The rebound release of growth hormone (GH) following somatostatin infusion in rats involves hypothalamic GH-releasing factor release

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ABSTRACT
We have studied the rebound secretion of GH following short-term somatostatin (SS) infusions in conscious rats, using an automatic sampling system for withdrawing frequent microsamples of blood. Intravenous infusions of SS (5–50 µg/h per rat) inhibited spontaneous GH secretion, but when SS was withdrawn there was a large burst of rebound GH secretion. A sub-anæsthetic dose of urethane reduced such rebound bursts of GH, suggesting a hypothalamic involvement in rebound GH secretion. Passive immunization with an antibody against rat GH-releasing factor (GRF) attenuated the rebound GH secretory response to the withdrawal of an SS infusion (GH concentration during rebound secretion was 26 ± 21 µg/l vs 475 ± 127 µg/l (mean ± s.e.m.), after 0.5 ml anti-GRF serum or non-immune serum respectively). The inhibition of GH rebound secretion was related to the dose of anti-GRF serum administered. Intravenous infusions of human GH (20–100 µg/h per rat) also reduced the size of the rebound GH secretion following SS withdrawal, in both male and female rats. We suggest that the rebound GH secretion that follows SS withdrawal in vivo is caused mainly by a hypothalamic release of GRF. Exogenous GH inhibits SS-induced rebound GH secretion in the conscious rat, possibly by inhibiting hypothalamic GRF release.


INTRODUCTION
The release of growth hormone (GH) from the anterior pituitary is known to be regulated by at least two opposing hypothalamic peptides, somatostatin (SS) (Brazeau, Vale, Burgus et al. 1973) and GH-releasing factor (GRF) (Guillemin, Brazeau, Böhlen et al. 1982; Rivier, Spiess, Thorner & Vale, 1982), as well as by a number of other peripheral factors, including nutrients, thyroid hormones, and gonadal and adrenal steroids (Tannenbaum, Rosrsta & Brazeau, 1979; Evans, Birnberg & Rosenfeld, 1982; Wehrenberg, Baird & Ling, 1983; Jansson, Eden & Isaksson, 1985). Although it is likely that GH secretory patterns chiefly reflect a changing balance between SS and GRF release (Tannenbaum & Ling, 1984; Tannenbaum, 1985), there is little or no direct information about the hypothalamic secretory patterns of GRF or SS in the conscious animal. In the conscious male rat GRF releases GH intermittently (Clark & Robinson, 1985), suggesting that a cyclically varying release of SS into hypophysial portal blood is responsible for the intermittent responsiveness of the anterior pituitary to exogenous GRF injections.

Somatostatin inhibits the effect of GRF on GH release (Fukata, Diamond & Martin, 1985), but does not inhibit the stimulation of GH gene transcription caused by GRF (Barinaga, Bilezikjian, Vale et al. 1985), so that pituitary GH stores will increase if both GRF and SS are present. Recent studies have also suggested that SS can inhibit its own release (Peterfreund & Vale, 1984; Richardson & Twente, 1986), and injections of SS directly into the cerebral ventricles lead to a paradoxical increase in GH release (Lumpkin, Negro-Vilar & McCann, 1981).

A striking feature of the effect of SS on GH release...
is that the inhibition is short-lived, since SS has a short half-life in vivo (Patel & Wheatley, 1983), and the abrupt removal of SS invariably leads to a rebound secretion of GH (Cowan, Chow & Kraicer, 1983; Clark & Robinson, 1988). This rebound GH release is much more pronounced in vivo than in vitro (Stachura, 1976; Kraicer, Cowan & Sheppard et al., 1986), suggesting that there may be an active hypothalamic component stimulating GH release upon removal of SS, rather than simply a removal of SS inhibition at the pituitary level.

We have developed a novel method for withdrawing multiple serial microsamples of blood from conscious freely moving rats (Clark, Chambers, Lewin & Robinson, 1986; Clark, Carlsson & Robinson, 1987), and have recently described the effects of i.v. SS, given continuously or in an intermittent pattern, on the endogenous GH secretory profile (Clark & Robinson, 1988). We now present the results of further studies of the mechanism of the in-vivo GH rebound response to SS withdrawal. These lend support to the idea of an active hypothalamic mechanism involving GRF. Some of these results have been presented in preliminary form (Carlsson, Clark & Robinson, 1986).

MATERIALS AND METHODS

Sprague–Dawley rats (150–200 g) from our own animal colony were equipped with chronic indwelling jugular venous cannulae under halothane anaesthesia, and housed individually in metabolic cages. Double-bore cannulae were used to allow simultaneous infusion and withdrawal of blood, using an automatic sampling system (Clark et al., 1986). All infusions were given by means of a computer-controlled multichannel peristaltic pump (Clark, Jansson, Isaksson & Robinson, 1985).

Somatostatin (Ferring AB, Malmö, Sweden; SS1-14, 5–50 μg/h) was infused either continuously or in a square-wave pattern (2.5 h on: 30 min off). Human GRF(1–29) amide was obtained from KabiVitrum AB (Stockholm, Sweden). Human GH (hGH; Crescormon; KabiVitrum) which does not cross-react in the assay for rat GH, was infused i.v. at 20–100 μg/h. The infusion vehicle was phosphate-buffered 0.9% (w/v) NaCl (pH 7.4) containing 5 units heparin/ml and 0.1 mg rat albumin/ml. In one group of animals, a sub-anaesthetic dose of urethane was injected (200 mg/kg i.v., followed immediately by 250 mg/kg i.p.).

Rat GRF antiserum production was as previously described for human GRF(1–40) (Rafferty & Schulster, 1985). Briefly, 300 μg rat GRF(1–43) (Bachem UK, Saffron Walden, Essex, U.K.) were conjugated to 1.5 mg bovine serum albumin (BSA; Sigma Chemical Company Ltd, Poole, Dorset, U.K.) with glutaraldehyde. After emulsification with Freund’s complete adjuvant (1:3, v/v), 2 ml emulsion, containing approximately 60 μg GRF, were injected at multiple intradermal sites in each of three rabbits. After 12 weeks, i.m. booster injections of half the amount of conjugated GRF in incomplete adjuvant were commenced: the interval between successive boosts was 1–2 months. After assessment of titre and avidity of binding of 125I-labelled GRF, antiserum RGSBM was selected for use in immunoneutralization studies. When tested in vitro, the antiserum had an apparent average affinity constant of 16 litres/nmol, and bound 50 ng rat GRF per ml serum at a final dilution of 1:25 000. The antiserum also blocks spontaneous GH secretory pulses when injected i.v. into conscious rats (R. G. Clark, B. Rafferty & I. C. A. F. Robinson, unpublished data).

Blood samples (10–20 μl) were assayed for rat GH by radioimmunoassay using reagents generously supplied by the NIADDK, as previously described (Clark & Robinson, 1985; Clark et al., 1986). Results are expressed in terms of the rat GH RPI standard, and analysed by analysis of variance (ANOVA) and Student’s t-test where appropriate.

RESULTS

An i.v. infusion of SS (50 μg/h) was given to eight male rats for 4 h. Blood samples were taken at 15-min intervals before and during the SS infusion, and at 5-min intervals for the 2 h immediately after stopping the SS infusion, for measurement of rat GH (Fig. 1). Four of the animals received a sub-anaesthetic dose of urethane 1 h before stopping the SS infusion, the other four animals serving as controls. During SS infusion there were no surges of GH above the detection limit of this assay (3 μg/l). Immediately following termination of the SS infusion in the control animals, there was a large multicomponent rebound secretion of GH, with blood concentrations rising more than 100-fold within 20 min (Fig. 1, left panel). The animals which had been given urethane were sedated but still maintained their righting reflexes. This dose of urethane markedly blunted the rebound GH secretory response to the withdrawal of the SS infusion (Fig. 1, right panel).

In the next experiment, a series of rebound secretory bursts of GH were elicited in eight conscious male rats by an i.v. infusion of SS (30 μg/h) which was interrupted for 30 min every 3 h over a 12-h period. Blood samples were taken at 15-min intervals during SS infusion, and the sampling rate increased to 12/h during the period of SS withdrawal, in order to define
Using a similar experimental design of intermittent SS infusions, the effect of an infusion of hGH on the GH rebound secretion following SS withdrawal was investigated. Figure 3 shows an experiment in seven conscious male rats given three consecutive interrupted infusions of SS (20 μg/h). During one of the SS infusions, hGH (100 μg/h) was included in the infusate. Blood samples were withdrawn every 10 min before and during SS infusions, and every 5 min after SS withdrawal. Spontaneous GH secretion was present initially in all the rats, and was blocked by SS infusions. As before, large rebound bursts of GH secretion followed the withdrawal of SS. Incorporation of hGH into the infusate dramatically reduced the size of the subsequent GH rebound whether or not the hGH was given during the second or third period of SS infusion (Fig. 3a and b respectively). Similar results were obtained in other experiments in both normal and ovariectomized female rats, in which hGH infusions (20–100 μg/h) blocked GH rebound secretion following SS withdrawal (Table 2).

**DISCUSSION**

Since the discovery of SS as a potent inhibitor of GH secretion, several studies have confirmed a role for this peptide, together with GRF, in regulating the secretory pattern of GH (Brazeau, Rivier, Vale & Guillemin, 1974; Wehrenberg, Ling, Böhlen et al. 1982; Tannenbaum & Ling, 1984; Wehrenberg, 1986). Although SS is potent in suppressing secretion of GH in vitro, very large doses are required in vivo to achieve a similar inhibition of GH release, even when the peptide is infused directly into the blood stream over long periods (Cowan, Gaul, Moor & Kraicer, 1984; Clark & Robinson, 1988). This may be due to the difficulty of maintaining high concentrations of SS similar to those found in hypophysial portal blood (Chihara, Arimura & Schally, 1979; Plotsky & Vale, 1985), given the rapid clearance of the peptide from the circulation (Patel & Wheatley, 1983), and the relatively short duration of action of SS(1–14) (Tannenbaum, Ling & Brazeau, 1982).

A characteristic feature of the effects of prolonged exposure to SS is that immediately following SS removal, there is a large and rapid release of GH (Cowan et al. 1983, 1984; Clark & Robinson, 1988). A rebound secretion of GH occurs in vitro after exposure of pituitary cells to SS (Stachura, 1976; Cowan et al. 1983), which probably reflects a build-up of readily releasable GH which is secreted after the removal of the SS inhibition (Kraicer et al. 1986). However, the amount of GH released during an in-vitro rebound is much smaller than that observed in vivo. In the rat, the peak heights of plasma GH reached during

![Figure 1. Rebound secretion of GH following short-term somatostatin (SS) infusions in conscious rats, and its attenuation by urethane. Serial blood samples were withdrawn automatically from eight conscious male rats and assayed for GH. An i.v. infusion of somatostatin (50 μg/h) was given for 4 h (solid bars), after which the blood-sampling rate was increased from 4/h to 12/h and continued for a further 2 h. Four of the animals were given a sedative dose of urethane (450 mg/kg; right panel, arrowed).](image-url)
TABLE 1. Blockade of somatostatin (SS)-induced GH rebound secretion by anti-rat GH-releasing factor (GRF) serum in the conscious rat. Values are means ± S.E.M. for four rats in each group

<table>
<thead>
<tr>
<th>Serum</th>
<th>Rebound 1 (µg/l)</th>
<th>Rebound 2 (µg/l)</th>
<th>Rebound 3 (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 µl NRS</td>
<td>285 ± 62</td>
<td>273 ± 62</td>
<td>329 ± 101</td>
</tr>
<tr>
<td>125 µl anti-GRF</td>
<td>224 ± 32</td>
<td>174 ± 39</td>
<td>310 ± 82</td>
</tr>
<tr>
<td>250 µl NRS</td>
<td>633 ± 304</td>
<td>322 ± 136</td>
<td>622 ± 210</td>
</tr>
<tr>
<td>250 µl anti-GRF</td>
<td>576 ± 269</td>
<td>136 ± 30</td>
<td>111 ± 12*</td>
</tr>
<tr>
<td>500 µl NRS</td>
<td>281 ± 35</td>
<td>475 ± 127</td>
<td>340 ± 30</td>
</tr>
<tr>
<td>500 µl anti-GRF</td>
<td>690 ± 248</td>
<td>26 ± 21**</td>
<td>105 ± 59**</td>
</tr>
</tbody>
</table>

A series of regular rebound secretions of GH were induced in conscious male or female rats by i.v. infusions of SS (25 or 30 µg/h), interrupted every 3 h for 30 min. Serial blood samples were withdrawn at 5-min intervals following each SS withdrawal, and assayed for GH. Between rebounds 1 and 2, animals were given an i.v. injection of either non-immune rabbit serum (NRS) or anti-rat GRF serum RG5BM. The rebound GH response was expressed as the average GH concentration measured in the six samples withdrawn during the period of SS removal on each occasion in each rat.

*P < 0.05, **P < 0.02 compared with NRS control (Student’s t-test).


post-SS rebound secretion can exceed the normal endogenous GH pulse height and approach those elicited by maximal doses of exogenous GRF in the rat (Clark et al. 1985, 1986).

We have recently shown that the rebound GH response to SS withdrawal in the rat can be repeatedly induced by using infusions of SS interrupted for a short period at regular intervals. Moreover, maintaining a pulsatile pattern of GH release in female rats given chronic intermittent SS infusions paradoxically stimulated body-weight gain and did not induce an inhibition of bone growth, which was seen when the same dose of SS was given as a continuous infusion (Clark & Robinson, 1988). However, the magnitude of the GH rebound responses suggested that they were not simply due to a disinhibition at the pituitary level, but probably reflected an acute change in hypothalamic output of GRF or SS.

In order to investigate the possibility of a neural involvement in the rebound response, we used conscious animals several days after surgery, and which
were then given a sedative dose of an anaesthetic. We chose urethane as it does not elicit a large release of GH, unlike barbiturate or steroid anaesthetics (Chihara et al. 1979). Urethane strongly attenuated the SS-induced GH rebound. This was not likely to be due to a direct effect of this small dose of urethane depressing the somatotrophs directly, as rats fully anaesthetized with a large dose of urethane respond to GRF. It was more likely that urethane inhibited the GH rebound either by stimulating SS release (Chihara et al. 1979) or by inhibiting GRF release.

Immunoneutralization with anti-SS sera does increase GH levels due to blockade of endogenous SS (Arimura, Smith & Schally, 1976; Tannenbaum & Ling, 1984), but the levels reached are nowhere near those produced in our experiments by the sudden removal of exogenous SS. The profiles of GH release during a rebound secretory event were similar to the profiles obtained in response to GRF, and we therefore suspect that GRF released from the hypothalamus was responsible for the rebound GH secretion. To test this directly, animals were passively immunized with an antibody against rat GRF.

Previous studies of passive immunization with antisera to GRF have shown that it is possible to inhibit the effects of endogenous GRF and thus block both normal secretory pulsing and slow down normal
somatic growth in rats (Wehrenberg et al. 1982; Wehrenberg, Bloch & Phillips, 1984; Wehrenberg, 1986). In our experiments, anti-GRF serum reduced the rebound release of GH following SS withdrawal in a dose-dependent manner, implicating the presence or release of endogenous GRF as the major mediator of SS-induced rebound GH secretion in vivo. The residual small GH rises seen in some animals after SS withdrawal in GRF-blocked animals could reflect incomplete immunoneutralization, or more likely, represent the rebound response intrinsic to the somatotroph. These results accord well with other studies (Thomas, Groot & Arimura, 1985; Miki, Ono & Shizume, 1988) which showed that anti-GRF reduced the rise in plasma GH caused by withdrawal of endogenous SS tone by immunoneutralization with anti-SS sera.

We do not know the mechanism whereby exogenous SS removal triggers GH secretion, and there are several possible explanations. Continuous exposure to high levels of exogenous SS could suppress endogenous SS release so that the normal hypothalamic GRF release is suddenly left unopposed as the exogenous SS is withdrawn. Alternatively, high levels of exogenous SS could stimulate GRF release directly, which only releases GH when the pituitary SS blockade is removed. A third possibility is that a rapid fall of SS per se could induce a release of GRF (Miki et al. 1988). One must also bear in mind that the doses of exogenous SS required to induce a GH rebound are high and that this peptide has many other actions besides inhibition of GH. Thus, other effects of SS (e.g. inhibition of insulin or glucagon release, gastrointestinal motility or nutrient absorption) could indirectly affect the release of endogenous GRF, perhaps by altering hypothalamic glucose supply (Painson & Tannenbaum, 1985).

The notion that SS could act at a hypothalamic level is supported by the studies of Lumpkin et al. (1981) which showed that central administration of SS paradoxically stimulates GH release, possibly by inhibiting its own secretion from hypothalamic neurones (Peterfreund & Vale, 1984; Richardson & Twente, 1986). It will be necessary to measure hypophysial portal blood levels of GRF and SS in the conscious animal undergoing an SS-induced rebound in order to demonstrate this directly.

The rebound GH secretion induced by SS withdrawal was also strongly attenuated by concomitant infusions of exogenous GH. This follows on from our previous work (Carlsson et al. 1986; Clark, Carlsson & Robinson, 1989) in which infusions of exogenous GH were shown to suppress spontaneous GH release in conscious rats, and is in line with earlier studies suggesting an inhibitory feedback effect of GH at a hypothalamic level (Kruilich & McCann, 1966; Tannenbaum, 1980; Willoughby, Menadue, Zeegers et al. 1980; Richman, Weiss, Hochberg & Florini, 1981; Abe, Molitch, van Wyk & Underwood, 1983; Nakamoto, Gertner, Press et al. 1986). Since SS-induced rebound secretion (which involves hypothalamic GRF release) is attenuated by exogenous GH, the GH feedback mechanism may involve a reduction in GRF release, as also suggested by the studies of Conway, McCann & Kruilich (1985) on clonidine-induced GH secretion in the rat. Of the other possibilities, a direct effect of GH at the pituitary or a stimulation of endogenous SS release by GH (Chihara, Minamitani, Kaji et al. 1981) do not explain our results in the conscious rat, since GRF readily stimulates GH release during such hGH infusions (Clark et al. 1988).

Rebound GH release was suppressed by exogenous hGH even though the hGH was withdrawn simultaneously with SS. In other experiments, single injections of GH 40 min before SS withdrawal failed to suppress the GH rebound secretion (L. M. S. Carlsson, R. G. Clark & I. C. A. F. Robinson, unpublished data). Taken together, the evidence suggests that relatively large amounts of GH present for some time are necessary for an effective suppression of GH release. However, it takes approximately 1 h for an i.v. infusion of GH to reach a steady-state plateau concentration in plasma, and the concentrations achieved by our GH infusions are not greater than those seen endogenously in the normal male rat (Tannenbaum & Martin, 1976; Clark et al. 1986).

These results suggest that hypothalamic GRF and SS secretion can be influenced by varying the levels of GH or SS in the peripheral bloodstream. How such peripherally infused peptides or proteins reach a hypothalamic site of action from the bloodstream is unclear. They could act on the terminals of GRF neurones which lie outside the blood-brain barrier in the median eminence, or even access other hypothalamic sites such as the arcuate nucleus which has a rich blood supply and is a major site for the production of GRF.

Our observations might have some clinical relevance if a release of GRF is involved in SS-induced rebound GH secretion in man. A short-term infusion of SS which is abruptly terminated would offer a simple means of revealing endogenous GRF release, and it might be possible to assess hypothalamic GRF secretory capability by performing short-term SS infusions in children with suspected hypothalamic GRF dysfunction and a normal pituitary GH response to exogenous GRF. On the other hand, the inevitable side-effects on insulin secretion and hence blood sugar may preclude a widespread application of such a test of SS dynamics in children.

Currently, newer analogues of SS, with a greatly extended duration of action, are receiving clinical attention (Bauer, Briner, Doepfner et al. 1982; Veber, 1988).
Saperstein, Nutt et al. (1984). These smaller and more hydrophobic SS analogues may penetrate the hypothalamus and affect the balance between endogenous GRF and SS release more effectively than natural SS, although the much longer plasma half-life of such stable SS analogues would not occasion such an abrupt rebound secretion of GH, seen on withdrawal of the natural SS sequence. Nevertheless, the possible effects on hypothalamic GRF release of prolonged exposure to exogenous SS and its analogues warrant further study.

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