

## EXPANDED ABSTRACT

# Anion secretory functions of acinar and intralobular duct cells in the rat parotid gland

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To clarify difference of anion secretory function between acinar and intralobular duct cells in the rat parotid gland, we investigated anion currents with the gramicidin-perforated patch recording method. Anions are supplied only by the cells themselves and released through anion channels in the gramicidin-perforated patch configuration. Accordingly, the anion currents measured with the present method reflect the anion-supplying activity and the anion channel activity of the cells (1). Furthermore, anion conductance, which reflects the anion channel activity, can be measured by superimposition of brief 5 mV pulses on the holding potential separately from anion currents. Thereafter the driving force, which reflects intracellular anion concentration, can be estimated.

In the acinar cells, carbachol (CCh), a Ca<sup>2+</sup>-increasing agent, induced an oscillatory anion current, of which amplitude became rather steady in 5 min after the CCh addition. cAMP-increasing agents, isoproterenol (IPR) and forskolin, evoked no marked current and reduced the CCh-induced oscillatory current (2). Bumetanide suppressed the CCh-induced oscillatory current in the steady state, suggesting that the oscillatory current in the steady state is driven mainly by Cl<sup>-</sup> uptake activity of the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter. Superimposition of brief 5 mV pulses on the holding potential, -80 mV, under the suppression of K<sup>+</sup> channels by blockers revealed that anion conductance was oscillatory. The Ca<sup>2+</sup> ionophore, A23187, induced a nonoscillatory inward

current in the acinar cells. The A23187-induced current and the driving force in the steady state were bumetanide-sensitive, but membrane conductance was not very sensitive to bumetanide. These are consistent with the effect of bumetanide on the CCh-induced oscillatory current.

In the intralobular duct cells, both CCh and IPR induced nonoscillatory currents with nonoscillatory increases in membrane conductance. The Ca<sup>2+</sup> ionophore, A23187, mimicked the CCh-induced current and the A23187-induced current in the steady state was blocked by diphenylamine-2-carboxylate. Forskolin mimicked the IPR-induced current and the forskolin-induced current in the steady state was sensitive to glibenclamide (3) and CFTR<sub>inh</sub>-172. All these currents during the steady state were inhibited by HCO<sub>3</sub><sup>-</sup> removal and addition of methazolamide and 5-(N,N-dimethyl)amiloride (DMA). The driving force, estimated from currents and membrane conductance, was sensitive to methazolamide and DMA, but membrane conductance was not. These suggest that the duct cells secrete HCO<sub>3</sub><sup>-</sup> with coordinated activities of the carbonic anhydrase and the Na<sup>+</sup>-H<sup>+</sup> exchanger, through a kind of Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channel and the CFTR Cl<sup>-</sup> channel activated by Ca<sup>2+</sup> and cAMP signals, respectively.

We conclude that Cl<sup>-</sup> secretion in acinar cells depends on an oscillatory increase in anion conductance and the driving force produced by the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter during the Ca<sup>2+</sup> signaling, while HCO<sub>3</sub><sup>-</sup> secretion in intralobular duct cells is maintained by nonoscillatory increases in anion conductance and the driving force generated by the carbonic anhydrase with the support of the Na<sup>+</sup>-H<sup>+</sup> exchanger during both Ca<sup>2+</sup> signaling and cAMP signaling.

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## REFERENCES

1. Hirono C, Shiba Y : Analyses of anion secretion by the gramicidin-perforated patch recording in secretory epithelial cells. *Recent Res Devel Membr Biol* 1 : 11-21, 2002
2. Shintani T, Hirono C, Sugita M, Iwasa Y, Shiba Y : Suppression of carbachol-induced oscillatory Cl<sup>-</sup> secretion by forskolin in rat parotid and submandibular acinar cells. *Am J Physiol Gastrointest Liver Physiol* 294 : G738-G747, 2008
3. Hirono C, Nakamoto T, Sugita M, Iwasa Y, Akagawa Y, Shiba Y : Gramicidin-perforated patch analysis on HCO<sub>3</sub><sup>-</sup> secretion through a forskolin-activated anion channel in rat parotid intralobular duct cells. *J Membr Biol* 180 : 11-19, 2001