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Modulation of N-type Ca^{2+} currents by moxonidine via imidazoline I_1 receptor activation in rat superior cervical ganglion neurons

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ABSTRACT

Moxonidine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_1 receptors (IR_1) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_1-induced inhibition of noradrenaline (NA) release is still unknown. We therefore, investigated the effect of IR_1 activation on voltage-dependent Ca^{2+} channels which is known to play an pivotal role in regulating NA in rat superior cervical ganglion (SCG) neurons, using the conventional whole-cell patch-clamp method. In the presence of rauwolscine (3 μM), which blocks α_2-adrenoceptor ($R_{\alpha 2}$), moxonidine inhibited voltage-dependent Ca^{2+} current (I_{Ca}) by about 30%. This moxonidine-induced inhibition was almost completely prevented by efaroxan (10 μM) which blocks IR_1 as well as $R_{\alpha 2}$. In addition, ω-conotoxin (CgTx) GVIA (1 μM) occluded moxonidine-induced inhibition of I_{Ca}, but, moxonidine-induced I_{Ca} inhibition was not affected by pertussis toxin (PTX) nor shows any characteristics of voltage-dependent inhibition. These data suggest that moxonidine inhibit voltage-dependent N-type Ca^{2+} current (I_{Ca-N}) via activating IR_1. Finally, moxonidine significantly decreased the frequency of AP firing in a partially reversible manner. This inhibition of AP firing was almost completely occluded in the presence of ω-CgTx. Taken together, our results suggest that activation of IR_1 in SCG neurons reduced I_{Ca-N} in a PTX-and voltage-insensitive pathway, and this inhibition attenuated repetitive AP firing in SCG neurons.

1. Introduction

Imidazoline derivatives such as moxonidine and rilmenidine suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mainly mediated by a central mechanism which is related activation of imidazoline I_1 receptors (IR_1) as well as α_2-adrenoceptors ($R_{\alpha 2}$) in rostral ventrolateral medulla (RVLM) [1,2]. In addition, it has also been described that moxonidine inhibit sympathetic outflow via a peripheral mechanism. Namely, moxonidine induces hypotension by suppressing sympathetic outflow via acting at postganglionic sympathetic neurons [3–8]. Recently, it is reported that activation of IR_1 is involved in moxonidine-induced inhibition of peripheral sympathetic outflow [5–8]. However, the precise mechanism of IR_1-induced inhibition of NA (noradrenaline) release in the peripheral sympathetic nerve terminal has not yet been elucidated.

Ca^{2+} influx through N-type Ca^{2+} channels plays an important role in membrane excitability and neurotransmitter release in sympathetic nervous system [9–15] and various transmitter receptors such as NA, acetylcholine, adenosine, somatostatin modulate N-type Ca^{2+} channels (for reviews, see [16]) and thus regulate NA release at nerve terminals in peripheral sympathetic neurons [17,18].

On this basis, It is possible that IR_1-induced inhibition of NA release in peripheral sympathetic nerve terminals may be mediated through the inhibition of N-type Ca^{2+} current (I_{Ca-N}) resulting in decreased intracellular Ca^{2+} concentrations. To investigate this possibility, we tested the effect of IR_1 activation on I_{Ca} in superior cervical ganglion (SCG) neurons. Our results provide evidence that activation of IR_1 significantly inhibited I_{Ca-N} acting through a voltage, pertussis toxin (PTX)-independent pathway in rat SCG neurons.

2. Materials and methods

This study was conducted in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee.

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2.1. Preparation of superior cervical ganglion neurons

SCG neurons were enzymatically dissociated according to a previously described, modified method [19]. The neurons were plated on poly-l-lysine-coated 12 mm glass cover slips and incubated in a humidified incubator with 95% air, 5% CO_2. Neurons were used within 12 h after plating. If necessary, neurons were incubated with 500 μg/L PTX for 14–18 h.

2.2. Electrophysiology

I_{Ca} was recorded using conventional whole-cell techniques. Electrode resistance varied from 3 to 5 MΩ when filled with internal solution. We performed measurements using an Axopatch 200A patch-clamp amplifier (Molecular Devices, Sunnyvale, CA). Voltage and current commands and digitization of membrane voltages and currents were controlled using a Digidata 1322 A interfaced with Clampex 9.2 of the pClamp software package (Molecular Devices, Sunnyvale, CA) on an IBM-compatible computer. We analyzed data using Clamfit (Molecular Devices, Sunnyvale, CA) and Prism 4.0 (GraphPad, San Diego, CA). Currents were low-pass filtered at 2 kHz using the four-pole Bessel filter in the amplifier. Capacitance (Cm) values were taken from automatically calculated recordings by pClamp 9.2 software. Action potentials were recorded in the current-clamp mode. Membrane potential measurements were low-pass filtered at 10 kHz. Only cells with resting membrane potential < −50 mV were included in the analysis. Multiple independently controlled syringes served as reservoirs for a gravity-driven fast drug perfusion system. Switching between solutions was accomplished by manually controlled valves. All experiments were conducted at room temperature.

2.3. Solutions and drugs

The internal (pipette) solution contained the following (in mM): 140 $CsCl_2$, 1.2 $MgCl_2$, 4 MgATP, 0.4 Na_2 GTP, 10, phosphocreatine, 10 HEPES, and 10 EGTA; the solution was adjusted to pH 7.2 with CsOH. The external (bath) solution contained 155 tetraethylammonium (TEA)-Cl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 14 glucose, and 10.5 HEPES; the solution was adjusted to pH 7.4 with TEA-OH. The external solution for current-clamp contained the following (in mM): 143 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, and 10 HEPES; the solution was adjusted to pH 7.4 with NaOH. The pipette solution used for current-clamp recordings contained the following (in mM): 113 K-gluconate, 30 KCl, 1.2 $MgCl_2$, 4 MgATP, 0.4 Na_2GTP, 10 phosphocreatine, 10 HEPES, and 0.05 EGTA; the solution was adjusted to pH 7.2 with KOH.

Moxonidine, ω-conotoxin (CgTx) GVIA, and efaroxan were purchased from Tocris (Tocris Cookson Inc., Bristol, UK). All other drugs were purchased from Sigma–Aldrich Chemicals. All drugs were dissolved in distilled water as stock solutions (1–100 mM).

2.4. Data analysis

Data are presented as the means ± SEM, with the number of experiments given within parentheses. The concentration–response curves of moxonidine for I_{Ca} inhibition were calculated by fitting to a single-site binding isotherm with least-squares nonlinear regression using Prism 4.0 (GraphPad, San Diego, CA). We used unpaired Student's *t* tests to compare two groups. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. Inhibition of I_{Ca} by moxonidine

I_{Ca} was evoked by 100 ms depolarizing step pulses to a test potential of 0 mV from a holding potential of −80 mV. The average membrane capacitance of SCG neurons was 23 ± 1 pF ($n = 58$). Application of 30 μM moxonidine significantly and reversibly inhibited I_{Ca} by 49 ± 4% ($n = 6$) (Supplementary Fig. 1(A),(B)). We generated concentration–response curves for moxonidine-induced I_{Ca} inhibition (Supplementary Fig. 1C). The degree of inhibition was estimated as the ratio of decreased current to control current elicited by test pulses at 0 mV, starting from −80 mV. The concentration at which moxonidine inhibited I_{Ca} in SCG neurons by 50% was about 30 μM (Supplementary Fig. 1C).

Moxonidine have been reported to bind $R_{\alpha 2}$ as well as IR_1 [20,21]. Thus, to determine an IR_1-mediated effect of moxonidine, it is necessary to exclude the α_2-adrenergic effect of moxonidine from IR_1-activating effect. According to our previous report [19], 1 μM NA inhibited I_{Ca} by about 50%, which is same level of I_{Ca} inhibition by 30 μM moxonidine. This NA-induced I_{Ca} inhibition was almost completely blocked by pretreatment with 3 μM rauwolscine (a selective $R_{\alpha 2}$ antagonist) [19]. We therefore performed all our experiments below using 30 μM moxonidine in the presence of 3 μM rauwolscine to rule out an α_2-adrenergic effect of moxonidine from IR_1-activating effect unless mentioned otherwise.

3.2. Moxonidine inhibited I_{Ca-N} through activation of IR_1 in rat SCG neurons

We investigated the characteristics of moxonidine-induced I_{Ca} inhibition in rat SCG neurons. Moxonidine (30 μM) inhibited I_{Ca} in rat SCG neurons in a reversible manner (35.7 ± 4.4%, $n = 6$) (Fig. 1A, left and middle). Moxonidine inhibited I_{Ca} over a potential range from −40 mV to +40 mV according to the current–voltage (*I–V*) relationship (Fig. 1A, right). This moxonidine-induced I_{Ca} inhibition was almost completely blocked by pretreatment with 10 μM efaroxan, a mixed IR_1 and $R_{\alpha 2}$ antagonist [5,6] (8.1 ± 2.0%, $n = 6$, $p < 0.01$) (Fig. 1B).

Next, we determined whether N-type Ca^{2+} channels were modulated by moxonidine using an ω-CgTx GVIA occlusion experiment. Consistent with previous results [22–24], rat SCG neurons displayed large I_{Ca}, with about 80% attributed to ω-CgTx GVIA-sensitive N-type Ca^{2+} channels (79.8 ± 4.0%, $n = 6$) (Fig. 1C, right and middle) . Moxonidine (30 μM)-induced I_{Ca} inhibition was almost completely occluded by application of ω-CgTx GVIA (9.6 ± 2.4%, $n = 6$, $p < 0.01$) (Fig. 1C, middle and left).

Taken together, these results suggest that moxonidine inhibited ω-CgTx GVIA-sensitive I_{Ca-N} mainly through activation of IR_1 in rat SCG neurons.

3.3. Characteristics of moxonidine-induced I_{Ca-N} inhibition

We determined the involvement of G protein in moxonidine-induced I_{Ca-N} inhibition using GDPβS, a hydrolysis-resistant GDP analog known to prevent G protein activation [25,26]. As shown in Fig. 2A, Moxonidine (30 μM)-induced I_{Ca-N} inhibition was nearly completely blocked by dialysis of GDPβS (2 mM) into the internal solution (Control group, 34.8 ± 1.6%, $n = 6$; GDPβS group, 9.5 ± 1.9%, $n = 6$, $p < 0.01$) (Fig. 2A).

To elucidate the nature of G protein coupling between IR_1 and I_{Ca}, SCG neurons were incubated for 16–18 h in a medium containing PTX (500 ng/mL) [19,27,28]. Moxonidine-induced I_{Ca} inhibition was still significant even after PTX pretreatment (Control group, 34.9 ± 1.6%, $n = 6$; PTX group, 31.0 ± 3.0%, $n = 6$, $P > 0.05$) (Fig. 2B).