Effect of Somatostatin on the Release of Gonadotropins in Male Rats

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Abstract. **Since somatostatin, the growth hormone release–inhibiting hormone, has inhibitory actions in many cell types and is delivered to the anterior pituitary gland *via* the hypophysial portal vessels, as well as being synthesized by cells within the gland, we tested the hypothesis that it might inhibit the release of gonadotropins from anterior pituitaries *in vitro.* Consequently, the effect of somatostatin on gonadotropin release from incubated anterior pituitaries of male rats with and without the stimulatory action of luteinizing hormone–releasing hormone (LHRH) was studied. After a preincubation period of 1 hr, hemipituitaries from adult male rats were incubated in fresh Krebs-Ringer bicarbonate (KRB) buffer in a Dubnoff incubator with an atmosphere of 95% O_2–5% CO_2 at 37°C for 3 hr. Incubation with somatostatin (10^{-6}, 10^{-7}, and 10^{-8} *M*) had no effect on basal release of either LH or follicle-stimulating hormone (FSH). However, somatostatin (10^{-6}–10^{-8} *M*) suppressed LHRH (1.7×10^{-8} *M*)-induced release of LH ($P < 0.01$ to $P < 0.0001$), but not FSH. Furthermore, somatostatin antiserum (1:1000) had no significant effect on basal LH or FSH release, whereas incubation with the antiserum plus LHRH (1.7×10^{-9} or 1.7×10^{-8} *M*) increased LH ($P = 0.015$ and $P = .005$, respectively), but not FSH release. In summary, our results suggest that somatostatin exerts a physiologically significant inhibitory effect on LH but not FSH release in the presence of LHRH *in vitro.* Presumably, somatostatin is secreted *in vitro* by pituitary cells since not only have anterior pituitaries of rats been shown to contain somatostatin, but also somatostatin mRNA. Somatostatin then diffuses to the LH gonadotropes, where it exerts its inhibitory action. However, the release of somatostatin is insufficient to alter basal *in vitro* release. On the other hand, at least at the concentrations employed, there was no significant effect either of somatostatin or the antiserum to alter basal or stimulated FSH release.** [P.S.E.B.M. 1997, Vol 214]

Somatostatin was originally detected by Krulich *et al.* (1–4) during the purification of growth hormone (GH)–releasing factor from sheep hypothalami. They named it growth hormone–inhibiting factor (GIF), purified it, and localized it within the hypothalamus. In 1973, Brazeau *et al.* (5) characterized GIF, a tetradecapeptide, and renamed it somatostatin. Somatostatin is widely distributed throughout the central nervous system (CNS) and is also found in various other organs (6–8), such as the pancreas, where it inhibits insulin and glucagon release (9). This peptide, acting as a hormone and as a neurotransmitter or neuromodulator, has physiological effects both in the periphery and in the CNS (6–8). In the pituitary gland, it dramatically inhibits the release of GH from somatotrophs (10) and blocks the release of thyroid-stimulation hormone (TSH) (10–12), prolactin (10, 11, 13), and adrenocorticotropic hormone (ACTH) under certain conditions (14, 15). It was hypothesized that somatostatin could block release of all pituitary hormones with sufficient dosage (6).

Therefore, in this study we examined the possibility that somatostatin might inhibit gonadotropin release from anterior pituitaries of male rats *in vitro* in the presence or absence of luteinizing hormone–releasing hormone (LHRH) (16).

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Materials and Methods

Adult male rats (200–220 g) were purchased from Harlan (Sprague-Dawley strain; Madison, WI) and housed under controlled conditions of photoperiod (lights on: 0500–1900 hr) and temperature (23°–25°C) with ad libitum food (rat pellets) and water. They were used for *in vitro* experiments 1 week after arrival.

On the day of experiment, the rats were decapitated and their anterior pituitaries were quickly removed, bisected longitudinally, and randomized among incubation flasks containing 1 ml of Krebs-Ringer bicarbonate (KRB) buffer adjusted to pH 7.4, such that each flask contained one hemipituitary. After a 1-hr preincubation in a Dubnoff shaker (50 cycles/min) in an atmosphere of 95% O_2–5% CO_2 at 37°C, the medium was replaced with 1 ml of fresh KRB medium containing the test substances, and the incubation was continued for 3 hr. In the first experiment, somatostatin (10^{-6}, 10^{-7}, and 10^{-8} *M*) was tested in the 3-hr-incubation system with and without the presence of LHRH (1.7×10^{-8} *M*).

To determine the possible physiologic significance of somatostatin on gonadotropin release, we examined the effect of somatostatin antiserum in the second experiment. Normal rabbit serum (NRS) (1:1000) or somatostatin antiserum (Anti-SRIF, 1:1000) was added to the medium in the presence or absence of LHRH (1.7×10^{-9} or 1.7×10^{-8} *M*) or incubated for 3 hr as described above. At the termination of the incubation, media were removed and stored frozen (–20°C) for later radioimmunoassays (RIA). All experiments were replicated.

Somatostatin was purchased from Peninsula Laboratories, Inc. (Belmont, CA). Anti-SRIF was courteously supplied by Dr. Louis De Palatis (Dow Chemical Co., Midland, MI).

The concentration of LH and follicle-stimulating hormone (FSH) in the incubation medium was measured by the RIA kits supplied by NIDDK, and hormone values were expressed in terms of NIDDK-rLH-RP-3 and NIDDK-rFSH-RP-2 standards, respectively. All samples were assayed in duplicate.

The results were analyzed by analysis of variance (ANOVA) with repeated measures and subsequent Newman-Keuls test. The significance of differences between two groups was determined by Student's *t* test.

Results

In the first experiment, somatostatin itself (10^{-6}, 10^{-7}, and 10^{-8} *M*) had no effect on basal LH release from hemipituitaries in the 3-hr incubation (Fig. 1). Incubation with LHRH itself (1.7×10^{-8} *M*) significantly stimulated LH release from the hemipituitaries. When these two peptides were added together, somatostatin (10^{-6}–10^{-8} *M*) uniformly suppressed LHRH-induced release of LH ($P < 0.01$ to $P < 0.001$). Moreover, incubation of hemipituitaries with LHRH (1.7×10^{-8} *M*) increased the release of FSH significantly, while incubation with somatostatin (10^{-6} to 10^{-8} *M*) had no effect on either basal or LHRH-induced release of FSH in the 3-hr incubation (Fig. 2).

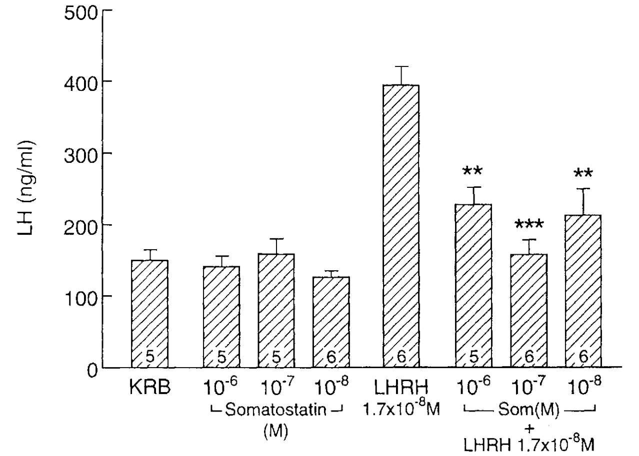

Figure 1. Somatostatin (Som) had no effect on basal LH release, but it suppressed the LHRH-induced LH release from hemipituitary incubation. However, the suppression was not dose dependent. Values are expressed as mean ± SEM in this and subsequent figures. Samples sizes are shown within each bar. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus LHRH-treated group.

To test the physiological significance of somatostatin on LH release *in vitro,* we added somatostatin antiserum in the hemipituitary incubation. Incubation with anti-SRIF (1:1000) or NRS (1:1000 as control) for 3 hr had no significant effect on basal LH release. However, incubation with the antiserum (1:1000) plus LHRH (1.7×10^{-9} or 1.7×10^{-8} *M*) significantly increased LH release compared with that in the MRS plus LHRH–treated groups (Fig. 3). Furthermore, incubation with anti-SRIF (1:1000) or NRS (1:1000) had no effect on basal FSH release, and anti-SRIF failed to alter LHRH-induced release of FSH during the 3-hr incubation of hemipituitaries (Fig. 4).

Discussion

Somatostatin has not been reported to affect gonadotropin release (6–8), even though it suppresses the release of the other anterior pituitary hormones. However, during

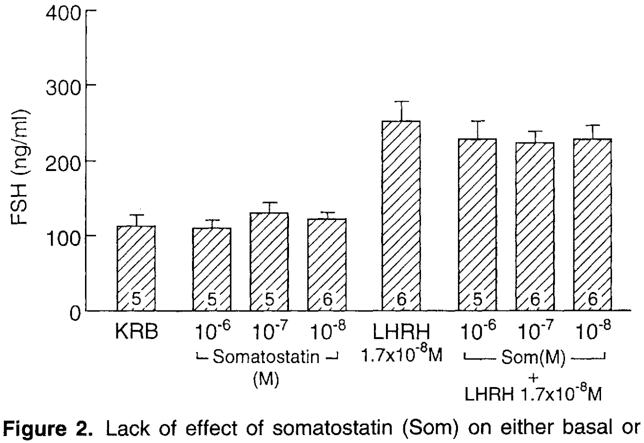

Figure 2. Lack of effect of somatostatin (Som) on either basal or LHRH-induced release of FSH.

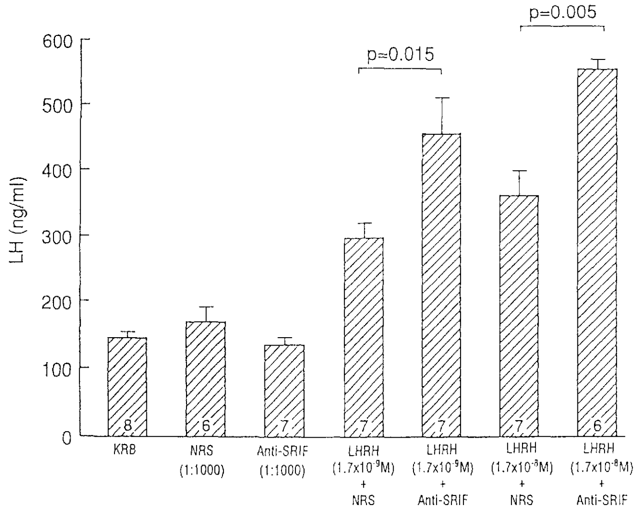

Figure 3. Effect of somatostatin antiserum (anti-SRIF, 1:1000) or (nrs) (1:1000) on basal and LHRH-stimulated LH release. Incubation with the antiserum (1:1000) plus LHRH (1.7×10^{-9} or 1.7×10^{-8} *M*) increased LH release ($P = 0.015$ and $P = 0.005$, respectively) versus the NRS plus LHRH–treated group.

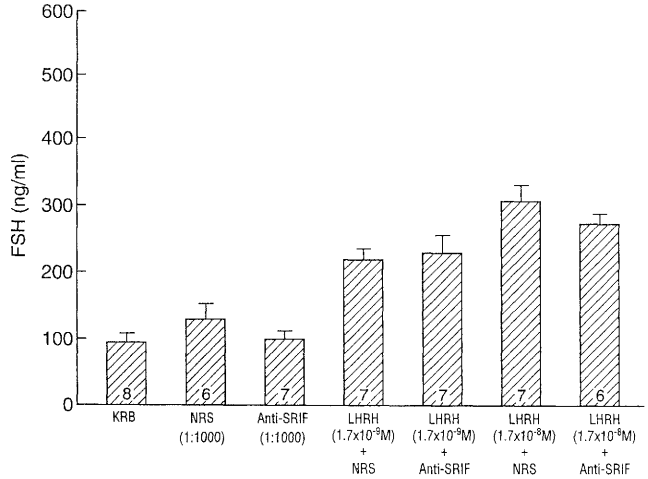

Figure 4. Ineffectiveness of somatostatin antiserum (anti-SRIF) or NRS on basal or LHRH-induced release of FSH.

purification of FSH-releasing factor from sheep median eminence (ME) extracts, we discovered that certain fractions from the Sephadex column inhibited FSH release (Yu *et al.*, unpublished data). We speculated that this inhibitory activity might be caused by somatostatin; however, the inhibitory zone was devoid of this peptide. The elution of somatostatin did overlap that of LHRH, and we hypothesized that somatostatin might diminish the pituitary response to LHRH. We performed these experiments to test this hypothesis.

Indeed, our results showed that somatostatin suppressed LHRH-induced LH, though not FSH release, but had no effect on basal release of either gonadotropin, suggesting that somatostatin may inhibit LHRH activity by combining with somatostatin receptors on LH gonadotropes. The lack of a dose-response relationship may be the result of using doses at or above the maximal effective dose. Incubation of the glands with anti-SRIF failed to alter release of either FSH or LH. However, when the incubation was carried out in the presence of effective concentrations of LHRH, the release of LH was markedly increased in the presence of the anti-SRIF, but not the control NRS. On the other hand, there was no effect of the antiserum on the lesser stimulation of FSH release evoked by LHRH.

The failure of the somatostatin or the anti-serum against it to alter basal secretion of either gonadotropin indicates that the quantity of somatostatin in the interstices of the gland was not sufficient to alter basal release, which is difficult to decrease from hemipituitaries by any means, and many represent, at least in part, passive release from damaged cells in the glands. On the other hand, the ability of somatostatin, at concentrations that could possibly exist in the gland, and the somatostatin anti-serum to have inhibitory and augmenting effects on LH release, respectively, argues for a secretion of somatostatin from pituitary cells *in vitro*. Alternatively, some somatostatin might remain in the gland previously delivered to it *via* the portal vessels *in vivo*. This seems a very unlikely possibility in view of the likely degradation during the 3-hr incubation period of any somatostatin present. It has been shown that the anterior pituitary contains immunoreactive somatostatin (17) localized in somatotropes and thyrotropes (18). Therefore, it would appear that in these *in vitro* conditions, somatostatin is actually secreted and diffuses to the LH gonadotrophs where it interacts with its receptors (19–22) to suppress the response of the gonadotrophs to LHRH. Other paracrine actions of incubation hormones within the anterior pituitary have been previously demonstrated (23).

Moreover, the mRNA of all five somatostatin receptor subtypes (rsstr 1–5) has recently been reported in somatotrophs, thyrotrophs, mammotrophs, corticotrophs, and gonadotrophs of rat anterior pituitaries (22). The mRNAs of rsstr 1 and 2 were dominant in the LH gonadotrophs, whereas the mRNA of rsstr 3 was predominantly expressed in the FSH-containing cells of the gland. The data are consistent with the concept that somatostatin plays a role in regulation of LH release through interaction with either the rsstr 1 or 2, or both of these receptors, whereas for some reason the rsstr 3 receptor on the FSH gonadotrophs is not affected, at least by the concentrations of somatostatin employed here. The affinity for somatostatin of the rsstr 3 receptors is significantly less than that of rsstr 1 and 2. Therefore, we hypothesize that this accounts for the failure of the FSH gonadotrophs to respond to somatostatin.

The mechanisms by which somatostatin acts to inhibit LH release after combination with its receptors on the LH-gonadotropes requires further study. Somatostatin lowers pituitary cAMP concentration by inhibiting adenyl cyclase and this may block LH release. Further work is needed to resolve these points.

Interestingly, it was reported that somatostatin receptors were present in a human pituitary tumor, a gonadotropinoma, which release gonadotropins (24). Our results point to the physiological significance of somatostatin in the inhibition of the response of the LH gonadotropes to LHRH *in vitro*. However, *in vivo* experiments are required to determine if this has any relevance *in vivo*. *In vivo,* not only would somatostatin be presented to the tissue by the pituitary cells themselves, but also it would be delivered in pulsatile fashion *via* the portal vessels after its secretion in the median eminence (25). Therefore, the concentrations of somatostatin, particularly at the peak of pulsatile somatostatin release, might actually be higher *in vivo* than in these *in vitro* experiments. *In vivo* experiments with somatostatin antagonists or antiserum should resolve this important issue.

That pharmacological concentrations of somatostatin can act *in vivo* in humans to suppress the pituitary response to LHRH is indicated, since somatostatin suppressed the amplitude of LH pulses but not FSH pulses in normal humans (26). Furthermore, this effect has also been seen with administration of somatostatin analogs in patients with polycystic ovarian disease and elevated plasma LH (27). The size of a human gonadotropin-secreting adenoma was also diminished by treatment of the patient with agonist somatostatin analogs (28). Therefore, the action of somatostatin to suppress the response of LH-gonadotropes to LHRH has clinical relevance.

In conclusion, our results indicate that somatostatin exerts a physiologically significant inhibitory effect *in vitro* on LH but not FSH release in the presence of LHRH.

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