Involvement of TTX-resistant Na^+ currents and protein kinase C in the action of GHRH on primary cultured somatotropes from GH-GFP transgenic mice

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Growth hormone (GH) secretion is primarily mediated by two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin. It is well-established that GHRH depolarizes the cell membrane of somatotropes and increases Ca^{2+} influx through voltage-gated Ca^{2+} channels, leading to an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and GH secretion. Three major cation channels in somatotropes, Ca^{2+}, and Na^+, are involved in the regulation of cell excitability which, in turn, regulate GH secretion. It has been suggested that GHRH increases the membrane Na^+ permeability *via* Na^+ channels, which are not blocked by tetrodotoxin (TTX-resistant or TTX-R) but sensitive to cAMP levels, leading to a depolarization of the membrane and Ca^{2+} influx (Kato & Sakuma, 1997). This TTX-R Na^+ channel has not been characterized in somatotropes to date. In this study, we demonstrate the presence of TTX-R Na^+ current and its modification by GHRH in Green Fluorescent Protein (GFP)-GH transgenic mice somatotropes, using the nystatin-perforated whole-cell patch-clamp recording configuration. The TTX-R Na^+ current was recorded from a holding potential of -70 mV in the presence of Ca^{2+}, K^+, and TTX-sensitive Na^+ channel blockers; tetraethylammonium (20 mM), Co^{2+} (3 mM), and TTX (1 μM), respectively, in bath solution. GHRH (100 nM) was applied directly onto the cell and it caused a significant increase in the TTX-R Na^+ current, which was reversible with removal of GHRH. The GHRH-induced increase in TTX-R Na^+ current was, however, not affected by cAMP antagonist Rp-cAMP (100 μM), PKA inhibitor KT5720 (0.1 μM) or H89 (0.1 μM). In addition, the GHRH-induced increase in TTX-R Na^+ current was not affected by elevated cAMP levels; 8-bromo cAMP (0.1 mM), forskolin (1 μM, adenylyl-cyclase activator) and IBMX (0.5 mM, phosphodiesterase inhibitor), although these agents alone increased TTX-R Na^+ current, that is, in the absence of GHRH. U-73122 (5 μM, a PLC inhibitor) totally abolished the TTX-R Na^+ current response to GHRH. PKC inhibitors, Gö-6983 (1 μM) and chelerythrine (3 μM) also blocked the effect of GHRH. PDBu (phorbol dibutyrate, 0.5 μM, a PKC activator) increased TTX-R Na^+ current, but additional GHRH had no further effect on the current. These results suggest that the GHRH-induced increase in the TTX-R Na^+ current in mouse somatotropes is mediated by the PKC system. An increase in the TTX-R Na^+ current may depolarize the membrane, enhance Ca^{2+} influx, and lead to GH secretion from somatotropes.

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