Somatostatin partially reverses desensitization of somatotrophs induced by growth hormone-releasing factor

R. N. Clayton and L. C. Bailey*
Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ
*Department of Medicine, University of Birmingham, Edgbaston, Birmingham B15 2TH
RECEIVED 17 April 1986

ABSTRACT

The effect of somatostatin on GH-releasing factor (GRF)-induced desensitization of somatotrophs was studied in vitro. Primary cultures of rat anterior pituitary cells pretreated for 4 or 18 h with GRF(1–40) (100 nmol/l) showed a 50% or greater reduction in maximal GH release when rechallenged with 10 nmol GRF/l. Rechallenge GRF dose–response curves were either very flat, making accurate measurement of the dose giving 50% maximum stimulation (ED50) impossible, or the ED50 concentration was increased from 0.3 nmol/l (untreated) to 2 nmol/l (GRF pretreated). Although GRF pretreatment reduced cellular GH content by 40–50%, correction for this did not restore GRF responsiveness measured in terms of maximal GRF-stimulated/unstimulated GH release (maximal/basal ratio), or the GRF ED50 concentration. Maximal/basal GH release per 4 h from GRF-pretreated cells was reduced when cells were rechallenged with forskolin (5 μmol/l) or calcium ionophore (A23187; 10 μmol/l), to the same extent as when rechallenged with 10 nmol GRF/l. Although this might be explained by a reduction in the pool of releasable GH, an alternative explanation is that pretreatment with GRF disrupts the GH release mechanism(s) at a common step(s) beyond cyclic AMP generation and Ca2+ influx.

Co-incubation of cells with somatostatin and GRF (100 nmol/l) partially reversed the desensitizing action of GRF during both 4- and 18-h pretreatments in a dose-dependent manner, with 1 μmol somatostatin/l being most effective. Maximal GRF (100 nmol/l)-stimulated/basal GH release was 4.4 ± 1.0 (mean ± s.e.m., n = four experiments), 1.55 ± 0.09 and 2.43 ± 0.1 for control, GRF-pretreated (4 h) and GRF plus somatostatin-pretreated cells respectively. Comparable values for cells pretreated for 18 h were 3.66 ± 0.44 (n = three experiments), 1.78 ± 0.28 and 3.04 ± 0.04 for control, GRF- and GRF plus somatostatin-pretreated cells. Somatostatin reduced the 50% depletion of cellular GH caused by GRF pretreatment to 15–20%, as well as attenuating GH released during the pretreatment period by 40 ± 5% (mean ± s.e.m., n = seven experiments). Somatostatin restored somatotroph sensitivity of GRF-desensitized cells indicating that, in addition to reversing depletion of the releasable pool of GH, the counter-regulatory hormone also prevents disruption of post-receptor cellular biochemical events which remain to be identified. These results add to the list of GRF actions inhibited by somatostatin and suggest a potentially important role for somatostatin in vivo to maintain somatotroph responsiveness to GRF.


INTRODUCTION

(Harwood et al. 1984). While GRF and somatostatin interact with somatotrophs through separate receptors (Srikant & Patel, 1982; Siefert, Perrin, Rivier & Vale, 1985), the precise biochemical step(s) on which their separate interactions converge is not entirely clear, although cAMP generation is highly likely since this cellular signal is a major ‘second messenger’ for GRF action. Exposure of anterior pituitary cells to GRF renders somatotrophs partially or totally refractory to subsequent rechallenge with the hormone, a process termed ‘desensitization’ which seems to be a common feature amongst the different anterior pituitary cell types (e.g., thyrotrophin-releasing hormone–thyrotroph; gonadotrophin-releasing hormone–gonadotroph). Desensitization, although described in animals in vivo (Wehrenberg, Brazeau, Ling et al. 1984), is more readily observed in pituitary cells in vitro (Bilezikjian & Vale, 1984; Ceda & Hoffman, 1985), though this has not been a universal finding (Diequez, Foord, Shewing et al. 1984). This raises the possibility that a mechanism(s) exists in vivo whereby desensitization is partially or completely prevented. Two recent studies in man lend support to this view. It was observed that GH secretion was episodic during prolonged continuous infusion of GRF (Vance, Kaiser, Evans et al. 1985) or of an equipotent agonist analogue of GRF (Davis, Sheppard, Shakespear et al. 1986), rather than reaching a plateau and then steadily declining. Based on these findings it has been suggested (Vance et al. 1985; Davis et al. 1986) that GRF-stimulated GH release caused increased secretion of somatostatin which transiently antagonized GRF action with the consequent fall in serum GH levels. The low GH levels would then remove somatostatin suppression of the somatotroph and once more allow full expression of the GH-releasing ability of GRF. Thus, in vivo, another aspect of somatostatin counter-regulation of GH secretion might be the prevention of somatotroph desensitization, at least in the short-term (24 h). To obtain direct evidence for this hypothesis we have determined the effect of somatostatin on GRF-induced desensitization in cultured rat pituitary cells. Under our experimental conditions somatostatin was able to partially attenuate the desensitizing action of GRF.

MATERIALS AND METHODS

Hormones

Growth hormone-releasing factor(1–40) was purchased from Bachem UK (Saffron Walden, Essex) and stored at −20 °C as a 10 μmol/l solution, in phosphate-buffered saline (PBS; pH 7-4). Somatostatin(1–14) was purchased from Sigma Chemical Co. (Poole, Dorset) and stored at −20 °C in PBS at 100 μmol/l. Tri-iodothyronine (T₃) and dexamethasone were also from Sigma and stored at 4 °C as solutions of 1 mmol/l in absolute ethanol. Calcium ionophore (A23187), dibutyryl cyclic 3'5'-monophosphate (dbcAMP) and bacitracin were from Sigma and forskolin was from Calbiochem (Cambridge). All other reagents were of analytical grade.

Radioimmunoassay (RIA) of GH

The GH content of medium and cells was measured by RIA, using reagents supplied by the National Hormone and Pituitary Agency, Baltimore, MA, U.S.A., as described previously (Clayton, Bailey, Abbot et al. 1986). All samples from one experiment were analysed in the same assay in which the intra-assay coefficient of variation was between 5 and 10%.

Cell cultures and treatments

Pituitary cells from adult female rats were prepared by collagenase, DNase and hyaluronidase digestion as described in detail by Young, Naik & Clayton (1984). This method yielded 0.5–1 x 10⁶ viable cells/pituitary (assayed by trypan blue exclusion). Cells were seeded at a density of 2 x 10⁶/well into Corning multiwell plates in a total volume of 2 ml Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% horse serum and 2.5% fetal calf serum (Gibco, Paisley, Strathclyde), supplemented with antibiotics (Young et al. 1984), 30 pmol T₃/l and 5 nmol dexamethasone/l. Cells were incubated at 37 °C in an atmosphere of 95% air and 5% CO₂ for 3–4 days before use. Before treatment, cells were washed twice with 2 ml DMEM without serum and then incubated with hormones at appropriate concentrations in 1 ml DMEM containing bacitracin (1 mmol/l), T₃ (30 pmol/l), dexamethasone (5 nmol/l) and antibiotics, but without serum, for 4 h or overnight (18 h) (pretreatment period). The pretreatment medium was harvested and stored at −20 °C for RIA of GH. Cells were then washed twice with 2 ml DMEM without serum and then reincubated with or without GRF (10 fmol/l–30 nmol/l or 10 nmol/l) in 1 ml DMEM without serum but with T₃, dexamethasone, bacitracin and antibiotics for 4 h (rechallenge). In some experiments the 4-h rechallenge was with other GH secretagogues which were dissolved in DMEM to give the final concentrations shown in Tables 1 and 2. At the end of the rechallenge period, medium was removed and stored for RIA of GH, and cells were washed twice with PBS before the addition of 1 ml Tris–Triton buffer (Tris–HCl (10 mmol/l; pH 7.6) containing 0.1% Triton X-100) to the wells, which were then frozen at −20 °C. After thawing, refreezing and thawing again, cells were scraped from the wells, debris was
removed by centrifugation at 600 g and the resultant supernatant assayed for GH to provide a measure of cellular GH content. Triplicate or quadruplicate wells/groups were used for all treatments in an experiment. Each experiment was performed three or more times, with different cell preparations.

Growth hormone released into the medium during the 4-h rechallenge period in response to a maximally stimulating dose of GRF (10 nmol/l produced maximal GH release) was used as a measure of desensitization. Because unstimulated (basal) GH release during the rechallenge period was reduced in GRF-pretreated cells and also varied between experiments, the response to GRF has been expressed in terms of the maximal/basal ratio of GH released/4 h. Although providing only one value per experiment, this allows pooling of data from several experiments. In representative experiments, absolute values for GH released/4 h are given. Statistical differences between treatments were determined by analysis of variance and Student’s t-test, with P<0·05 considered to be significant.

RESULTS

GH responses to GRF in cells pretreated with GRF

To ensure adequate desensitization with GRF, initial experiments used a high dose (100 nmol/l) for a long period (18-h pretreatment). The subsequent response of cells to increasing concentrations of GRF is shown in Fig. 1. In this experiment there was essentially no response of GRF-pretreated cells to rechallenge with GRF, in contrast with a sixfold stimulation of GH release by GRF in untreated cells. For the untreated cells the GRF concentration for 50% of maximal GH release (ED50) was 0·34 nmol/l and maximal release was achieved with 10 nmol GRF/l. In another experiment in which cells were pretreated with 100 nmol/l for 18 h, maximal GH release on rechallenge with GRF was 2·3 times the basal release compared with 3·7 times for untreated cells. In the second experiment in GRF-pretreated cells, the ED50 concentration was 2 nmol/l, whereas in control cells it was 0·3 nmol/l. On the basis of these results a GRF concentration of 10 nmol/l was used for the determination of maximal GH secretion from GRF-pretreated cells in subsequent experiments.

There was no difference in maximal/basal GH release during the subsequent rechallenge period with 10 nmol GRF/l from cells pretreated for either 4 or 18 h with 100 nmol GRF/l (4-h pretreatment, maximal/basal GH release = 4·09±0·8 (s.e.m.) untreated vs 1·61±0·09 GRF-pretreated (five experiments with three of four replicates/treatment); 18-h pretreatment, maximal/basal GH release = 3·21±0·4 untreated vs

\[1·56±0·08\] GRF-pretreated (eight experiments with three or four replicates/treatment)). Although there was an indication that GRF responsiveness was better in cells incubated in medium alone for only 4 h, this was not significant. The dependence of desensitization on pretreatment GRF concentration was therefore assessed after 4 rather than 18 h, and the results are shown in Fig. 2a. This shows a dose-dependent effect of GRF, with 0·1 nmol/l being marginally effective and increasing GRF concentrations progressively reducing the maximal/basal GH release during the subsequent rechallenge with 10 nmol GRF/l, such that 100 nmol GRF/l produced a similar desensitization as that described above.

In one experiment, cells were exposed to GRF (100 nmol/l), gonadotrophin-releasing hormone (GnRH; 1 μmol/l), or thyrotrophin-releasing hormone (TRH; 1 μmol/l) for 18 h before challenge with 10 nmol GRF/l. Maximal/basal GH release/4 h was 2·4, 1·8, 2·6 and 2·5 for untreated, GRF (100 nmol/l)-pretreated, GnRH-pretreated and TRH-pretreated cells respectively. Release of GH during the 18-h pretreatment period was 0·34±0·02 (n=four wells), 1·59±0·01 (P<0·001 vs untreated), 0·29±0·02 and 0·3±0·03 mg/l for untreated, GRF-, GnRH- and TRH-pretreated cells respectively. Thus other hypothalamic releasing factors did not impair somatotroph responsiveness to subsequent GRF challenge.

GH responses to forskolin and A23187 in cells pretreated with GRF

The effect of GH secretagogues, which do not act through the GRF receptor, on GH release

\[\text{FIGURE 1. Release of GH during a 4-h rechallenge with increasing concentrations of GH-releasing factor (GRF) from cultured rat pituitary cells exposed for 18 h to 100 nmol GRF(1-40)/l (○) or to medium alone (●). Values are means ± s.e.m.; n = three wells/point.}\]
from desensitized somatotrophs was also examined. Forskolin, a direct activator of the catalytic subunit of adenylate cyclase, was chosen because its mechanism of action resembles that of GRF, though bypassing the receptor. The calcium ionophore, A23187, stimulates GH release by raising intracellular free Ca$^{2+}$ concentration, and thus probably acts independently of changes in cellular cAMP. For these experiments, cultured cells were treated with 100 nmol GRF/I for either 4 or 18 h before rechallenge with GRF (10 nmol/I), forskolin (5 µmol/I) or A23187 (10 µmol/I). These concentrations of forskolin (Clayton et al. 1986) and A23187 (Young, Naik & Clayton, 1985) are effective GH secretagogues and are non-toxic to cells. The results of these treatments are shown in Table 1 (pooled data from four or five experiments). In untreated cells the GH-releasing ability of 5 µmol forskolin/I and 10 µmol A23187/I was similar to that of 10 nmol GRF/I. The decrease in GH responsiveness of cells pretreated with GRF was also similar for all three secretagogues, especially when expressed as a percentage of responsiveness in untreated cells (Table 1).

**TABLE 1.** Effect of pretreatment with GH-releasing factor (GRF; 4 or 18 h) on GH release from cultured rat pituitary cells in response to rechallenge (4 h) with GRF (10 nmol/I), forskolin (5 µmol/I) or the calcium ionophore A23187 (10 µmol/I). Values are maximal/basal GH release during rechallenge for 4 h with the respective agents after pretreatment. Values are means ± S.E.M. for the number of experiments shown in parentheses in the first column (three or four replicates/treatment within each experiment).

<table>
<thead>
<tr>
<th></th>
<th>Maximal/basal GH release</th>
<th>Pretreatment with GRF (100 nmol/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rechallenge</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GRF (4)</td>
<td>2·93 ± 0·4</td>
<td>1·6 ± 0·16* (54)</td>
</tr>
<tr>
<td>Forskolin (5)</td>
<td>3·4 ± 0·6</td>
<td>2·2 ± 0·44* (65)</td>
</tr>
<tr>
<td>A23187 (5)</td>
<td>3·77 ± 0·81</td>
<td>2·3 ± 0·29* (61)</td>
</tr>
</tbody>
</table>

* $P < 0·01$ compared with respective untreated group (ANOVA and Student’s $t$-test).

**GH content of cells pretreated with GRF with or without somatostatin**

Reduced responsiveness of GRF-pretreated cells to rechallenge with GRF or other GH secretagogues could be related to cellular depletion of GH during the pretreatment period. The GH content of cells pretreated with GRF and with GRF plus somatostatin before rechallenge was expressed as a percentage of that in untreated cells to enable pooling of data from several experiments. Pretreatment with GRF, whether

---

for 4 or 18 h, caused significant depletion of cellular GH content in all experiments, the degree of depletion being similar for both pretreatment periods (18-h pretreatment, 40 ± 6% of untreated (n = six experiments); 4-h pretreatment, 57 ± 3% (n = three experiments)). Pretreatment with GRF plus somatostatin did not significantly reduce cellular GH content, values being 86 ± 10% of that in untreated controls after 4 h of pretreatment (n = 3) and 80 ± 11% (n = 6) after 18 h. These values, however, were significantly (P < 0.001) greater than corresponding values after treatment with GRF alone. Correction of GH release for GH content increases both unstimulated and maximal GRF-stimulated absolute GH release by the same proportion, such that the maximal/basal ratio is unchanged. Likewise, this correction does not alter the GRF ED₅₀ concentration in experiments where GRF-pretreated cells were rechallenged with a range of GRF concentrations.

**Effect of somatostatin combined with GRF on subsequent somatotroph responses to GRF**

The results of a representative experiment in which cells were treated with increasing concentrations of somatostatin (10 nmol/l–1 μmol/l) added simultaneously with GRF (100 nmol/l) for 18 h are shown in Fig. 3. Untreated cells showed a 4.4-fold increase in GH release during the 4-h rechallenge period, while GRF-pretreated cells responded poorly (1.6 times basal). There was a dose-dependent attenuation of GRF-induced desensitization by somatostatin. With 1 μmol somatostatin/l the absolute GH release on rechallenge with GRF was not significantly different from that of untreated cells, but the maximal/basal ratio (3.0) was reduced by virtue of the higher basal (unstimulated) release of GH from the somatostatin plus GRF-pretreated cells (Fig. 3). Because a tenfold molar excess of somatostatin to GRF was most effective at restoring GH responsiveness, 1 μmol somatostatin/l was used in all subsequent experiments.

The results of another experiment where cells were exposed to increasing concentrations of GRF and a fixed concentration of somatostatin (1 μmol/l) for 4 h before rechallenge with 10 nmol GRF/l are shown in Fig. 2b. Compared with cells pretreated with GRF alone (Fig. 2a), absolute maximal and basal GH release on rechallenge with GRF was reduced by somatostatin in all groups. However, the maximal/basal ratio was increased significantly (P < 0.01) with the highest concentration of GRF used for pretreatment (100 nmol/l) which produced the most marked desensitization. In this experiment, somatostatin did not completely restore responsiveness to GRF.

Combined somatostatin (1 μmol/l) plus GRF (100 nmol/l) pretreatment was compared with GRF (100 nmol/l) pretreatment alone, using different cell preparations, four times for 4 h of exposure and three times for 18 h. The results, expressed as maximal/basal ratio of GH release on rechallenge with GRF...
TABLE 2. Effect of somatostatin (SST; 1 μmol/l) on GH-releasing factor (GRF)-induced refractoriness of rat somatotrophs to rechallenge with GRF (10 nmol/l). Values are maximally stimulated (10 nmol GRF/l)/basal (unstimulated) ratios of GH release. There were three or four replicates per treatment within each experiment. Numbers in parentheses represent responses in GRF- or GRF plus SST-pretreated cells as a percentage of the response in untreated cells.

<table>
<thead>
<tr>
<th>Pretreatment time (h)</th>
<th>Maximal/basal GH release</th>
<th>Pretreatment</th>
<th>GRF (100 nmol/l)</th>
<th>GRF (100 nmol/l) + SST (1 μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (Expt 1)</td>
<td>3.92</td>
<td>1.56 (40)</td>
<td>2.54 (65)</td>
<td></td>
</tr>
<tr>
<td>4 (Expt 2)</td>
<td>7.33</td>
<td>1.47 (20)</td>
<td>2.48 (34)</td>
<td></td>
</tr>
<tr>
<td>4 (Expt 3)</td>
<td>3.29</td>
<td>1.79 (54)</td>
<td>2.56 (78)</td>
<td></td>
</tr>
<tr>
<td>4 (Expt 4)</td>
<td>3.04</td>
<td>1.39 (46)</td>
<td>2.13 (70)</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.39 ± 1.0</td>
<td>1.55 ± 0.09 (40 ± 7)</td>
<td>2.43 ± 0.1 (62 ± 10)</td>
<td></td>
</tr>
<tr>
<td>18 (Expt 1)</td>
<td>2.9</td>
<td>1.37 (47)</td>
<td>3.13 (108)</td>
<td></td>
</tr>
<tr>
<td>18 (Expt 2)</td>
<td>4.42</td>
<td>1.65 (37)</td>
<td>3.0 (68)</td>
<td></td>
</tr>
<tr>
<td>18 (Expt 3)</td>
<td>3.68</td>
<td>2.33 (65)</td>
<td>3.0 (81)</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>3.66 ± 0.44</td>
<td>1.78 ± 0.28 (50 ± 8)</td>
<td>3.04 ± 0.04 (86 ± 12)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

These results confirm those of Bilezikjian & Vale (1984) and Ceda & Hoffman (1985) in showing that pretreatment of cultured rat pituitary cells with GRF causes a dose-dependent reduction in GH responsiveness to subsequent rechallenge with GRF. This is manifest by reduction in both sensitivity to GRF (increased ED$_{50}$ concentration) and absolute GH release in response to a maximal GRF stimulus. Indeed, in some experiments there was minimal GH response to GRF rechallenge and an accurate ED$_{50}$ concentration could not be determined. We have also shown that a 4-h pretreatment period with GRF is as
effective as overnight pretreatment (18 h) at inducing desensitization, which agrees with the results of Ceda & Hoffman (1985). Although we have shown that GRF desensitization of somatotrophs was dependent upon the concentration, we required a tenfold higher amount than Ceda & Hoffman (1985) and 20-fold more than Bilezikjian & Vale (1984). Furthermore, our cells were not as sensitive to GRF, since the ED50 concentration (0.3 nmol/l) was about tenfold higher than that reported previously (Bilezikjian & Vale, 1984; Harwood et al. 1984; Ceda & Hoffman, 1985).

The explanation for this discrepancy is not clear, but may be related to differences in the GRF preparation, culture conditions, cells themselves or unknown factors. Despite the above discrepancies of GRF dose–response characteristics, the qualitative results are very similar and demonstrate that GRF, like all other hypothalamic peptides studied to date, desensitizes rat pituitary target cells in vitro.

One obvious explanation for the reduction in maximum responsiveness of desensitized somatotrophs to rechallenge with GRF is a marked depletion in intracellular hormone content, rather than, or in addition to, disruption of the stimulus–secretion coupling mechanism(s) (i.e. true desensitization). Indeed, some observations in this study suggest that this is possible: (1) the extent of cellular GH depletion was very similar to the degree of reduction in maximal responsiveness to GRF and (2) maximal GH release in response to non-GRF secretagogues (forskolin and A23187) was reduced by a similar extent to that in response to GRF rechallenge (Table 1). That this is not the only mechanism involved is apparent from evidence in this and other studies. First, reduction in cellular GH content would not be expected to increase the ED50 concentration of GRF (this study; Bilezikjian & Vale, 1983). Indeed, correction for reduced GH content did not restore full responsiveness to GRF either in terms of maximum GH release or ED50 concentration. A similar conclusion regarding correction for hormone depletion was reached by Smith, Perrin & Vale (1983) and by Young et al. (1985) for GnRH desensitization of gonadotrophs. Secondly, depletion of cellular GH by pretreatment with dbcAMP did not alter the ED50 concentration of GRF in subsequent dose–response studies (Bilezikjian & Vale, 1984). Moreover, although not demonstrated here, but shown by Ceda & Hoffman (1985), desensitized somatotrophs responded as well as untreated cells to dbcAMP and forskolin. Thirdly, somatostatin prevented GRF-induced depletion of GH stores, but did not fully restore responsiveness to GRF (Fig. 2b and Table 2), especially after pretreatment for 4 h. Collectively, the aforementioned observations indicate that mechanisms in addition to depletion of readily releasable GH stores are involved in GRF desensitization of somatotrophs. Although no data have yet been reported, GRF receptor down-regulation may contribute to this desensitization, a situation analogous to that pertaining in GnRH-desensitized gonadotrophs (Clayton, 1982; Young et al. 1985).

Uncoupling of the GRF receptor from adenylate cyclase is suggested by the data of Ceda & Hoffman (1985), though Bilezikjian & Vale (1984) showed less effect of GRF pretreatment on subsequent cAMP generation than on GH release, implying that disruption of steps distal to cAMP production contribute to the desensitization processes. Although cAMP production was not measured in the present study, our data with forskolin and A23187 would be consistent with a defect late in the pathway of GH release which is shared by cAMP and Ca2+.

Of particular interest is our consistent observation that somatostatin can partially reverse GRF-induced desensitization of somatotrophs. While this finding may be partly related to partial prevention of cellular GH depletion, restoration of somatroph sensitivity to GRF implies an action at the level of the effector mechanism(s). Since somatostatin partially antagonizes GRF stimulation of adenylate cyclase (Bilezikjian & Vale, 1983; Michel et al. 1983; Harwood et al. 1984), it is tempting to invoke this as a possible site for attenuation of desensitization. As previously stated, however, cAMP production is not markedly disrupted in GRF-desensitized somatotrophs (Bilezikjian & Vale, 1984), raising the possibility that other post-receptor reactions, such as membrane polyphosphoinositide turnover and protein kinase C activation, might be interrupted during desensitization and that these remain intact in somatotrophs concurrently treated with somatostatin. Edwards, Diequez, Peters et al. (1986) reported that somatostatin largely prevented the GRF-induced reduction in maximal GH response to rechallenge, although somatostatin did not reduce the ED50 concentration of GRF. While the latter observation is at variance with our present results, their general conclusions are consistent with ours regarding the cellular mechanisms of desensitization.

These observations with somatostatin in vitro have important implications for interpretation of in-vivo studies with long-term GRF treatment. If, as seems likely, somatostatin attenuates GRF desensitization as well as acute GH release in vivo (Davies et al. 1985), this could explain the observations of Vance et al. (1985) and Davis et al. (1986) that episodic GH secretion continues during prolonged GRF infusion (up to 24 h), rather than falling progressively. In this regard it is perhaps pertinent that the refractoriness to prolonged GRF infusion in animals has been demonstrated in rats treated concurrently with a
somatostatin antiserum (Wehrenberg et al. 1984), although these authors did not include a comparison with animals in which endogenous somatostatin action was preserved. Thus, in animals with intact somatostatin secretion and action, much larger amounts of GRF for longer periods than have been used hitherto may be required to desensitize the somatotroph completely and eliminate GH secretion.

In conclusion, we have shown that GRF-induced desensitization of somatotrophs can be partially reversed by the GH counter-regulatory hormone, somatostatin. This is unlikely to be entirely caused by restoration of releasable GH stores but by interaction with other cellular mechanisms responsible for GRF desensitization, which remain to be defined.

REFERENCES


