Agonist-induced chromogranin A secretion coincides with redistribution of IP_3 receptor and compound exocytosis in granular duct cell of rat submandibular gland.

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Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

The granular duct cells of the rodent submandibular gland have unique functions: the cells synthesize transforming growth factor a (TGF-□), hepatocyte growth factor, erythroid differentiation factor, endothelin, insulin-like growth factor including nerve growth factor, and kallikreins[1]. In the granular the duct cells, we have shown that chromogranin A-like immunoreactivity (CgA-like IR) is stored as well, and that highly concentrated CgA-like IR (approximately 1 mM) is secreted by compound exocytosis into saliva in response to 1 μM noradrenaline (NAd)[2]. The secretory response to 3 mM phenylephrine, an □-adrenergic agonist, was hardly inhibited in Ca-deficient environment, and was almost completely inhibited by 100 μM 2-aminoethoxydiphenyl borate (2APB), an inhibitory modulator of inositol 1,4,5 trisphosphate (IP_3)-mediated Ca release from intracellular stores[3]. These results are compatible with a view that mobilization of Ca from IP_3-sensitive pool may preferentially be involved in the secretory response to □-adrenergic agonist. The present study was thus carried out to obtain direct evidence that the duct cells exhibiting the strongest IP_3 receptor type 2 immunoreactivity (IP_3R2 IR) are the granular duct cells, which contain CgA-like IR, that NAd causes compound exocytosis of the secretory granules, and that the compound exocytosis may result in dynamic subcellular redistribution of localized IP_3R2 IR[4].

METHODS

Isolated and perfused preparation of the rat submandibular gland was used. Male Wistar rats were anesthetized and the submandibular gland was isolated and vascularly perfused. The secreted saliva was collected at an interval of 2 min. CgA-like IR was measured by a region-specific enzyme immunoassay (EIA) for rat CgA using anti-rat $CgA_{(359-389)}$ developed in our institute. Cryostat sections, 12 μm thick, were incubated with anti-rat $CgA_{(359-389)}$ serum followed by Cy3-labeled donkey anti-rabbit IgG, and then incubated with mAb against IP_3R2, followed by FITC-labeled donkey anti-mouse IgG (Jackson ImmunoResearch). The immunostained sections were obtained under laser scanning microscope.

RESULTS AND DISCUSSION

The 1 μM NAd-induced secretion of highly concentrated CgA-like IR (~1 mM) was diluted and facilitated by adding 0.1 μM acetylcholine (ACh), which has been known to cause substantial increase in flow rate with indiscernible secretion of CgA-like IR and protein. The first 4 min stimulation induced significant maximum increases in secretory responses (CgA-like IR secretion, protein secretion and flow; $p<0.01$ respectively) within 2 min followed by steep decays during the continuous stimulation. The secretory responses to the second stimulation were smaller than the corresponding first responses: the rate of CgA-like IR secretion, protein secretion and flow were 54%, 44%, and 33% of the corresponding first responses, respectively. The secretory responses to the third stimulation were much smaller than the corresponding responses to the first stimulation. The decay in the responsiveness during the continuous stimulation is, by definition, desensitization. The progressive reduction in the second and third response may also be due to continuous desensitization. In the control sections prepared from resting state, an intense IP_3R2 IR was detected at the apical pole of granulated duct cell containing IP_3R2 IR.

Ultrastructure of the granular cell in the resting state showed that the cells stored numerous membrane-bound granules in the apical cytoplasm. The combined stimulation with 1 μM NAd and 0.1 μM acetylcholine caused immediate maximum increase in secretory responses. When the sections were prepared from the gland at the peak of secretory responses, the apically converged IP_3R2 IR became indistinguishable. Ultrastructure of the maximally stimulated cells exhibited extensive compound exocytosis in the apical half of the granular duct cells. The secretory responses to the stimulation were significantly inhibited by 100 μM-2APB, and the intense IP_3R2 IR was well preserved at the apical pole of granular duct cells containing CgA-like IR.

The NAd-induced extensive compound exocytosis at the apical pole may be initiated by marked increase in local concentration of Ca^{2+} ion, which may be released from the intracellular Ca^{2+} store during activation of IP_3R2. It is recently shown that the activation of IP_3R may drastically be potentiated by the Ca^{2+} storage protein CgA[5],

and the extensive compound exocytosis in the granulated duct cells may be induced by the synergic interaction of IP_3R2 in the membrane of Ca^{2+} store and CgA in the granule.

The divergence of apically localized IP_3R coincides with the development of compound exocytosis may result in desensitization, and thus provides a novel paradigm for the desensitization of G protein-coupled signaling system[4].

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