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Noradrenergic inhibition and voltage-dependent facilitation of ω-conotoxin-sensitive Ca channels in insulin-secreting RINm5F cells

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We found that, besides dihydropyridine-sensitive Ca channels, insulin-secreting RINm5F cells also contain a minority (15–25%) of ω-conotoxin (ω-CgTx)-sensitive channels that show a high-affinity binding to [^{125}I]ω-CgTx (K_d 51 pM). Noradrenaline (NA, 10 μM) slows down Ca-channel activation in these cells and produces a sizeable reduction of Ca currents that is relieved by strong pre-conditioning depolarizations (facilitation). The action of NA is mimicked by intracellular application of GTP-γ-S and is prevented by pertussis toxin (PTX) or by cell pre-incubation with ω-CgTx. This suggests specific noradrenergic inhibition of ω-CgTx-sensitive Ca channels that is modulated by membrane potentials and PTX-sensitive G-protein activation.

Insulin-secreting cell; Ca channel; GTP-analog; Noradrenaline; ω-Conotoxin

1. INTRODUCTION

Catecholamines inhibit insulin secretion in pancreatic β-cells through the activation of α_2-adrenoreceptors [1,2]. A number of modulatory pathways may underly this action, including Ca^{2+}-entry diminution [3], opening of K^+ channels [4], stimulation of prostaglandin synthesis [5], and reduction of both cyclic AMP production [6] and GTP-dependent exocytosis [7]. Here we describe a novel mechanism by which noradrenaline (NA) can interfere with Ca^{2+} influx through voltage-operated Ca channels in the rat insulinoma cell line RINm5F. NA (10 μM) prolongs the activation kinetics and decreases the size of Ba currents through ω-CgTx-sensitive Ca channels, that are a minority (15–25%) of the overall high-threshold Ca channels expressed by these cells [8]. NA-inhibition is mediated by α_2-adrenergic receptors [9,10] and requires the activation of a PTX-sensitive G-protein. As in peripheral neurons, Ca-channels inhibition by NA [11] or intracellular GTP-γ-S [12–15] can be partially prevented by strong positive potentials. This might explain the relief of NA-induced inhibition of insulin release during sustained depolarizations and prolonged β-cell spike activity following glucose-level elevation in the plasma [16,17].

2. MATERIALS AND METHODS

The experiments wre performed on the rat insulinoma cell line RINm5F [7] (kindly provided by Dr. C.B. Wollheim). After trypsinization the cells were plated in plastic Petri dishes and grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, in an atmosphere of 5% CO_2 in air, at 37°C.

The cells were patch-clamped 2–5 days after plating as already described [15,18]. The bath solution (23°C) contained (in mM): 10 $BaCl_2$, 125 cholineCl, 1 $MgCl_2$, 10 Cs-Hepes (pH 7.3). Patch pipettes were filled with (in mM): 110 CsCl, 30 TEACl, 2 $MgCl_2$, 10 Cs-Hepes (pH 7.3). ω-CgTx fraction GVIA (Peninsula Labs) was dissolved in a 2 mM Ca^{2+} solution and applied acutely at 6.4 μM for 1 to 2 min. Alternatively the cells were pre-incubated with a 3.2 μM toxin concentration for 30 min before recording. Ca currents were filtered at 3 kHz and digitized by a 12 bit A/D converter (Tecmar Lab Master, 125 kHz) interfaced with an AT-compatible computer (Hyunday 386-20). Stimulation, acquisition and off-line data analysis were done using p-Clamp programs (version 5.5; Axon Instruments).

[^{125}I]ω-CgTx binding assays and saturation studies were performed in crude cell homogenates, as already described [19]. [^{125}I]ω-CgTx binding curves were obtained by adding increasing amounts of the radiolabeled toxin (5 to 250 pM) to a fixed amount of cell homogenate. Non-specific binding was determined in parallel tubes in the presence of 100 nM unlabeled toxin. The binding reaction was terminated by washing the samples three times with an ice-cold buffer [19].

3. RESULTS

High-voltage activated (HVA) Ba currents in RINm5F cells showed the same kinetic features of Ba currents in pancreatic β-cells [20–22] and peripheral neurons (see [8,23] for a review). They activated at around −30 mV, reached maximal amplitude between −10 and 0 mV and inactivated slowly during pulses of 100 ms duration. Similar findings were reported from the same cells using Ca^{2+} as a charge carrier [24]. External application of ω-CgTx (6.4 μM) decreased the amplitude of these currents (22 ± 6%, mean ± SD, $n = 18$) with little or no effect on their activation–inac-

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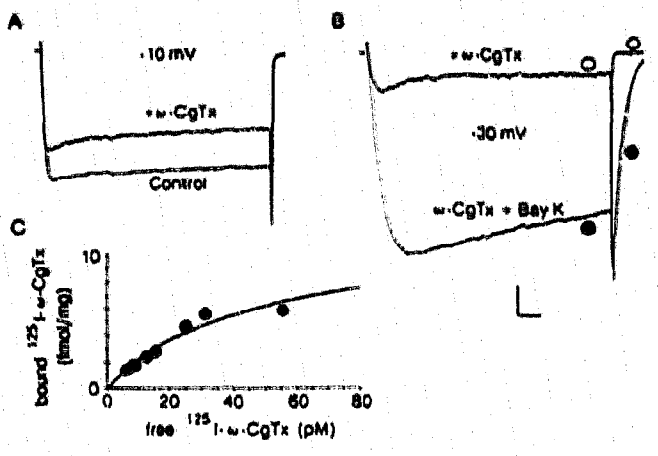

Fig. 1. A. Blocking action of ω-CgTx (3.2 μM) on HVA Ba currents in a RINm5F cell. The currents were recorded at −10 mV from −90 mV holding potential (V_h). Tail currents were recorded on returning to −60 mV. B. Ba currents recorded at −30 mV from the same cell of panel A after ω-CgTx-treatment (empty circle) and during application of 1 μM Bay K 8644 (filled circles). Horizontal and vertical scale: 20 ms and 200 pA. C. [125I]ω-CgTx binding to RINm5F cell homogenates. Non-specific binding was determined as described elsewhere [19]. The saturation curve has a K_d of 34 pM and a maximal bound [125I]ω-CgTx of 10.53 fmol/mg of protein (B_{max}).

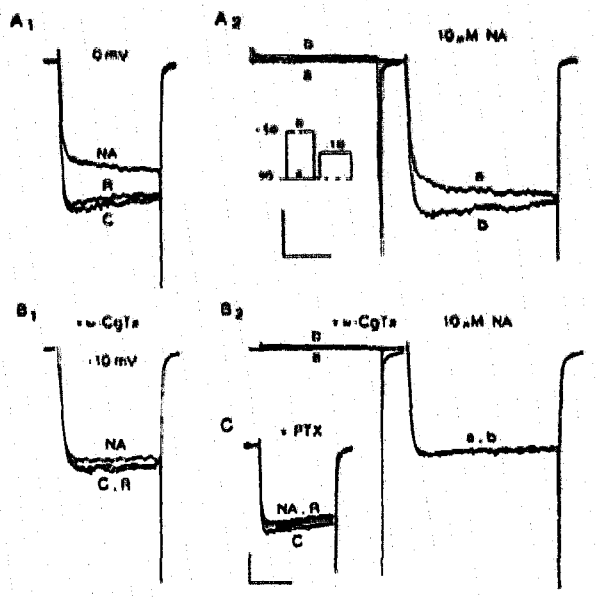

Fig. 2. A_1. HVA Ba currents recorded before (C), during (NA) and after (R) application of 10 μM NA on a RINm5F cell. Depolarizations to 0 mV; V_h −90 mV. A_2. The inhibitory action of NA (10 μM) on HVA currents (trace a) is relieved by a conditioning pre-pulse to +50 mV (trace b). B_1 and B_2. HVA Ba currents recorded from a ω-CgTx-treated RINm5F cell. The inhibitory action of NA (10 μM) and the facilitatory effect of the pre-pulse were nearly absent. V_h −90 mV. Pulse protocol in B_2 was as in A_2. Vertical scales: 80 pA (A_1,B_1,B_2), 200 pA (A_2). Horizontal scale: 20 ms. C. Effects of NA (20 μM) on a cell pre-incubated with PTX (0.5 μg/ml for 4 h). Depolarizations to 0 mV. V_h −90 mV. Vertical and horizontal scales: 50 pA and 20 ms.

tivation kinetics (Fig. 1A). At the concentration used, the toxin required about two min to reach steady-state levels of block and had no action on both the current-voltage characteristics and current reversal potential. Noteworthy, the residual ω-CgTx-resistant Ba current was found highly sensitive to dihydropyridines. Bay K 8644 (1 μM) had a marked agonistic action on ω-CgTx-resistant Ba currents that resulted in a 5-fold amplitude increase at −30 mV and a prolongation of HVA channel deactivation at −60 mV (Fig. 1B).

The existence of ω-CgTx-sensitive Ca channels in RINm5F cells is also supported by the finding that radiolabeled [125I]ω-CgTx binds specifically and with high affinity to these cells (Fig. 1C). Non-specific binding increased linearly with increasing [125I]ω-CgTx concentrations and represented 30–50% of the total binding near the K_d. From the analysis of 5 saturation curves we could estimate an average K_d of 51 ± 23 pM and a maximum number of receptor sites of 13.6 ± 1.92 fmol/mg of protein that are comparable to those reported for peripheral neurons [8,19].

NA had a depressive action on HVA Ba currents in RINm5F that varied from cell to cell. In 50% of the cells tested (n = 135) NA produced a reversible slowdown and a sizeable reduction of Ba currents that altogether caused a 20–30% depression of the total current. An example of NA-induced inhibition is shown in Fig. $2A_1$. Ba currents turned on quickly soon after the onset of the pulse to 0 mV and then more slowly. The slow turn-on of the current was less visible at more positive potentials. In the other 50% of the cells, NA had either no action or caused a small reduction of the currents (<10%) with little change to the Ca channel activation kinetics. The action of NA was found dose-dependent (K_d 0.9 μM), mimicked by clonidine (10 μM) and partially prevented by yohimbine (10 μM) (n = 18, not shown), suggesting that NA inhibition of HVA Ca channels in RINm5F cells is likely to be mediated by α_2-adrenoreceptors [9,10].

The inhibitory action of NA on Ba currents (trace a in Fig. $2A_2$) is partially relieved by applying strong pre-conditioning depolarizations to +50 mV followed by short repolarizations to resting potential (−90 mV). Pre-depolarizations were particularly effective in removing the slow activation of Ba currents induced by NA (trace b in Fig. $2A_2$). This suggests that NA inhibition of Ba currents merely represents a slow recovery of HVA channels which are inhibited by catecholamine at a resting potential, rather than a steady-state block of a transient Ca channel subtype as earlier proposed for neurons [10]. Strong depolarizations to +50 mV accelerate the relief of NA inhibition, giving rise to quickly activating Ba currents of larger amplitude at −10 mV. The inhibitory action of NA and the corresponding facilitatory effect of conditioning pre-pulses were dramatically reduced in cells pre-incubated for 30 min with ω-CgTx (3.2 μM, n = 23, Fig. 2). In these cells there was no more than a 5% reduction of HVA Ba currents

with NA (10 μM, Fig. 2B$_1$) and no sign of facilitation by pre-pulses (Fig. 2B$_2$). Thus, in RINm5F cells noradrenergic inhibition appears confined to ω-CgTx-sensitive Ca channels.

The action of NA was prevented by cell incubation with PTX (0.5 μg/ml for 3-6 h, $n = 10$) (Fig. 2C) or by intracellular application of GDP-β-S (500 μM, $n = 8$; not shown). We also tested the effects of intracellular GTP-γ-S on RINm5F cells ($n = 25$). Like NA, GTP-γ-S (100 μM) caused a prolongation and a partial depression of HVA Ba currents (Fig. 3A$_1$) that was relieved by strong conditioning depolarizations (trace b in Fig. 3A$_2$). Unlike NA, however, the action of GTP-γ-S was progressive, irreversible and effective also on ω-CgTx-resistant Ba currents (Fig. 3B).

4. DISCUSSION

Our data show that, besides dihydropyridine (DHP)-sensitive Ca channels [24], insulin-secreting RINm5F cells also possess a minority (15-25%) of ω-CgTx-sensitive channels that are characteristic of neuronal tissues (see [8,23]). As in other cell preparations, ω-CgTx- and DHP-sensitive channels in RINm5F cells can hardly be distinguished in terms of their range of voltage activation, inactivation kinetics and sensitivity to holding potentials [18,23]. Nevertheless, the two sets of channels appear to have sharply different sensitivity toward NA, that inhibits preferentially ω-CgTx-sensitive channels sparing the predominant ω-CgTx-resistant fraction. As clonidine and GTP-γ-S mimic the action of NA and yohimbine, and GDP-β-S and PTX prevent inhibition by the catecholamine, we conclude that NA modulates ω-CgTx-sensitive Ca channels through the activation of α_2-adrenoreceptors coupled to a PTX-sensitive G-protein. The unselective action of GTP-γ-S on both ω-CgTx-sensitive and ω-CgTx-resistant Ca channels may just be the consequence of a broader activation of internally accessible G-proteins coupled to the different HVA Ca-channel subtypes [15].

A selective inhibition of HVA Ca channels by NA has already been proposed for several neurons [9-11,14]. In bullfrog sympathetic neurons [10], the slowdown of Ca-channel activation by NA has been attributed to a block of a transient N-type Ca channel that unmasks a more slowly activating HVA current component. In contrast, our data show that the selective action of NA on ω-CgTx-sensitive Ca channels merely represents a voltage-dependent release of NA-induced inhibition that speeds up with increasing membrane depolarizations. A similar modulation has recently been described for a variety of neurons [11-15,23]. This, however, is the first report demonstrating that a voltage-dependent NA inhibition of Ca channels may occur also in endocrine cells. According to this mechanism, membrane voltage and cell activity would play a key role in modulating the potency of α-adrenergic inhibition of HVA channels in insulin-secreting cells. NA inhibition of Ca^{2+}-entry through ω-CgTx-sensitive channels would be fully effective at rest but quickly relieved during sustained depolarizations and prolonged spike activity following glucose-level elevation in the plasma [3]. This may also explain the glucose-induced relief of catecholamine inhibition on β-cells' electrical activity [25] and insulin secretion [16-17].

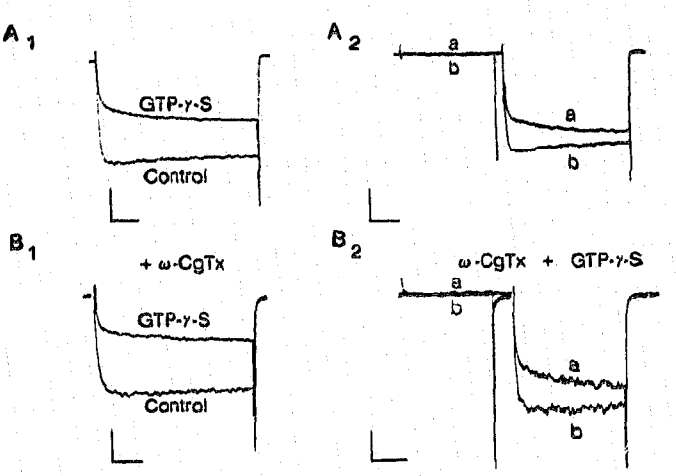

Fig. 3. Intracellularly applied GTP-γ-S (100 μM) mimics the action of NA on RINm5F cells (A$_1$, trace a in A$_2$) and its inhibitory action is relieved by conditioning pre-pulses (trace b in A$_2$). ω-CgTx-resistant Ba currents are also inhibited by GTP-γ-S (100 μM) (B$_1$, trace a in B$_2$) and the inhibition is relieved by conditioning pre-pulse (trace b in B$_2$). Conditioning and test pulses were as in Fig. 2A$_2$. V_h = −90 mV. Vertical scales: 100 pA (A$_1$,A$_2$,B$_2$), 80 pA (B$_1$). Horizontal scales: 10 ms (A$_1$,B$_1$), 20 ms (A$_2$,B$_2$).

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