Simultaneous monitoring of Ca^{2+} response and salivary secretion in rat submandibular gland in live animals

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[Background] Effects of Ca^{2+} responses on the activation of ion channels have been studied extensively, while the Ca^{2+} responses during the salivary secretion are still unknown. Thus, we do not even know the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) required for inducing fluid secretion. To address these questions, we developed a method for real time imaging of Ca^{2+} responses with simultaneous monitoring of salivary fluid secretion in live animal. [Methods] We expressed the genetically encoded Ca^{2+} indicator, YC-Nano50, following gene transfer via retrograde ductal injection of adenovirus vectors through the orifice of rat submandibular gland (SMG). To perform Ca^{2+} imaging in the YC-Nano50-expressing SMG, we used the multi-purpose zoom fluorescence microscope. Salivary secretion from SMG was measured using the fiber optic pressure sensor. [Results] An intravenous infusion of acetylcholine (ACh) increased $[\text{Ca}^{2+}]_i$ throughout the SMG. ACh-induced Ca^{2+} responses and salivary flow rate in SMGs were increased in a dose-dependent manner (720 nmol/min). The apparent ED50 values for ACh stimulation on $[\text{Ca}^{2+}]_i$ rise and salivary secretion were 74 and 118 nmol/min, respectively. Repetitive infusion of ACh induced similar levels of salivary secretion, associated with a similar rise in $[\text{Ca}^{2+}]_i$. Simultaneous monitoring of Ca^{2+} response and salivary secretion revealed a clear time lag between the onset of rise in $[\text{Ca}^{2+}]_i$ and that of salivary secretion. The time lag with the lowest dose of ACh (30 nmol/min) was 106 s, which shortened to 19 s with the dose used for maximum salivary secretion (360 nmol/min). Based on the assumption that the resting $[\text{Ca}^{2+}]_i$ in SMGs is 37 nM, the estimated $[\text{Ca}^{2+}]_i$ at the onset of salivary secretions were 57 nM, irrespective of ACh dose. These results indicate that low $[\text{Ca}^{2+}]_i$ is sufficient to trigger fluid secretion in the rat SMG *in vivo*. Interestingly, we found that low doses of ACh (< 120 nmol/min) induced Ca^{2+} oscillations with tissue-wide synchronization in SMG. These Ca^{2+} oscillations were associated with the oscillatory changes in blood flow. [Conclusions] We demonstrate Ca^{2+} responses during ACh-induced salivary secretions using a novel *in vivo* simultaneous monitoring of Ca^{2+} and salivary secretions.