

Involvement of somatostatin receptor subtypes in membrane ion channel modification by somatostatin in pituitary somatotropes

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Summary

1. Growth Hormone (GH) secretion from pituitary somatotropes is mainly regulated by two hypothalamic hormones, GH-releasing hormone (GHRH) and Somatotrophin Releasing Inhibitory Factor (SRIF).

2. SRIF inhibits GH secretion *via* activation of specific membrane receptors, somatostatin receptors (SSTRs) and signaling transduction systems in somatotropes.

3. Five subtypes of SSTRs, SSTR1, 2, 3, 4, and 5, have been identified, with receptor 2 divided into SSTR2A and SSTR2B. All SSTRs are G-protein coupled receptors (GPCRs).

4. Voltage-gated Ca^{2+} and K^{+} channels on the somatotrope membrane play an important role in regulating GH secretion, and SRIF modifies both channels to reduce intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and GH secretion.

5. Using specific SSTR subtype-specific agonists, it has been found that reduction in Ca^{2+} currents by SRIF is mediated by SSTR2, and an increase in K^{+} currents is mediated by both SSTR2 and SSTR4, in rat somatotropes.

Introduction

Growth Hormone (GH), which is a single peptide of 191 amino acids, is an anabolic hormone that is essential for normal linear growth, and also regulates various physiological processes in the body, such as aging, metabolism, immune system, and reproductive system. GH is synthesized, stored, and secreted by the pituitary somatotrope cells¹ which are located mainly in the lateral wings of the anterior pituitary and comprise 40 to 50% of anterior pituitary cells. GH is transported through the circulation by at least two binding proteins, GH-binding protein-1 (GHBP-1) and GHBP-2.² The regulation of GH secretion from the anterior pituitary gland is under the reciprocal control of two hypothalamic hormones, a stimulatory hormone, GH-releasing hormone (GHRH), and an inhibitory hormone, somatostatin, and an endogenous GH secretagogue, ghrelin, which stimulates GH secretion. Somatostatin, known as Somatotrophin Releasing Inhibitory Factor (SRIF), inhibits GH secretion from the anterior pituitary.³ SRIF is a cyclic peptide that is distributed widely through the body and regulates both endocrine and exocrine secretion.⁴ SRIF is synthesized in the hypothalamus, and is released into and transported by the hypothalamo-hypophyseal portal blood vessels, which enables direct delivery of SRIF to the anterior pituitary

gland, where it inhibits the release of GH. In addition to its effects on hormone secretion, SRIF inhibits proliferation of various cell lines including pituitary cells^{5,6} and pituitary tumours.^{7,8} SRIF is also produced throughout the Central Nervous System, where it acts as neurotransmitter and neuromodulator, and in many peripheral organs such as in the gastrointestinal tract and pancreas.^{4,9,10} Some of the effects of SRIF, such as the inhibition of GH secretion from both normal pituitaries and GH-secreting tumours,^{4,11,12} as well as basal and stimulated secretion from other endocrine and exocrine cells,^{13,14} and the inhibition of cell proliferation,^{15,16} are targets for specific therapeutic agents. They may be of considerable pathophysiological importance in several human diseases, including the cognitive functions of Alzheimer's disease and the movement control of Parkinson's disease.¹⁷⁻¹⁹ These SRIF regulatory effects are mediated by specific, high-affinity membrane bound SRIF receptors (SSTRs) on target tissues. So far, five subtypes of SSTRs, SSTR1, -2, -3, -4, and -5, have been identified and all are expressed in somatotropes,^{20,21} and each displays a seven α helical transmembrane domain, which is typical of G-protein coupled receptors.^{19,22} Activation of SSTRs is associated with a reduction in intracellular cAMP levels and Ca^{2+} concentration, and stimulation of protein tyrosine phosphatase.²¹ SSTRs are coupled to several types of Ca^{2+} and K^{+} channels. The inhibition of Ca^{2+} and activation of K^{+} currents causes hyperpolarization of the membrane and a decrease in Ca^{2+} currents, leading to a decrease in the frequency and amplitude of action potentials, resulting in a reduction in intracellular Ca^{2+} concentration.^{23,24} Non-peptide agonists of each of the five SSTRs have been identified (SSTR1, L-797,591; SSTR2, L-779,976; SSTR3, L-796,778; SSTR4, L-803,087; SSTR5, L-817,818) and each agonist shows high affinity for its specific SSTR subtype.²⁵

Ion channels in somatotropes are involved in the regulation of cell excitation which leads to hormone secretion. Ca^{2+} , K^{+} and Na^{+} channels, which are the main cation channels, regulate the electrical activities in somatotropes. GH secretion from somatotropes is stimulated by an increase in intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) which is mainly regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels in the plasma membrane.²⁴ Na^{+} and K^{+} channels are involved in the modification of the somatotrope function *via* their effect on membrane potential and action potential duration and frequency, and hence cytoplasmic Ca^{2+} levels.²⁴

The inhibitory effect of SRIF could be explained by

Table 1. The nomenclature of somatostatin receptor and size of amino acids for human and rat. From Reisine & Bell, 1995.²²

Receptors	Gene cloning		Size (amino acids)		Homology between Human & Rat
	Human	Rat	Human	Rat	
SSTR1	Yamada <i>et al.</i> 1992 ³⁵	Li <i>et al.</i> 1992 ⁸⁸	391	391	97%
SSTR2A	Yamada <i>et al.</i> 1992 ³⁵	Kluxen <i>et al.</i> 1992 ²⁹	369	369	92%
SSTR2B	Patel <i>et al.</i> 1993 ³¹	Vanetti <i>et al.</i> 1992 ³⁰		346	
SSTR3	Yamada <i>et al.</i> 1992 ³⁵	Meyerhof <i>et al.</i> 1992 ⁸⁹	418	428	86%
SSTR4	Rohrer <i>et al.</i> 1993 ⁹⁰	Bruno <i>et al.</i> 1992 ⁹¹	388	384	89%
SSTR5	O'Carroll <i>et al.</i> 1994 ⁹²	O'Carroll <i>et al.</i> 1992 ³³	364	363	81%

the decrease in Ca²⁺ current and increase in K⁺ current, so that the action potential duration and frequency are reduced, hence the reduction in Ca²⁺ influx, leading to reduced GH secretion.^{23,24,26} The present review will mainly discuss SSTR subtypes and ion channels, and the involvement of SSTR subtypes and ion channel modification in pituitary somatotropes, with experimental evidence.

The SSTR subtypes

The physiological actions of SRIF are initiated by its interaction with specific membrane-bound high-affinity receptors, SSTRs, on the surface of responsive cells. Schonbrunn & Tashjian²⁷ were the first to demonstrate high-affinity functional SSTRs in GH4C1 cells, a rat pituitary tumour cell line that synthesizes and secretes GH and prolactin.²⁷ Five different SSTR subtypes have been cloned and characterized using a recent molecular cloning technique. Two major approaches have been used to isolate and identify receptor cDNA clones: polymerase chain reaction (PCR)-based strategies for cloning new G-protein coupled receptors; and the use of [¹²⁵I-Tyr11]-SRIF-14 and [¹²⁵I-Tyr3]-octreotide (SRIF analogue) to screen a cDNA library expressed in cells in rat.^{28,29} Alternative splicing has revealed two forms of SSTR2, called SSTR2A and SSTR2B,³⁰ the difference being the length of their cytoplasmic tail.³¹ The sequences of the five different SRIF receptor subtypes, SSTR1 – SSTR5, from different species have been reported. The amino acid sequences of human and rat SSTR1-5, mouse SSTR1-3, and bovine SSTR2 have been reported from the analysis of cDNA and/or genomic sequences. Five SSTR proteins show highly conserved in size and structure, especially human SSTRs vary in size from 356-391 amino acids, and show 55-70% sequence identity between the subtypes.²¹ Table 1 shows the nomenclature of human and rat SSTRs.

A remarkable degree of structural conservation across species has been reported. SSTR1 is the most highly conserved with 97% identity between human and rat, and the sequence of SSTR5 is the most divergent with 81% identity between human and rat. There is 92%, 86% and 89% identity between human and rat SSTR2, -3, and -4 respectively.^{22,32} A variety of pharmacological studies have probed the binding properties of the five SSTRs. Most

commonly SRIF-14, SRIF-28, and SRIF analogues MK678 and octreotide, have been used to study binding properties. Both SRIF-14 and SRIF-28 show high affinity to all SSTR subtypes, while octreotide and MK678, which have been used in clinical trials, are selective, high-affinity ligands for SSTR2 and SSTR5, with an intermediate affinity for SSTR3. There appears to be some selectivity of SRIF-28 towards SSTR5.^{33,34} The tissue distribution of SSTRs has been examined using several procedures, including Northern blotting, RNase protection, reverse transcriptase-PCR amplification of cellular RNA and *in situ* hybridization histochemistry. The mRNA for the five SSTRs is expressed widely in human and rat tissues and they have distinct but overlapping patterns of expression.^{28,33,35,36} All five receptors are expressed in the CNS and hypothalamus. *In situ* hybridization studies³⁶⁻⁴¹ have shown that SSTR1-4 mRNAs are present in high levels throughout the neocortex, the hippocampus and amygdale. In addition, they are present in the piriform cortex and the primary olfactory cortex in the rat. There are also high levels of SSTR3 mRNA in the olfactory tract, and levels of SSTR2 and SSTR4 mRNA are especially high in the habenula. SSTR1-5 are expressed in the hypothalamus. SSTR mRNA has also been identified in the tissues of peripheral organs such as the gastrointestinal tract, kidney, heart and lung. Table 2 shows the distribution of SSTRs in various organs in the rat. Distribution in the pituitary is shown in the shaded area, with high expression of SSTR2 and SSTR5, and low expression of SSTR4.

The signalling systems of the five SSTRs have been widely examined. SSTR1 is involved in inhibition of Adenylyl cyclase (AC) *via* pertussis toxin (PTX)-sensitive G-protein,⁴² and has also been shown to mediate PLC activation and IP3 production in CHO and COS monkey kidney cells.^{43,44} SSTR2 is involved in inhibition of AC, and also mediates the activation of PLC in COS, GH4C1, and F4C1 cells.^{43,45,46} F4C1 cells showed a particular involvement of PTX-sensitive and -insensitive G-proteins, biphasic responses, by SSTR2.⁴⁶ Also, stimulation of MAP kinase signalling pathways through SSTR2 has been demonstrated in the rat.⁴⁷ SSTR3 is involved in inhibition of AC through PTX-sensitive G-proteins; for example, stimulation of SSTR3 in CHO and HEK293 cells decreased AC *via* G α i protein.^{48,49} SSTR4 is involved in inhibition of

Table 2. Tissue specific expression of SSTR genes in rat. From Patel et al. 1995.³¹

	<i>SSTR1</i>	<i>SSTR2</i>	<i>SSTR3</i>	<i>SSTR4</i>	<i>SSTR5</i>
BRAIN					
Cortex	++++	++++	++	++	++
Striatum	±	+	++	+	++
Hippocampus	++	++	++	++	++
Amygdala	+++++	++	+++	+	++
Olfactory Bulb	++	++	++	++	++
Thalamus	++	+	+	±	++
Hypothalamus	++	++	+	±	+++++
POA	+	+	+	+	+++++
Cerebellum	±	±	+++++	-	-
Midbarin	+	+	+	±	++
Pons	+	+	+	±	-
PERIPHERY					
Pituitary	++	+++	++	+	+++++
Pancreas	-	+	-	-	-
Islets	++	++++	+	++	+
Stomach	+	+	+	+	-
Small intestine	++	-	+	+	++
Liver	-	-	++	-	-
Lung	-	-	-	++	-
Kidney	-	+	+	+	-
Heart	+	-	+	++++	-
Spleen	++	+	++++	+	+
Adrenals	+	++++	+	-	-

AC via PTX-sensitive G-protein,⁴² and it was shown that PLC and IP₃ production was stimulated in COS cells via SSTR4.⁵⁰ Stimulation of MAP kinase via SSTR4 was observed in human.⁵¹ SSTR5 is involved in inhibition of AC through PTX-sensitive G-protein.⁴² Activation of PLC and IP₃ production was observed in transfected COS cells,⁵⁰ and reduction in intracellular cGMP formation was observed in CHO cells expressing the SSTR5.⁵² Inhibition of MAP kinase was reported through a mechanism involving inhibition of cGMP in CHO cells.⁵² The signaling pathways employed by each receptor have not yet been fully elucidated as different tissues and cells express different subtypes of receptors, most cells have more than one subtype of receptor, and the availability of specific agonists is limited.

The subtypes of SSTRs regulate different functions by various mechanisms. SSTR2 inhibits voltage-dependent Ca²⁺ channels in certain cells, such as GH12C1, RINm5F, and GH3 cells.^{20,46,53} Also SSTR2 stimulates voltage dependent K⁺ channels,²⁰ and regulates inhibition of cell growth and induction of apoptosis.⁵⁴⁻⁵⁶ SSTR1 inhibits Ca⁺ current in GH12C1 rat pituitary tumor cells,⁴⁶ but not in GH3 cells.²⁰ It is also involved in cell growth regulation.⁵⁷ SSTR3 regulates apoptosis⁵⁸ and SSTR4 modifies voltage-gated K⁺ current.²⁰ SSTR5 inhibits cell growth and proliferation,⁵² and seems to be involved in K⁺ current regulation in xenopus oocytes,⁵⁹ but not in other cell types. SSTR5 is also important in cancer growth regulation as one

of the most potent inhibitory receptors.⁶⁰

Non-peptide agonists, L-compounds, of each of the five SSTRs have been identified by the Merck Research Laboratory, and agonists activation of SSTR was done in several experiments. Using L-779,976 and L-817,818, the SSTR2 and SSTR5 agonists respectively, the study showed that the SSTR2 and SSTR5 subtypes together regulate GHRH-stimulated GH release from rat pituitary cells. Both agonists potentially inhibited GHRH-stimulated GH release, but the SSTR5 agonist showed approximately 10-fold less potency in inhibiting GH release compared with the SSTR2 agonist.^{61,62} In cultured monolayer of E17-18 rat embryonic cortical neurons, SRIF inhibited 10⁻⁶ M forskolin-stimulated cAMP accumulation by 37%, a level of inhibition that was mimicked by L-797,591, a potent and selective agonist of SSTR1.⁶² The role of SSTR2 and SSTR4 in limbic seizures and glutamate-mediated neurotransmission in mouse hippocampus has been investigated using the SSTR2 agonist L-779,976 and SSTR4 agonist L-803,087.⁶³ Investigation of homo- and heterodimerization of SSTR2 and SSTR3 was performed using the SSTR2 agonist L-779,976 and SSTR3 agonist L-796,778.⁶⁴ Ligand activation by SSTR is associated with a reduction in intracellular cAMP and [Ca²⁺]_i, mainly via membrane ion channels.

Membrane ion channels in somatotropes

It is well known that GH secretion is directly related to $[Ca^{2+}]_i$, which is primarily regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels, stimulated by GHRH and inhibited by SRIF^{24,65-70}. It is also well documented that a number of neuropeptides, especially those from the hypothalamus, exert their regulatory role of somatotrope secretion through the modification of transmembrane ion channels.⁷¹⁻⁷³ GHRH and SRIF provide the main driving force in maintaining normal GH secretion status in all species including humans. Both are capable of regulating somatotrope activity by firstly binding to their G-protein coupled receptors on the cell membrane, which then sets in train the various intracellular second messenger systems.^{74,75} Studies using patch-clamp in conjunction with Ca^{2+} imaging techniques have demonstrated that GHRH and SRIF regulate $[Ca^{2+}]_i$ *via* modification of Ca^{2+} , K^+ , and Na^+ channels.²³ Second messenger systems, including intracellular cAMP, protein kinase A (PKA) and PKC, are particularly important in mediating the GH release by these hypothalamic peptides.⁶⁹ Despite all these achievements, a comprehensive understanding of the mechanisms by which ion channels are involved in the regulation of GH secretion in somatotropes still needs to be addressed. It has been shown that GHRH-induced/SRIF-suppressed GH secretion is a Ca^{2+} -regulated process involving modification of Ca^{2+} and K^+ channels, and subsequent change in $[Ca^{2+}]_i$. GHRH depolarizes the cell membrane, allowing significant Ca^{2+} influx *via* voltage-gated Ca^{2+} channels. In contrast, SRIF hyperpolarizes the cell membrane, decreasing the Ca^{2+} influx through voltage-gated Ca^{2+} channels and increasing K^+ outflow *via* voltage-gated K^+ channels.^{23,73} In most somatotropes, when intracellular Ca^{2+} stores are activated by GHRH, biphasic Ca^{2+} oscillations can be recorded, with an initial sharp increase in $[Ca^{2+}]_i$ resulting from Ca^{2+} release from reservoirs within the cell, followed by a moderate, long-lasting $[Ca^{2+}]_i$ rise due to the influx of Ca^{2+} through voltage-gated Ca^{2+} channels in the plasma membrane.^{76,77} In spite of the mobilization of Ca^{2+} from intracellular Ca^{2+} storage pools, the major contribution to the regulation of $[Ca^{2+}]_i$ is caused by Ca^{2+} influx *via* Ca^{2+} channels. GH secretion in response to GH factors is abolished by blockade of membrane Ca^{2+} channels.⁷⁸⁻⁸⁰ In addition, ovine pituitary somatotropes show an increase in $[Ca^{2+}]_i$ in response to GH releasing peptide-2 mainly through the influx of Ca^{2+} *via* voltage-gated Ca^{2+} channels, without detectable Ca^{2+} release from intracellular Ca^{2+} stores.⁸¹

In somatotropes, the major Ca^{2+} channels are of the voltage-gated T- (transient) and L- (long-lasting) type, and the currents through these channels have been characterized in somatotropes.⁷³ In rat somatotropes, the L-type current contributes by far the largest proportion (60-70%) to the total Ca^{2+} channel current. A moderate proportion (around 20%) of T-type Ca^{2+} currents are involved, but contribution of T- and L-type currents varies across species.^{26,69,82} Our studies of rat pituitary somatotropes (GH3 cells, the rat pituitary tumour cell lines), have shown that there are large

T- and L-type Ca^{2+} currents with a small involvement of N-(neural) type current. Few studies have reported the involvement of N-type Ca^{2+} current in pituitary cells, but our experiments and previous studies show a small proportion of N-type is involved.⁸³

The ion channels which are involved in the depolarization of somatotrope membranes have not been defined. It appears that Na^+ channels do not play a major role in the response to GHRH and SRIF. Although there was a report showing that GHRH activates Na^+ current and that SRIF partially suppressed this current,⁸⁴ most studies on the mechanism of SRIF action have not targeted Na^+ channels. K^+ channels are important and voltage-gated K^+ current has been characterized in rat pituitary cell lines. The majority of voltage-gated K^+ currents were composed of transient outward (I_A) and delayed rectifying (I_K).²⁰ Different proportions of each type of voltage-gated K^+ current were recorded in different species. A large proportion of I_A was observed in rat pituitary, but I_A was only a small component of the total K^+ current in sheep somatotropes.^{26,74} A small proportion of I_K and a large proportion of I_A were observed in our study using the rat pituitary tumour cell line, GH3.²⁰ There is a report that a rat pituitary tumour cell has low levels of Ca^{2+} -activated K^+ current, but in our study Ca^{2+} -activated K^+ current seems to be absent, so cannot be involved in SRIF action on pituitary tumour cells.²⁰

Modification of ion channels by somatostatin

Ca^{2+} channels play a key role in mammalian cells in all species. As we have mentioned, our experiment showed a large proportion of T- and L-type Ca^{2+} currents in GH3 cells. Although there was a report showing low T-type (about 10%) and high L-type in GH3 cells,^{61,19} T- and L-types seem to be the major contributor of voltage-gated Ca^{2+} channels in somatotropes. But T-type current was not altered by SRIF (10^{-7} M) application, while L-type was significantly reduced. Our study and previous reports show a small proportion of N-type current is involved in GH3 somatotropes, which was decreased by SRIF application.⁸³

K^+ channels may also be involved, because a reduction in K^+ current in response to GHRH and an increase in K^+ current in response to SRIF has been reported in rat and ovine somatotropes.^{23,20,81} A small proportion of I_K and a large proportion of I_A were observed in our study using the rat pituitary tumour cell line, GH3, and both I_K and I_A currents were increased by SRIF.²⁰

In summary, SRIF inhibits voltage-gated Ca^{2+} channels, decreasing Ca^{2+} current. In contrast, SRIF stimulates voltage-gated K^+ channels, increasing K^+ current, leading to hyperpolarization of the membrane and reduction in action potential frequency and duration, resulting in decreased GH secretion.

SSTR subtypes and ion channels in somatotropes

It is well established that SSTRs 1-5 are G-protein coupled receptors,¹⁹ and binding of SRIF activates G-protein and various down-stream second messenger systems

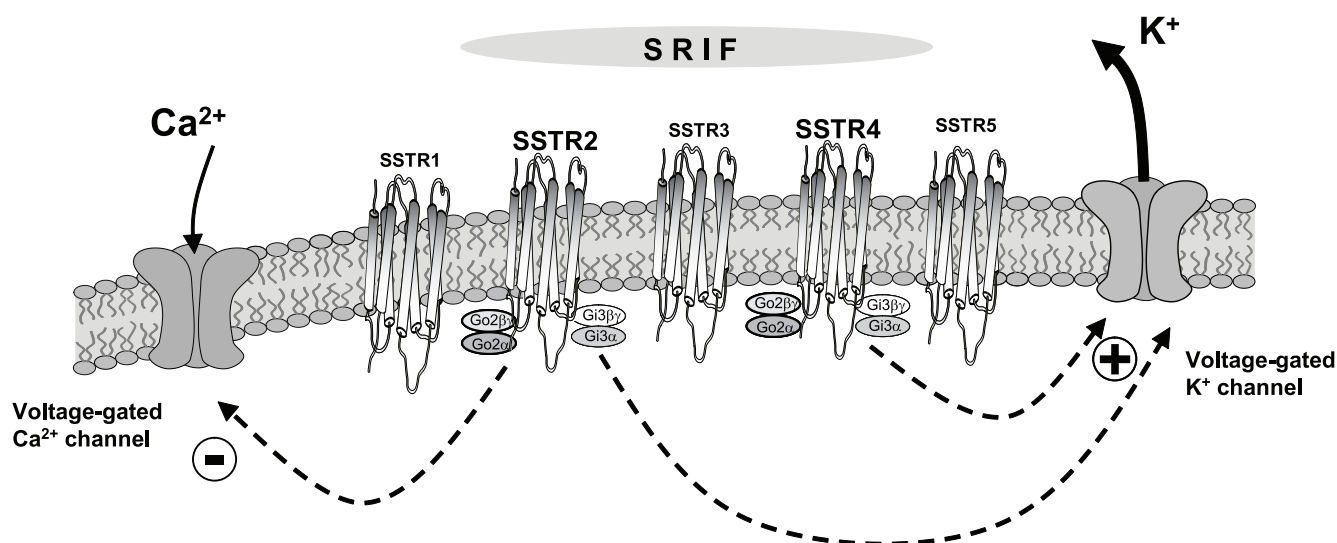


Figure 1. Signalling pathways employed by SRIF via SSTRs on voltage-gated Ca²⁺ and K⁺ channels. When SRIF binds to SSTRs, SSTR2 and 4 activate voltage-gated K⁺ channels and only SSTR2 inactivates voltage-gated Ca²⁺ channels to increase K⁺ outflow and decrease Ca²⁺ influx, which subsequently leads to inhibition of GH secretion.

including inhibition of cAMP formation.⁸⁵ This stimulates voltage-gated K⁺ current and inhibits voltage-gated Ca²⁺ current and consequent suppression of Ca²⁺ influx.^{75,86} The experiment was performed to investigate the involvement of K⁺ channels and SSTR subtypes in the rat pituitary tumour cell line using five SSTR agonists (SSTR1, L-797,591; SSTR2, L-779,976; SSTR3, L-796,778; SSTR4, L-803,087; SSTR5, L-817,818) and the patch-clamp technique. SSTR2 and SSTR4 increased the voltage-gated K⁺ current.²⁰ SSTR1 and SSTR5 partially increased K⁺ current, but because of the potentially non-specific effect of SSTR1 and SSTR5 agonists on SSTR2 and SSTR4, the involvement of SSTR1 and SSTR5 is unclear. The SSTR1 agonists can activate SSTR4, although at 100 times lower affinity than for SSTR1, and the SSTR5 agonist showed about 10 times higher affinity for SSTR5, compared with SSTR1, and was 130 times higher than SSTR2.²⁵ But it seems SSTR2 and SSTR4 are the main receptors which respond to SRIF, activating a functional cascade, and inhibiting the GH secretion in somatotropes. SSTR2 is the most abundantly expressed and SSTR4 is the least expressed receptor in pituitary somatotropes,^{22,38,87} so it was suggested that SSTR2 and SSTR4 may undergo dimerization with activation of either receptor causing activation of voltage-gated K⁺ channels to increase K⁺ currents. Another experiment was done to investigate the involvement of Ca²⁺ current and SSTR subtypes in the rat pituitary tumour cell line. Among five receptors, only SSTR2 modified voltage-gated Ca²⁺ current. SSTRs are G-protein coupled receptors, and K⁺ channels were mediated by G_i-protein, while Ca²⁺ channels were mediated by G_o-protein.^{57,61} Because SSTRs influence K⁺ and Ca²⁺ currents by different G-proteins, SSTR2 may be coupled to two different G-proteins to mediate the effect on voltage-gated K⁺ and Ca²⁺ currents respectively.

In summary, SSTR2 and SSTR4 are the main receptors which activate voltage-gated K⁺ current, and SSTR2 is the main receptor inhibiting voltage-gated Ca²⁺ current (Figure 1).

Conclusion

The inhibitory effect of SRIF could be explained, at least partially, by the fact that SRIF hyperpolarizes the cell membrane through the increase in K⁺ currents through SSTR2 and SSTR4, and the decrease in Ca²⁺ currents through SSTR2 (Figure 1), so that the frequency and duration of action potentials are reduced, which subsequently leads to a reduction in [Ca²⁺]_i and inhibition of GH release.

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