The Cytosolic Acidification in Rat Parotid Cells Is Associated with an Increase in Cytosolic Ca\(^{2+}\) Concentration

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ABSTRACT—A transient decrease in cytosolic pH ([pH]\(_{cyt}\)) in rat parotid cells was evoked by the addition of carbachol (CCh), phenylephrine, or substance P, whereas isoproterenol and dibutyryl cyclic AMP had little or no effect on [pH]\(_{cyt}\). The decrease in [pH]\(_{cyt}\) induced by the Ca\(^{2+}\)-mobilizing agonists was also observed in Ca\(^{2+}\)-free medium, but not when the intracellular Ca\(^{2+}\) stores were previously depleted. Ionomycin and thapsigargin elicited a decrease in [pH]\(_{cyt}\) with an increase in cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{cyt}\)). The protein kinase C activator and inhibitor had no effect on the agonist-induced decrease in [pH]\(_{cyt}\). These results suggest that the cytosolic acidification is associated with an increase in [Ca\(^{2+}\)]\(_{cyt}\).

Keywords: pH (cytosolic), Ca\(^{2+}\) concentration (cytosolic), Parotid cell (rat)

A decrease in cytosolic pH ([pH]\(_{cyt}\)) during cell stimulation has been observed in a variety of cell types. In vascular smooth muscle cells (1) and mesangial cells (2), an increase in cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{cyt}\)) is necessary for cytosolic acidification, while the change in [pH]\(_{cyt}\) in neutrophils (3) and renal epithelial cells (4) seems to be regulated by protein kinase C (PKC) and cyclic AMP (cAMP), respectively. Thus, the mechanisms for producing cytosolic acidification may vary in different cell types.

In rat parotid acinar cells, stimulation of muscarinic cholinergic, \(\alpha\)-adrenergic and substance P receptors causes phosphoinositide breakdown, leading to Ca\(^{2+}\) mobilization and activation of PKC (5). On the other hand, \(\beta\)-adrenergic receptor activation evokes cAMP accumulation, but does not cause a detectable increase in [Ca\(^{2+}\)]\(_{cyt}\) (6, 7). It has been reported that muscarinic stimulation induces a rapid decrease in [pH]\(_{cyt}\) in rat parotid cells (8, 9). The cytosolic acidification is speculated to be due to a rapid loss of intracellular HCO\(_3^-\), because the acidification is inhibited by removal of HCO\(_3^-\) and by carbonic anhydrase inhibitors (8, 9). However, it is not fully clarified whether the acidification results from an increase in [Ca\(^{2+}\)]\(_{cyt}\) or from PKC activation. In this study, we examined the relationship between a decrease in [pH] and an increase in [Ca\(^{2+}\)]\(_{cyt}\), in response to agonists. Furthermore, to assess the involvement of PKC in the acidification, the effects of an activator and an inhibitor of PKC on [pH]\(_{cyt}\) were examined.

Male Wistar-strain rats, weighing about 300 g, were anesthetized with diethyl ether and killed by cardiac puncture. Parotid acinar cells were prepared by enzyme digestion with trypsin and collagenase as previously described (7). The dispersed cells were suspended in a physiological salt solution (PSS) containing: 115 mM NaCl, 5 mM KCl, 1 mM MgC\(_2\), 1 mM CaC\(_2\), 25 mM NaHCO\(_3\), 10 mM glucose, 10 mM HEPES, and 0.2% bovine serum albumin, pH 7.4. The [pH] was monitored using a pH-sensitive fluorescent dye, 2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). The cells were incubated with 2 \(\mu\)M BCECF acetoxymethyl ester (Dojin Laboratories, Kumamoto) for 30 min at 37°C. The BCECF-loaded cells were washed twice, resuspended in PSS and shaken at 32°C until use. Immediately before measurements, the cells were washed by centrifugation and resuspended in fresh PSS. Measurements of fluorescence were made at 37°C in a stirred cuvette in a Hitachi F-2000 spectrofluorimeter (Hitachi, Tokyo), with excitation at 440 and 500 nm and emission at 530 nm. The [pH] values were calculated from the ratio of fluorescence by the nigericin/K\(^+\) method (10). To check leakage of BCECF into extracellular medium, dye-loaded cells were centrifuged (150 x g, 30 sec), and the fluorescence in the supernatant was measured. The dye leakage from cells was 0.8-1.2%/min at 37°C.

To determine [Ca\(^{2+}\)]\(_{cyt}\), dispersed cells were incubated with 2 \(\mu\)M fura-2 acetoxymethyl ester (Dojin Labora-
tories) for 45 min at 37°C and kept at 32°C after washing with PSS. Fura-2 fluorescence was measured with excitation at 340 and 380 nm and emission at 510 nm, and [Ca$$^{2+}$$], was calculated from the ratio of fluorescence as described by Grynkiewicz et al. (11).

The resting [pH], of rat parotid cells was 7.26±0.08 (mean±S.D., n=40). The [pH], increased slightly with time, probably due to the dye leakage from the cells. Figure 1a shows the effects of carbachol (CCh) and substance P (SP) on [pH], and [Ca$$^{2+}$$], in the presence of extracellular Ca$$^{2+}$$. The cholinergic agonist CCh (10 μM) caused a rapid decrease in [pH], (0.07±0.01 pH units, n=6) in addition to an increase in [Ca$$^{2+}$$],, and the decreased [pH], returned to the resting value within 2 min. When the CCh stimulation was terminated by the muscarinic receptor antagonist atropine (Atr), subsequent addition of SP (10 nM) caused changes in [Ca$$^{2+}$$], and [pH], similar to those observed with CCh (Fig. 1a).

The acidification and the [Ca$$^{2+}$$], response were also evoked by 10 μM phenylephrine, an α-adrenergic agonist (data not shown). On the other hand, the β-adrenergic agonist isoproterenol (1 μM) and the cAMP analogue dibutyryl cAMP (2 mM), which do not cause a rise of [Ca$$^{2+}$$], in rat parotid cells (6, 7), had little or no effect on [pH], (data not shown). These results lead to the assumption that the cytosolic acidification is associated with an increase in [Ca$$^{2+}$$].

To assess the Ca$$^{2+}$$-dependence of the cytosolic acidification further, the effects of CCh and SP on [pH], were examined in a Ca$$^{2+}$$-free medium containing 1 mM EGTA. CCh caused a decrease in [pH], with a transient increase in [Ca$$^{2+}$$], (Fig. 1b). When the CCh stimulation was terminated by Atr after the return of [Ca$$^{2+}$$], to the resting level, subsequent addition of SP evoked neither a decrease in [pH], nor an increase in [Ca$$^{2+}$$], (Fig. 1b). When, however, Atr was added before the return of [Ca$$^{2+}$$], to the resting level, subsequent addition of SP evoked small changes in [pH], and [Ca$$^{2+}$$], (Fig. 1c). These data indicate that the depletion of intracellular Ca$$^{2+}$$ stores resulted in the disappearance of the [pH], response to SP stimulation and that the Ca$$^{2+}$$ mobilization from intracellular stores was sufficient to cause the acidification.

In addition, we examined the effects of the Ca$$^{2+}$$ ionophore ionomycin and the microsomal Ca$$^{2+}$$-ATPase inhibitor thapsigargin on [pH],. These reagents are expected to bypass the receptor-mediated elevation of [Ca$$^{2+}$$], (12–14). Ionomycin (0.5 μM) and thapsigargin (1 μM) evoked a slow decrease in [pH], correlated with an increase in [Ca$$^{2+}$$], (Fig. 2). This finding supports the view that the agonist-induced decreases in [pH], are closely linked with increases in [Ca$$^{2+}$$].

To confirm that the cytosolic acidification does not affect [Ca$$^{2+}$$], 30 mM sodium propionate, which evokes a rapid decrease in [pH], as a result of the permeation of propionic acid into the cytoplasm (9), was added to the fura-2- or BCECF-loaded cells. The addition did not
cause any changes in $[\text{Ca}^{2+}]_i$, although a rapid decrease in $[\text{pH}]_i$ was observed (data not shown).

$\text{Ca}^{2+}$-mobilizing agonists, such as CCh, SP, and phenylephrine, would activate PKC via formation of diacylglycerol. To assess whether PKC plays a role in the agonist-induced decrease in $[\text{pH}]_i$, the effects of the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) and the PKC inhibitor staurosporine on $[\text{pH}]_i$ were examined. The addition of TPA (100 nM) did not cause any change in $[\text{pH}]_i$ (Fig. 3a), although the concentration used here induces the maximum response of amylase release without increasing $[\text{Ca}^{2+}]_i$ (13). In addition, pretreatment with TPA did not potentiate the CCh-induced decrease in $[\text{pH}]_i$ (Fig. 3a). Also, the acidification was not inhibited by 100 nM staurosporine (Fig. 3b), which is a sufficient concentration for inhibition of PKC (13). These results suggest that the activation of PKC is not involved in the agonist-induced decrease in $[\text{pH}]_i$.

The present study suggests that the cytosolic acidification in rat parotid cells is closely linked with a rise of $[\text{Ca}^{2+}]_i$. Our data are compatible with the previous report (9) that the decrease in $[\text{pH}]_i$ was blocked by chelation of intracellular $\text{Ca}^{2+}$. Melvin et al. (9) suggest that CCh causes the rapid efflux of intracellular $\text{HCO}_3^-$ via anion channels localized in the apical membrane of rat parotid cells. The rapid loss of intracellular $\text{HCO}_3^-$ would result in an accumulation of intracellular $\text{H}^+$ leading to a transient decrease in $[\text{pH}]_i$. The opening of apical anion channels is thought to be mediated by $\text{Ca}^{2+}$ (15). Thus, it is reasonable to presume that the cytosolic acidification induced by the $\text{Ca}^{2+}$-mobilizing agonists is the result of a rapid loss of intracellular $\text{HCO}_3^-$ via an increase in $[\text{Ca}^{2+}]_i$.

REFERENCES

1 Berk, B.C., Brock, T.A., Gimbrone, M.A., Jr. and Alexander, R.W.: Early agonist-mediated ionic events in cultured vascular smooth muscle cells. J. Biol. Chem. 262, 5065–5072 (1987)
2 Mené, P., Dubyak, G.R., Scarpa, A. and Dunn, M.J.: Regulation of cytosolic pH of cultured mesangial cells by prostaglandin E2 and thromboxane A2. Am. J. Physiol. 260, C159–C166 (1991)
3 Grinstein, S. and Furuya, W.: Cytoplasmic pH regulation in phorbol ester-activated human neutrophils. Am. J. Physiol. 251, C55–C65 (1986)
4 Harada, H., Kanai, Y., Anzai, M. and Suketa, Y.: cAMP activates $\text{Cl}^-/\text{HCO}_3^-$ exchange for regulation of intracellular pH in renal epithelial cells. Biochim. Biophys. Acta 1092, 404–407 (1991)
5 Baum, B.J.: Regulation of salivary secretion. In The Salivary System, Edited by Sreebny, L.M., pp. 123–134, CRC Press, Boca Raton (1987)
6 Hughes, A.R., Takemura, H. and Putney, J.W., Jr.: Does β-adrenoceptor activation stimulate Ca²⁺ mobilization and inositol trisphosphate formation in parotid acinar cells? Cell Calcium 10, 519–525 (1989)
7 Tanimura, A., Matsumoto, Y. and Tojyo, Y.: Evidence that isoproterenol-induced Ca²⁺-mobilization in rat parotid acinar cells is not mediated by activation of β-adrenoceptors. Biochim. Biophys. Acta 1055, 273–277 (1990)
8 Nauntofte, B. and Dissing, S.: Cholinergic-induced electrolyte transport in rat parotid acini. Comp. Biochem. Physiol. 90A, 739–746 (1988)
9 Melvin, J.E., Moran, A. and Turner, R.J.: The role of HCO₃⁻ and Na⁺/H⁺ exchange in the response of rat parotid acinar cells to muscarinic stimulation. J. Biol. Chem. 263, 19564–19569 (1988)
10 Thomas, J.A., Buchsbaum, R.N., Zimniak, A. and Racker, E.: Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. Biochemistry 18, 2210–2218 (1979)
11 Grynkiewicz, G., Poenie, M. and Tsien, R.Y.: A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450 (1985)
12 Takemura, H., Hughes, A.R., Thastrup, O. and Putney, J.W., Jr.: Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. J. Biol. Chem. 264, 12266–12271 (1989)
13 Tojyo, Y., Matsui, S., Tanimura, A. and Matsumoto, Y.: Relationship between cytosolic Ca²⁺ concentration and amylase release in rat parotid acinar cells following muscarinic stimulation. Biochim. Biophys. Acta 1134, 278–284 (1992)
14 Tojyo, Y., Tanimura, A., Matsui, S. and Matsumoto, Y.: Effect of thapsigargin on cytosolic Ca²⁺ level and amylase release in rat parotid acinar cells. Cell Struct. Funct. 17, 223–227 (1992)
15 Young, J.A., Cook, D.I., Van Lennep, E.W. and Roberts, M.: Secretion by major salivary glands. In Physiology of the Gastrointestinal Tract, Edited by Johnson, L.R., Vol. 1, pp. 773–815, Raven Press, New York (1987)