Effect of GRF and Somatostatin on 7B2 Secretion by Rat GH₁ Cells

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Abstract

A novel pituitary protein "7B2" was secreted by GH₁ cells. The secretion of 7B2 was increased in the presence of human GRF in a dose-responsive manner. In contrast, a somatostatin analog, SMS 201-995, revealed the inhibitory effects on the basal- and GRF-induced secretion of 7B2 at the concentration of 10⁻⁷M. These findings suggest that 7B2 is a secretory protein of rat GH₁ cells under certain conditions.

A novel protein "7B2", initially isolated from human and porcine pituitary glands (Hsi *et al.*, 1982; Seidah *et al.*, 1983), is distributed widely in the rat, with high concentrations in the neuroendocrine tissues (Iguchi *et al.*, 1984). Immunocytochemical studies revealed the presence of 7B2 in the gonadotrophs and thyrotrophs of the rat pituitary gland (Marcinkiewicz *et al.*, 1987). Release of 7B2 induced by high K⁺ and/or LHRH was also demonstrated using cultured cells of the rat pituitary gland (Iguchi *et al.*, 1984; Deng *et al.*, 1986). These observations indicate that 7B2 is a secretory protein in the rat pituitary gland. The presence of 7B2 was also noted in the

human GH-producing pituitary adenoma and an increase in plasma 7B2 after an administration of GRF was found in some patients with acromegaly but not in normal subjects (Natori *et al.*, 1988). These findings suggest that 7B2 could be secreted by human GH-producing pituitary adenoma and its secretion is regulated by GRF.

This work was undertaken in order to investigate 7B2 secretion under certain conditions by the rat adenomatous GH secreting cells (GH₁ cells).

Materials and Methods

Cell culture

GH₁ cells, established from rat GH secreting pituitary tumor, were obtained from American Type Culture Collection (Rockville, Maryland, USA) and maintained in T-flasks (Corning Glass Works, Corning, NY, USA) as a monolayer cul-

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ture in Ham's F-10 medium supplemented with horse serum (15%) and fetal bovine serum (FBS) (2.5%). Culture reagents were purchased from Gibco (Grand Island, NY, USA). Human GRF (1-44)NH₂(hGRF) was purchased from Peptide Institute (Minoh, Japan) and SMS 201-995 was a gift from Sandoz Ltd. (Basel, Switzerland). The cells were detached from the surface of the T-flasks by the trypsin treatment and replated in the 35 mm-dishes (Corning) at a density of 5×10^5 cells/dish. On the 3rd day after replating, the release experiment was performed. The medium was removed and the cells were washed twice with 2 ml Ham's F-10 containing 2.5% FBS. Then, 1 ml Ham's F-10 containing 2.5% FBS with KCl (50 mM), hGRF (10^{-7} , 10^{-6} , 10^{-5} M), SMS 201-995 (10^{-9} , 10^{-7} , 10^{-5} M) and hGRF (10^{-6} M) + SMS 201-995 (10^{-9} , 10^{-7} , 10^{-5} M) were added and the cells were allowed to incubate for 1 h at 37°C under 5% CO₂-95% air. The medium was collected at the termination of incubation and centrifuged at $200 \times g$ for 5 min to remove desquamated cells, and the supernatant was stored at -20°C until assay. The cells in each dish were scraped into 1 ml of 1 M acetic acid and extracted by sonication. After centrifugation, the supernatant was stored at -20°C until assay.

Protein concentrations of the cell extracts were determined with a Bio-Rad protein assay kit (Richmond, CA, USA) with BSA as a standard.

Gel permeation chromatography

Gel permeation chromatography of the culture medium and cell extract was performed on a Sephadex G-100 column (95 $\times$ 1.4 cm). Samples (2 ml) were layered onto the column and eluted with 1 M acetic acid at 7 ml/h at 4°C. Each fraction (1.3 ml) was dried with a centrifugal concentrator (Taiyo VC-36, Taiyo Scientific Industrial Co. LTD., Tokyo Japan) and reconstituted with RIA-buffer before assay. The column was calibrated with protein markers (Vo, catalase; 43K, ovalbumin; 25K, chymotrypsinogen A; 13.7K, ribonuclease).

RIA

Immunoreactive (IR)-7B2 was measured by RIA as described previously (Iguchi *et al.*, 1984). A 7B2 antiserum was raised against a synthetic fragment of 7B2, corresponding to amino acids 23-39 of authentic porcine 7B2 (7B2 23-39), coupled to bovine thyroglobulin in rabbits. The antiserum did not cross-react

with POMC-related peptides, GH, LH, FSH, TSH, PRL, AVP, oxytocin, neurophysin, CRF, GRF, somatostatin, calcitonin, insulin, glucagon, pancreatic polypeptide, secretin, substance P, dynorphin 1-13, Met⁵-enkephalin and thyroglobulin. 7B2 23-39 and ¹²⁵I-7B2 23-39 were used as standard and tracer, respectively. Intra- and interassay coefficients of variation were 9% and 12%, respectively (n=5). Dilution curves of the culture medium and cell extract revealed a parallel displacement with a RIA standard curve (data not shown).

GH was measured by double-antibody RIA using materials provided by the NIADDK Rat Pituitary Hormone Distribution Program. Intra- and interassay coefficients of variation were less than 15% (n=3).

Statistics

Student's *t*-test was performed to assess the significant difference between medium concentrations of IR-7B2 or GH in each group.

Results

Contents of IR-7B2 and GH in GH₁ cells

Cell contents of IR-7B2 and GH (mean $\pm$ SD, n=8) were 943 ± 276 and 2127 ± 177 fmol/mg protein, respectively.

Effects of high K⁺ and hGRF on the secretion of IR-7B2 and GH by GH₁ cells

IR-7B2 was secreted by GH₁ cells and the secretion of IR-7B2 was increased about 3-fold by high K⁺-induced depolarization (data not shown). GH was also secreted by high K⁺ but the magnitude of the increase was less than that of IR-7B2 (data not shown). The medium concentration of IR-7B2 was significantly increased in the presence of 10^{-6} M ($P < 0.01$) and 10^{-5} M hGRF ($P < 0.01$), but secretion of IR-7B2 remained unchanged in the presence of 10^{-7} M hGRF (Fig. 1). The medium concentration of GH was also increased in the presence of 10^{-5} M hGRF ($p < 0.05$) (Fig. 1).

In the presence of 10^{-7} and 10^{-6} M hGRF the medium GH concentrations tended to increase, but the increase was not statistically significant (Fig. 1).

Effects of SMS201-995 on the basal and GRF(10^{-6} M)-induced secretion of IR-7B2 and GH by GH₁ cells

As shown in Fig. 2, the addition of SMS 201-995 caused a significant decrease

Fig. 1. Effects of GRF (10^{-7} , 10^{-6} and 10^{-5} M) on the secretion of IR-7B2 and GH by GH₁ cells. Each bar represents the mean ($\pm$ SD) concentrations of IR-7B2 (hatched) and GH (white) in the medium after the 1h-incubation with or without GRF in the four replicate dishes. * $P < 0.05$, ** $P < 0.01$ (vs. paired control)

Fig. 2. Effects of SMS 201-995 (10^{-9} , 10^{-7} and 10^{-5} M) on the basal secretion of IR-7B2 and GH by GH₁ cells. Each bar represents the mean ($\pm$ SD) concentrations of IR-7B2 (hatched) and GH (white) in the medium after the 1h-incubation in the presence or absence of SMS 201-995 in the four replicate dishes. * $P < 0.01$ (vs. paired control)

Fig. 3. Effects of SMS 201-995 (10^{-9} , 10^{-7} and $10^{-5}M$) on the GRF ($10^{-6}M$)-induced secretion of IR-7B2 and GH by GH₁ cells. Each bar represents the mean ($\pm$ SD) concentrations of IR-7B2 (hatched) and GH (white) in the medium after the 1h-incubation with or without SMS 201-995 in the presence of $10^{-6}M$ GRF. Four dishes were used for each group.

* $P < 0.05$, ** $P < 0.01$ (vs. paired control)

in the basal secretion of IR-7B2 at the concentration of $10^{-7}M$ ($P < 0.01$), however, the maximal inhibition was obtained at $10^{-7}M$ and the higher dose was toxic to the cells. Such inhibition by SMS 201-995 of GH secretion was also noted, but it was not statistically significant (Fig. 2). Similarly, hGRF ($10^{-6}M$)-induced secretion of IR-7B2 was significantly decreased in the presence of SMS201-995 at the concentration of $10^{-7}M$ ($P < 0.01$) and $10^{-5}M$ ($P < 0.05$), and this inhibitory effect of SMS 201-995 was maximum at $10^{-7}M$ (Fig. 3). GH secretion in the presence of $10^{-6}M$ hGRF was significantly inhibited by $10^{-7}M$ SMS 201-995 ($P < 0.05$) although $10^{-6}M$ hGRF did not cause a significant increase in the medium GH concentration (Fig. 3).

Gel permeation chromatography

Fig. 4 depicts the elution profiles of the cell extract and culture medium of GH₁ cells on a Sephadex G-100 column. One peak of IR-7B2 with an apparent mol wt of 45K was found in the cell extract,

whereas a major peak of IR-7B2 with an apparent mol wt of 45K and a minor peak with an apparent mol wt of 13K were found in the culture medium (Fig. 4).

Discussion

7B2 is a secretory protein of the neuro-endocrine tissues of several species (Iguchi *et al.*, 1984; Deng *et al.*, 1986; Iguchi *et al.*, 1987; Natori *et al.*, 1988; Venetikou *et al.*, 1988). 7B2 is present in human plasma and an increase in plasma 7B2 was noted in some patients with various endocrine tumors (Suzuki *et al.*, 1986; Iguchi *et al.*, 1987). Increased plasma 7B2 in these patients could be attributed to the secretion of 7B2 by the tumors, although the origin of plasma 7B2 in normal humans is uncertain. Recently, Natori *et al.* (1988) observed an increase in plasma 7B2 in response to GRF *in vivo* and a high K^{+} -induced release of 7B2 *in vitro* in acromegalic patients. However, little is known of the

Fig. 4. Gel permeation chromatography of the cell extract (upper panel) and culture medium (lower panel) of GH₁ cells on a Sephadex G-100 column (95 × 1.4 cm). Samples (2ml) were layered onto the column and eluted at 7 ml/h at 4 C. Each fraction was assayed for 7B2. Molecular markers are Vo, catalase; 43 K, ovalbumin; 25 K, chymotrypsinogen A and 13.7 K, ribonuclease.

mechanism regulating 7B2 secretion from these tissues. Clonal lines of rat pituitary tumor cells (GH cells) are widely used in studies of the mechanism regulating of pituitary function. Inhibition of membrane adenylate cyclase activity by somatostatin was demonstrated in GH₁ cells (Heindel *et al.*, 1978), but GH release from the cells induced by GRF has not been reported to my knowledge. In GH₃ cells, on the other hand, hGRF did not affect either the secretion of GH by the cells or the cellular GH-mRNA concentration (Zeytin *et al.*,

1984). Thus, we used GH₁ cells and studied the responsiveness of these cells to hGRF or somatostatin. IR-7B2 was secreted by GH₁ cells and this secretion of IR-7B2 was increased by adding hGRF in a dose-responsive manner. On the other hand, the somatostatin analog SMS201-995 inhibited the basal secretion of IR-7B2 as well as the hGRF-induced IR-7B2 secretion. These findings suggest that 7B2 is a secretory protein of the rat GH₁ cells and that this secretion of 7B2 is under certain control. In human subjects, on the other hand, an

increase in plasma 7B2 after an i. v. bolus infusion of GRF was noted only in patients with acromegaly (Natori *et al.*, 1988). This suggests the GRF-induced secretion of 7B2 by the GH secreting pituitary adenoma. 7B2 has been shown to be present in gonadotrophs and thyrotrophs of the rat pituitary gland (Marcinkiewicz *et al.*, 1987). However, present studies together with the findings described by Natori *et al.* (1988) suggest the presence of 7B2 in the adenomatous GH secreting cells in the human and rat. 7B2 may be applicable to a clinical utility as a marker for acromegaly.

A significant increase in the medium GH concentration after an addition of 10^{-5} M hGRF was also noted, although its effect on hGRF was weak. This suggests that GH release is induced by hGRF even in GH₁ cells. Such a weak effect of hGRF on the GH secretion by the cells may be due to species' difference of GRF. The magnitude of the increase or decrease in IR-7B2 and GH in the presence of hGRF or a somatostatin analog were different. This may be attributed to the localization of IR-7B2 and GH in the different secretory granules.

In the analysis of the molecular form of IR-7B2 on gel permeation chromatography, different elution profiles were obtained, i. e. only a 45K-peak in the cell extract and 45K- and 13K-peaks in the culture medium. Earlier studies revealed that the 45k-peak of IR-7B2 obtained by gel permeation chromatography shifted to 20–22K in the analysis on SDS-PAGE under the reduced condition (Iguchi *et al.*, 1984). The mol wt of 7B2, deduced from the human pituitary cDNA, is 20793 (Martens, 1988). Thus, the 45K-7B2 could be a dimer. On the other hand, the smaller mol wt form of IR-7B2 (13K) was found only in the culture medium. A 7B2 molecule contains three sites for possible proteolytic cleavage (Martens, 1988) and the N-terminal amino acid sequence of 13K-7B2 was

identical with that of 22K-7B2 in the human tissues (Marcinkiewicz *et al.*, 1986). The 13K-7B2 found in the culture medium in the present study is likely to be a flanking peptide after proteolytic cleavage. A possible explanation for the presence of 13K-7B2 only in the culture medium is that a 7B2 molecule is processed into small fragments including 13K-7B2 in the secretory granules just before secretion. We cannot exclude the possibility that the 13K-7B2 is a degradation product produced during storage.

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