Differential effect of insulin-like growth factor-I and growth hormone on hypothalamic regulation of growth hormone secretion in the rat


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ABSTRACT. Pharmacological administration of either growth hormone (GH) or insulin-like growth factor I (IGF-I) were reported to inhibit endogenous GH release in humans and in the laboratory animal. We have evaluated the short-term differential mechanisms whereby the two hormones affect hypothalamic regulation of GH secretion. Wistar male rats (90 days old) were injected i.p. with either GH (recombinant GH NIAMDD, Baltimore, MD, USA), rIGF-I (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) or saline. Animals were sacrificed at 15, 30, 60 and 120 minutes following injection. Hypothalami were dissected and extracted immediately and the levels of growth hormone-releasing hormone (GHRH) and somatostatin were determined using specific antisera. Trunk blood was collected for GH and IGF-I determination by RIA. Administration of IGF-I or GH markedly decreased hypothalamic somatostatin stores by 77% and 54% respectively, within 15 minutes. Concomitantly, the wide range of GH levels found in the control group was reduced in the IGF-I treated group suggesting that the pulsatile pattern of GH secretion was suppressed. Growth hormone administration induced an increase in hypothalamic GHRH stores (60% at 120 minutes). During this period serum IGF-I levels were not altered. It is suggested that short term modulation of hypothalamic neurohormones by GH and IGF-I is mediated by rapid stimulation of somatostatin release by both hormones, and inhibition of GHRH release is induced only by GH.

INTRODUCTION

The complex mechanism regulating growth hormone (GH) secretion involves three different pathways which affect the synthesis and release of hypothalamic GH-releasing hormone (GHRH) and somatostatin. The input for this regulation arrives from: a) central neurotransmitter systems (1-3); b) peripheral signals induced by circulating hormones, such as GH and insulin-like growth factor-I (IGF-I) either by a direct effect at the pituitary level, or at the median eminence, which is incompletely covered by the blood brain barrier (4-6); c) intra-hypothalamic short-loop transmission interacting between GHRH and somatostatin neurons (7-9). Previous studies have demonstrated that acute administration of GH or IGF-I to normal man and laboratory animals inhibited endogenous GH release, as reflected by a reduced responsiveness to provocative stimuli such as GHRH or clonidine (10-11) or by impairment of the pulsatile release of GH (12). Studies performed in the conscious rat support the major role of GHRH rather than of somatostatin in the autofeedback effect of GH (13), while hypothalamic somatostatin seems to be involved in the suppressive effect of IGF-I (14). In view of the wide clinical use of recombinant GH for therapy of various conditions in subjects without any evidence of GH deficiency, and the introduction of replacement therapy with IGF-I in children with GH insensitivity (Laron Syndrome = LS) (15-17) and in some conditions of diabetes associated with insulin resistance, it seemed of interest to study the differential short term effects of IGF-I and GH on hypothalamic GHRH and somatostatin content and on plasma GH levels.

MATERIALS AND METHODS

Ninety-day-old Wistar male rats weighing approximately 250gr were used. The animals were kept in...
controlled conditions, with a light/dark cycle (14:10 hrs) and were fed ad libitum. Recombinant IGF-I (FK 780 Lot 104982K - Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) and rat GH (recombinant GH B-11, the gift of NIAMDD, Baltimore, MD, USA) were injected i.p. Animals injected with the vehicle, 0.9% saline, served as controls.

Two types of experiments were performed:
1. Time effect: IGF-I (0.1 mg/kg) GH (40 μg/kg) or 0.9% saline were administered ip and the animals were sacrificed 15, 30, 60 and 120 minutes later.
2. Dose effect: IGF-I was injected at increasing doses of 0.03, 0.1 and 0.3 mg/kg and animals sacrificed 30 minutes later.

The rats were killed by decapitation and trunk blood was collected. After separation, the serum was kept at -20°C until determination of GH and IGF-I. Brains were immediately removed. Medial basal hypothalamus bounded by the optic chiasm, the lateral grooves and the mamillary bodies were dissected as a block weighing 16±2 mg and immersed in boiling phosphate buffered saline (PBS, 0.05 mol/l pH 7.1) for 2 minutes. The tissue was then homogenized, reboiled and centrifuged. The supernatant was collected and frozen until assayed for GHRH and somatostatin. This procedure has been demonstrated adequate for the quantitative extraction of hypothalamic neuropeptides (18).

Growth hormone releasing hormone was determined in 100 μl of the extracted hypothalamic tissue by a double antibody RIA using rat GHRH 1-43 (Peninsula, St. Helens, UK) as standard and specific antiserum kindly donated by Prof. E.E. Müller (Inst. of Pharmacology, Milan, Italy). 125I labelled GHRH 1-44 (Amersham International, Amersham Bucks, UK) was used as tracer (100% cross-reactivity was found between the antiserum and human GHRH 1-44). No cross-reactivity was found with glucagon, vasoactive intestinal peptide, thyrotropin releasing hormone, somatostatin or GH.

The sensitivity of the assay was 30 pg/tube and the intra- and inter assay variations were 12% and 18% respectively. Recovery of GHRH added before extraction was 90-95%.

Rat GH determination was performed using antigen and antiserum that were kindly provided by NIAMDD (Baltimore, MD, USA), using recombinant GH RP-2 as the standard. The sensitivity of the assay was 1.2 ng/ml and inter- and intra-assay variations were 7% and 9% respectively.

Insulin-like growth factor-I was measured by RIA. Serum samples were extracted using the cryoprecipitation technique as described by Brier et al (20) prior to RIA determinations of IGF-I. Rabbit polyclonal antiserum to IGF-I (B01066S), and rIGF-I reference standard (FR 61780) were the products of Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan. The sensitivity of the assay was 0.03 ng, and intra- and interassay variations were 4.7% and 7.0% respectively.

Statistical evaluations were carried out by using one-way ANOVA with the Duncan multiple range test. Values of p < 0.05 were considered significant.

RESULTS

Figure 1 demonstrates the results of the time-dependent effects of GH or rIGF-I administration on GH, somatostatin and GHRH levels. Control animals treated with 0.9% saline did not show any significant difference at all time points in either hypothalamic content of GHRH or of somatostatin, and therefore all control groups were combined. Plasma GH levels of the control groups showed a high range of variability due to physiological pulsatility. Bolus administration of IGF-I (0.1 mg/kg, ip) induced a marked and rapid depletion in hypothalamic somatostatin (77%), GHRH (34%) stores (p<0.001), with nadir levels at 15 minutes for both neurohormones. Hypothalamic somatostatin content remained low for at least 2 hours. Hypothalamic GHRH levels fluctuated between normal (30 minutes) and slightly depressed levels (60 minutes). The normal pulsatility in GH secretion was abolished at 15 and 30 minutes after IGF-I administration.

Plasma GH levels were significantly lower at 15 and 30 minutes after IGF-I administration (Fig. 1). A similar lowering of plasma GH levels due to IGF-I administration was also observed (Fig. 2) in the other set of experiments dealing with the dose-dependent effect of IGF-I (doses of 0.1 and 0.3 mg/kg). Serum glucose levels showed were not changed at 15 and 30 minutes after IGF-I administration (0.1 μg/kg). Only 60 minutes after IGF-I administration a slight but significant hypoglycemic state was observed (12.3±0.9 nmol/l vs 15.4±0.4 nmol/l; p < 0.01).