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Biochemical Pharmacology 69 (2005) 841–854
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Contrasting the effects of nifedipine on subtypes of endogenous and recombinant T-type Ca^{2+} channels

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Received 1 October 2004; accepted 30 November 2004

Abstract

There is evidence that nifedipine (Nif) – a dihydropyridine (DHP) Ca^{2+}-channel antagonist mostly known for its L-type-specific action – is capable of blocking low voltage-activated (LVA or T-type) Ca^{2+} channels as well. However, the discrimination by Nif of either various endogenous T-channel subtypes, evident from functional studies, or cloned $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ T-channel α1 subunits have not been determined. Here, we investigated the effects of Nif on currents induced by $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ expression in *Xenopus* oocytes or HEK-293 cells ($I_{\alpha 1G}$, $I_{\alpha 1H}$ and $I_{\alpha 1I}$, respectively) and two kinetically distinct, ''fast'' and ''slow'', LVA currents in thalamic neurons ($I_{LVA,f}$ and $I_{LVA,s}$). At voltages of the maximums of respective currents the drug most potently blocked $I_{\alpha 1H}$ (IC_{50} = 5 μM, max block 41%) followed by $I_{\alpha 1G}$ (IC_{50} = 109 μM, 23%) and $I_{\alpha 1I}$ (IC_{50} = 243 μM, 47%). The mechanism of blockade included interaction with $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ open and inactivated states. Nif blocked thalamic $I_{LVA,f}$ and $I_{LVA,s}$ with nearly equal potency (IC_{50} = 22 μM and 28 μM, respectively), but with different maximal inhibition (81% and 51%, respectively). We conclude that $Ca_v3.2$ is the most sensitive to Nif, and that quantitative characteristics of drug action on T-type Ca^{2+} channels depend on cellular system they are expressed in. Some common features in the voltage- and state-dependence of Nif action on endogenous and recombinant currents together with previous data on T-channel α1 subunits mRNA expression patterns in the thalamus point to $Ca_v3.1$ and $Ca_v3.3$ as the major contributors to thalamic $I_{LVA,f}$ and $I_{LVA,s}$, respectively.

Keywords: Endogenous and cloned T-type Ca^{2+} channels; Nifedipine; Thalamic neurons; *Xenopus* oocytes

1. Introduction

Low voltage-activated (LVA) Ca^{2+} channels, otherwise known as T-type, lack specific ligands, which essentially impeded the progress in their molecular cloning compared to the high voltage-activated (HVA) counterparts. Moreover, native T-type Ca^{2+} channels display quite variable tissue-specific pharmacological sensitivity, suggesting the existence of their numerous functional subtypes. Recent cloning of three different T-channel pore-forming α1 subunits, α1G ($Ca_v3.1$), α1H ($Ca_v3.2$) and α1I ($Ca_v3.3$), through the use of in silico strategies [1–4] has provided a molecular basis for T-channel diversity. A logical extension of these findings is to correlate endogenous T-channel subtypes to specific cloned α1 subunits. In this respect determining tissue-specific expression of respective mRNAs or even proteins may not be sufficient, as their presence is no guarantee of functional channel. Therefore, comparison of functional properties of endogenous LVA channels and heterologously expressed α1 subunits is also required. Generally, the properties of endogenous channels depend not only on the nature of the primary pore-forming

Abbreviations: A_{max}, maximal percentage of inhibition; DMSO, dimethyl sulfoxide; HVA, high voltage-activated; IC_{50}, half-inhibitory concentration; $I_{\alpha 1G}$, current through heterologously expressed α1G ($Ca_v3.1$) subunit of low voltage-activated calcium channel; $I_{\alpha 1H}$, current through heterologously expressed α1H ($Ca_v3.2$) subunit of low voltage-activated calcium channel; $I_{\alpha 1I}$, current through heterologously expressed α1I ($Ca_v3.3$) subunit of low voltage-activated calcium channel; $I_{LVA,f}$, ''fast'' low voltage-activated current in thalamic neurons; $I_{LVA,s}$, ''slow'' thalamic low voltage-activated current; *I–V*, current–voltage relationship; *k*, slope factor; LD, laterodorsal; LVA, low voltage-activated; *p*, cooperativity coefficient; $V_{1/2}$, potential of half-maximal activation or inactivation; V_m, membrane potential

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doi:10.1016/j.bcp.2004.11.024

α1 subunit but also on the presence of potential auxiliary subunits and/or other cell-specific regulatory factors. Thus, comparative studies of endogenous LVA currents in different cell types and the currents induced by heterologous expression of α1 subunits can be useful in providing information on both molecular organization of endogenous LVA channels as well as tissue-specific regulation of various α1 subunits.

The hallmark paper of Akaike et al. [5] gave rise to the notion that LVA Ca^{2+} channels in central neurons are more sensitive to the block by representatives of three classes of L-type Ca^{2+}-channel antagonists, phenylalkylamines (verapamil, D-600), dihydropyridines (DHPs—nifedipine, nicardipine) and benzothiazepines (diltiazem) as well as to the neuroleptic flunarizine compared to the ones in peripheral tissues [6]. We have recently identified two kinetically distinct, ''fast'' and ''slow'', endogenous LVA Ca^{2+}-channel subtypes in the neurons from rat laterodorsal (LD) thalamic nucleus [7–9]. In addition to the differences in the major biophysical properties, these channels were characterized by specific developmental pattern of the expression on neuronal surface and pharmacology. Interestingly, based on their sensitivity to nifedipine, flunarizine and La^{3+}, the pharmacology of only the ''fast'' thalamic LVA Ca^{2+} channel could be attributed to the ''central'' type, whereas the ''slow'' channel was characterized by distinct features of ''peripheral'' pharmacology manifested by the preference to amiloride and Ni^{2+} [7,9]. A limitation to these studies was that block was only studied at a single concentration of the agents, and dose–response relationships for the blockade of each of the channel subtype are still missing. In addition, there are no dose–response relationships for the action of these agents on the cloned T-channel α1 subunits either.

In the present study, we used pharmacological criteria, namely nifedipine sensitivity, in the attempt to distinguish LVA Ca^{2+} currents in thalamic neurons and shed some light on α1-subunit composition of underlying channels. To do so, we broke down the overall LVA Ca^{2+} current in isolated neurons from LD thalamic nucleus of 14–17-day-old rats onto two components based on the difference in the rate of inactivation and examined the effects of nifedipine on each of them. These were then compared to the effects of nifedipine on three subtypes of recombinant LVA Ca^{2+}-channel α1 subunits functionally expressed in *Xenopus* oocytes. Our results showed that $Ca_v3.2$ is the most sensitive to nifedipine, and that the mechanism of drug action is characterized by common for all subunits preferential interaction with activated and inactivated states. The dose–response relationships for the endogenous currents did not match any of those for the expressed ones, suggesting either the presence of yet unknown T-channel α1 subunit(s) or distinct regulation of pharmacological sensitivity of the existing subunits in thalamic neurons. Nevertheless, some common features in state-dependence of nifedipine action, together with previous reports on predominant $Ca_v3.1$ and $Ca_v3.3$ mRNA expression in the thalamus, allowed us to cautiously conclude that $Ca_v3.1$ and to a lesser extent $Ca_v3.2$ most likely contribute to the population of ''fast'' channels, whereas ''slow'' channels may be primarily composed of $Ca_v3.3$.

2. Materials and methods

2.1. Preparation of isolated thalamic neurons

Fourteen to seventeen days old rats were anesthetized with ether and decapitated. The brain was quickly removed from cranium and placed for 3–4 min in cold (4 °C) physiological saline 120 mM NaCl, 2.2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 24 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 15 mM glucose, pH 7.4 bubbled with carbogen (mixture of 95% O_2 and 5% CO_2). It was then separated into two hemispheres that were cut in the sagittal plane into a 300 μM thick slices using a razor blade. The slices that according to the rat brain stereotaxic atlas contained LD thalamic nucleus were selected and subjected to the enzymatic treatment with 20 mg/ml of pronase (Sigma) in physiological saline continuously bubbled with carbogen at room temperature for 30 min. After the treatment the slices were transferred into the bubbled enzyme-free saline. Isolated neurons were obtained by pipetting the slices. The suspension of the cells was transferred onto the cover slip and placed in the recording chamber.

2.2. Preparation of cRNA, isolation, maintenance and injection of Xenopus oocytes

Capped $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ complementary RNA (cRNA) was prepared from linearized plasmid (pSP73 for $Ca_v3.3$, pGEM-HEA for $Ca_v3.1$ and $Ca_v3.2$) containing respective cDNA sequences using T7 mMessage mMachine in vitro transcription kit (Ambion).

Stage V and VI oocytes from adult female *Xenopus laevis* frogs were used for the recombinant T-type Ca^{2+}-channel expression. The procedures for oocyte isolation, maintenance and injection did not differ from those detailed elsewhere [10]. The volume of injected $Ca_v3.1$, $Ca_v3.2$ or $Ca_v3.3$ cRNA solution (0.2 μg/μl) was usually 50 nl per oocyte. The injection was performed using a semiautomatic nanoliter-range injector (Bibigon, Kyiv, Ukraine). Oocytes were used for the experiments 5 days after cRNA injection, since this time was required for maximal T-type Ca^{2+}-channel current expression (data not shown).

2.3. Electrophysiology and solutions

Macroscopic Ca^{2+}-channel currents in isolated thalamic neurons were measured using the whole-cell patch-clamp technique. To provide the best voltage-clamp performance,