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Sulfhydryl oxidation induces rapid and reversible closure of the ATP-regulated K^+ channel in the pancreatic β-cell

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Effects of sulfhydryl modification on the ATP regulated K^+ channel (K_{ATP} channel) in the pancreatic β-cell were studied, using the patch clamp technique. Application of the sulfhydryl oxidizing agents thimerosal and 2,2′-dithio-bis(5-nitropyridine) (DTBNP), in micromolar concentrations, caused complete inhibition of the K_{ATP} channel, in inside-out patches. The inhibition was rapid and was reversed by the disulfide reducing agents dithiothreitol and cysteine. Thimerosal, which is poorly membrane permeable, inhibited channel activity, only when applied to the intracellular face of the plasma membrane. In contrast, DTBNP, which is highly lipophilic, caused closure of the K_{ATP} channel and consequent depolarization of the membrane potential, also when applied extracellularly. Our results indicate the presence of accessible free SH groups on the cytoplasmic side of the K_{ATP} channel in the pancreatic β-cell. These SH groups are essential for channel function and it is possible that thiol-dependent redox mechanisms can modulate K_{ATP} channel activity.

ATP-regulated K^+ channel; Sulfhydryl reagent; Thimerosal; Pancreatic β-cell

1. INTRODUCTION

K^+ channels characterized by their sensitivity to intracellular ATP (K_{ATP}), play an important role in the regulation of insulin secretion from the pancreatic β-cell [1–3]. Under resting conditions, at glucose concentrations less than 5 mM, the K_{ATP} conductance dominates and therefore determines the membrane potential of the β-cell [4]. A key event in the glucose stimulation of insulin secretion is the closure of this channel. Closure of the K_{ATP} channel results in depolarization of the cell, Ca^{2+}-influx through the voltage-gated Ca^{2+} channel, increase in the cytoplasmic free Ca^{2+} concentration and insulin secretion [3,5]. The K_{ATP} channel is also the target for sulfonylureas, a class of drugs which inhibits channel activity, and are used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) [6]. The precise signals that generate from glucose metabolism and control the activity of the K_{ATP} channel are still unknown. Currently, a change in the intracellular concentration of ATP or ATP/ADP ratio is believed to be the most important link between fuel metabolism and depolarization of the cell [2,7]. However the regulation of the channel appears to be more complex than that and may involve modulation by protein kinase C, G proteins and changes in the redox potential of the cell. [8–13]. At present little is known about the structure of the K_{ATP} channel protein, as well as about the molecular basis of its regulation.

Many biologically active proteins contain critical cysteine residues. The function of these proteins often depends on the oxidation state of sulfhydryl (thiol) groups (SH groups) [14]. Some proteins are active only when their specific SH groups remain in the reduced form, whereas for the activity of others the disulfide redox state is essential [15,16]. Selective modification of SH groups, has been extensively used to ascertain the relationship between structure and function of many biomolecules. Different types of ion channel proteins also contain SH groups, modification of which may affect channel activity [17,18]. The sulfhydryl reagent thimerosal and some 'reactive disulfides' open intracellular Ca^{2+} channels by oxidizing critical SH groups [18,19]. There is evidence to suggest, that the K_{ATP} channel of mouse skeletal muscle contains functionally important SH groups [20]. The role of SH groups in regulating the activity of the K_{ATP} channel in the pancreatic β-cell is unknown, although it is known since long that many sulfhydryl reagents stimulate insulin secretion [21–24]. In the present study we demonstrate that the sulfhydryl oxidizing agents, thimerosal and 2,2′-dithio-bis(5-nitropyridine) (DTBNP) induce rapid and reversible closure of the K_{ATP} channel in the pancreatic β-cell, indicating that this channel contains SH groups essential for the channel activity.

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