PROCEEDING

Characterization of neurokinin A-evoked salivary secretion in the perfused rat submandibular gland

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Abstract : Neurokinin A (NKA) evokes salivary secretion. Despite such reports, the direct effect of NKA on salivary secreteion in submandibular gland has not been clarified. Here we studied characterization of salivary fluid secretion induced by NKA in the perfused submandibular grand (SMG) of the rat. NKA (3-100 nM) stimulated salivary fluid secretion in a dose-dependent manner. The profile of secretion induced by NKA consisted of two phases, transient and sustained phases. When the gland was perfused with Lucifer yellow (LY)-containing perfusate buffer and stimulated by NKA, concentration of LY in saliva was increased. In the absence of Ca^{2+} in the perfusate, NKA induced only a transient salivary fluid and a transient LY secretion. When the gland was treated with BAPTA, NKA failed to induce both salivary fluid secretion and LY secretion. These results suggest that NKA induces salivary secretion *via* both transcellular and paracellular pathways, which depends on intracellular Ca^{2+} mobilization. J. Med. Invest. 56 Suppl. : 278-280, December, 2009

Keywords **:** *neurokinin A, salivary fluid secretion, submandibular gland*

INTRODUCTION

The tachykinins are a family of peptides that share a common carboxyl-terminal sequences and are extensively distributed in central nervous system and their periphery nerves, includes salivary glands (1, 2). The tachykinins exert their physiological activities through activation of three subtypes of NK receptors, NK1, NK2 and NK3 receptors (3). Neuronkinin A (NKA) is a member of the tachykinins and induces saliva secretion mediated by NK1 receptors in the rat submandibular gland (SMG) (4, 5). Despite such reports, the direct effect of NKA on salivary secretion in SMG has not been clarified. This is because the tachykinins including NKA were administrated to animals *via* intravenous or intraperitoneal injection in previous studies (6-9). Here we studied characterization of salivary fluid secretion induced by NKA in the perfused SMG of the rat.

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Ca^{2+}-DEPENDENT NKA-EVOKED SALIVA FLUID SECRETION

The procedure for isolating the SMG of the rat has been described previously (10). NKA (3-100 nM) stimulated salivary secretion in a dose-dependent manner. The profile of secretion induced by NKA consisted of two phases, transient and sustained phases.

It has been considered that the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is essential for

salivary fluid secretion (11). Therefore we checked the effect of NKA on $[Ca^{2+}]_i$ mobilization in the dispersed SMG cells (12, 13). In the fura2-loaded dispersed SMG cells, NKA (3-100 nM) provoked an increase in $[Ca^{2+}]_i$. In Ca^{2+}-free medium, NKA only induced transient increase of $[Ca^{2+}]_i$. and quickly returned to the basal level. Thus, transient increase of $[Ca^{2+}]_i$. was depended on intracellular Ca^{2+} stores and sustained phase was correlated to extracellular Ca^{2+} entry.

When the gland was perfused with saline without Ca^{2+} and stimulated by NKA, the transient phase was clearly reduced and the sustained phase disappeared. In the presence of BAPTA-AM (Dojindo, Japan), a membrane permeable Ca^{2+}-specific chelator, in the perfusate buffer, NKA failed to induce the salivary fluid secretion. These results suggest that NKA-induced salivary fluid secretion is depended on intracellular Ca^{2+} mobilization.

NKA-STIMULATED PARACELLULAR SECRETION

It is considered that saliva is secreting *via* two pathways, transcellular and paracellular pathways (10). Therefore, we next examined the contribution of the paracellular pathway to salivary secretion induced by NKA using Lucifer Yellow (LY), a low MW fluorescent substance that can pass through the paracellular pathway but not the transcellular pathway. When the gland was perfused with LY-containing perfusate buffer and stimulated by NKA, concentration of LY in saliva was increased. In the absence of Ca^{2+} in the perfusate, NKA induced only a transient LY secretion. When the gland was treated with BAPTA, LY secretion was completely abolished. These results suggest that NKA-induced salivary fluid secretion *via* the paracellular pathways is also depended on intracellular Ca^{2+} mobilization.

In conclusion, NKA stimulates salivary fluid secretion *via* transcellular and paracellular pathways, in which Ca^{2+} mobilization is coupled to both pathway functions in rat SMG.

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