Biochimica et Biophysica Acta 1778 (2008) 2437–2443

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Spermine biphasically affects N-type calcium channel currents in adult dorsal root ganglion neurons of the rat

Ilaria Cino [1], Alessandro Formenti *

Institute of Human Physiology II, University of Milan, Via Mangiagalli, 32, Milan, I-20133, Italy

ARTICLE INFO

Article history:
Received 5 February 2008
Received in revised form 18 June 2008
Accepted 9 July 2008
Available online 16 July 2008

Keywords:
N-type Ca^{2+}channel
Spermine
Polyamine
Calcium current
Sensory neuron
Adult rat

ABSTRACT

Spermine (Spe) is a polyamine co-secreted with neurotransmitters. In this work its effects on N-type Ca^{2+} channel ($Ca_V2.2$) have been studied on adult sensory neurons of the rat by means of whole-cell patch-clamp. Spe exerted biphasic effects when added to the external solution: at 500 μM decreased N-type Ca^{2+} channel currents, reducing the maximum whole-cell conductance, shifting the activation curve to the right on the voltage axes and decreasing its slope; conversely, at lower concentration (500 nM) Spe induced completely opposite effects. In 62% of the neurons the inhibitory effects were accompanied by a slowing down of the activation kinetics relieved by a conditioning pre-pulse to +50 mV. The biphasic effects and their rapid onset and offset time course may be explained if multiple sites of action with a different affinity for Spe are present directly on the channel. The effects of Spe on HVA Ca^{2+} currents were strongly dependent on $[Ca^{2+}]_{ext}$, high $[Ca^{2+}]$ powerfully reducing Spe effects. This may be explained if we take into account that as Spe has four positive charges at physiological pH; it may compete with divalent cations for some negatively charged regulatory sites. In these experiments, Spe was effective at concentrations possibly reached in physiological conditions.

1. Introduction

The polyamines putrescine, spermidine and spermine are present in almost all cells. These organic polycations, protonated at physiological pH, appear to play important roles in protein synthesis, cell growth and cell differentiation, and their synthesis and degradation are tightly controlled by several enzymes which are regulated by cellular activity [See Fig. 4 in ref. 1].

Aizenman and coworkers [2] have shown that neuronal activity induces expression of ornithine decarboxylase, an enzyme which controls the rate-limiting step in mammalian polyamine synthesis, resulting in additional production of spermine (Spe). In turn, Spe can interact with various ion channels. It blocks the inward-rectifier K^+ channels from the intracellular side, and acts at extracellular sites on neurons to potentiate the activity of NMDA receptors. Intracellular Spe has also been shown to control rectification and the total amount of current flow in some subtypes of AMPA and kainate receptors [3,4], and to block non-selective cation channels [5]. This polyamine has also been found to modulate high-voltage activated (HVA) Ca^{2+} currents in DRG neurons, although at very high extracellular concentrations [6], and modulate L-type Ca^{2+} currents from the inside as well as the outside the cell membrane in smooth muscle cells [7–9].

Spe is co-secreted with neurotransmitters and may itself be a putative neurotransmitter or neuromodulator itself [10–12]. Little is known about the effects of Spe on HVA N-type Ca^{2+} channels ($Ca_V2.2$). These channels are expressed in endocrine cells and primarily in neurons, where they initiate neurotransmission at many fast synapses and mediate Ca^{2+} entry into cell bodies and dendrites [For a review of the literature see ref. 13]. Spe can bind to their $\alpha_2\delta$ subunit [14], and may modulate the actions of ω-conotoxin GVIA, specific N-type channel blocker, through a mutually exclusive binding process [15]. In adult rat dorsal root ganglia (DRG) neurons, N-type current component represent about 50% of the total somatic HVA Ca^{2+} currents [16]. The aim of this work is to investigate the effects of Spe on N-type Ca^{2+} channel currents in adult DRG neurons of the rat.

2. Materials and methods

2.1. Cell preparation

The methods used in this study are similar to those previously reported [16]. DRG were dissected from Wistar adult rats (175–200 g; 6–7 weeks old). The animals were killed following the instructions of the ethics committee of the University of Milan. The animals were deeply anaesthetized with diethyl ether (Carlo Erba, Italy) and killed by cervical dislocation. The dorsal column was excised. After laminectomy the spinal cord was removed and the ganglia were collected in a cool Ca^{2+}-free Tyrode solution. The

* Corresponding author.
E-mail address: alessandro.formenti@unimi.it (A. Formenti).
[1] Present address: Vita-Salute University, IVF Unit, H S Raffaele, Via Olgettina 60, 20132 Milan, Italy.

0005-2736/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2008.07.006

ganglia were then minced and treated enzymatically in a Ca^{2+}-free Tyrode solution, containing 1% trypsin (Sigma type XI-S) and 0.015% collagenase (Sigma type I-S) at 37 °C for 12 min. The fragments of the ganglia were then gently minced in a solution containing 0.1% DNAse (Sigma type II). After centrifugation, the cells were placed on uncoated Petri dishes (Nunc), in Tyrode solution at room temperature. Recordings were carried out in the 6 h which followed.

2.2. Patch-clamp recordings

Standard whole-cell patch-clamp technique was utilized. The cells with a diameter ranging between 15 and 25 μm (small cells) were voltage-clamped using patch pipettes of 3–4 MΩ resistance. An Axopatch 200B integrating patch-clamp (Axon Instruments, USA) was used interfaced with a PC. Analog signals were low-pass filtered at 5 kHz and the data were digitized at a sampling rate of 100 μs using a Digidata 1200 Series Interface (Axon Instruments). A home-built two barrel fast perfusion exchange system was utilized to determine time course of the onset and offset of drug effects. Solution changes were typically complete within <10 ms.

2.3. Data acquisition and analysis

Data acquisition and analysis took place using pClamp programs. The linear component of the leakage current was calculated and subtracted from the current traces. In some cells capacitive transients and leakage currents were compensated by subtracting residual Cd^{2+}-insensitive currents recorded after adding 500 μM Cd^{2+} to the external medium. The effects on Ca^{2+} channel currents were taken into account only when the recovery was at least partially observed after washing out the drug. The cells with low-voltage-activated calcium currents were not used. Data were presented as mean values±SEM. Where specified the results were analyzed for statistical differences with a t-test or using two-way ANOVA method followed by Bonferroni post test, with GraphPad software Prism 4.

2.4. Stimulation protocols

To distinguish between effects on the currents due to changes in whole-cell Ca^{2+} conductance and those due to a shift of the current activation curve along the potential axis, a ramp of increasing potentials was adopted. This stimulus protocol provides information

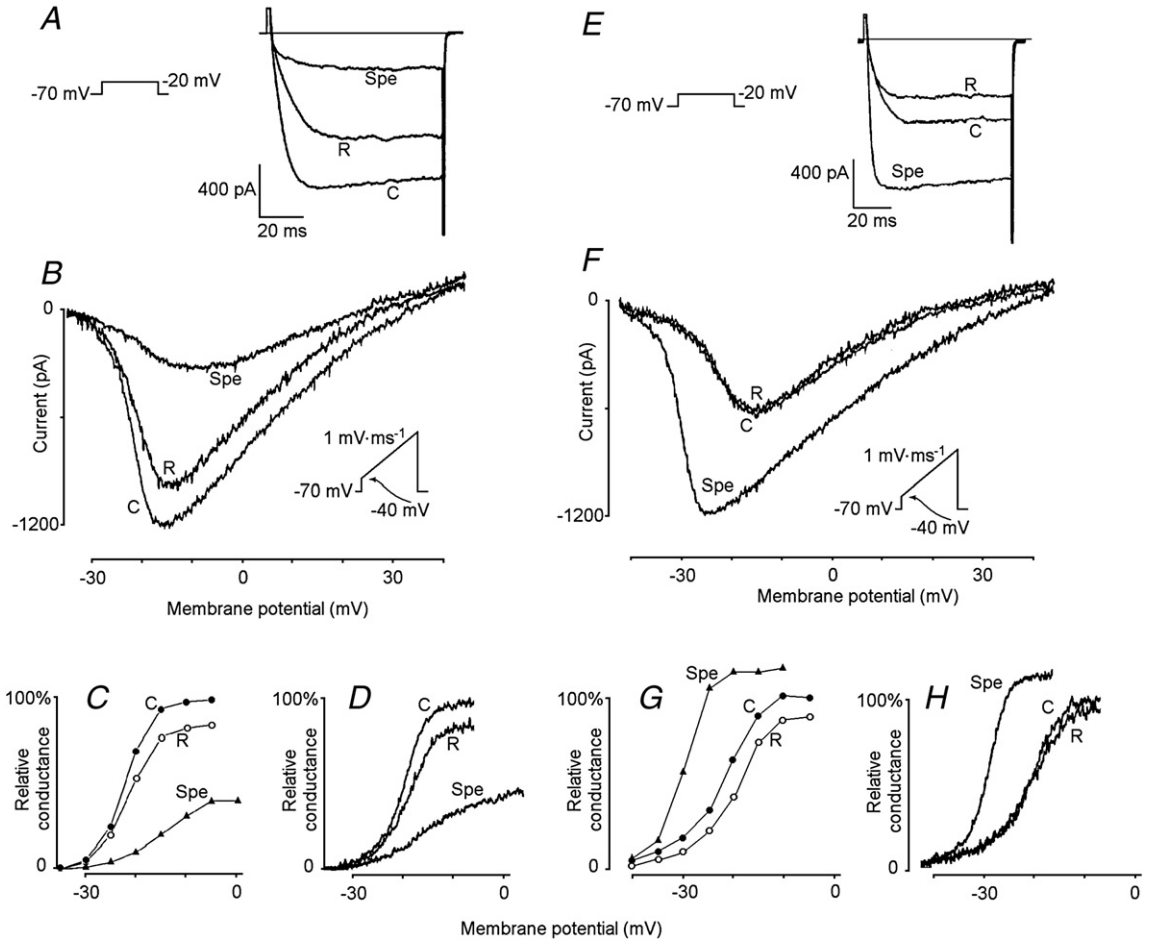

Fig. 1. Biphasic effects of Spe at different concentrations on N-type Ca^{2+} channel currents in rat DRG neurons. N-type current component was isolated by adding 10 μM nifedipine and 0.2 μM ω-agatoxin IVA to the extracellular solution. Experiments were carried out in patch-clamp whole-cell configuration with 3 mM Ba^{2+} as a charge carrier. Currents evoked by means of depolarizing steps (only the step to −20 mV is shown for clarity) from a holding potential of −70 mV were inhibited by Spe 500 μM (A). Conversely, when Spe was superfused at lower concentration (500 nM), the currents were markedly increased (E). In all the panels, C stands for control and R for recovery upon washing of the drug. The same effects as those illustrated above were also investigated by means of a ramp protocol (insets in panels B and F). Ramps of potential (1 mV 1 ms^{-1}) were utilized to quickly obtain current to voltage (I–V) relationships (B and F). From these recordings, we can see that Spe changes the voltage to peak, indicating that the effects on the current intensity seen above are the result of a combined effect of Spe on whole-cell conductance and voltage-dependent characteristics of N-type Ca^{2+} channels. Panels C and G show the current activation vs. voltage, measured from the tail currents from the step experiments in A and E after leakage and capacitive transient subtraction. Note that no relevant differences are present if compared with panels D and H, where the relative conductance is calculated from the currents (I) shown in B and F respectively (relative conductance $= I/(V_m - E_{inv})$ where V_m is the membrane potential and E_{inv} is the potential at which the currents are inverted). All traces are normalized, with the control trace being set to 100%. Note that Spe exerts its effects on the whole-cell maximum conductance, shifts the activation curves along the potential axis, and changes their slopes.