PROCEEDING

The thiol-oxidizing agent diamide reduces isoproterenol-stimulated amylase release in rat parotid acinar cells

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Abstract : In parotid acinar cells, activation of β-adrenergic receptors provokes exocytotic amylase release *via* the accumulation of intracellular cAMP. Cellular redox status plays a pivotal role in the regulation of various cellular functions. Cellular redox imbalance caused by the oxidation of cellular antioxidants, as a result of oxidative stress, induces significant biological damages. In this study, we examined effect of diamide, a thiol-oxidizing reagent, on amylase release in rat parotid acinar cells. In the presence of diamide, isoproterenol (IPR)-induced cAMP formation and amylase release were partially reduced. Diamide had no effect on amylase release induced by forskolin and mastoparan, an adenylate cyclase activator and heterotrimeric GTP binding protein activator, respectively. In the cells pretreated with diamide, the binding affinity of [^{3}H]dihydroalprenolol to β-receptors was reduced. These results suggest that oxidative stress results in reduction of binding affinity of ligand on β-receptor and consequently reduces protein secretory function in rat parotid acinar cells. J. Med. Invest. 56 Suppl. : 284-286, December, 2009

Keywords **:** *oxidative stress, β-receptor, diamide, amylase release, parotid acinar cells*

INTRODUCTION

The generation of reactive oxygen species (ROS) is part of physiologically metabolic processes in cells. Since the rodex state of cells is determined by the balance of generation of ROS and the capacity of antioxidant systems, cellular redox imbalance caused by the oxidation of cellular antioxidants, as a result of oxidative stress, induces significant biological damages. Diamide is a specific oxidant, which reacts specifically with intracellular thiols, both low-molecular-mass thiols and protein sulfhydryls (1, 2). Diamide has been reported to have many effects on the function and the morphology of mammalian cells.(3).

In parotid acinar cells, it is well known that activation of β-adrenergic receptors induces intracellular cAMP accumulations and consequently (4). Dysfunction of acinar cells results in decrease of secreteory function in diseases of salivary glands such as sialadenitis and Sjögren syndrome. In the present study, we demonstrated thiol oxidant courses to reduce IPR-induced amylase release in rat parotid acinar cells.

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METHODS

Rat parotid acinar cells were dispersed by using trypsin and collagenase as previously described

(5). Amylase activity was measured according to Bernfeld's method (6). Intracellular cAMP levels were determined by using cAMP Enzyme immunoassay system (Amersham). For β-adrenergic receptor binding assay, binding of [^{3}H] dihydroalprenolol hydrochloride ([^{3}H] DHA) to the cells preincubated with diamide was examined at 4°C. Specific binding of [^{3}H] DHA was calculated by subtracting the amount of bound [^{3}H] DHA in the presence of propranolol (nonspecific binding) from total bound [^{3}H] DHA.

RESULTS AND DISCUSSION

To examine the effect of diamide on IPR-induced amylase release in rat parotid acinar cells, the cells were incubated with or without 1 μM IPR for 20 min in the presence or absence of diamide (35-100 μM). Diamide had no effect on the non-stimulated amylase release, whereas this thiol oxidant reduced IPR-induced amylase release in dose and time dependent manner.

It is well known that intracellular cAMP accumulation is essential for IPR-induced amylase release in rat parotid acinar cells. Therefore, we examined the effect of diamide on IPR-induced cAMP accumulation. In the presence of diamide, IPR-induced cAMP formation was reduced. Diamide had no effect on cAMP levels in non-stimulated cells. These observations suggest that the target of diamide is in the upstream pathway of cAMP formation.

β-adrenergic receptors promote stimulation of the heterotrimeric GTP-binding protein G_s, which in turn activates adenylate cyclase for cAMP formation. Then we examined the effect of diamide on amylase release induced by forskolin and mastoparan, adenylate cyclase and GTP-binding protein activators, respectively. However, diamide had no effect on forskolin- and mastparan-induced amylase release in rat parotid acinar cells. Because these results imply that β-adrenergic receptors are affected by diamide, we examined the effect of diamide on β-adrenergic receptor characteristics in parotid acinar cells.

To assess the change of β-receptor characterestics in the presence of diamide, the ability of [^{3}H] DHA to bind to parotid acinar cells was examined. In the cells pretreated with diamide, the Kd values, but not the density of β-receptors, were clearly decreased compared with the control, indicating that diamide induces the reduction of affinity for agonist to β-receptors.

The extracellular domains of β-adrenergic receptors contain thiol residues capable of forming disulfide bridges (7, 8). Because of the thiol group, cysteine is the most chemically reactive natural amino acid found in cells. Cysteine residues within G protein-coupled receptors have been identified as important for inducing and maintaining the three-dimensional receptor conformation by forming critical intermolecular and intramolecular disulfide bond (9). Therefore, a reaction of diamide with the cysteine residues appears to reduce the ligand-receptor binding.

In conclusion, oxidative stress by diamide reduced IPR-induced amylase release, which caused by the reduction of ligand binding ability to β-adrenergic receptors in rat parotid acinar cells.

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