Different Expression of Synaptotagmin Isoforms in Mouse and Rat Parotid Acini

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Abstract

Different Expression of Synaptotagmin Isoforms in Mouse and Rat Parotid Acini

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(Directed by Prof. Heung-Kyu Son, D.D.S., Ph. D.)

Synaptotagmin is a Ca$^{2+}$ sensing protein that triggers a fusion of synaptic vesicles in neuronal transmission. It is not known whether mouse and rat parotid acinar cells have a similar type of synaptotagmin isoforms and the contribution of synaptotagmin to the release of secretory vesicles in exocrine gland cells. We investigated the type of synaptotagmin and Ca$^{2+}$ signaling and amylase secretion in rat and
mouse parotid acinar cells using RT-PCR, microfluorometry, and amylase assay. Mouse showed more sensitive amylase release in response to muscarinic stimulation than rat. However, the increases in \([\text{Ca}^{2+}]_i\) were identical, suggesting that the expression or activity of \(\text{Ca}^{2+}\) sensing signaling proteins is different between a rat and mouse. Whereas in a rat parotid acinar cells synaptotagmin 2, 5 and 6 were not expressed, mice showed the expression of all kinds of \(\text{Ca}^{2+}\)-dependent synaptotagmin from 1-7. These results might indicate that different expression of synaptotagmin contributes to the release of secretory vesicles in parotid acinar cells.

Key words : Synaptotagmin, Calcium Signaling, Parotid Acinar Cells, Exocytosis
I. Introduction.

Mammalian salivary protein secretion is mainly evoked by the β-adrenergic stimulation, which generates intracellular second messenger, cAMP, followed by the activation of cAMP dependent protein kinase (PKA) (Quissel D et al. 1993; Takuma T et al. 1994). Salivary fluid secretion is regulated by intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) increases by cholinergic and α-adrenergic stimulation, to evoke fluid secretion to open Ca\(^{2+}\)-activated Cl\(^-\) channel at luminal membrane (Fig.1) (Turner R et al. 2002). Most regulated exocytosis is regulated by the elevation of [Ca\(^{2+}\)]\(_i\), such as synaptic transmission, the release of insulin and pancreatic enzymes (Levius O et al. 1997). In some cases intracellular cAMP concentration [cAMP]\(_i\) increases induce exocytosis of secretory proteins such as glucagons and luteinizing hormone. In parotid acinar cells most exocytosis is regulated by [cAMP]\(_i\) increases without affecting [Ca\(^{2+}\)]\(_i\) and PKA activation is essential for cAMP-
dependent exocytotic secretion (Levius O et al. 1997; Quissel D et al. 1993; Takuma T et al. 1994). Therefore, parotid gland acini are regarded as a good model for investigating cAMP-dependent exocytotic secretion. But muscarnic stimulation also evokes the release of secretory vesicle without affecting [cAMP]i, although the amount of protein secretion by Ca²⁺ is lower than by cAMP (Butcher F et al. 1980; Sugiya H et al. 1989). However, the exact mechanism of Ca²⁺-dependent exocytotic pathway in salivary protein secretion has not been clearly understood.

The mechanism of Ca²⁺-dependent exocytotic pathway in neuronal cells has been known well and is mediated by SNAREs (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors). Ca²⁺-dependent exocytosis was investigated in neuronal cells where Söller et al. proposed the ‘SNARE hypothesis’, namely that synaptic vesicles docking to a target membrane through the interaction of the
vesicles and target membrane protein referred to as SNAP receptors (Söller T et al. 2003). In neuronal cells, the assembly of a core complex composed of three proteins mediate exocytosis: VAMP2 is a vesicle-associated SNARE (v-SNARE), and syntaxin1 and SNAP-25 are target membrane SNAREs (t-SNARE). These proteins spontaneously assemble to into a functional complex and are sufficient to mediate exocytosis. In neuronal cells, the family of the Ca\textsuperscript{2+} binding proteins, synaptotagmins, are believed to consist, or at least be part of the block in Fig 2. A (Chapman E et al. 2002). The synaptotagmin family consists of at least 15 members that can be grouped into three subclasses (Fukuda M et al. 2003). In addition, synaptotagmins family can be grouped into Ca\textsuperscript{2+}-dependent or independent type (Table1). Genetic and biochemical evidence showed that synaptotagmin I functions as a low affinity Ca\textsuperscript{2+} sensor (Geppert M et al. 1998). Synaptotagmin III may have other Ca\textsuperscript{2+}-dependent functions in
neuronal cells (Butz S et al., 1999), but it appears to serve as a high affinity Ca\textsuperscript{2+} sensor for exocytosis in neuroendocrine cells, such as pancreatic islet β-cells (Brown H et al., 2000; Gao Z et al., 2000; Mizuta M et al., 1997). The role of the other synaptotagmins in cell function is not known at present.

In non-neuronal cells, syntaxin 4 and SNAP-23 are the plausible candidate for t-SNAREs. Both of them were detected in rat parotid acinar cells (Fig 2. B), but they were not coimmunoprecipitated with VAMP-2, suggesting that their interactions with VAMP2 may be weak (Takuma T et al., 2000). In the previous study, synaptotagmin I was detected and copurifies with rat parotid acinar cells (Levious O et al. 1997). But recently Imai et al. (2001) could not find synaptotagmin I except synaptotagmin III and IV using RT-PCR. Regardless of the existence of synaptotagmin I, parotid acinar cells might secrete proteins in Ca\textsuperscript{2+} dependent manners like neuronal cells, because the
protein secretion occurred in parotid acinar cells by increase of \([\text{Ca}^{2+}]\).

Stimulation with muscarinic, substance P, peptidergic or \(\alpha\)-adrenergic receptors also elicits significant amylase release from the rat parotid, albeit at levels that are significantly lower than those observed from a \(\beta\)-adrenergic receptor-mediated response. The stimulation of these receptors activates phosphatidyl inositide metabolism and induces an increase in intracellular \(\text{Ca}^{2+}\) concentration without acting intracellular cAMP levels (Butcher F et al. 1980; Sugiya H et al. 1989).

Alteration in \([\text{Ca}^{2+}]\), is a key regulator of many cellular process (Berridge MJ et al. 2003). To allow precise regulation of \([\text{Ca}^{2+}]\), and a diversity of signaling by this ion, cells possess many mechanism by which they are able to control \([\text{Ca}^{2+}]\), both globally and at subcellular level. The elevated \(\text{Ca}^{2+}\) give an effect to the factor related to the protein secretion in the parotid acinar cells. Accordingly, synaptotagmin would be the plausible candidate of \(\text{Ca}^{2+}\) dependent protein secretion.
in parotid acinar cells.

In the present work, we investigated the type of synaptotagmin and 
Ca\(^{2+}\) signaling in parotid acinar cells, because it is not known whether 
mouse and rat parotid acinar cells have which kind of synaptotagmins 
and the contribution of synaptotagmin to the release of secretory 
vesicles.
Fig 1. Schematic regulation mechanism of salivary secretion. The cellular mechanism whereby adrenergic, muscarinic, and substance P stimulation evoke salivary secretion. β-adrenergic receptor increases intracellular levels of cAMP and exocytosis is regulated by [cAMP], increases and PKA activation is essential for cAMP-dependent exocytotic secretion. Muscarinic stimulation increases intracellular Ca²⁺ level to open Ca²⁺-activated Cl⁻ channel at luminal membrane. AC, adenynyl cyclase; PLC, phospholipaseC; ER, endoplasmic reticulum; TJ, tight junction
Fig 2. SNARE complex in neuronal cells (A) and in parotid acinar cells (B). In neuronal cells, elevation of intracellular Ca\(^{2+}\) level induces exocytosis, and synaptotagmin is a candidate for the calcium sensor that changes its conformation and triggers membrane fusion. In parotid acinar cells, the expression and function of SNAREs such as synaptotagmin and other SNAREs proteins is not clearly known.
Table 1. Isotypes of synaptotagmin and its calcium dependency

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<th>Calcium dependency</th>
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<td>Class 1</td>
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</tr>
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<td>Class 2</td>
<td>Synaptotagmin 7</td>
<td>Brain ubiquitous; localized to active zone and plasma membrane and in a kidney</td>
</tr>
<tr>
<td>Class 3</td>
<td>Synaptotagmin 3, 5, 6, and 10</td>
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<td>Synaptotagmin 4 and 11</td>
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<td>Class 6</td>
<td>Synaptotagmin 8, 12, and 13</td>
<td>Brain ubiquitous</td>
</tr>
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</table>

Calcium-dependent type

| I | II | III | IV | V | VI | VII |

Calcium-independent type

| VIII | XII | XIII | XV |
II. Materials and Methods

1. Preparation of parotid acinar cells from mice and rats

Male sprague-Dawley rats (150-250g) and ICR strain mice (23-28g) were sacrificed by cervical dislocation method. The cells were prepared from the parotid of SD rats and ICR mice by limited collagenase digestion as described previously (Zeng W et al. 1997). For pure isolation of acinar cells, density gradient centrifugation was performed with Accudenz and pure acinar cells were confirmed under light microscope. After isolation, the acini were resuspended in an extracellular physiologic salt solution (PSS) as follows: NaCl, 140 mM; KCl, 5mM; MgCl₂, 1 mM; CaCl₂, 1 mM; HEPES, 10 mM; and glucose, 10 mM titrated to pH 7.4 with NaOH. The osmolality of the extracellular solution (measured with a FISKE 110 osmometer), was 310 mOsm (Hong J et al. 2004).
2. \([\text{Ca}^{2+}]_i\) measurement

Cells were incubated for 40 mins in PSS containing 5 \(\mu\text{M}\) fura2-acetoxymethyl ester (Teflabs inc. Austin. USA) in the presence of Pluronic F-127 to enhance dye loading. Changes in \([\text{Ca}^{2+}]_i\) were measured by means of fura2 fluorescence, with excitation wavelengths of 340 nm and 380 nm, respectively, and an emission wavelength of 510 nm at room temperature. Background fluorescence was subtracted from the raw signals at each excitation wavelength before calculating the fluorescence ratio as follows:

\[
\text{Ratio} = \frac{F_{340}}{F_{380}}.
\]

The emitted fluorescence was monitored with a CCD camera (Photon Technology International Inc, Lawrenceville, NJ) attached to an inverted microscope. Fluorescence images were obtained at 2 s intervals. The each cell was stimulated by carbachol in a dose-dependant manner (Hong J et al. 2004).
3. Amylase assay

The animals were allowed water but were starved for 24hr before experiment. Each acinar cells was stimulated equal concentrations used in [Ca\(^{2+}\)], measurement study. Acinar cells were incubated with carbachol during 20 min in shaking incubator at 37°C and 60 rpm. The carbachol concentrations were followed dose dependent manner. Acinar cells were lysed by sonication. The lysates were clarified by centrifugation at 13,000 rpm for 10 min. The total amylase content or that released into the medium was determined by the method described by Bernfeld et al. (1951) using starch suspension as the substrate. Aliquots of the incubation medium and of the supernatants of the homogenized glands were incubated at 37°C with a 0.5% starch suspension during 10 min. Absorbance was measured at a wavelength of 540 nm. Amylase activity in the medium was expressed as a percentage of the total activity.
4. Western blotting

Protein extracts were prepared from parotid acinar cells as follows. Pure acinar cells were washed with ice-cold PBS and then lysed by adding Tris-HCl, NaCl, and EDTA buffer (1% NP-40, 10mM of Tris HCl [pH7.8], 150mM NaCl, 1mM of EDTA, 2mM of Na3VO4, 10mM of NaF, 10µg/ml of aprotinin, 10µg/ml leupeptine, 10µg/ml of PMSF) The lysates were clarified by centrifugation at 13,000 rpm for 10 min. Samples were separated by 12% SDS–PAGE. Proteins in the gel were transferred to Nitrocellulose membrane (Schleicher and Schuell Bioscience, Dassel, Germany) for 1h at current of 200mA. The Nitrocellulose membrane was blocked by incubation in 6% skim milk in buffer TTBS [1X TBE solution + 0.1% Tween 20]. Then the membrane was probed overnight at 4 °C with each primary antibody, anti-α amylase (Sigma, Saint Louise, USA) and anti-synaptotagmin I (Alomone Labs, Jerusalem, Israel) polyclonal antibody (1:2,000,
1:1000). After the membrane was washed three times with buffer TTBS, it was incubated with horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, USA) buffer TTBS containing 3% skim milk for 1 h at room temperature and washed with buffer TTBS. Detection was performed using an ECL detection system (Amersham Biosciences, Uppsala, Sweden) and immunoreactive bands were visualized using Medical X-Ray film (AGFA).

5. RT-PCR

Total RNA was isolated from brain and acinar cells. Brain was homogenized using homogenizer with Liquid Nitrogen. Total RNA was isolated using Trizol reagent (Sigma, Saint Louise, USA), chroloform, and isoprophanol. Relative RT-PCR was permformed to measure gene expression of synaptotagmin isoforms. The specific primers for each synaptotagmin isoforms are described in Table 2. Polymerase Chain
Reactions were performed on a T gradient 96 PCR machine (Biometra Co., Gottingen, Germany) using 1~2nM of cDNA, 5 pmoles each oligonucleotide primer, 200µM of each dNTP, 1 unit of Taq polymerase (Applied Biosystems, California, USA) and 10 X Taq polymerase buffer in a 50µl volume. The PCR program initially started with 94°C denaturation for 30 sec, followed by 40 cycles of 94°C/30sec, Ta/1min, 72°C/1min (Ta, annealing temperature; 56°C to 61°C).
Table 2. The PCR primers encoding Ca$^{2+}$-dependent synaptotagmin

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<td>U201</td>
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*Accession numbers of GeneBank/EMBL data bank*
6. Data analysis and statistics

The results are expressed as the mean ± S.E.M. The statistical significances of differences between the groups were determined using the Student T-test. In statistical tests, the p value < 0.05 was considered to be significant.
III. Results

1. Ca$^{2+}$-dependent exocytosis is more sensitive to agonist in cells from mice.

To compare the amylase release between parotid acinar cells from rats and mice, amylase release assay was performed. Fig.3 shows typical amylase secretion curve with stimulation of carbachol, muscarinic agonist from the range of 0.1µM to 100µM in parotid acinar cells from rats and mice. Mice showed that up to 3 µM carbachol, amylase release increased in a dose-dependent manner, but at 100 µM amylase release was slightly decreased: 2.33 ± 0.48% at 0.1 µM, 6.22 ± 0.93% at 0.3 µM, 9.71 ± 0.85% at 1 µM, 10.7 ± 0.81% at 3 µM, and 9.15 ± 0.86% at 100 µM (n=4). In Rats increases of agonist concentration evoked dose-dependent increases in amylase release: 0.55 ± 0.3% at 0.1 µM, 3.83 ± 0.56% at 0.3 µM, 6.68 ± 0.48% at 1 µM, 8.2 ± 0.66% at 3 µM, and 9.01 ± 0.41% at 100 µM (n=4). Ca$^{2+}$-
triggered exocytosis from mouse acinar cells occurred with the apparent affinity of ~ 0.26 ± 0.03 µM (n=4). In contrast, Ca^{2+}-triggered exocytosis from rat acinar cells occurred with the apparent affinity of ~ 0.48 ± 0.02 µM (n=4). Thus, Ca^{2+}-dependent exocytosis was ~ 2-fold more sensitive to agonist in cells from mice. It is of note that the extent of exocytosis at optimal concentration of 100 µM carbachol was the same in two cell types.
Fig 3. Concentration-response curve of muscarinic agonist, carbachol, on amylase release by parotid gland acinar cells from rats and mice. Amylase releases were measured with stimulation of carbachol, muscarinic agonist from the range of 0.1µM to 100µM in parotid acinar cells from rats and mice. Results are expressed as the mean ± S.E.M. of four experiments in each group. * P<0.01
Because amylase assay of Fig. 3 indicates the relative release of amylase (% total), the amount of amylase protein in parotid acinar cells cannot indicate. Therefore, we investigated amylase release using a different protocol. First, parotid acinar cells were treated with each concentration of carbachol for 20 min and then, cells were centrifuged at 14,000rpm for 5-10 sec and Western blotting were performed with cell pellets. Each lane contained 50µg of extracted proteins and remaining amylase contents were measured with pAb against α-amylase. Fig. 4A shows that in resting state mice and rats have a similar content of amylase in remained proteins and with the increases of carbachol concentration from 0.3µM, the intensity decreased, indicating that remnant rate of amylase in rat; 1.09 ± 0.08 at 0.1 µM, 0.96 ± 0.16 at 0.3 µM, 0.83 ± 0.14 at 1 µM, 0.62 ± 0.11 at 3 µM, and 0.49 ± 0.08 at 100 µM (n=8); in mouse 0.99 ± 0.04 at 0.1 µM, 0.78 ± 0.01 at 0.3 µM, 0.51 ± 0.10 at 1 µM, 0.43 ± 0.01 at 3 µM, and 0.27 ±
0.06 at 100 μM (n=5). Although we could not detect any changes in amylase contents at 0.1μM of carbachol, mice showed more sensitive amylase release than rats from 0.3μM to 100μM of carbachol in Fig. 4B.
Fig 4. Concentration-response curve of muscarinic agonist carbachol on the remaining amylase contents. A, After parotid acinar cells were treated with each concentration of carbachol for 20 min, cells were centrifuged at 14,000rpm and Western blotting were performed with cell pellets. Remaining amylase contents were measured with pAb against amylase. Each lane contains 50 µg of proteins. B, concentration-response curve of Western blot data. Results are expressed as the mean ± S.E.M. of four to five experiments in each group. * P<0.05
2. $[\text{Ca}^{2+}]_i$ increases with muscarinic stimulation in rats and mice parotid acini are identical.

Amylase release depends on $[\text{cAMP}]_i$, and $[\text{Ca}^{2+}]_i$ increases in parotid acinar cells. In both cells amylase release with the same concentration of muscarinic stimulation was different. Therefore, we investigated whether the same concentration of muscarinic stimulation evoked the same $[\text{Ca}^{2+}]_i$ increases in both cells or not. The fluorescence of fura2-loaded cells was measured at the relative ratio of $[\text{Ca}^{2+}]_i$ in response to above-mentioned concentrations of carbachol. Although we did not detect the changes in $[\text{Ca}^{2+}]_i$ at 0.1 and 0.3 µM of carbachol, 1 µM, 3 µM, and 100 µM carbachol evoked $[\text{Ca}^{2+}]_i$ increases in a dose-dependent manner (Fig. 5A and B). The amplitude of each $[\text{Ca}^{2+}]_i$ increases in rats were 0.26 ± 0.05 at 1 µM, 0.44 ± 0.05 at 3 µM, and 0.88 ± 0.06 at 100 µM (n=7); in mice 0.24 ± 0.08 at 1 µM, and 0.38 ± 0.1 at 3 µM, and 0.89 ± 0.06 at 100 µM (n=7). Interestingly rat and mouse cells show identical $[\text{Ca}^{2+}]_i$ increases with the same
concentration of agonist, suggesting that both animals have the similar 

$[\text{Ca}^{2+}]_{i}$, increasing machineries.
Fig 5. Intracellular calcium measurements with muscarinic stimulation.
A. Intracellular Ca\(^{2+}\) concentration [Ca\(^{2+}\)], in parotid acinar cells was measured with the relative ratio of fura2 fluorescence in response to each concentration of carbachol. [Ca\(^{2+}\)], increased rapidly with agonist and slowly decreased. The amplitude of [Ca\(^{2+}\)], increases shows a dose-dependent manner. The traces are representatives of 7 different experiments. B, relative [Ca\(^{2+}\)], ratio in response to carbachol from 1 μM, 3 μM, and 100 μM. The amplitudes between rats and mouse were not significantly different. Results are expressed as the mean ± S.E.M. of seven experiments in each group.
3. Different expression of Ca\(^{2+}\)-sensing protein synaptotagmin

Since there was no difference of Ca\(^{2+}\) response between rat and mouse parotid acinar cells, we hypothesized the expression of Ca\(^{2+}\)-sensing protein differed from both cells. It is generally accepted that a protein related to Ca\(^{2+}\)-triggering exocytosis is a synaptotagmin, which has 15 different isoforms and 7 Ca\(^{2+}\)-dependent types from 1 to 7 (Südhof T et al. 2002). Imai (2001) reported parotid acinar cells from rats express 3 and 4 type of synaptotagmin, whereas rat brain all expresses 7 Ca\(^{2+}\)-dependent types. In addition, the existence and type of synaptotagmin in mouse parotid acinar cells are not reported. Fig. 6A shows the result of parotid acinar cells from rats expressing 3 and 4 type of synaptotagmin from Imai et al. As a positive control, RT-PCR for specific 7 Ca\(^{2+}\)-dependent types was performed in rat and mouse brain, which is known to have all kinds of synaptotagmin. Especially in rat experiments, we used the same PCR-primers used before by Imai
et al. (2001). In Fig.6B and C (n=7), mice and rats brain expressed 7 Ca$^{2+}$-dependent types of synaptotagmin from 1 to 7. Whereas rats did not express 2, 5, 6 type of synaptotagmin in parotid acinar cells, mice express Ca$^{2+}$-dependent types of synaptotagmin.
Fig 6. RT-PCR products encoding synaptotagmins. A, RT-PCR of synaptotagmin family from rats. b: brain, p: parotid (adapted from Imai et al., 2001, Archives of Oral biology), lane numbers indicate synaptotagmin isoforms. B, RT-PCR results of synaptotagmin family in rats with the same PCR-primer by Imai et al (2001). C, RT-PCR results of synaptotagmin family in mice. Note that parotid and brain from mice have all kinds of synaptotagmin isoforms, whereas parotid from rats expresses 1, 3, 4, and 7 synaptotagmin isoforms. Each data is the representative of five to eight different experiments.
Table 3. Expression of synaptotagmin and expected size of PCR products in rat and mouse brain and parotid acinar cells

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<tr>
<td>synaptotagmin</td>
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<td>4</td>
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<td>301</td>
<td>363</td>
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<tr>
<td>Brain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Parotid</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</table>
4. Synaptotagmin I is expressed in exocrine gland cells.

Since there was a debate about the expression of synaptotagmin 1 in rat parotid gland acinar cells, we did Western blot experiment in brain, parotid, submandibular, and pancreatic acinar cells using pAb against synaptotagmin 1. Western blot data shows that synaptotagmin 1 was expressed all kinds of epithelial cells in rats and mice (n=4).
**Fig 7. Western blot in brain, parotid, submandibular, and pancreatic acinar cells.** SDS-PAGE and western blot were performed with pAb against synaptotagmin I. Brain contains 40µg and parotid, submandibular, and pancreatic acinar cells contain 400µg of proteins in each lane.
IV. Discussion

In the present work, we studied the type of synaptotagmin in rat and mouse parotid acinar cells, which expression was mainly reported in brain tissues (Südhof T et al. 2002). Synaptotagmin acts as a $\text{Ca}^{2+}$ sensor in neuronal cells, which cytoplasmic domain contains two $\text{C}2$ repeats capable of binding phospholipids in a $\text{Ca}^{2+}$ dependent manner (Chapman E et al. 2002). Among the synaptotagmins, synaptotagmin I mainly found in nervous system has been intensively studied and found especially in synaptic vesicles and dense-core vesicles and serves as a major $\text{Ca}^{2+}$ regulator of neurotransmitter release in fast release of synaptic vesicles (Südhof T et al. 2002). In this study we have shown that synaptotagmin I is present in parotid acini with RT-PCR and western blot. For RT-PCR, we used the same design of primers for encoding synaptotagmin I from Imai et al (2001), which group reported the expression of synaptotagmin III and IV except
synaptotagmin I. Therefore, we verified presence of it with pAb against synaptotagmin I. Moreover, we found submandibular, pancreatic acinar cells also express synaptotagmin I with the same Ab, suggesting that most of exocrine gland acini has synaptotagmin I and it may be generally involved in regulating exocytosis of secretory vesicles. Interestingly, parotid and submandibular acini have ~1% of synaptotagmin I and pancreatic acini ~0.2% compared to brain tissues. Basically there was no difference of expression level in brain tissues between rats and mice.

The remarkable finding of our work is that rats and mice have different synaptotagmin isoforms and this could be related to the ability of exocytosis of salivary proteins such as amylase. RT-PCR results show that mice express all kinds of Ca^{2+}-dependent synaptotagmins and rats express type I, III, IV and VII. This different expression of it can explain the reason of left shift of amylase releases in respond to
muscarinic stimulation in mouse parotid acinar cells in Fig. 4. Additionally, we confirmed more sensitive amylase releases in mouse parotid acini with Western blot in Fig. 5. However, we don’t have any direct evidence that absence of synaptotagmins evoked different amylase releases in both cells. It is possible to investigate if we use knock-out mice of each synaptotagmin or permeablization technique with specific Abs against synaptotagmins.

Ca\textsuperscript{2+} is a key regulator of many cellular process. In parotid acinar cells, cholinergic or \(\alpha\)-adrenergic receptors are seven trans-membrane-spanning, G-protein coupled receptors, that activate Gq to release G\(\alpha_q\) and G\(\beta\gamma\). G\(\alpha_q\) activates phospholipase C\(\beta\) to generate 1,4,5-tris-inositolphosphate in the cytosol to release Ca\textsuperscript{2+} from the ER. Ca\textsuperscript{2+} release from the ER leads to activation of store-operated Ca\textsuperscript{2+} channels (SOCs) in the plasma membrane and Ca\textsuperscript{2+} release and influx increase [Ca\textsuperscript{2+}]. Subsequently, the plasma membrane Ca\textsuperscript{2+} pump
(PMCA) removes Ca\textsuperscript{2+} from the cytosol to reduce Ca\textsuperscript{2+} towards resting levels until [Ca\textsuperscript{2+}]\textsubscript{i} stabilizes at a plateau, the level of which depends on the relative ratio between the activity of SOCs and PMCA (Kiselyov K et al. 2003). In parotid acinar cells muscarinic stimulation directly indicates [Ca\textsuperscript{2+}]\textsubscript{i} increases. The difference of amylase releases in Fig. 4 promptly made us to investigate [Ca\textsuperscript{2+}]\textsubscript{i} in response to different concentration of muscarinic stimulations. Interestingly, both cells showed identical [Ca\textsuperscript{2+}]\textsubscript{i} increases in Fig. 5, indicating that both animals have the similar [Ca\textsuperscript{2+}]\textsubscript{i} increasing and decreasing machineries.
V. Conclusion

Mouse showed more sensitive amylase releases in response to muscarinic stimulation than rat. However, the increases in [Ca$^{2+}$]$_i$ were identical, suggesting that the expression or activity of Ca$^{2+}$ sensing signaling proteins is different between rats and mice. Whereas in a rat parotid acinar cells synaptotagmin 2, 5 and 6 were not expressed, mice showed the expression of all kinds of Ca$^{2+}$-dependent synaptotagmin from 1-7. These results might indicate that different expression of synaptotagmin contributes to the release of secretory vesicles in parotid acinar cells.
VI. Reference


Butz s., Fernandez C.R., Schmitz F., Jahn R., Sudohf T.C. The subcellular localization of atypical synaptotagmin III and VI.


국문요약

흰쥐와 생쥐 이하선세포에서 다르게 발현되고 있는 Synaptotagmin의 각 아형들

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Synaptotagmin은 신경전달기전에 관여하는 절전 신경세포에서 세포내 칼슘신호를 감지하며, 신경전달물을 함유하고 있는 분비과정의 세포막에 융합하여 분비되는 과정을 촉진시키는 중요 단백 물질이다. 그러나 신경세포이외 다른 조직이나 세포에서 synaptotagmin의 발현과 역할에 대한 보고는 거의 없는 상태이다. 특히 타액선세포인 이하선세포에서 synaptotagmin의 각 아형에 따른 발현, 그리고 synaptotagmin의 정확한 역할에 대하여는 알려져 있지 않다. 이에 본 연구에서는 흰쥐와 생쥐 이하선세포에서 RT-PCR, 미세형광분석법, 아밀라제 분석법, 그리고 Western blot을 통해 두 세포에서의 synaptotagmin의 각 아형에 따른 발현과 역할을 알아보고자 하였다. 무수카린성
자극시 이하선세포는 농도에 의존적으로 아밀라제 분비를 촉진시켰는데, 생쥐는 흰쥐에 비해 같은 농도의 자극에 대해 보다 많은 아밀라제를 분비시켰다. 무수카린성 자극에 의한 아밀라제 분비는 세포내 칼슘농도의 증가에 의존적이기 때문에 두 세포간의 무수카린성 자극시 나타나는 칼슘농도의 증가 크기를 비교한 결과 동일함을 발견하였다. 이는 칼슘증가와 연관된 칼슘신호 단백들의 발현과 활성도는 차이가 없음을 의미하며, 칼슘신호를 감지하는 기전에 차이가 있을 가능성을 나타낸다. 이에 가장 대표적으로 알려진 칼슘신호 감지 단백인 synaptotagmin 중 칼슘의존성 아형에 따른 발현을 비교하였다. 이하선세포에서 생쥐는 칼슘의존성 synaptotagmin인 I-VII에 걸친 아형을 발현시키고 있었으나 흰쥐는 synaptotagmin I, III, IV, 그리고 VII만을 발현시키고 있었다. 더불어 그간 논란이 되어온 흰쥐에서의 synaptotagmin I의 발현을 Western blot을 통해 확인하였다. 이상의 실험결과로 미루어 이하선세 포는 칼슘의존성 synaptotagmin을 발현하고 있으며 이들이 분비과정의 분비에 관여하고 있을 가능성을 나타내고 있다.

핵심되는 말 : Synaptotagmin, 칼슘신호전달, 이하선세포, Exocytosis