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Calbindin-D_{28k} decreases L-type calcium channel activity and modulates intracellular calcium homeostasis in response to K^+ depolarization in a rat beta cell line RINr1046-38

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Abstract

Calbindin-D_{28k}, acts as a modulator of depolarization induced calcium transients in the pancreatic beta cell. However, specific mechanisms have not been defined. Here we show for the first time that the calcium binding protein calbindin-D_{28k} acts by affecting calcium influx through voltage-dependent calcium channels in RIN pancreatic beta cells. Whole-cell patch–clamp recordings revealed that Ca^{2+} current amplitudes of calbindin-D_{28k} expressing RINr1046-38 beta cells were smaller than the Ca^{2+} current amplitudes in control cells in response to depolarizing pulses. The peak current was observed at +20 mV and the average amplitude was ~50 pA in the calbindin expressing cells compared to ~250 pA in control cells. In calbindin-D_{28k} expressing cells, the channels had enhanced sensitivity to Ca^{2+} dependent inactivation and currents decayed much more rapidly than in control cells. The Ca^{2+} channels affected by calbindin were found to have biophysical properties consistent with dihydropyridine-sensitive L-type calcium channels. In response to depolarizing concentrations of K^+, calbindin expression caused a five-fold decrease in the rate of rise of $[Ca^{2+}]_i$ and decay was slower in the calbindin expressing cells. Application of verapamil resulted in a drop in the $[Ca^{2+}]_i$ signal to pre-stimulation levels indicating that the Ca^{2+} channel responsible for the depolarization evoked Ca^{2+} entry, modulated by calbindin, is the L-type. Co-immunoprecipitation and GST pull-down assays indicate that calbindin-D_{28k} can interact with the α_1 subunit of $Ca_v1.2$. We thus conclude that calbindin-D_{28k} can regulate calcium influx via L-type calcium channels. Our findings suggest a role for calbindin-D_{28k} in the beta cell in modulating Ca^{2+} influx via L-type voltage-dependent calcium channels.

Keywords: L-type calcium channel; Calbindin-D_{28k}; Pancreatic beta cell

1. Introduction

Changes in the concentration of intracellular calcium control a number of cellular events including neurotransmitter release, hormone secretion, differentiation and apoptosis [1,2]. $[Ca^{2+}]_i$ levels can be altered by regulation of calcium influx which is mediated by voltage dependent, ligand gated and other plasma membrane ion channels. An increase in $[Ca^{2+}]_i$ can also occur by release from internal stores. Mechanisms of reducing $[Ca^{2+}]_i$ levels include the calcium extrusion systems such as Ca^{2+}ATPase and Na^+/Ca^{2+} exchanger and uptake into organelles, particularly endoplasmic reticulum and mitochondria [1,2]. Thus, an interplay among membrane components, such as calcium pumps, ion channels and intracellular organelles exists. Although calcium signaling is complex and incorporates multiple factors, it has been suggested that the ability of the calcium ion to interact with a family of high affinity calcium binding proteins, known as EF hand proteins, can play an important role in the transduction of the calcium signal into a biological response [3]. The EF

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hand is a Ca^{2+} coordinating helix–loop–helix structural motif [4]. The EF hand is a recurring motif in the family of calcium binding proteins which includes calmodulin, parvalbumin, the S100 proteins and the calbindins [3,4]. Calbindin-D_{28k} is a 28,000M_r (molecular weight) high affinity calcium binding protein present in highest concentrations in avian intestine and in avian and mammalian kidney, brain and pancreas [5,6]. In some cells, the concentration of calbindin-D_{28k} has been reported to be as high as 1 mM [7,8]. Calbindin is thought to act as a facilitator of calcium diffusion in intestine and kidney where it is regulated by vitamin D [5,6]. In brain, calbindin-D_{28k} is present in most neuronal groups and has been reported to act as a calcium buffer to modulate intracellular calcium transients and evoked calcium signals [9–11]. Calbindin, unlike calmodulin, does not undergo major changes in conformation upon calcium binding [12–14]. In the nervous system it has been suggested that neuronal calbindin, by buffering calcium, can regulate intracellular responses to physiological stimuli and in addition can protect neurons against calcium mediated neurotoxicity [5,10,11]. A protective role for calbindin-D_{28k} has been observed not only in apoptosis susceptible cells in the central nervous system but also in human embryonic kidney cells (HEK293), osteoblasts and pancreatic beta cells [15–18]. Thus, calbindin-D_{28k} can modulate intracellular calcium transients and in addition has an important role in protecting against apoptotic cell death.

The requirement for an elevation of cytoplasmic Ca^{2+} concentration for pancreatic beta cell function is well established [19–21]. Glucose is metabolized by beta cells, increasing the ATP/ADP ratio which in turn closes ATP dependent K^+ channels. This causes membrane depolarization and the opening of voltage-dependent calcium channels (VDCC), resulting in an increase in intracellular calcium, fusion of secretory granules with the plasma membrane and release of insulin. Although both high voltage and low voltage activated channels have been detected in beta cells, electrophysiological studies of calcium currents reveal predominant L-type channels in pancreatic beta cells [22]. In addition, insulin secretory granules and L-type VDCC are co-localized in beta cells and pharmacological studies have demonstrated that the L-type Ca^{2+} channel is the most important channel for triggering insulin release [23–25]. Although the L-type Ca^{2+} channels represent the final common pathway for insulin secretion, the cellular and molecular mechanisms involved in the modulation of intracellular calcium in the beta cell are not yet clearly understood. It has been suggested that calcium binding proteins can contribute to the regulation of intracellular calcium responses in beta cells. Calmodulin is a calcium binding protein in addition to calbindin-D_{28k} and calcyclin present in the pancreatic beta cells [26,27]. Direct binding of calmodulin to L-type calcium channels has been reported to be a key step in the autoregulation of L-type channels [28,29]. Recent studies have shown the colocalization of calbindin-D_{28k} and L-type VDCC in pancreatic beta cells [30]. In addition, immunocytochemical studies mapping calbindin in the brain noted the similarity between the distribution of calbindin immunoreactivity and the distribution of L-type calcium channels mapped using autoradiography [9]. In our continuing efforts to understand the role of calbindin-D_{28k}, in this study we examined possible mechanisms involved in the modulation of intracellular calcium by calbindin in the rat beta cell line RINr1046-38 (RIN-38; 31). Although RIN cells have reduced insulin content compared to islet beta cells, they were chosen as an experimental model since rat insulinoma cells have been reported to retain similarities with normal pancreatic beta cells with regard to responses to secretagogues and membrane signaling [31]. Insulin secretagogues cause membrane depolarization and activate VDCC activity in these cells [32]. RIN cells have been widely used to study the role of L-type VDCC in the functioning of the beta cell [33,34]. The gating kinetics and single channel conductance of VDCCs in RIN cells are indistinguishable from those in beta cells. In addition, the α_{1c} subunit, which has been shown to be required for insulin secretion [35], is a major constituent of the L-type calcium channel in RIN cells [36]. In this study we show for the first time that calbindin-D_{28k} acts in the RIN beta cells by affecting calcium influx through voltage-gated calcium channels. The calcium channels affected by calbindin are of the L-type. In the presence of calbindin-D_{28k} the channels had enhanced sensitivity to calcium dependent inactivation and our results indicate that calbindin-D_{28k} can interact with the α_1 subunit of $Ca_v1.2$. In addition, in calbindin expressing RIN cells there is a decrease in the rate of rise of intracellular calcium and altered kinetics of decay. Thus, our findings suggest that calbindin, in addition to affecting intracellular calcium homeostasis, can also act to modulate calcium influx through L-type gated Ca^{2+} channels.

2. Materials and methods

2.1. Plasmids and probes

cDNA prepared from rat renal tubular mRNA was used to isolate calbindin-D_{28k} cDNA via PCR. The calbindin-D_{28k} insert was confirmed by sequencing and subsequently cloned into the plasmid pREP4 (Invitrogen) to create the expression plasmid pREP4-CB28 as described [37].

2.2. Stable transfection of RIN cells and clonal selection

Stable transfection was performed using lipofectin (GIBCO/BRL) on approximately 50% confluent RIN-38 cells (pass >50, grown in RPMI 1640 media; 31) in T-75 flasks. Ten micrograms of both pREP 4 (empty vector) and pREP4-CB28 were mixed with 50–75 μl of lipofectin and added to serum free media. The solutions were incubated for 18 h at 37 °C. Serum was added on the following day. RPMI 1640 medium, containing hygromycin (400 μg/ml) was added to the cells on the third day after transfection. Colonies were then hand picked under sterile conditions after