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Somatostatin-induced control of cytosolic free calcium in pituitary tumour cells

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1 In rat pituitary tumour cells (GC cells), spontaneous oscillations of the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) induce growth hormone (GH) secretion that is inhibited by octreotide, a somatostatin (SRIF) agonist which binds to SRIF subtype (sst) receptor 2. The effects of its functional activation on the control of $[Ca^{2+}]_i$ were investigated using fluorimetric measurements of $[Ca^{2+}]_i$.

2 SRIF decreases the basal $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ rise in response to forskolin (FSK) through the inhibition of L-type voltage-dependent Ca^{2+} channels.

3 Pretreatment with octreotide or with L-Tyr^{8}Cyanamid 154806, a sst_2 receptor antagonist, abolishes the SRIF-induced inhibition of $[Ca^{2+}]_i$. Octreotide is known to operate through agonist-induced desensitization, while the antagonist operates through receptor blockade.

4 sst_1 and sst_2 receptor-immunoreactivities (-IRs) are localized to cell membranes. sst_2, but not sst_1 receptor-IR, internalizes after cell exposure to octreotide.

5 SRIF-induced inhibition of basal $[Ca^{2+}]_i$ or FSK-induced Ca^{2+} entry is blocked by pertussis toxin (PTX).

6 FSK-induced cyclic AMP accumulation is only partially decreased by SRIF or octreotide, indicating that sst_2 receptors are coupled to intracellular pathways other than adenylyl cyclase (AC) inhibition.

7 In the presence of H-89, an inhibitor of cyclic AMP-dependent protein kinase (PKA), SRIF-induced inhibition of basal $[Ca^{2+}]_i$ is still present, although reduced in amplitude.

8 SRIF inhibits $[Ca^{2+}]_i$ by activating sst_2 receptors. Inhibition of AC activity is only partly responsible for this effect, and other transduction pathways may be involved.

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Abbreviations: AC, adenylyl cyclase; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; FSK, forskolin; GC cells, rat tumour somatotrophs; Ω-CgTX, Ω-conotoxin GVIA; GH, growth hormone; GHRH, growth hormone-releasing hormone; H-89, H-89 dihydrochloride; IBMX, 3-isobutyl-1-methylxanthine; IP_3, inositol 1,4,5-trisphosphate; PI, phosphatidyl inositol; PKA, cyclic AMP-dependent protein kinase; PKG, cyclic GMP-dependent PK; PLC, phospholipase C; PTP, phosphotyrosine phosphatase; PTX, pertussis toxin; SRIF, somatotrophin release inhibitory factor; SRIF receptor-IR, SRIF receptor-immunoreactivity; sst receptor, SRIF subtype receptor

Introduction

Modulation of the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is critical for somatotroph function (Lussier *et al*., 1991a; Cuttler *et al*., 1992). Indeed, the release of pituitary growth hormone (GH) appears to be critically dependent on changes in $[Ca^{2+}]_i$. In particular, in rat somatotrophs, growth hormone-releasing hormone (GHRH) generates $[Ca^{2+}]_i$ oscillations that may periodically trigger GH release (Kwiecien *et al*., 1997). Somatostatin (SRIF, somatotrophin release inhibitory factor) plays an important role in inhibiting rhythmic $[Ca^{2+}]_i$ transients (Kwiecien *et al*., 1997) either by directly decreasing extracellular Ca^{2+} influx (Lussier *et al*., 1991c) or by increasing K^+ conductance, and thereby secondarily decreasing Ca^{2+} influx (Lussier *et al*., 1991b). SRIF ability to lower $[Ca^{2+}]_i$ is responsible for its inhibitory action on GH release (Lussier *et al*., 1991c).

The GC cell line is derived from a rat pituitary tumour (subclone of the GH_3 mammosomatotroph strain) and represents a homogeneous *in vitro* model of tumour somatotrophs (Mounier *et al*., 1995; Kwiecien *et al*., 1998). In contrast to GH_3 cells, GC cells release GH but not prolactin. As normal somatotrophs, GC cells exibit rhythmic $[Ca^{2+}]_i$ oscillations resulting mainly from Ca^{2+} entry through L-type Ca^{2+} channels (Kwiecien *et al*., 1998). In contrast to normal somatotrophs, however, $[Ca^{2+}]_i$ transients do not depend on GHRH, but they occur spontaneously. The function of pacemaker activity in GC cells allows GH secretion that is inhibited by the application of octreotide, a long-lasting SRIF agonist (Mounier *et al*., 1995).

SRIF has been shown to play its multiple roles by interacting with specific SRIF subtype (sst) receptors (see for review Meyerhof, 1998). Five receptors have been identified to date and designated sst_1 through sst_5 receptors, each originating from a distinct gene (see for reference Hoyer *et al*., 1995). Splice variants of the mouse sst_2 receptor, $sst_{2(a)}$ and $sst_{2(b)}$, have been cloned (Vanetti *et al*., 1992). These two isoforms that originate from alternative splicing of the sst_2 receptor mRNA differ in their coupling efficiency to adenylyl cyclase (AC) and in agonist-induced receptor desensitization (Vanetti *et al*., 1993). In contrast, equivalent rat splice variants display broadly similar pharmacological properties (Schindler *et al*., 1998).

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