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ORIGINAL ARTICLE

Window current through the T-type Ca^{2+} channel triggers the mechanism for cellular apoptosis via mitochondrial pathways

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Abstract We hypothesized that Ca^{2+} entry through the window T-type Ca^{2+} current causes apoptosis. To test this hypothesis, we transfected human embryonic kidney (HEK) 293 cells to express recombinant $Ca_v3.2$ T-type Ca^{2+} channels (hereafter called HEK-$Ca_v3.2$ cells). After incubation in media containing a high concentration (7.2 mM) of Ca^{2+}, intracellular Ca^{2+} levels increased in HEK-$Ca_v3.2$ cells without electrical stimulation but not in untransfected HEK293 cells. In quiescent HEK-$Ca_v3.2$ cells exposed to high Ca^{2+} media, apoptosis, as indicated by the appearance of hypodiploid cells, loss of mitochondrial transmembrane potential, and activation of caspases-3 and -9 was observed, while caspase-8 was not activated. These apoptosis-associated changes were blunted by pretreatment with the R(−)-isomer of efonidipine, a selective blocker of T-type Ca^{2+} channels. High Ca^{2+} did not induce apoptosis in untransfected HEK293 cells. Our findings show that Ca^{2+} entry through the steady-state window current of T-type Ca^{2+} channels causes apoptosis via mitochondrial pathways, and suggests that T-type Ca^{2+} channels may be novel therapeutic targets for several diseases associated with abnormal apoptosis.

Keywords T-type Ca^{2+} channel · Window current · $Ca_v3.2$ · Apoptosis · Mitochondrial pathways

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Introduction

T-type Ca^{2+} channels, a subtype of voltage-gated Ca^{2+} channels, are characterized by their activation at low voltage, rapid inactivation, and slow deactivation [1]. These channels have been shown to be inhibited by nickel, mibefradil, and efonidipine, and to be resistant to representative L-type Ca^{2+} channel blockers such as nifedipine, diltiazem, and verapamil [2]. Three different cDNAs for the pore-forming α1 subunits of T-type Ca^{2+} channels, i.e., $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}), and $Ca_v3.3$ (α_{1I}), have been cloned [3]. The channels are expressed in a variety of tissues including heart, brain, smooth muscle, kidney, and several endocrine organs, and it is thought that they participate in the regulation of cardiac automaticity [4], neuronal excitability [5], vascular tone [6], renal microcirculation [7], and the secretion of various hormones [8]. In addition, increases in the expression of T-type Ca^{2+} channels have been demonstrated in some pathological states in the heart such as ventricular hypertrophy [9], myocardial infarction [10], and atrial fibrillation [11]. Thus, the remodeling of T-type Ca^{2+} channels may play a role in both physiological and pathophysiological conditions of various organs and tissues.

Apoptosis is an active process that leads to cell death. It is morphologically and biochemically distinct from necrosis. Apoptosis occurs under both physiological and pathophysiological conditions [12]. The activation of apoptosis under pathological conditions is now thought to contribute to a variety of disease processes including in carcinomatous, autoimmune, neurodegenerative, and cardiovascular diseases [13]. During apoptosis, changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) are known to play a critical role [14]. Excessive $[Ca^{2+}]_i$ has been linked to apoptosis via activation of the major sources of Ca^{2+} influx, including L-type Ca^{2+} channels [15], ryanodine receptors [16], and Na^+/Ca^{2+}