Acta Pharmacologica Sinica 2006 Jul; 27 (7): 843–847

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Invited review

Cytosolic calcium oscillations in submandibular gland cells[1]

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Key words

submandibular gland; secretory axis; calcium oscillation

[1] Project supported by grants from NSFC (39825112, 30472048, 30540420524), Ministry of Education (No 104186), and NSF Beijing (No 6062014).
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Received 2006-04-16
Accepted 2006-04-28

doi: 10.1111/j.1745-7254.2006.00376.x

Abstract

Calcium oscillations can, by default, encode diverse and specific signals by different modes of modulation. Frequency modulation is illustrated by the activation of calcium/calmodulin-dependent protein kinase II at unit Hz, and of calcineurin at 10 mHz frequencies, respectively. The submandibular gland secretory axis is characterized by both potassium and osmolarity gradients from the luminal side of the secretory cells. Such gradients may play significant physiological roles through the feedback modulation of cholinergic stimulation. High potassium transforms plateau calcium increases induced by cholinergic stimulation of the submandibular acinar cells into oscillatory calcium increases. The ductal cells may have similar mechanisms of feedback modulation both by high potassium and by hypo-osmolarity. Such feedback mechanisms could modulate the decision-making process for determining which secretory products are selectively released after nerve stimulation.

Introduction

An increase in cytosolic calcium concentration ($[Ca^{2+}]_c$) is among the earliest events that occur after stimulation of many different types of cells by endogenous signaling molecules such as hormones, neurotransmitters, and reactive oxygen species (ROS) including singlet oxygen[1,2]. The $[Ca^{2+}]_c$ increase is frequently in the form of oscillations. Oscillations can, by default, be encoded by at least three independent modes of modulation: amplitude modulation (AM), frequency modulation (FM), and individual calcium spike-shape modulation (SM). Thus the simplest divalent cation, by temporal oscillation, encodes numerous, diverse and specific signals to modulate various vital body functions such as oocyte fertilization[3], cell secretion[4–6], muscle contraction[7], neuronal migration and neurite growth[8,9], development[10], and apoptosis[11]. As such, calcium oscillation is a multi-functional and universal signal.

Typical FM-encoded signaling and the corresponding specific activation of cellular function or protein functional modulation are exemplified by the calcium/calmodulin dependent protein kinases and phosphatases. The multifunctional calcium/calmodulin-dependent protein kinase II (CaM Kinase II) has been extensively investigated because of its involvement in many cellular functions such as neuronal plasticity and memory formation[12]. Detailed studies have delimited the specific calcium oscillating frequency that this enzyme is sensitive to. It was found that this enzyme is maximally activated by calcium oscillations in the frequency range of a few Hz[13]. This is in sharp contrast with the frequency modulation of calcium/calmodulin-dependent protein phosphatase IIB or calcineurin (CN). This enzyme is a vital component in the modulation of transcription factor NFAT and is mainly modulated by calcium increases in the cytosol[14,15]. Calcineurin is optimally activated by calcium oscillations in the frequency range of about 10 mHz[16,17]. A comparison of the specific activation frequency of CaM Kinase II and calcineurin is illustrated in Figure 1.

The above examples are only the beginning of probably a very long list of such frequency-encoding cases. This list is likely to be growing at a fast rate in the next few years as more detailed works are carried out to investigate the involvement of calcium/calmodulin-dependent cytosolic proteins in specific cellular functions, which are modulated by calcium oscillating frequencies. It may be possible sometime in the future to group these proteins to provide a modulation spectrum, based on frequencies, similar to the light spectrum.

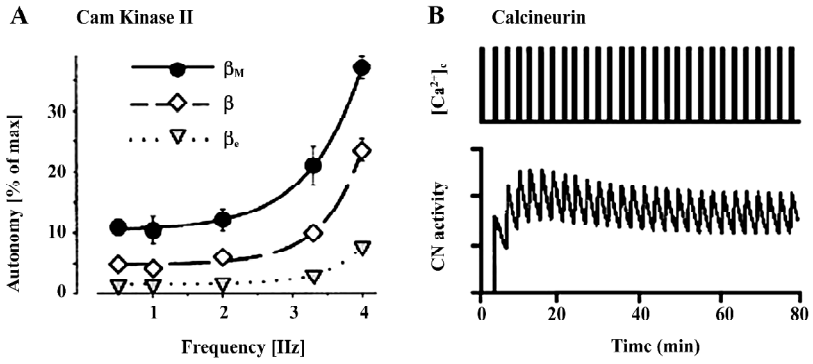

Figure 1. Frequency-modulation (FM) of calcium oscillations. Calcium oscillation can encode diverse and specific activating signals. Calcium/calmodulin-dependent protein kinase II (CaM kinase II) is activated at a frequency of a few Hz (A), whereas calcineurin (CN) is activated at a frequency of about 10 mHz (*B*). β_M, β, β_e, refer to different splice isoforms of CaM Kinase II β. Modified with permission from Bayer KU, de Koninck P & Schulman H. (© 2002 Nature Publishing Group)[13] and Tomida *et al* (© 2003 Nature Publishing Group)[17] .

Other than frequency modulation, it would also be interesting to see protein-specific amplitude modulations. Although most of the cell types after stimulation regularly oscillate at the level of 600–700 nmol/L such as pancreatic acinar cells[18], brown adipocytes[19], the magnitude of calcium oscillation spikes increases with increasing concentrations of the stimuli[18]. It would also be interesting to analyze the on- and off-rates of each individual calcium spike in different cells and under different circumstances, which determine the particular shape of the individual spike. Relative densities of IP_3R/calcium channels, and SERCA/PMCA isoforms and their activity status, such as phosphorylation status will shape the overall profile for each spike. The off rate, for example, may determine the speed at which a particular enzyme returns to its de-activated state.

During the course of our studies investigating the mechanisms of pace-making activities for calcium oscillations in non-excitable cells, we noted that some isolated exocrine cells *in vitro* did not typically respond to hormone or neurotransmitter stimulation by oscillatory increases in $[Ca^{2+}]_c$. The rodent submandibular gland acinar cells are noted to increase $[Ca^{2+}]_c$ not in the form of oscillations, especially at low stimulating concentrations, but rather in the form of graded plateaus. The rat submandibular acinar cells responded to noradrenaline or acetylcholine stimulation by plateau increase in $[Ca^{2+}]_c$, even at the minimal or threshold stimulating concentrations used[20]. All of these works have been carried out on the isolated submandibular acini bathed in a Kreb's-like buffered solution. But, in fact, these acinar cells and other components of the secretory axis in submandibular gland *in vivo* may actually be bathed in extracellular medium with very different composition.

Submandibular gland: microanatomy, potassium and osmolarity gradients along the secretory axis

The rat submandibular gland structure is illustrated in Figure 2. The secretory axis starts with acinus, a few acinar cells form a spherical acinus structure. The luminal side of the acinus is connected to the rather narrow intercalated duct. Intercalated duct cells are the smallest cells in the secretory axis. The intercalated duct is connected to the convoluted granular tubules (GCT), striated duct, excretory duct, and ends with the main excretory duct in the mouth. Acinar cells, cells in GCT and striated ducts are 20–30 μm in diameter, the intercalated duct is only about 10 μm[21].

The salivary gland cells have a large portion of their surface bathed in the saliva, which is different from ordinary extracellular fluid. The usual inorganic compositions of extracellular fluids are rather similar to that of serum, with potassium at approximately 5 mmol/L and total osmolarity at 310 mOsm. Most of the cells in the body are bathed in such extracellular fluids. But the salivary cells are different in that at least one side of the cells is bathed in saliva. In fact in the submandibular gland, some authors reported that the acinar cells are nearly encircled by the extended acinar lumen system[22].

Saliva formation is divided into two steps. Primary saliva is formed at the site of acini/intercalated ducts, with a composition similar to that of serum and extracellular fluid but with obviously enhanced potassium: Na^+ 126–136 mmol/L, K^+ 8.4–11.9 mmol/L, osmolarity 310 mOsm[21]. The potassium concentration increases and total osmolarity decreases progressively along the secretory axis. The osmolarity drops to 307 mOsm at the intercalated duct, 229 mOsm at the main excretory duct, and 89 mOsm at the main excretory duct orifice, which opens to the oral cavity[23]. At the main excretory duct, K^+ increases to 130 mmol/L[21,23]. To take into consideration of this peculiarity, some early researchers raised the potassium concentration in the Kreb's buffer to 15 mmol/L; these authors noted that such a raised potassium concentration was needed to maintain acinar structure [24]. But the effects of the significant changes in inorganic components of the saliva along the secretory axis (K^+ from 8.4 to

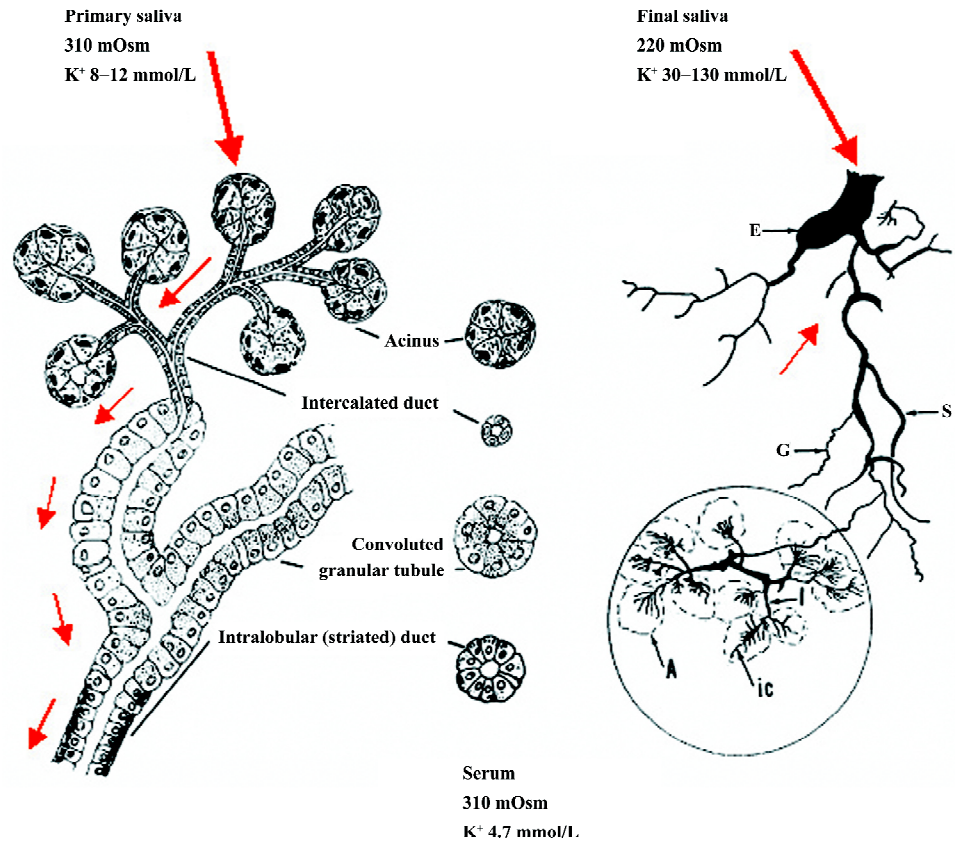

Figure 2. Rat submandibular gland. Microanatomy, potassium and osmolarity gradients. Heavy arrows indicate place of formation of primary and final saliva. Thin arrows indicate direction of saliva flow. In the right panel, the relative locations of acini (A), intercellular canaliculi (ic), intercalated ducts (I), granular tubules (G), striated ducts (S), and excretory ducts (E) are indicated. Modified with permission from: Schneyer, Young & Schneyer (© 1972 the American Physiological Society)[21].

30–130 mmol/L, osmolarity from 310 to 220 mOsm)-on the functions of the acinar and different ductal cells, and the effects of any disturbances of such ordered gradients on both the physiology and pathology, have never been systematically investigated at the cellular and molecular levels. The fact that large amounts of potassium ion are secreted after stimulation of the salivary glands has long been noted, but the physiological significance of the secreted potassium has never been properly addressed[20,25–27].

Calcium oscillations along the secretory axis in submandibular gland

The combination of AM, FM and SM of calcium oscillations as mentioned above ensures both complexity and diversity, and probably also specificity in the encoded calcium signals. In rat submandibular gland acinar cells, both ACh and noradrenaline induced plateau increases in cytosolic calcium concentrations, rather than oscillatory increases (Figure 3). To trace the possible source of this unique property, we looked for typical differences in salivary acinar cells and other exocrine acinar cells and found significant differences in the extracellular fluid that bathe the cells and the gradual changes in saliva composition. Yoshida *et al*[26] made the initial discovery that when the potassium concentration in Kreb's buffer was raised (to 30 mmol/L), ACh-induced calcium increases changed dramatically from plateau increases to oscillatory increases. This discovery was confirmed in our laboratory (Figure 3). In addition, we found that this transformation was applicable to cholinergic stimulation, but not to adrenergic stimulation[20]. This provides a cellular basis for the differences in sympathetic and parasympathetic stimulation of saliva secretion in the salivary glands (Figure 3).

The intercalated ducts are rather small. The GCT or granulated duct cells are the largest in the submandibular secretory axis and may be up to 30 μm in diameter (Figure 2). Granulated ductal cells are typical exocrine cells, but surprisingly very little is known about these cells, especially about their calcium signal-encoding and molecular mechanisms of exocytosis. Submandibular granulated duct cells are known to contain multiple secretory products, such as tissue kallikrein[28], growth factors epidermal growth factor (EGF) and nerve growth factor (NGF)[29], carbonic anhydrase[30], insulin-like proteins[31], angiotensin[32], and chromogranins[33]. Fine-tuning may be required for specific release of desired secretory products after nerve stimulation of the subman-

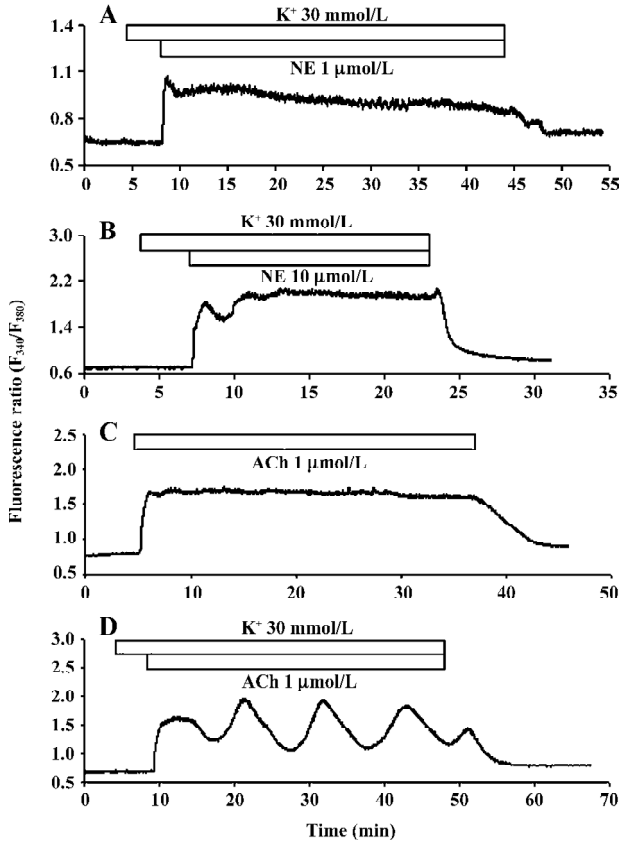

Figure 3. High-potassium transforms parasympathetically-induced plateau increases in $[Ca^{2+}]_c$ into oscillatory increases, but did not influence sympathetically-induced plateau increases, in the freshly isolated rat submandibular acini. High potassium (K^+ 30 mmol/L), noradrenaline (NE 1 or 10 μmol/L), and acetylcholine (ACh, 1 μmol/L) were added as indicated by the horizontal bars. NE at 1 μmol/L (A), and at 10 μmol/L (B) induced plateau increases in $[Ca^{2+}]_c$ in the presence of high K^+ 30 mmol/L. ACh (1 μmol/L) induced plateau increase in $[Ca^{2+}]_c$ in the absence of high K^+ (C), but induced oscillatory increases in $[Ca^{2+}]_c$ in the presence of high K^+ 30 mmol/L (D). $[Ca^{2+}]_c$ is expressed as 340/380 ratios, which were measured in a Photon Technology Incorporation (PTI, New Jersey, USA) calcium measurement system (DeltaScan). Reprinted with permission from Ma, Chen & Cui (© 2004, Elsevier)[20]

dibular gland. But works on isolated mouse GCT so far observed only graded plateau increases after cholinergic and adrenergic stimulations (JIA & CUI,unpublished). This is in contrast with works reported in a human submandibular gland ductal cell line (HSG) where carbachol induced regular calcium oscillations[34]. To clarify whether oscillatory calcium increases occur in rodent GCT cell *in vivo*, *in situ* imaging or sophisticated *in vitro* maneuverings in terms of the extracellular microenvironments are required.

Potassium modulation of other neurotransmitter stimulations, such as purinergic stimulation, is not known at the moment. Neither is it known whether such potassium modulation applies to the ductal cells in the intercalated duct, granulated duct, and striated duct. The effects of hypo-osmolarity remain to be determined also. These works may have important implications for salivary diseases such as cystic fibrosis, and Sjogren's syndrome.

Preliminary data indicated that high potassium-modulation of calcium signals in the submandibular gland may be related to sodium/calcium exchanging activity (MA and CUI, unpublished). NCX1 has been found to exist in submandibular gland[27,35–37], possibly working in the reverse mode[27,38], although detailed distribution along the secretory axis is not known. Future works involving primary cultures of granulated and striated duct cells, and iRNA downregulation of NCX1 will more definitively elucidate the involvement of NCX1 in high potassium- and low osmolarity-induced transformations of calcium increases.

In conclusion, the submandibular gland is unique in that it has all the secretory components in the secretory axis: acinus, intercalated duct, granular convoluted tubules, striated duct, and excretory duct. The potassium and osmolarity gradients in saliva may play a feedback role in regulating neurotransmitter-induced calcium increases. High potassium transformation of cholinergically-induced plateau increase into oscillatory increase serves as a typical example of such feedback regulation. Additional works will be needed to reveal whether this is a general rule along the secretory axis, especially in the intro- and inter-lobular ducts.

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