Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
The International Journal of Biochemistry & Cell Biology 37 (2005) 876–886
IJBCB
www.elsevier.com/locate/biocel

Phosphodiesterases 1 and 2 regulate cellular cGMP level in rabbit submandibular gland cells

Hiromi Michikawa[a,b,*], Hiroshi Sugiya[a,b], Tomoyoshi Yoshigaki[a], Junko Fujita-Yoshigaki[a,b], Shunsuke Furuyama[a,b]

[a] *Department of Physiology, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-nishi, Matsudo, Chiba 271-8587, Japan*
[b] *Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-nishi, Matsudo, Chiba 271-8587, Japan*

Received 20 February 2004; received in revised form 22 October 2004; accepted 9 November 2004

Abstract

In rabbit salivary glands, stimulation of muscarinic cholinergic receptors causes production of cGMP through intracellular Ca^{2+} and nitric oxide. In this study, we investigated a role of cyclic nucleotide phosphodiesterase (PDE) in regulating the cellular cGMP level by using cells dispersed from the submandibular gland. Methacholine, a cholinergic agonist, rapidly elevated the cGMP level. The elevation was greatly enhanced by IBMX, a non-specific inhibitor for most isoforms of the 11 PDEs. The cGMP level was also elevated by MM-IBMX and EHNA, which inhibit the activities of PDE1 and PDE2, respectively. The elevation by the simultaneous application of the two drugs corresponded to 90% of that by IBMX. Therefore, PDE1 and PDE2 are the main PDEs that act to degrade cGMP in methacholine-stimulated cells. The presence of the two PDEs was confirmed by assaying their activities of the cell lysate. In unstimulated cells, the cGMP level was elevated by MM-IBMX and little elevated by EHNA. While the PDE2 activity was thus low, it was estimated that methacholine increases its activity approximately 50-fold. The strong activation can be explained by the elevation of the cGMP level because PDE2 is a cGMP-stimulated PDE. SNAP, a nitric oxide donor, causes production of cGMP without a receptor-operated increase in intracellular Ca^{2+} concentration. In SNAP-stimulated cells, MM-IBMX elevated the cGMP level higher than in methacholine-stimulated cells although the PDE1 activity is dependent on Ca^{2+}/calmodulin. Besides Ca^{2+}, other factors may regulate the PDE1 activity in living cells.

Keywords: cGMP; Phosphodiesterase; Submandibular gland; Cholinergic agonist; Ca^{2+}

1. Introduction

Cyclic guanosine-3′,5′-monophosphate (cGMP) is an intracellular second messenger that functions in many cases such as smooth muscle relaxation, intestinal secretion and retinal phototransduction (Waldman

* Corresponding author. Tel.: +81 47 360 9326; fax: +81 47 360 9327.
E-mail address: mitikawa@mascat.nihon-u.ac.jp (H. Michikawa).

1357-2725/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2004.11.008

& Murad, 1987). Acetylcholine, carbachol and methacholine stimulate secretion of salivary gland cells through cholinergic receptors (Putney, 1986). It has been reported that they evoke production of cGMP in the parotid gland of rabbit and rat (Butcher, McBride, & Rudich, 1976; Glenert & Nauntofte, 1988; Wojcik, Grand, & Kimberg, 1975) and the submandibular gland of guinea pig and rat (Albano, Bhoola, Heap, & Lemon, 1976; Schultz, Hardman, Schultz, Baird, & Sutherland, 1973; Spearman & Pritchard, 1979). Therefore, it is likely that cGMP is involved in the secretory process of salivary glands.

We previously reported on the signaling pathway of cGMP production in rabbit salivary gland cells (Michikawa et al., 1998; Sakai, Michikawa, Furuyama, & Sugiya, 2002; Sugiya et al., 2001). Stimulation of muscarinic cholinergic receptors increases the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ activates Ca^{2+}/calmodulin-dependent nitric oxide synthase (NOS) to cause generation of nitric oxide (NO). Then, NO activates soluble guanyl cyclase to elevate the cGMP level. When cells dispersed from the parotid and submandibular glands of rabbit were stimulated by the same concentration of methacholine, time-dependent profiles of the cGMP concentration were different between the two glands (Michikawa et al., 1998; Sakai et al., 2002). While the cGMP concentration increased and reached a plateau in the submandibular gland, it greatly increased and then gradually decreased in the parotid gland. The peak value of the cGMP level was approximately 10 times greater in the parotid gland than in the submandibular gland. However, $[Ca^{2+}]_i$ increased to almost the same level in the parotid and submandibular glands (Sakai et al., 2002; Sugiya et al., 2001). In addition, the activity of NOS was 1.5–2.5 times greater in the parotid gland than in the submandibular gland (Michikawa et al., 1998; Mitsui, Yasuda, Furuyama, & Sugiya, 1997; Sakai et al., 2002). These data lead to the idea that the difference in the cGMP level between the two glands is mainly caused by the degradation system of cGMP, not by the production system.

Cyclic nucleotide phosphodiesterase (PDE) catalyzes the hydrolysis of cAMP and cGMP. PDEs have been classified into 11 families on the basis of substrate and inhibitor specificities, allosteric properties and amino acid sequences (Beavo & Brunton, 2002; Conti & Jin, 1999; Francis, Turko, & Corbin, 2001; Houslay, 2001; Manganiello & Degerman, 1999; Soderling & Beavo, 2000). In salivary glands, the activities of PDE1 (Imai, Nashida, & Shimomura, 1999; Yokoyama, Murota, Negishi, Saito, & Furuyama, 1983), PDE2 (Imai et al., 1999) and PDE5 (Komine & Shimomura, 2002; McPherson, Pereira, Lloyd Mills, Murray, & Dormer, 1999) were detected. Ca^{2+}/calmodulin binds to and allosterically activates PDE1, so it is called a Ca^{2+}/calmodulin-dependent PDE (Beavo, 1995; Francis et al., 2001; Kakkar, Raju, & Sharma, 1999). While PDE2 has a lower K_m for cGMP than for cAMP, the hydrolysis of cAMP by PDE2 is stimulated by binding of cGMP to its allosteric cGMP-binding site (Beavo, 1995; Conti & Jin, 1999; Francis et al., 2001; Juilfs, Soderling, Burns, & Beavo, 1999). So PDE2 is called a cGMP-stimulated PDE. PDE5 hydrolyzes only cGMP and contains a non-catalytic cGMP-binding domain similar to that found in PDE2 (Conti & Jin, 1999; Francis et al., 2001; Juilfs et al., 1999). PDE5 is called a cGMP-binding cGMP-specific PDE. In the submandibular gland, PDE1 (Komine & Shimomura, 2002; Yokoyama et al., 1983) and PDE5 (Komine & Shimomura, 2002; McPherson et al., 1999) were detected in rat on the basis of activation by Ca^{2+}, sensitivity to inhibitors and immunoblot analyses. In the parotid gland, PDE1 and PDE2 were detected in several rodents (Imai et al., 1999). Recently, mRNA expression of PDE11 was reported in human salivary glands (Fawcett et al., 2000; Yuasa et al., 2000). While much information has been thus obtained about PDE isoforms in salivary glands, it remains to be elucidated how they regulate the cGMP level in living cells of the glands. In this report, we studied the degradation system of cGMP by using cells dispersed from the submandibular gland of rabbit.

2. Materials and methods

2.1. Materials

Collagenase A and bovine serum albumin (BSA) were purchased from Boehringer Mannheim (Germany). Dipyridamole, *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA), trypsin (type III), trypsin inhibitor (type I-S), leupeptin and *Crotalus atrox* snake venom were obtained from Sigma (MO, USA). Methacholine (*O*-acetyl-β-methylcholine) was obtained from Wako (Japan), *S*-nitroso-*N*-acetyl-DL-