the labeled cells, and miniature excitatory postsynaptic currents (mEPSCs) were examined. Cevimeline induced the inward currents dose-dependently and increased the frequency of mEPSCs. Therefore, it is suggested that cevimeline enhances the excitability *via* post- and presynaptic muscarinic receptors in the rat SS neurons. In conclusion, cevimeline may enhance the excitability of the SS neurons. J. Med. Invest. 56 Suppl. : 267-269, December, 2009

Keywords **:** *cevimeline, muscarinic acetylcholine receptor, superior salivatory neurons*

INTRODUCTION

Activation of the parasympathetic neurons induces the production of abundant watery saliva via mAChRs (1-4). Cevimeline ($C_{10}H_{17}NSO$: (±)-cis-2-methylspiro[1,3-oxathiolane-5,3'-quinuclidine]) is an agonist of mAChRs, and it directly stimulates peripheral mAChRs of the salivary glands as a therapeutic drug for xerostomia (5, 6). In a whole-body autoradiographic study, [^{14}C] cevimeline was distributed in the rat brain after oral administration. Therefore it is possible that cevimeline affects the central nervous system. However, it is not known whether cevimeline enhances the excitability of the SS neurons which control the submandibular and sublingual salivary glands.

In the present study, we focused on the SS neurons which control submandibular salivation, and examined the effects of cevimeline on the SS neurons using the whole-cell patch-clamp technique in neonatal rat brain slices.

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Address correspondence and reprint requests to Ryuji Matsuo, Department of Oral Physiology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Shikata-cho 2-5-1, kita-ku, Okayama 700-8525, Japan and Fax : +81-86-235-6644.

MATERIALS AND METHODS

Retrograde labeling of the SS neurons

In neonatal Wistar rats (6-10 days), the SS neurons were retrogradely labeled with 5-10% Dextran-Texas Red-lysine (in physiological saline, 3,000 MW, Molecular Probes, Eugene, OR, USA) applied to the chorda-lingual nerve.

Slice preparation and recording

Two days after injection, the animals were deeply anesthetized with halothane and decapitated. The brain was rapidly removed from the skull and kept for 5 min in sucrose-based artificial cerebrospinal fluid (ACSF) (pH 7.4) bubbled with 95% O_2 and 5% CO_2 at < 4°C. Sucrose-based ACSF contained : 234 mM sucrose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$, and 11 mM glucose. Sagittal brainstem slices (200 μm thick) were obtained by cutting with a tissue slicer (DTK-2000, Dosaka EM, Kyoto, Japan). The slices were preincubated in standard ACSF (pH 7.4) bubbled with 95% O_2 and 5% CO_2 at room temperature (21-24°C) for more than 1 h before recording. Standard ACSF contained : 115 mM NaCl, 5 mM KCl, 1.6 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose. After preincubation, the slices were transferred to a recording chamber and perfused with standard ACSF at room temperature at a rate of 2-3 ml/min using a peristaltic pump (Minipuls 2, Gilson, Villiers, France). Whole-cell recordings were obtained from labeled cells with a patch-clamp amplifier (EPC-8, HEKA Elektronik, Lambrecht/Pfalz, Germany). To examine mEPSCs, all recordings were performed at a holding potential of -70 mV in normal ACSF containing TTX (tetrodotoxin, 0.5 μM). Muscarine or cevimeline were applied to the SS neurons by employing a Y tube technique (7).

RESULTS AND DISCUSSION

Muscarine induced inward currents

Muscarine (300 μM) induced inward currents in the rat SS neurons (n=4). These inward currents were completely inhibited in the presence of atropine (3 μM, n=3). These findings indicate that mAChRs exist on the postsynaptic membranes of the SS neurons.

Cevimeline induced inward currents

Cevimeline induced inward currents in all the SS neurons (10, 30, 50, 100, 300, 500, and 1000 μM, n= 5 at each concentration). This indicates that cevimeline affects postsynaptic mAChRs in the SS neurons. Additionally, cevimeline increased the frequency of mEPSCs in approximately 60% of neurons (n=8/ 14). This indicates that cevimeline affects presynaptic mAChRs in some SS neurons.

CONCLUSION

In this study, we found that cevimeline enhances the excitability of the rat SS neurons via mAChRs. This suggests that cevimeline affects not only peripheral salivary glands, but also the central nervous system.

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