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Biophysical properties of voltage-gated Na^+ channels in frog parathyroid cells and their modulation by cannabinoids

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Summary

The membrane properties of isolated frog parathyroid cells were studied using perforated and conventional whole-cell patch-clamp techniques. Frog parathyroid cells displayed transient inward currents in response to depolarizing pulses from a holding potential of –84 mV. We analyzed the biophysical properties of the inward currents. The inward currents disappeared by the replacement of external Na^+ with $NMDG^+$ and were reversibly inhibited by 3 μmol l^{-1} TTX, indicating that the currents occur through the TTX-sensitive voltage-gated Na^+ channels. Current density elicited by a voltage step from –84 mV to –24 mV was –80 pA pF^{-1} in perforated mode and –55 pA pF^{-1} in conventional mode. Current density was decreased to –12 pA pF^{-1} by internal GTPγS (0.5 mmol l^{-1}), but not affected by internal GDPβS (1 mmol l^{-1}). The voltage of half-maximum ($V_{1/2}$) activation was –46 mV in both perforated and conventional modes. $V_{1/2}$ of inactivation was –80 mV in perforated mode and –86 mV in conventional mode. Internal GTPγS (0.5 mmol l^{-1}) shifted the $V_{1/2}$ for activation to –36 mV and for inactivation to –98 mV. A putative endocannabinoid, 2-arachidonoylglycerol ether (2-AG ether, 50 μmol l^{-1}) and a cannabinomimetic aminoalkylindole, WIN 55,212-2 (10 μmol l^{-1}) also greatly reduced the Na^+ current and shifted the $V_{1/2}$ for activation and inactivation. The results suggest that the Na^+ currents in frog parathyroid cells can be modulated by cannabinoids *via* a G protein-dependent mechanism.

Key words: parathyroid, voltage-gated Na^+ channel, G protein, activation, inactivation, cannabinoid, frog.

Introduction

Parathyroid hormone (PTH) regulates extracellular free Ca^{2+} concentration ($[Ca^{2+}]_o$) in cooperation with 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and calcitonin (CT). On the other hand, $[Ca^{2+}]_o$ regulates the secretion of PTH from parathyroid cells through extracellular Ca^{2+}-sensing receptor (CaR; Brown et al., 1993; Hofer and Brown, 2003). High $[Ca^{2+}]_o$ inhibits and low $[Ca^{2+}]_o$ enhances PTH secretion. It is believed that extracellular Ca^{2+} inhibits the secretion of PTH *via* the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). However, the molecular mechanism by which $[Ca^{2+}]_i$ regulates PTH secretion is not well elucidated.

Several electrophysiological studies have been performed in mammalian parathyroid cells. Those using classical intracellular microelectrodes indicated that rodent parathyroid cells display a deep resting potential (about –70 mV), which is depolarized by increasing $[Ca^{2+}]_o$ (Bruce and Anderson, 1979; López-Barneo and Armstrong, 1983). Later, the patch-clamp technique was applied on bovine, human and rodent parathyroid cells (Castellano et al., 1987; Jia et al., 1988; Komwatana et al., 1994; Kanazirska et al., 1995; McHenry et al., 1998; Välimäki et al., 2003). These studies showed that parathyroid cells possess some types of K^+ channels. Other studies suggested the presence of voltage-gated Ca^{2+} channels in bovine and goat parathyroid cells (Sand et al., 1981; Chang et al., 2001). However, voltage-gated Na^+ channels could not be found in any of the aforementioned studies.

Ion channels are regulated by neurotransmitters and hormones *via* G protein-coupled receptors (GPCRs; Wickman and Clapham, 1995; Dascal, 2001). GPCRs dissociate heterotrimeric G proteins (Gαβγ) to Gα-GTP and Gβγ. Both subunits can regulate a variety of ion channels directly (*via* physical interactions between G protein subunits and the channel protein) or indirectly (*via* second messengers and protein kinases).

In the present study, we report that frog parathyroid cells possess voltage-gated Na^+ channels and that their activity may be modulated by cannabinoids.