Sympathetic Voltage-Independent Regulation of Voltage-Gated Calcium Channels in Pancreatic β -Cells

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Abstract

Voltage-gated calcium (Ca_v) channels are regulated by G proteins via voltage-dependent and independent pathways. Voltage-independent regulation of calcium channels is important for intracellular calcium concentration and insulin secretion. Voltage dependence of each pathway can be elucidated by a prepulse facilitation protocol. Using this experimental approach, we compared Ca_v regulation by GTPyS and noradrenaline (NA) in rat pancreatic β -cells and rat superior cervical ganglion (SCG) neurons. The SCG neuron is a model in which the bases of Ca_v channel regulation by G proteins have been established. Ca_v channel regulation through activation of the sympathetic nervous system has been poorly studied in native insulin-secreting cells. We recorded Ca_v channel currents by means of the patch-clamp technique in the whole-cell configuration. We found that application of both GTPyS (a nonspecific activator of G proteins) by cell dialysis and noradrenaline (NA)-exposure reduced Ca_v current amplitude in pancreatic β -cells and in SCG neurons. However, the inhibition of Ca_v channel currents in GTPyS-dialyzed SCG neurons was relieved by a strong depolarizing pulse. By contrast, in pancreatic β -cells, the inhibition was maintained after a strong depolarizing pulse. In SCG neurons, the Ca_v channel inhibition by NA is predominantly voltage-dependent, whereas in pancreatic β -cells it is only 40%. Thus, it appears that Ca_v channels in rat pancreatic β -cells are regulated mainly through a voltage-independent pathway. The signaling pathway for Ca_v channel regulation by NA in pancreatic β -cells appears to differ from the classic signaling pathway described in SCG neurons. Therefore, voltage-independent regulation of Ca^{2+} entry through Ca_v channels is a critical step in understanding the pathophysiology of type 2 diabetes.

Keywords: G proteins; pancreatic β -cell; voltage-dependent calcium channel; voltage-dependent pathway; voltage-independent pathway.

Introduction

The islets of Langerhans are innervated by the sympathetic nervous system [1,16]. Secretion of insulin by cells in these islets is inhibited by the neurotransmitter noradrenaline (NA) through activation of α_2 -adrenergic receptors coupled to G proteins [1,19]. NA has been shown to regulate voltage-gated calcium (Ca_v) channels in insulin-secreting cell lines, however, this has not been observed in native pancreatic β -cells [3,13,19].

G protein regulation of Ca_v channels has been studied extensively in neurons of the superior cervical ganglion (SCG), and the biophysical properties of Ca_v channels are differentially expressed via voltage-dependent and -independent pathways [11,20]. Both pathways reduce the Ca_v channel current amplitude and regulate Ca^{2+} -dependent processes, such as neurotransmitter release. NA-induced inhibition of Ca_v channels in neurons is a fast voltage-dependent mechanism [6]. The voltage-dependent pathway is thought to be delimited to the plasma membrane by G protein $\beta\gamma$ subunits; the voltage-independent pathway is less understood. There is evidence that the membrane lipid PIP_2 is the molecule responsible for the voltage-independent inhibition of Ca_v channels triggered by M_1R activation [9,10,15]. Furthermore, some other voltage-independent pathways have been documented [20].

Voltage-independent regulation of calcium channels governs intracellular calcium concentration and insulin secretion. The voltage-dependent and -independent pathways can be elucidated by a prepulse protocol that consists of two identical voltage pulses separated by a strong depolarizing pulse [8]. The strong depolarizing pulse is commonly used as a tool to differentiate the two mechanisms. It can be mimicked by action potential burst firing and thus it is physiologically relevant to study the voltage-dependence of Ca_v channel regulation [4,17].

Pancreatic β -cells and SCG neurons express α_2 -adrenergic receptors, therefore, we examined NA regulation of Ca_v channels. We used rat SCG neurons, a model in which signaling pathways have been elucidated, so that we could compare NA regulation of Ca_v channels in pancreatic β -cells against NA regulation of Ca_v channels in SCG neurons. In order to compare G protein regulation in both cell types, endogenous G proteins were activated with GTPyS. A prepulse protocol was used to isolate the voltage-dependent and -independent pathways.

Materials and Methods

Cell Culture

Wistar rats were provided by the Universidad

Nacional Autónoma de México School of Medicine's animal breeding facility and were handled according to the Mexican Official Norm for Use, Care and Reproduction of Laboratory Animals (NOM-062-ZOO-1999).

Neurons were dissociated from the SCG by mechanical and enzymatic methods, as previously described [14]. Pancreatic β -cells were also obtained as previously described [5]. SCG neurons and pancreatic β -cells were plated and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂ for 16–24 hours before electrophysiological recordings.

Electrophysiological study

Recordings of Ca_v currents in SCG neurons and pancreatic β -cells were obtained at room temperature (22–24°C) by the patch-clamp technique in the whole-cell configuration and using an EPC-9 amplifier (HEKA Electronic, Lambrecht, Germany). Voltage protocols were generated, and current responses were digitized and stored by means of the Patchmaster software (HEKA Electronic). Pipettes were pulled from borosilicate glass capillaries with a horizontal patch electrode puller (Sutter Instrument, Novato, CA, USA), and SCG neurons were filled with internal solution consisting of (in mM) 140 CsCl, 20 TEA-Cl, 10 HEPES, 0.1 BAPTA-tetra cesium, 5 MgCl₂, 5 Na₂ATP, 0.3 Na₂GTP, and 0.1 leupeptin; the solution was adjusted to pH 7.2 with CsOH. Resistance of the pipettes was 1.8–2.0 M Ω . Pancreatic β -cells were filled with internal solution consisting of (in mM) 140 CsCl, 32 TEA-Cl, 10 HEPES, 0.1 BAPTA-4 Cs, 1 MgCl₂, 3 Na₂ATP, 3 Na₂GTP, and 0.1 leupeptine; the solution was adjusted to pH 7.4 with CsOH. Resistance of the pipettes was 2.5–3.5 M Ω .

SCG neurons and pancreatic β -cells were superfused (1–2 mL/min) with external solution designed to isolate Ba²⁺ currents (I_{Ba}) through Ca_v channels. For SCG neurons, this solution consisted of (in mM) 165 TEA-Cl, 2 BaCl₂, 10 HEPES, 8 glucose, 1 MgCl₂, and 0.0002 TTX; the solution was adjusted to pH 7.4 with TEA-OH. For pancreatic β -cells, this solution consisted of (in mM) 125 NaCl, 5 MgCl₂, 10 HEPES, 10 glucose, 10 BaCl₂, 2 H₂O, and 0.0001 TTX, the solution was adjusted to pH 7.4 with NaOH. Series resistance was compensated to >70% and did not exceed 10 M Ω ; capacitance of SCG neurons was 60–80 pF, and that of pancreatic β -cells was 6–10 pF. Steady-state currents were sampled at 20 kHz. Current recordings were filtered at 2.9 kHz.

RPMI medium and Antibiotic-Anti mycotic (100 $\times$) were obtained from Life Technologies (Grand Island, NY, USA), bovine serum albumin was obtained from Microlab (México City, México), and all other reagents used were obtained from Sigma (St. Louis, MO, USA).

Data Analysis

Inhibition of the current induced by GTP γ S or NA was calculated as the amplitude of the steady-state current under GTP γ S dialysis or during NA exposure minus the current amplitude under control conditions. Thereafter, it was divided by the current amplitude under control conditions (thus reported as a percentage). The facilitation index was calculated by measuring the peak current amplitude at P2 divided by the peak current amplitude at P1. Time constants (τ) were calculated from an exponential equation. Data are shown as mean $\pm$ SEM.

Statistical differences were analyzed by t-test. $P < 0.05$ was considered significant.

Results

Inhibition of Ca_v Current by GTP γ S

We first examined the fraction of voltage-dependent and -independent regulation by activation of endogenous G proteins with GTP γ S. Although both types of regulation were seen in SCG neurons, the voltage-dependent pathway was predominant under G protein activation [7]. Representative Ca_v currents evoked by the prepulse protocol in control and in GTP γ S-dialyzed SCG neurons and in pancreatic β -cells are shown in Figure 1A and B, respectively. The Ca_v current amplitude was reduced by GTP γ S in both cell types, however, this inhibition was released after a strong depolarizing pulse only in SCG neurons (Figure 1A and B). In SCG neurons the time course of the reduction in Ca_v current amplitude by GTP γ S was biphasic (Figure 1C; $\tau_1 = 17.2 \pm 8.8$ seconds and $\tau_2 = 29.9 \pm 6.09$ seconds), whereas in pancreatic β -cells it was monophasic (Figure 1D; $\tau = 31.2 \pm 2.6$ seconds). Ca_v current inhibition was greater in SCG neurons (P1 = $88 \pm 2.3\%$ and P2 = $28 \pm 4.3\%$) than in pancreatic β -cells (P1 = $53 \pm 4.6\%$ and P2 = $50 \pm 5.6\%$) (Figure 1E). The facilitation index was used to evaluate the fraction of voltage-dependent and voltage-independent regulation. Facilitation indices in SCG cells were 1.08 ± 0.06 (control) and 2.82 ± 0.026 (GTP γ S), whereas in pancreatic β -cells, they were 1.04 ± 0.01 and 0.99 ± 0.042 , respectively. In summary, these data support that Ca_v currents are reduced by endogenous G protein activation. However, voltage-dependent regulation is predominant in rat SCG neurons, whereas voltage-independent regulation does the same in rat pancreatic β -cells.

Voltage-Independent Inhibition of Ca_v Current by NA in Pancreatic β -Cells

Ca_v current in sympathetic neurons has been shown to be reduced by NA mainly through a voltage-dependent pathway [6]. Thus, we hypothesized that Ca_v currents in rat pancreatic β -cells are regulated by NA in a similar manner. We showed recently that Ca_v channels are regulated in SCG neurons and pancreatic β -cells via similar pathways when M₁R is activated. We used a prepulse protocol and characterized a specific time course as a hallmark of a voltage-dependent pathway. Representative P1 and P2 Ca_v current traces elicited in control and NA-treated sympathetic neurons and pancreatic β -cells are shown in Figure 2A and B, respectively. Ca_v current was reduced by NA in both cell types, however, the current inhibition was released by the prepulse only in sympathetic neurons. The time course of I_{Ba} amplitude in NA-treated sympathetic neurons and pancreatic β -cells is shown in Figure 2C and D, respectively. Notably, there was a reduction in Ca_v current amplitude in treated SCG neurons ($87 \pm 8.8\%$ in P1 and $25 \pm 3.5\%$ in P2) and treated pancreatic β -cells ($44.7 \pm 4\%$ in P1 and $34.5 \pm 4.4\%$ in P2) (Figure 2). However, the reduced current was scarcely restored by a depolarizing prepulse in pancreatic β -cells compared to SCG neurons (Figure 2F: SCG control = 1.1 ± 0.06 , SCG NA = 2.6 ± 0.42 and pancreatic β control = 1.02 ± 0.02 , pancreatic β NA = 1.4 ± 0.03 cells). Taking together, these data show that NA reduces the Ca_v current amplitude in

Figure 1: Inhibition of Ca_V current amplitude by $\text{GTP}\gamma\text{S}$ via a voltage-independent pathway in rat pancreatic β -cells. (A) and (B) Representative current traces elicited by a prepulse protocol, which consisted of a pair of 10-ms depolarizing pulses to -5 mV (P1, P2) from a holding potential of -80 mV and separation of P1 and P2 by a prepulse (PP) of $+125$ mV during a 25-ms period. Left panel shows overlapping P1 and P2 current traces from rat superior cervical ganglion (SCG) neurons under control and $\text{GTP}\gamma\text{S}$ conditions. Right panel shows overlapping P1 and P2 current traces from rat pancreatic β -cells under control and noradrenaline (NA)-treatment. Time course of I_{Ba} relative (P1) in a $\text{GTP}\gamma\text{S}$ -dialyzed neuron (C) and a pancreatic β -cell (D). Summary of the percentage inhibition of P1 and P2 (E) and facilitation index (F) in rat SCG neurons and rat pancreatic β -cells.

rat pancreatic β -cells through a voltage-independent pathway and suggest that this Ca_V current regulation by NA is significantly different from that observed in SCG neurons.

Discussion

We showed in rat pancreatic β -cells that Ca_V channels are inhibited by G proteins mainly via voltage-independent pathways. Voltage-independent regulation of calcium channels determines

intracellular calcium concentration and insulin secretion. The macroscopic Ca_V channel current was reduced to 55% by $\text{GTP}\gamma\text{S}$ dialysis. Similarly, in mouse pancreatic β -cells, $\text{GTP}\gamma\text{S}$ dialysis has been shown to reduce Ca^{2+} currents by 30% [2]. Inhibition in both mouse and rat pancreatic β -cells is minimal compared to that in SCG cells. The Ca_V channel inhibition in the mouse model is partially released by a strong depolarizing pulse, suggesting a predominant voltage-dependent pathway under endogenous G protein activation [2].

On the contrary, Ca_v channel inhibition was not relieved despite the application of a strong depolarizing pulse in GTP γ S-dialysed rat pancreatic β -cells. According to Elmslie and colleagues [8], the voltage-dependent regulation by G proteins in SCG neurons is released by a strong depolarizing pulse, and this relief is the hallmark of G protein regulation in neurons. Our data suggest that regulation of Ca_v channels by G protein-coupled receptors in insulin-secreting pancreatic cells could be mediated by pathways that have no relationship to the classic regulatory pathways described in SCG neurons [8].

In SCG neurons, the NA signal is transmitted mainly through α_2 -adrenergic receptors coupled to Gi proteins. This signaling pathway is voltage-dependent and requires the action of G $\beta\gamma$ subunits [9,10]. Similarly, α_2 -adrenergic receptor activation by NA reduces insulin release in rat pancreatic β -cells [18,19].

Ca_v channels are thought to be similarly inhibited by activation of α_2 -adrenergic receptors in human β -, RINm5F, and HIT cells [13,19]. The regulation in RINm5F is predominantly voltage-dependent, whereas in human β -cells the inhibition is voltage-independent [13]. Our data suggest that NA inhibits Ca_v currents via a voltage-independent pathway, in a similar way to human β -cells. Interestingly, α_2 -adrenoreceptor stimulation does not inhibit L-type calcium channels in mouse pancreatic β -cells [3]. Our study in primary cultured cells is the very first providing evidence that NA inhibits Ca_v channels in pancreatic β -cells. The classic understanding is that voltage-independent regulation is mediated by G $_{q/11}$ proteins involving PIP $_2$ hydrolysis, however, there is no evidence that activation of α_2 -adrenergic receptors hydrolyzes PIP $_2$. The signaling cascade and specific

activities of G protein subunits and their link to α_2 -adrenergic receptors in the modulation of β -cell Ca_v channels remain to be elucidated.

Inhibition of the insulin secreting process by activation of the sympathetic nervous system has been reported [18,19]. Our findings are consistent with this notion. We showed that Ca_v current diminishes in rat pancreatic β -cells treated with NA, resulting in a decreased Ca^{2+} entry. Although calcium influx through Ca_v channels is a known part of this process, little is known about Ca_v channel regulation that occurs via G-protein activation of the voltage-independent pathway. In conclusion, regulation of Ca_v channels by G proteins in pancreatic β -cells via a voltage-independent pathway appears to be an additional mechanism by which the Ca^{2+} concentration is controlled and a key step in such an important physiological process. Whatever the mechanism turns out to be, a voltage-independent mechanism is involved in pancreatic β -cells. However, an important question for future studies is whether or not a specific voltage-independent mechanism may account for the Ca_v current inhibition in pancreatic β -cells.

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Declarations

No conflicts of interest, financial or otherwise, are declared by the author(s).

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