Increased hypothalamic somatostatin mRNA following dexamethasone administration in rats

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There is increasing evidence to suggest that supraphysiological doses of glucocorticoids suppress growth hormone secretion in vivo by augmenting somatostatin release from the hypothalamus; previously, we reported an increase in hypothalamic somatostatin content in dexamethasone-treated rats. To further examine whether the production of somatostatin really is augmented, hypothalamic somatostatin mRNA levels were determined by the Northern blot technique in female rats receiving 330 μg of dexamethasone daily for three days. In two series of experiments, hypothalamic somatostatin mRNA levels in dexamethasone-treated rats were significantly (p<0.05) increased to 133±19 (mean±sd)% and 153±38% of the controls. In the dexamethasone-treated rats, plasma growth hormone levels were markedly suppressed compared with those of the controls. These results further support the hypothesis that pharmacological doses of glucocorticoids increase the production and release of somatostatin from the hypothalamus and thus inhibit growth hormone secretion, overriding the direct stimulatory effect of glucocorticoids on growth hormone production at the pituitary level.

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The effects of supraphysiological doses of glucocorticoids on growth hormone (GH) secretion are complicated; glucocorticoids have been generally considered as the inhibitor of GH secretion in vivo (1–6). However, the stimulation in the early phase (7–10) or diverse effects, depending on the time of day and/or the sort of stimuli (11), was also reported recently. On the other hand, when pituitary somatotropes are incubated with these steroids in vitro, the production of GH is consistently stimulated (12–15), though the release is initially inhibited (16–17).

We previously reported (18) that in vivo administration of pharmacological doses of dexamethasone for three days in rats caused the hypothalamic content of immunoreactive somatostatin to increase and of GHRH to decrease. If the changes in hypothalamic content parallel those in release from the hypothalamus, it can be postulated that these changes of hypothalamic hormones override the direct stimulatory effect of the steroid at the pituitary level, resulting in a net inhibitory effect in vivo.

The present study was carried out to confirm this hypothesis by examining the changes in hypothalamic somatostatin mRNA as a much more direct parameter of somatostatin production, in in vivo administration of dexamethasone to rats.

Materials and methods

In each of two series of experiments, 4–5 Wistar-strain female rats were given 400 μg sc of dexamethasone sodium phosphate (Decadron®, Nippon Merk Banyu Co, diluted 1:4 with physiological saline), equivalent to 330 μg of dexamethasone daily in two divided doses for three days. The same number of rats received the same volume of physiological saline in the same time schedule. The body weights of the rats at the beginning of the study were 282.2±14.2 g (mean±sd; controls) and 284.8±17.8 (dexamethasone-treated) in Experiment I and 268.0±19.2 (controls) and 266.8±18.7 (dexamethasone-treated) in Experiment II.

Four hours after the last injection, they were sacrificed by decapitation and the blood was collected. The hypothalami, delineated anteriorly by the optic chiasm, posteriorly by the mamillary bodies, laterally by the sulci formed with the temporal lobes, and superiorly by a plane 3 mm dorsal to the ventral surface of the median eminence, was removed and RNA immediately extracted by the method of Chomczynski and Sacchi (19). After electrophoresis on 1.5% agarose gel, one hypothalami extract in one well, the RNA was transferred to a nylon membrane (Hybond-N, Amershams, England).
The rat somatostatin cDNA probe was provided by Dr MR Montminy of the Salk Institute, CA. A $^{32}$P-labelled antisense cRNA probe was generated with SP6 RNA polymerase using an SP6/T7 transcription kit (Boehringer) and cytidine 5'-[alpha-$^{32}$P] triphosphate (Amersham, England).

The somatostatin mRNA on the membrane was hybridized with this labelled probe in the solution containing 50% deionized formamide, 5× standard saline citrate (SSC), 50 mmol/l TRIS–HCl buffer (pH 7.5), 0.1% sodium pyrophosphate, 1% sodium dodecyl sulphate (SDS), 0.2% polyvinylpyrrolidone, 0.2% ficoll, 5 mmol/l EDTA and 190 μg/ml denatured salmon sperm DNA at 65°C for 18 h. The membrane was rinsed twice in 2× SSC at room temperature for 15 min, twice in 2× SSC and 0.1% sds at 70°C for 15 and 20 min, respectively, and twice in 0.1× SSC and 0.1% sds at 70°C for 15 and 20 min, respectively. The autoradiography was performed and the density of the spots corresponding to approximately 0.8 kilo base pairs (kbp) (Fig. 1) was measured using the Tefco image analysis system (TIAS 100, ACI, Japan).

The blots were stripped in boiling 0.1× SSC and 0.1% sds, and reprobed with a $^{32}$P-labelled alpha-tubulin oligonucleotide probe at 43°C in the solution containing 50% deionized formamide, 0.65 mol/l NaCl, 0.1 mol/l Na-Pipes, 10% Denhardt’s solution, 0.1% sds, 5 mmol/l EDTA and 100 μg/ml denatured salmon sperm DNA. The labelled probe was prepared with an oligonucleotide 5’-end labelling system (NEN, Boston), alpha-tubulin oligonucleotide probe (958–987; NEN, Boston) and ATP tetra-triethylammonium salt [gamma-$^{32}$P] (NEN, Boston). The membrane was rinsed twice in the solution containing 2× SSC, 0.2% sodium pyrophosphate, 0.1% sds at room temperature and once in the same solution at 43°C, each for 20 min. The autoradiography and densitometry of the spots corresponding to approximately 1.7 kbp (Fig. 1) was carried out just as in the case of somatostatin mRNA.

To account for the variations in sample handling, the relative amount of somatostatin mRNA was expressed as the density for somatostatin mRNA divided by that for alpha-tubulin mRNA in each spot. The relative density was then expressed as a percentage of the mean value in the control group for each series.

Plasma rat GH levels were determined by the radioimmunoassay method reported previously (18). The significance of difference was tested by the unpaired t-test.

Fig. 1. Part of an autoradiogram of a Northern blot of hypothalamic RNA extract. Two autoradiograms, one for somatostatin mRNA the other for alpha-tubulin mRNA, are superimposed. C: Control. D: Dexamethasone-treated.

Fig. 2. Hypothalamic somatostatin (SS) mRNA (upper panel) and plasma GH levels (lower panel) in control (open bars) and dexamethasone treated (dotted bars) rats in experiments I and II. SS mRNA levels are expressed as percentages of the mean of the controls in each experiment. Horizontal bars indicate 1 se of the mean.
Results

Plasma GH levels were significantly suppressed after three-day in vivo administration of pharmacological doses of dexamethasone in both series of experiments: 26.6 ± 10.1 μg/l in the controls vs 5.6 ± 2.9 in the dexamethasone-treated rats (p < 0.01) in Experiment I and 34.3 ± 10.9 vs 8.2 ± 3.8 (p < 0.01) in Experiment II (lower panel of Fig. 2).

The hypothalamic contents of somatostatin mRNA of dexamethasone-treated rats were significantly increased compared with those of the controls in both series: 133 ± 19% of the controls (100 ± 14%; p < 0.05) in Experiment I and 153 ± 38% of the controls (100 ± 15%; p < 0.05) in Experiment II (upper panel of Fig. 2).

Discussion

We previously reported that the hypothalamic content of somatostatin was increased by three-day treatment with pharmacological doses of dexamethasone (18). The present study was undertaken to examine whether the change in content really reflects that of production, by measuring intracellular mRNA; the increase in hypothalamic somatostatin mRNA content by in vivo administration of dexamethasone was confirmed. It is possible that this increase does not reflect an increased production of the mature peptide and its release, as glucocorticoids might also affect translational and post-translational steps. However, the coincidental marked suppression of plasma GH levels suggests the appropriate increase of somatostatin production and release, although complete proof has to await the measurement of somatostatin concentration in hypophyseal portal blood.

Zeitler et al. (20) recently reported that hypothalamic somatostatin mRNA content varied reciprocally with the presumed secretion of somatostatin in ultradian rhythm in male rats. However, the relationship between content or release and production in such short cycles ought to be different from that in relatively long-term treatment, as in our present study.

Since our previous report, increasing evidence suggests an augmented somatostatin release from the hypothalamus with pharmacological doses of glucocorticoids; Wehrenberg et al. (21) reported that immunoneutralization of somatostatin in dexamethasone-treated conscious rats resulted in an enhanced GH response to GHRH, in contrast to a blunted response without anti-somatostatin antisemur injection. Giustina et al. (22) found that pyridostigmine, a presumed inhibitor of somatostatin release, blocked the inhibitory effect of ghrelin on GHRH-stimulated GH secretion, although another report (23) with a smaller dose of GHRH and a larger dose of ghrelin showed only a partial block. Furthermore, Corder et al. (24) reported that the depletion of hypothalamic GHRH by neonatal monosodium glutamate treatment revealed the inhibitory effect of betamethasone on GH secretion in adult rats.

The results presented here further support the hypothesis that supraphysiological doses of glucocorticoids augment hypothalamic somatostatin synthesis and release, and consequently suppress GH secretion in vivo, overriding the direct stimulatory effect of glucocorticoids on GH production at the pituitary level.

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