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 ($[Ca^{2+}]_i$) 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 cells[1] 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 ($[Ca^{2+}]_i$) 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 *et al.* 1992[35]	Li *et al.* 1992[88]	391	391	97%
SSTR2A	Yamada *et al.* 1992[35]	Kluxen *et al.* 1992[29]	369	369	92%
SSTR2B	Patel *et al.* 1993[31]	Vanetti *et al.* 1992[30]		346	
SSTR3	Yamada *et al.* 1992[35]	Meyerhof *et al.* 1992[89]	418	428	86%
SSTR4	Rohrer *et al.* 1993[90]	Bruno *et al.* 1992[91]	388	384	89%
SSTR5	O'Carroll *et al.* 1994[92]	O'Carroll *et al.* 1992[33]	364	363	81%

the decrease in Ca^{2+} current and increase in K^+ current, so that the action potential duration and frequency are reduced, hence the reduction in Ca^{2+} 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 & Tashjian[27] 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 [^{125}I-Tyr11]-SRIF-14 and [^{125}I-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 studies[36-41] 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* Gαi 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]

	SSTR1	***SSTR2***	***SSTR3***	***SSTR4***	***SSTR5***
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 Ca^{2+} 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 apoptosis[58] 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 potently 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 $[Ca^{2+}]_i$ 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

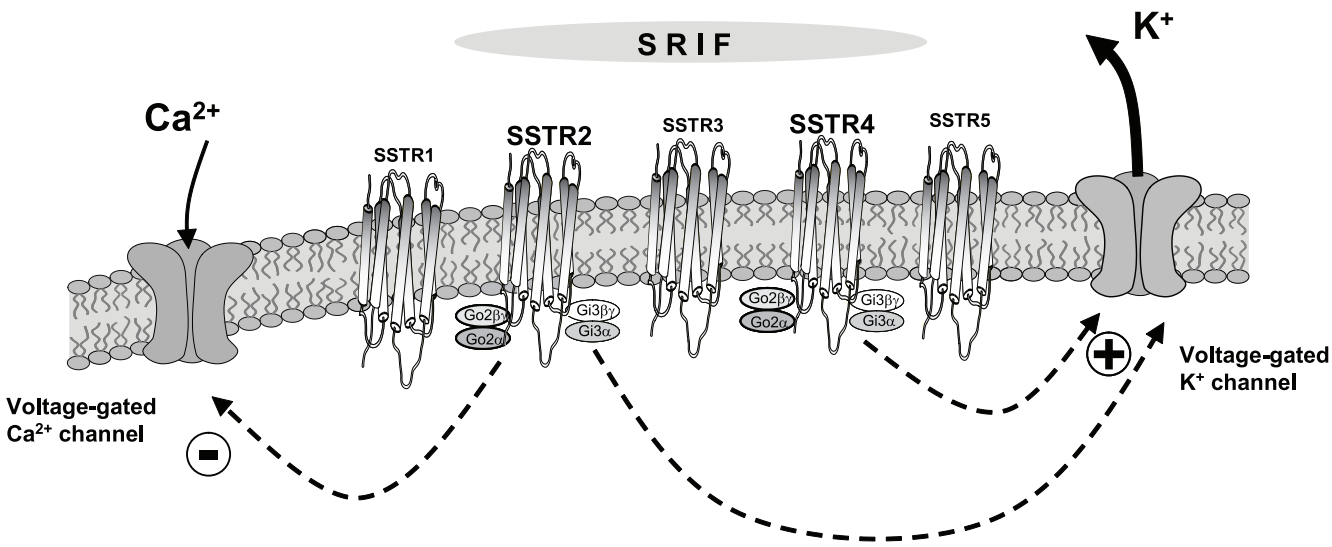

Figure 1. Signalling pathways employed by SRIF* via *SSTRs on voltage-gated Ca^{2+} and K^+ channels. *When SRIF binds to SSTRs, SSTR2 and 4 activate voltage-gated K^+ channels and only SSTR2 inactivates voltage-gated Ca^{2+} channels to increase K^+ outflow and decrease Ca^{2+} influx, which subsequently leads to inhibition of GH secretion.*

including inhibition of cAMP formation.[85] This stimulates voltage-gated K^+ current and inhibits voltage-gated Ca^{2+} current and consequent suppression of Ca^{2+} 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 Ca^{2+} current and SSTR subtypes in the rat pituitary tumour cell line. Among five receptors, only SSTR2 modified voltage-gated Ca^{2+} current. SSTRs are G-protein coupled receptors, and K^+ channels were mediated by G_i-protein, while Ca^{2+} channels were mediated by G_o-protein.[57,61] Because SSTRs influence K^+ and Ca^{2+} currents by different G-proteins, SSTR2 may be coupled to two different G-proteins to mediate the effect on voltage-gated K^+ and Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} currents through SSTR2 (Figure 1), so that the frequency and duration of action potentials are reduced, which subsequently leads to a reduction in $[Ca^{2+}]_i$ and inhibition of GH release.

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References

1. Ojeda SR, Jameson HE. Developmental patterns of plasma and pituitary growth hormone (GH) in the female rat. *Endocrinology* 1977; **100:** 881-9.
2. Herington AC, Tiong TS, Ymer SI. Serum binding proteins for growth hormone: origins, regulation of gene expression and possible roles. *Acta Paediatr Scand Suppl.* **1**991; **379:** 61-9.
3. Krulich L, Dhariwal AP, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. *Endocrinology* 1968; **83:** 783-90.
4. Reichlin S. Somatostatin. *N. Engl. J. Med.* 1983; **309:** 1495-1501.

5. Pelicci G, Pagliacci MC, Lanfrancone L, Pelicci PG, Grignani F, Nicoletti I. Inhibitory effect of the somatostatin analog octreotide on rat pituitary tumor cell (GH3) proliferation in vitro. *J. Endocrinol Invest.* 1990; **13:** 657-62.
6. Cheung NW, Boyages SC. Somatostatin-14 and its analog octreotide exert a cytostatic effect on GH3 rat pituitary tumor cell proliferation via a transient G0/G1 cell cycle block. *Endocrinology* 1995; **136:** 4174-81.
7. Lamberts SW, Hofland LJ, de Herder WW, Kwekkeboom DJ, Reubi JC, Krenning EP. Octreotide and related somatostatin analogs in the diagnosis and treatment of pituitary disease and somatostatin receptor scintigraphy. *Front. Neuroendocrinol.* 1993; **14:** 27-55.
8. Lopez F, Esteve JP, Buscail L, *et al*. Molecular mechanisms of antiproliferative effect of somatostatin: involvement of a tyrosine phosphatase. *Metabolism.* 1996; **45:** 14-16.
9. Patel YC, Reichlin S. Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat. *Endocrinology* 1978; **102:** 523-30.
10. Hokfelt T, Johansson O, Efendic S, Luft R, Arimura A. Are there somatostatin-containing nerves in the rat gut? Immunohistochemical evidence for a new type of peripheral nerves. *Experientia* 1975; **31:** 852-4.
11. Brazeau P, Vale W, Burgus R, *et al.* Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1973; **179:** 77-79.
12. Tannenbaum GS, Ling N, Brazeau P. Somatostatin-28 is longer acting and more selective than somatostatin-14 on pituitary and pancreatic hormone release. *Endocrinology* 1982; **111:** 101-7.
13. Mandarino L, Stenner D, Blanchard W, *et al.* Selective effects of somatostatin-14, -25 and -28 on in vitro insulin and glucagon secretion. *Nature* 1981; **291:** 76-77.
14. Brown M, Rivier J, Vale W. Somatostatin: analogs with selected biological activities. *Science* 1977; **196:** 1467-9.
15. Tahiri-Jouti N, Cambillau C, Viguerie N, *et al.* Characterization of a membrane tyrosine phosphatase in AR42J cells: regulation by somatostatin. *Am. J. Physiol.* 1992; **262**: G1007-14.
16. Buscail L, Delesque N, Esteve JP, *et al.* Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: mediation by human somatostatin receptor subtypes SSTR1 and SSTR2. *Proc. Natl. Acad. Sci. USA* 1994; **91:** 2315-19.
17. Dournaud P, Cervera-Pierot P, Hirsch E, *et al.* Somatostatin messenger RNA-containing neurons in Alzheimer's disease: an in situ hybridization study in hippocampus, parahippocampal cortex and frontal cortex. *Neuroscience* 1994; **61:** 755-64.
18. Schindler M, Humphrey PP, Emson PC. Somatostatin receptors in the central nervous system. *Prog. Neurobiol.* 1996; **50:** 9-47.
19. Patel YC. Molecular pharmacology of somatostatin receptor subtypes. *J. Endocrinol. Invest.* 1997; **20:** 348-67.
20. Yang SK, Parkington HC, Blake AD, Keating DJ, Chen C. Somatostatin increases voltage-gated K^+ currents in GH3 cells through activation of multiple somatostatin receptors. *Endocrinology* 2005; **146:** 4975-84.
21. Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H, Srikant CB. The somatostatin receptor family. *Life Sci.* 1995; **57:** 1249-65.
22. Reisine T, Bell GI. Molecular biology of somatostatin receptors. *Endocr. Rev.* Aug 1995; **16:** 427-442.
23. Chen C, Clarke IJ. Ion channels in the regulation of growth hormone secretion from somatotrophs by somatostatin. *Growth Regul.* 1992; **2:** 167-74.
24. Chen C, Vincent JD, Clarke IJ. Ion chennels and signal transduction pathways in the regulation of growth hormone secretion. *Trends Endocrinol. Metab.* 1994; **5:** 227-233.
25. Rohrer SP, Birzin ET, Mosley RT, *et al.* Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* 1998; **282:** 737-40.
26. Chen C, Zhang J, Vincent JD, Israel JM. Somatostatin increases voltage-dependent potassium currents in rat somatotrophs. *Am. J. Physiol.* 1990; **259:** C854-61.
27. Schonbrunn A, Tashjian H, Jr. Characterization of functional receptors for somatostatin in rat pituitary cells in culture. *J. Biol. Chem.* 1978; **253:** 6473-83.
28. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proc. Natl. Acad. Sci. USA* 1992; **89:** 251-5.
29. Kluxen FW, Bruns C, Lubbert H. Expression cloning of a rat brain somatostatin receptor cDNA. *Proc. Natl. Acad. Sci. USA* 1992; **89:** 4618-22.
30. Vanetti M, Kouba M, Wang X, Vogt G, Hollt V. Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). *FEBS Lett.* 1992; **311:** 290-4.
31. Patel YC, Greenwood M, Kent G, Panetta R, Srikant CB. Multiple gene transcripts of the somatostatin receptor SSTR2: tissue selective distribution and cAMP regulation. *Biochem. Biophys. Res. Commun.* 1993; **192:** 288-94.
32. Patel YC. Somatostatin and its receptor family. *Front. Neuroendocrinol.* 1999; **20:** 157-98.
33. O'Carroll AM, Lolait SJ, Konig M, Mahan LC. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. *Mol. Pharmacol.* 1992; **42:** 939-46.
34. Patel YC, Greenwood MT, Warszynska A, Panetta R, Srikant CB. All five cloned human somatostatin receptors (hSSTR1-5) are functionally coupled to adenylyl cyclase. *Biochem. Biophys. Res. Commun.*

1994; **198:** 605-12.
35. Yamada Y, Reisine T, Law SF, *et al.* Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. *Mol. Endocrinol.* 1992; **6:** 2136-42.
36. Yasuda K, Rens-Domiano S, Breder CD, *et al.* Cloning of a novel somatostatin receptor, SSTR3, coupled to adenylylcyclase. *J. Biol. Chem.* 1992; **267:** 20422-8.
37. Breder CD, Yamada Y, Yasuda K, Seino S, Saper CB, Bell GI. Differential expression of somatostatin receptor subtypes in brain. *J. Neurosci.* 1992; **12:** 3920-34.
38. Kaupmann K, Bruns C, Hoyer D, Seuwen K, Lubbert H. Distribution and second messenger coupling of four somatostatin receptor subtypes expressed in brain. *FEBS Lett.* 1993; **331:** 53-9.
39. Kong H, DePaoli AM, Breder CD, Yasuda K, Bell GI, Reisine T. Differential expression of messenger RNAs for somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3 in adult rat brain: analysis by RNA blotting and in situ hybridization histochemistry. *Neuroscience* 1994; **59:** 175-84.
40. Perez J, Rigo M, Kaupmann K, *et al.* Localization of somatostatin (SRIF) SSTR-1, SSTR-2 and SSTR-3 receptor mRNA in rat brain by in situ hybridization. *Naunyn Schmiedebergs Arch. Pharmacol.* 1994; **349:** 145-60.
41. Bito H, Mori M, Sakanaka C, *et al.* Functional coupling of SSTR4, a major hippocampal somatostatin receptor, to adenylate cyclase inhibition, arachidonate release and activation of the mitogen-activated protein kinase cascade. *J. Biol. Chem.* 1994; **269:** 12722-30.
42. Siehler S, Hoyer D. Characterisation of human recombinant somatostatin receptors. 3. Modulation of adenylate cyclase activity. *Naunyn Schmiedebergs Arch. Pharmacol.* 1999; **360:** 510-21.
43. Tomura H, Okajima F, Akbar M, Abdul Majid M, Sho K, Kondo Y. Transfected human somatostatin receptor type 2, SSTR2, not only inhibits adenylate cyclase but also stimulates phospholipase C and Ca^{2+} mobilization. *Biochem. Biophys. Res. Commun.* 1994; **200:** 986-92.
44. Kubota A, Yamada Y, Kagimoto S, *et al.* Multiple effector coupling of somatostatin receptor subtype SSTR1. *Biochem. Biophys. Res. Commun.* 1994; **204:** 176-86.
45. Hipkin RW, Wang Y, Schonbrunn A. Protein kinase C activation stimulates the phosphorylation and internalization of the sst2A somatostatin receptor. *J. Biol. Chem.* 2000; **275:** 5591-9.
46. Chen L, Fitzpatrick VD, Vandlen RL, Tashjian AH, Jr. Both overlapping and distinct signaling pathways for somatostatin receptor subtypes SSTR1 and SSTR2 in pituitary cells. *J. Biol. Chem.* 1997; **272:** 18666-72.
47. Sellers LA, Alderton F, Carruthers AM, Schindler M, Humphrey PP. Receptor isoforms mediate opposing proliferative effects through gβγ-activated p38 or Akt pathways. *Mol. Cell. Biol.* 2000; **20:** 5974-85.
48. Law SF, Yasuda K, Bell GI, Reisine T. $G_{i\alpha 3}$ and $G_{o\alpha}$ selectively associate with the cloned somatostatin receptor subtype SSTR2. *J. Biol. Chem.* 1993; **268:** 10721-27.
49. Law SF, Zaina S, Sweet R, *et al.* $G_{i\alpha 1}$ selectively couples somatostatin receptor subtype 3 to adenylyl cyclase: identification of the functional domains of this alpha subunit necessary for mediating the inhibition by somatostatin of cAMP formation. *Mol. Pharmacol.* 1994; **45:** 587-90.
50. Akbar M, Okajima F, Tomura H, *et al.* Phospholipase C activation and Ca^{2+} mobilization by cloned human somatostatin receptor subtypes 1-5, in transfected COS-7 cells. *FEBS Lett.* 1994; **348:** 192-196.
51. Smalley KS, Feniuk W, Sellers LA, Humphrey PP. The pivotal role of phosphoinositide-3 kinase in the human somatostatin sst_4 receptor-mediated stimulation of p44/p42 mitogen-activated protein kinase and extracellular acidification. *Biochem. Biophys. Res. Commun.* 1999; **263:** 239-43.
52. Cordelier P, Esteve JP, Bousquet C, *et al.* Characterization of the antiproliferative signal mediated by the somatostatin receptor subtype sst5. *Proc. Natl. Acad. Sci. USA* 1997; **94:** 9343-8.
53. Fujii Y, Gonoi T, Yamada Y, Chihara K, Inagaki N, Seino S. Somatostatin receptor subtype SSTR2 mediates the inhibition of high-voltage-activated calcium channels by somatostatin and its analogue SMS 201-995. *FEBS Lett.* 1994; **355:** 117-20.
54. Guillermet J, Saint-Laurent N, Rochaix P, *et al.* Somatostatin receptor subtype 2 sensitizes human pancreatic cancer cells to death ligand-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 2003; **100:** 155-60.
55. Kikutsuji T, Harada M, Tashiro S, *et al.* Expression of somatostatin receptor subtypes and growth inhibition in human exocrine pancreatic cancers. *J. Hepatobiliary Pancreat. Surg.* 2000; **7:** 496-503.
56. Rochaix P, Delesque N, Esteve JP, *et al.* Gene therapy for pancreatic carcinoma: local and distant antitumor effects after somatostatin receptor sst2 gene transfer. *Hum. Gene. Ther.* 1999; **10:** 995-1008.
57. Zatelli MC, Piccin D, Tagliati F, *et al.* Somatostatin receptor subtype 1 selective activation in human growth hormone (GH)- and prolactin (PRL)-secreting pituitary adenomas: effects on cell viability, GH, and PRL secretion. *J. Clin. Endocrinol. Metab.* 2003; **88:** 2797-802.
58. Sharma K, Patel YC, Srikant CB. Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol. Endocrinol.* 1996; **10:** 1688-96.
59. Kreienkamp HJ, Honck HH, Richter D. Coupling of rat somatostatin receptor subtypes to a G-protein gated inwardly rectifying potassium channel (GIRK1). *FEBS Lett.* 1997; **419:** 92-94.

60. Szepeshazi K, Schally AV, Halmos G, *et al.* Targeting of cytotoxic somatostatin analog AN-238 to somatostatin receptor subtypes 5 and/or 3 in experimental pancreatic cancers. *Clin. Cancer Res.* 2001; **7:** 2854-61.
61. Parmar RM, Chan WW, Dashkevicz M, *et al.* Nonpeptidyl somatostatin agonists demonstrate that sst2 and sst5 inhibit stimulated growth hormone secretion from rat anterior pituitary cells. *Biochem. Biophys. Res. Commun.* 1999; **263:** 276-80.
62. Blake AD. Somatostatin receptor subtype 1 (sst_1) regulates intracellular 3',5'-cyclic adenosine monophosphate accumulation in rat embryonic cortical neurons: evidence with L-797,591, an sst_1-subtype-selective nonpeptidyl agonist. *Neuropharmacology* 2001; **40:** 590-6.
63. Moneta D, Richichi C, Aliprandi M, *et al.* Somatostatin receptor subtypes 2 and 4 affect seizure susceptibility and hippocampal excitatory neurotransmission in mice. *Eur. J. Neurosci.* 2002; **16:** 843-9.
64. Pfeiffer M, Koch T, Schroder H, *et al.* Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst_3 receptor function by heterodimerization with sst_{2A}. *J. Biol. Chem.* 2001; **276:** 14027-36.
65. Chen C, Israel JM, Vincent JD. Electrophysiological responses to somatostatin of rat hypophysial cells in somatotroph-enriched primary cultures. *J. Physiol.* 1989; **408:** 493-510.
66. Chen C, Israel JM, Vincent JD. Electrophysiological responses of rat pituitary cells in somatotroph-enriched primary culture to human growth-hormone releasing factor. *Neuroendocrinology* 1989; **50:** 679-87.
67. Chen C, Zhang J, Vincent JD, Israel JM. Two types of voltage-dependent calcium current in rat somatotrophs are reduced by somatostatin. *J. Physiol.* 1990; **425:** 29-42.
68. Chen C, Zhang J, McNeill P, Pullar M, Cummins JT, Clarke IJ. Human growth hormone releasing factor (hGRF) modulates calcium currents in human growth hormone secreting adenoma cells. *Brain Res.* 1993; **604:** 345-8.
69. Chen C, Clarke IJ. Modulation of Ca^{2+} influx in the ovine somatotroph by growth hormone-releasing factor. *Am. J. Physiol.* 1995; **268:** E204-12.
70. Lussier BT, French MB, Moor BC, Kraicer J. Free intracellular Ca^{2+} concentration and growth hormone (GH) release from purified rat somatotrophs. III. Mechanism of action of GH-releasing factor and somatostatin. *Endocrinology* 1991; **128:** 592-603.
71. Mason WT, Rawlings SR. Whole-cell recordings of ionic currents in bovine somatotrophs and their involvement in growth hormone secretion. *J. Physiol.* 1988; **405:** 577-93.
72. Chen C, Xu R, Clarke IJ, Ruan M, Loneragan K, Roh SG. Diverse intracellular signalling systems used by growth hormone-releasing hormone in regulating voltage-gated Ca^{2+} or K channels in pituitary somatotropes. *Immunol. Cell. Biol.* 2000; **78:** 356-68.
73. Chen C, Heyward P, Zhang J, Wu D, Clarke IJ. Voltage-dependent potassium currents in ovine somatotrophs and their function in growth hormone secretion. *Neuroendocrinology* 1994; **59:** 1-9.
74. Chen C. Gi-3 protein mediates the increase in voltage-gated K^+ currents by somatostatin on cultured ovine somatotrophs. *Am. J. Physiol.* 1998; **275:** E278-84.
75. Chen C, Clarke IJ. G_o-2 protein mediates the reduction in Ca^{2+} currents by somatostatin in cultured ovine somatotrophs. *J. Physiol.* 1996; **491:** 21-29.
76. Kwiecien R, Hammond C. Differential management of Ca^{2+} oscillations by anterior pituitary cells: a comparative overview. *Neuroendocrinology* 1998; **68:** 135-51.
77. Kwiecien R, Robert C, Cannon R, *et al.* Endogenous pacemaker activity of rat tumour somatotrophs. *J. Physiol.* 1998; **508:** 883-905.
78. Wu D, Chen C, Zhang J, Katoh K, Clarke I. Effects in vitro of new growth hormone releasing peptide (GHRP-1) on growth hormone secretion from ovine pituitary cells in primary culture. *J Neuroendocrinol.* 1994; **6:**185-90.
79. Wu D, Chen C, Katoh K, Zhang J, Clarke IJ. The effect of GH-releasing peptide-2 (GHRP-2 or KP 102) on GH secretion from primary cultured ovine pituitary cells can be abolished by a specific GH-releasing factor (GRF) receptor antagonist. *J. Endocrinol.* 1994; **140:** R9-13.
80. Akman MS, Girard M, O'Brien LF, Ho AK, Chik CL. Mechanisms of action of a second generation growth hormone-releasing peptide (Ala-His-D-β Nal-Ala-Trp-D-Phe-Lys-NH2) in rat anterior pituitary cells. *Endocrinology* 1993; **132:** 1286-1291.
81. Chen C, Clarke IJ. Effects of growth hormone-releasing peptide-2 (GHRP-2) on membrane Ca^{2+} permeability in cultured ovine somatotrophs. *J. Neuroendocrinol.* 1995; **7:** 179-86.
82. Fox AP, Nowycky MC, Tsien RW. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.* 1987; **394:** 149-72.
83. Santi CM, Darszon A, Hernandez-Cruz A. A dihydropyridine-sensitive T-type Ca^{2+} current is the main Ca^{2+} current carrier in mouse primary spermatocytes. *Am. J. Physiol.* 1996; **271:** C1583-93.
84. Kato M, Sakuma Y. Regulation by growth hormone-releasing hormone and somatostatin of a Na^+ current in the primary cultured rat somatotroph. *Endocrinology* 1997; **138:** 5096-5100.
85. Schonbrunn A. Somatostatin action in pituitary cells involves two independent transduction mechanism. *Metabolism.* 1990; **39:** 96-100.
86. Yatani A, Codina J, Sekura RD, Birnbaumer L, Brown AM. Reconstitution of somatostatin and muscarinic receptor mediated stimulation of K^+ channels by

isolated GK protein in clonal rat anterior pituitary cell membranes. *Mol. Endocrinol.* 1987; **1:** 283-9.
87. Bruno JF, Xu Y, Song J, Berelowitz M. Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. *Endocrinology* 1993; **133:** 2561-67.
88. Li X-J, Forte M, North RA, Ross CA, Snyder SH. Cloning abd expression of a rat somatostatin receptor enriched in brain. *J. Biol. Chem.* 1992; **267:** 21307-21312.
89. Meyerhof W, Wulfsen I, Schonrock C, Fehr S, Richter D. Molecular cloning of a somatostatin-28 receptors and comparison of its expression pattern with that of a somatostatin-14 receptor in rat brain. *Proc. Natl. Acad. Sci. USA* 1992; **89:** 10267-19271.
90. Rohrer L, Raulf F, Bruns C, Buettner R, Hofstaedter F, Schule R. Cloning and characterization of a fourth human somatostatin receptor. *Proc. Natl. Acad. Sci. USA* 1993; 90: 4196-4200.
91. Bruno JF, Xu Y, Song J, Berelowitz M. Molecular cloning and functional expression of a novel brain specific somatostatin receptor. *Proc. Natl. Acad. Sci. USA* 1992; **89:** 11151-11155.
92. O'Carroll A-M, Raynor K, Lolait SJ, Reisine T. Characterization of cloned human somatostatin receptors SSTR5. *Mol. Pharmacol.* 1994; **48:** 291-298.

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