PROCEEDING

Pilocarpine-induced salivary fluid secretion in the perfused submandibular gland of the rat

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Abstract : Xerostomia is the symptom of dry mouth often seen in patients who receive head and neck radiation therapy or in patients who have Sjögren's syndrome. The primary treatment to relieve xerostomia symptom is oral administration of pilocarpine, a parasympathomimetic agent with muscarinic action. Increase in salivary secretion induced by systemic administration of pilocarpine is considered to be mediated by actions on muscarinic cholinergic receptors in the central nervous system and salivary glands. In this study, we investigated the direct effect of pilocarpine on salivary fluid secretion in the isolated, perfused rat submandibular gland. Pilocarpine provoked salivary fluid secretion in a dose-dependent manner. The Na^+-channel blocker tetrodotoxin had almost no effect on the pilocarpine-induced salivary fluid secretion, indicating that pilocarpine directly stimulates submandibular gland. Pilocarpine induced an increase in intracellular Ca^{2+} concentration in dispersed submandibular gland cells at 37°C, but not 25°C. The salivary fluid secretion induced by pilocarpine was consisted of a rapid and transient phase and a subsequent sustained phase, which profile was different from that evoked by carbachol, another typical muscarinic agonist. Pilocarpine also induced Lucifer yellow secretion via paracellular route. J. Med. Invest. 56 Suppl. : 281-283, December, 2009

Keywords **:** *pilocarpine, salivary fluid secretion, paracellular pathway, submandibular gland*

INTRODUCTION

Xerostomia (dry mouth) is a common symptom seen in the elderly patients who receive therapeutic irradiation of the head and neck cancer or in the patients who have Sjögren's syndrome (SS), a chronic multisystem immune-mediated disorder characterized by lymphocytic infiltration into the salivary and lacrimal glands (1). Decrease of salivary secretion can affect numerous aspects of oral function, contributing to pain, poor diet caries and oral infections.

In the past decade, pilocarpine, a parasympathomimetic agent with muscarinic action, has widely been used to relieve symptoms of oral dryness (2). Increase in salivary secretion induced by systemic administration of pilocarpine is considered to be mediated by actions on muscarinic cholinergic receptors in the central nervous system and salivary glands (3). However, despite the wide clinical use, the direct action of pilocarpine on salivary glands has not been defined in detail. In this study, we investigated the effect of pilocarpine on salivary fluid secretion in the isolated, perfused rat submandibular gland.

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CHARACTERISTICS OF PILOCARPINE-INDUCED SALIVARY FLUID SECRETION

We examined the effect of various dose of pilocarpine (1-1,000 μM) on salivary fluid secretion in the perfused rat submandibular gland prepared as described previously (4). Salivary fluid secretion rate and total saliva volume were increased by pilocarpine in a dose-dependent manner. The peak levels of salivary flow rate and total volume induced by 1, 10, 100 and 1000 μM pilocarpine were 35.69 ± 21.07, 73.83 ± 7.53, 89.74 ± 10.17 and 76.12 ± 15.87 μl/g/min (n=3) and 0.15 ± 0.09, 0.33 ± 0.05, 0.39 ± 0.07 and 0.07 ± 0.02 ml/g/5 min (n=3), respectively. The pattern of pilocarpine-induced salivary fluid secretion consisted of two phases, a sharply transient phase and a subsequently sustained phase. We also examined the effect of carbachol in the perfused rat submandibular gland. Although pilocarpine and carbachol have been reported to be the same muscarinic agonists, we found that the pattern of the salivary fluid secretion induced by pilocarpine was different from that by carbachol in some aspects : the concentration threshold of carbachol for salivary fluid secretion was lower (200 nM) than that of pilocarpine, and the maximal response on salivary fluid secretion induced by carbachol was higher than that by pilocarpine. Finally, after washout of stimuli from perfusion to terminate treatment, carbachol-stimulated fluid secretion was returned to the resting level much more rapidly than pilocarpine-stimulated one.

LESS EFFECT OF TETRODOTOXIN ON PILOCARPINE-INDUCED SALIVARY FLUID SECRETION

A whole submandibular gland used for our experiment contains some neuron fibers. To exclude effect of neurotransmitters released from neurons in the gland, effect of pilocarpine on salivary fluid secretion was examined in the presence of tetrodotoxin, a specific Na^+-channels blocker. When the submandibular gland was stimulated with pilocarpine after pretreatment with 1 μM tetrodotoxin for 20 min, pilocarpine induced almost complete response, indicating that pilocarpine directly stimulates submandibular gland.

In previous studies, the pilocarpine-evoked saliva fluid secretion was examined *in vivo* by intravenous or intraperitoneal injection or oral administration of pilocarpine. After administration of pilocarpine, the plasma concentration of pilocarpine ranging from 4 nM to 0.81 μM induces saliva flow (5-9). In such experiments, threshold is lower than that in our experiments. Pilocarpine induces saliva flow via activation of muscarinic receptors at both central nerve system and gland sites *in vivo*. Therefore, although pilocarpine directly stimulates submandibular gland, salivary fluid secretion appears to be induced by synergistic effect of pilocarpine on both central nerve system and gland.

PILOCARPINE-INDUCED INCREASE IN $[Ca^{2+}]_i$ IN DISPERSED SUBMANDIBULAR GLAND CELLS

It has been considered that the increase in $[Ca^{2+}]_i$ is essential for salivary fluid secretion. Therefore we examined the effect of pilocarpine on Ca^{2+} mobilization in the dispersed submandibular gland cells, which were dispersed by collagenase and hyaluronidase (10) and loaded with fura-2 (11). The cells were stimulated with 1-1000 μM pilocarpine, which induced saliva secretion. We first carried out such experiments at 25°C, but we failed to detect pilocarpine-induced Ca^{2+} mobilization in dispersed submandibular gland cells, whereas carbachol provoked Ca^{2+} mobilization at 25°C. However, when the temperature was shifted to 37°C, pilocarpine clearly induced the increase in $[Ca^{2+}]_i$ dose-dependently. These results suggest that Ca^{2+}-mobilizaing signaling pathway activated by pilocarpine is different from that by carbachol, which appears to cause the difference between effects of pilocarpine and carbachol on the mode of salivary fluid secretion.

PILOCARPINE-INDUCED LUCIFER YELLOW (LY) SECRETION

It has been considered that water and ions are supplied from plasma to saliva via two pathways, transcellular and paracellular pathways. Previous studies showed the LY is a good tracer that reflects salivary fluid flow via paracellular pathway, because LY traverses the paracellular pathway and not through transcellular route (12, 13). Then we examined the effect of pilocarpine on LY secretion when the gland was perfused with perfusate buffer with LY. Pilocarpine clearly provoked LY secretion, whereas no LY secretion was observed in the

absence of pilocarpine. These observations suggest that paracellular pathway contributes to pilocarpine-induced salivary fluid secretion.

IN CONCLUSION

We demonstrated that pilocarpine directly stimulates salivary fluid secretion in rat submandibular gland, in which paracellular pathway contributes to a part of it. Pilocarpine-induced Ca^{2+} mobilization was temperature-dependent, which was different from the effect of carbachol. The signaling pathway related to salivary fluid secretion induced by pilocarpine appears to be different from that by carbachol.

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