NOTE

Immunoreactive 7B2 Concentrations in Rats with Various Endocrine Conditions

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Abstract

Changes in 7B2 immunoreactivity in the pituitary as well as in the other brain regions and gut after various endocrine situations were investigated. Gonadectomy and neonatal monosodium glutamate (MSG) treatment resulted in an appreciable increase in the pituitary 7B2 concentration, though 7B2 content in the MSG treated pituitary was not significantly different when calculation was performed on a per pituitary gland basis. The 7B2 concentration in the cerebellum, midbrain and cortex in thyroxine treated rats showed a significant increase, which might indicate possible thyroid hormone involvement in 7B2 metabolism in the brain. The pituitary 7B2 concentration during the estrous cycle did not change significantly. These results suggest that pituitary 7B2 may correlate to the pituitary gonadotropins and that brain 7B2 content may be modulated by thyroid hormones.

The peptide 7B2 was first extracted from the porcine and human pituitary glands (Hsi *et al.*, 1982; Seidah *et al.*, 1983). Subsequent studies by radioimmunoassay (RIA) revealed its presence outside the pituitary glands, that is, in the brain (Iguchi *et al.*, 1984, 1985), spinal cord

(Suzuki *et al.*, 1985), pancreas (Suzuki *et al.*, 1987), gut, thyroid gland and adrenals (Iguchi *et al.*, 1984). This novel peptide 7B2 was also shown to be released by high potassium stimulation (Iguchi *et al.*, 1984; Deng *et al.*, 1986) or luteinizing hormone-releasing hormone (LH-RH) from the pituitary (Deng *et al.*, 1986) and a pheochromocytoma cell line (PC 12) *in vitro* (Suzuki *et al.*, 1986 a), suggesting that 7B2 may be a peptide secreted by these endocrine glands. Because of the lack of a pure synthetic peptide, the function of this novel peptide has not yet been known.

In this study, we intended to assess

Received December 9, 1988

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This work was supported in part by a Grant
in Aid for Scientific Research from the Ministry
of Education of Japan (No. 62770858).

whether there was any change in the 7B2 concentration in any of the specific conditions mentioned below.

Materials and Methods

Experimental animals

All animals used in this study were male and female Wistar-derived rats obtained from a commercial source. They were housed, at least two weeks prior to an experiment, in a room with controlled temperature ($25 \pm 1^\circ\text{C}$) and lighting (lights on 0800 to 2000 h), and given rat food and tap water *ad libitum*. The rats weighed between 200 and 300 g when used. Female rats of Sprague-Dawley strain were also employed to study whether any changes occurred in the pituitary or hypothalamic 7B2 concentration during the estrous cycle. Following repeated observation of vaginal smears taken early in the morning, rats which showed at least three regular 4-day cycles were selected. In the morning of the experiment, the final smears were taken from the selected rats, and the stages of the estrus cycle were determined (Schwartz & Bartosik, 1962). Rats which showed typical proestrus ($n=9$), estrus ($n=18$), metestrus ($n=8$) and diestrus ($n=15$) respectively, were immediately decapitated with a guillotine.

Endocrinological treatments

Thyroid dysfunction

To manipulate the thyroid status and to study 7B2 content, 7 male Wistar rats were injected with $20 \mu\text{g}$ l-thyroxine (Sigma Co. St. Louis, MO, USA) daily for 2 weeks. Nine rats had been given 0.2% methyl mercaptoimidazole (MMI; Sigma Co.) instead of water for 4 weeks. Six rats served as controls and were given food and water *ad libitum*.

Adrenalectomy

Twelve male rats were adrenalectomized. The adrenalectomized animals were given 1.0% saline *ad libitum* instead of water. All of the operated rats and 7 control rats were killed 2 weeks after the treatment.

Gonadectomy

Castrated ($n=11$) and ovariectomized ($n=8$) Wistar rats and control rats of each sex ($n=7$

of each) were killed 2 weeks after gonadectomy.

Neonatal monosodium l-glutamate (MSG) treatment

Male and female rats were mated and their offspring received a subcutaneous injection of 4 mg/g body weight MSG (Wako Chemicals Co., Tokyo, Japan) on alternate days for the first 10 days of life. Control pups received equivalent volume injections of 10% saline as an isotonic control. At 21 days of age, the offspring were weaned and placed in group cages according to sex and treatment. In the experiments, only male rats were used when they reached approximately 15 weeks of age.

Sample preparation

The animals were decapitated with a guillotine with a minimum of handling. The trunk blood was collected into tubes containing 1 mg of ethylenediamine tetraacetate (EDTA; Wako Chemicals) and 400 K.I.U. of aprotinin (Sigma Co.) per ml of blood. Pituitary glands were rapidly removed. The hypothalamus and some other brain regions (brainstem, midbrain, cerebellum, thalamus, striatum and cortex) were dissected according to methods described previously (Glowinski & Iversen, 1966; Heffner *et al.*, 1980). The pancreas and gastrointestinal (GI) tract (stomach, duodenum, ileum and colon) were also dissected so as to be as free as possible from fatty tissue. Each tissue sample was weighed and immersed in 1 ml of boiling 0.5 mole/L acetic acid for 10 min (Bryant & Bloom, 1982). The extracts were stored at -20°C until the assay.

Radioimmunoassays (RIA)

The RIA for 7B2 has been described in full elsewhere (Suzuki *et al.*, 1986b). 7B2 antiserum was raised against a synthetic segment of 7B2 (sequence positions 23–39; Cambridge Research Biochem., Cambridge, U.K.). The antibody exhibited 33% cross-reactivity on a molar basis with authentic porcine 7B2 and no cross-reactivity was observed for rat follicle stimulating hormone (rFSH), rat luteinizing hormone (rLH), beta-endorphin, somatostatin, rat growth hormone (rGH) or substance P (Igcuhi *et al.*, 1984; Suzuki *et al.*, 1986b; Deng *et al.*, 1986). The sensitivity of the assay was 0.3 fmol/assay tube using synthetic 7B2 (23–39) and iodinated 7B2 (23–39) as standard and tracer, respectively.

The intra- and inter-assay coefficients of variation were 7 and 12%, respectively.

RIAs of rLH and rTSH were performed by double-antibody procedures and using kits generously provided by the National Institute of Diabetes, Digestive and Kidney Disease (NIDDK), Bethesda, MD, USA, and calculated in terms of the standards for LH-RP-2 and TSH-RP-2. The minimal detectable doses of rLH and rTSH were 18 and 25 pg, respectively. The intra- and interassay coefficients of variations for these two RIAs were less than 10% and 15%, respectively. The RIAs were performed according to the methods described in the protocols. All peptides and proteins were iodinated with sodium ^{125}I using the chloramine T method (Hunter & Greenwood, 1962) or lactoperoxidase method (Miyachi *et al.*, 1972).

Plasma free thyroxine concentrations were assessed with a commercial RIA kit supplied by Japan Diagnostic Products Co., Tokyo, Japan.

Statistical analysis

The significance of difference between several groups of data was assessed by one way analysis of variance (ANOVA). A difference was considered significant if the resulting F value gave a probability of <0.05 . Differences between pairs of groups were then assessed by Student's unpaired *t*-test.

Results

The estrous cycles (Fig. 1)

No significant changes in pituitary or hypothalamic 7B2 concentrations were observed during the cycle. Plasma rLH concentrations were also determined. No significant difference was observed during the estrus cycle (proestrus, 0.48 ± 0.15 (mean $\pm$ SEM) ng/ml; estrus, 0.20 ± 0.06 ng/ml; metestrus, 0.54 ± 0.13 ng/ml; diestrus, 0.21 ± 0.05 ng/ml).

Endocrinological treatments

Tissue 7B2 concentrations under the endocrinological treatments are shown in Fig. 2 and Table 1 and 2.

Hyperthyroid and hypothyroid rats

Plasma free thyroxine concentrations were 11.3 ± 1.4 (hyperthyroid rats) and 0.6 ± 0.2 (hypothyroid rats) ng/100 ml, respectively, and these values were significantly different from the control value (1.9 ± 0.2 ng/100 ml; both $p < 0.01$). In hypothyroid rats, plasma

Fig. 1. Pituitary weight, and pituitary and hypothalamic 7B2 concentrations during the estrous cycle. Results are shown as mean (column) $\pm$ SEM (bar) in the proestrus (P), estrus (E), metestrus (M), and diestrus (D) of rat estrous cycle.

Fig. 2. Pituitary weight and 7B2 concentration in rats subjected to various endocrinological treatments. Abbreviations used in the figure are as follows; *: $p < 0.05$; **: $p < 0.01$ vs corresponding control value for each; Hyper: hyperthyroid rats treated with thyroxine; Hypo: hypothyroid rats treated with methyl mercaptoimidazole; Adx: adrenalectomized rats; Castrated: castrated rats; Ovx: ovariectomized rats; MSG: rats treated with monosodium glutamate neonatally.

Table 1. The effect of various endocrine treatments on 7B2 concentration in the rat brain.

	Cerebellum	Brainstem	Midbrain	Thalamus	Hypothalamus	Striatum	Cortex
T4 (7)	27.0±6.9*	36.3±7.1	152.1±11.1*	115.9±16.7	193.0±26.1	92.1±15.5	82.4±10.1*
MMI (9)	14.2±2.6	30.1±6.4	116.8±28.7	82.5±13.8	267.8±40.6	95.5±27.6	62.3±4.5
Control (6)	15.6±2.4	25.7±3.5	83.3±9.3	63.7±9.7	253.0±31.4	76.4±13.5	55.5±12.0
Adx (12)	12.8±2.4	21.7±4.6	129.7±24.7	92.7±13.3	206.8±19.7	83.4±10.5	68.6±6.1
Control (7)	17.6±3.9	24.7±3.0	95.8±9.3	70.8±8.0	239.4±21.3	80.4±11.9	48.6±8.3
Castrated (11)	16.7±2.3	17.2±3.0	94.6±8.7	57.9±10.8	252.6±14.1	88.2±10.3	62.0±8.4
Control (7)	19.5±3.2	25.0±4.1	97.2±6.7	76.5±8.0	242.3±21.4	91.5±10.9	46.7±6.4
Ovx (8)	16.4±2.8	23.9±6.9	106.4±18.6	68.5±6.6	225.5±15.4	78.2±11.3	36.8±7.7
Control (7)	16.3±2.9	27.0±4.0	112.5±17.1	73.8±9.8	248.7±15.2	109.2±18.4	44.9±5.3
MSG (11)	21.5±2.1	31.1±4.2	195.4±16.5**	117.4±19.3	354.5±11.2**	116.3±11.8	51.9±7.4
Control (8)	22.8±4.6	32.2±6.8	117.6±10.7	85.3±15.8	246.7±22.6	109.9±8.5	52.4±7.6

Results shown are mean±SEM and expressed as pmol/g wet weight of the tissue. Numbers in parentheses indicate the numbers of rats studied. Abbreviations used in the Table are as follows; T4: thyroxine-treatment; MMI: methyl mercaptoimidazole-treatment; Adx: adrenalectomy; Ovx: ovariectomy; MSG: neonatal monosodium glutamate-treatment (for details, see Materials and Methods).

*, $p < 0.05$ vs corresponding control value.

**, $p < 0.01$ vs corresponding control value.

Table 2. 7B2 concentrations in pancreas and gastrointestinal tissues in rats with various endocrine treatments.

	Stomach	Duodenum	Ileum	Colon	Pancreas
T4 (7)	6.7±1.3	6.8±1.2	7.6±1.5	4.4±0.5	13.1±2.2
MMI (9)	6.4±1.2	7.9±1.4	8.0±1.4	5.2±2.5	20.4±5.7
Control (6)	6.1±1.1	7.2±1.0	5.8±1.0	7.3±1.3	12.8±2.1
Adx (12)	7.9±1.7	5.3±0.9	4.7±1.0	5.4±1.5	13.2±3.4
Control (7)	6.9±1.0	6.1±0.5	5.4±2.2	7.3±1.7	11.3±2.4
Castrated (11)	6.1±3.3	7.9±2.1	4.7±1.0	5.4±1.5	15.0±3.1
Control (7)	5.2±2.2	8.4±1.2	4.8±1.2	5.7±1.3	13.9±3.0
Ovx (8)	7.1±1.9	7.1±1.8	6.9±2.0	6.3±1.7	11.8±2.5
Control (7)	6.6±1.2	8.9±3.0	5.7±1.2	4.4±0.9	13.6±2.7
MSG (11)	10.1±2.8	10.8±3.7	9.2±2.1	6.4±0.8	16.0±5.4
Control (8)	7.3±2.8	9.3±2.3	7.5±3.0	8.4±2.6	15.1±3.0

For details, see Table 1.

TSH was increased (hypothyroid; 14.45 ± 0.79 ng/ml; control 1.40 ± 0.52 ng/ml, $p < 0.01$). In hyperthyroid rats plasma TSH was rather depressed (0.31 ± 0.15 ng/ml). These results confirmed the success of the manipulation of thyroid hormone. As shown in Fig. 2 and Table 1, although no significant changes in the pituitary and hypothalamic 7B2 concentrations were observed, there were significant differences in 7B2 concentrations in some brain regions (cerebellum, midbrain, and cortex) in hyperthyroid rats. 7B2 concentrations in GI tracts did not change significantly among the groups (Table 2).

Adrenalectomized rats

No differences between 7B2 concentrations in adrenalectomized animals and controls were observed in the pituitary (Fig. 2). 7B2 concentrations in tissues from the brain and GI tract in adrenalectomized rats did not change significantly from the control values (Tables 1 and 2).

Gonadectomized rats

Plasma LH levels were significantly raised in castrated males and ovariectomized females compared with control values (castrated, 23.3 ± 6.0 ; male control, 0.26 ± 0.07 ;

ovariectomized, 19.1 ± 5.9 ; female control 0.33 ± 0.10 ng/ml; $p < 0.01$ for each sex). In gonadectomized rats, the weight of the pituitary glands increased significantly (castrated, 12.6 ± 0.5 ; male control, 10.3 ± 0.6 ; ovariectomized, 13.5 ± 0.5 ; female control, 10.4 ± 0.7 mg; $p < 0.01$ for each sex).

The pituitary 7B2 content in castrated and ovariectomized rats differed significantly ($p < 0.05$) from that in controls (Fig. 2), though no significant differences were observed in brain regions and GI tract (Tables 1 and 2).

Neonataly MSG treated rats

All MSG treated rats exhibited typical somatic features (obese, short tail and small testes) and the disappearance of the optic nerve (Olney, 1969). The pituitary glands were significantly smaller than those in controls (MSG, 6.5 ± 0.5 ; control, 10.1 ± 0.6 mg; $p < 0.01$). 7B2 content in the pituitary, hypothalamus and midbrain showed a significant increase ($p < 0.05$ or $p < 0.01$ vs control values, Fig. 2 and Table 1).

Discussion

Immunocytochemical studies revealed that a novel pituitary protein, 7B2, was

localized in endocrine cells of the rat anterior pituitary cells and particularly localized in gonadotropic cells (Marcinkiewicz *et al.*, 1985, 1986; Steel *et al.*, 1988). In human pituitaries and gonadotropinomas the coexistence of 7B2 and gonadotropin immunoreactivities was observed (Steel *et al.*, 1988). Furthermore, the release of 7B2 from gonadotropes has been demonstrated to be prompted by high potassium and LH-RH (Iguchi *et al.*, 1984; Deng *et al.*, 1986; Natri *et al.*, 1988). These observations were consistent with the possibility that 7B2 is a novel secretory protein and has a close relation to gonadotropes.

On the other hand, Marcinkiewicz *et al.* (1986) reported that 7B2 immunoreactivity was found to be present not only in gonadotropes but also in thyrotropes. Iguchi *et al.* (1987) found that there were significant correlations between plasma 7B2 levels in cord blood and the body weight of neonates and plasma growth hormone levels in cord blood. Natri *et al.* (1988) also demonstrated that plasma 7B2 levels in patients with GH producing pituitary adenoma were elevated after intravenous GH-RH stimulation and that high potassium induced the release of 7B2 from the human somatotrope adenoma cells *in vitro*.

These complicated results prompted us to study whether any changes could be observed in various endocrine situations. In this study, we failed to find any significant difference in the pituitary 7B2 concentrations during the estrous cycles. Although it was only of borderline significance, increased 7B2 concentrations were found in castrated and ovariectomized pituitaries. Steel *et al.* (1988) demonstrated that 7B2 immunoreactivity in ovariectomized pituitaries was more intense, more frequent and more extensive than that in control pituitaries. In contrast to the present study, Deng *et al.* (1986) observed neither a significant increase in 7B2 content in castrated rat pituitaries nor a significant dif-

ference in 7B2 release from castrated rat anterior pituitary cells *in vitro*. In man, we have demonstrated plasma 7B2 release after intravenous LH-RH administration (Kobori, 1989) and high plasma 7B2 in patients with Turner's or Klinefelter's syndrome (Suzuki *et al.*, in preparation). Because LH and FSH immunoreactive cells have been reported to increase after ovariectomy (Ibrahim *et al.*, 1986) and 7B2 immunoreactivity has been demonstrated to colocalize with gonadotropin immunoreactivities in the pituitary (Marcinkiewicz *et al.*, 1985; Steel *et al.*, 1988), an increase in 7B2 content in the pituitary after gonadectomy seemed to be reasonable. The discrepancy in pituitary 7B2 content in these gonadectomized rats (Deng *et al.*, 1986; Steel *et al.*, 1988), might be due to the difference in rate of secretion of pituitary 7B2 to the circulation modified by LH-RH or other unknown secretagogues.

In rat pituitaries from adrenalectomized rats and thyroxine or MMI treated rats, no significant change in 7B2 content was found and this result is in accordance with the results of Steel *et al.* (1988). The significant brain 7B2 increase found in thyroxine treated rats might, however, indicate that thyroid hormones could play a role in 7B2 metabolism in the brain.

Paraenteral administration of MSG to neonatal rodents has been reported to result in a central nervous system lesion, restricted largely to neurons of the arcuate nucleus and to the retina (Olney 1969). These rats show typical growth retardation, obesity and atrophy of optic nerve and endocrine glands. It had been reported that anterior pituitary weight and GH content in the anterior pituitary was reduced (Ieiri *et al.*, 1981). Recently, MSG has been reported to deplete immunoreactive growth hormone releasing factor (GRF) content in pituitary stalk-median eminence tissue (Bloch *et al.*, 1984; Wakabayashi *et al.*, 1986). Not only GH but gonadotropin abnormalities were

also demonstrated, though results were not uniform due to sex difference (Dada *et al.*, 1984; Dada & Blake 1985; Inkster *et al.*, 1985). Furthermore, many other peptides such as adrenocorticotropin (ACTH), beta endorphin (Krieger *et al.*, 1979), neuropeptide Y (Kererian & Pelletier 1986) and somatostatin (Depaolo & Steger 1985), etc. had been shown to be influenced by the treatment. A recent study demonstrated changes in 7B2 content in the hypothalamus and substantia nigra (Hashigami. 1989). In the present study, in addition to these observations, a significant increase in pituitary 7B2 content was demonstrated. As Dada *et al.* (1984) had reported for LH, the calculation of 7B2 content on the basis of the entire weight of the pituitaries showed no difference between the MSG treated rats and controls. This result suggest that the increase in 7B2 content per mg pituitaries might be of importance in maintaining the normal amount of 7B2 in small pituitaries. As the levels of the peptide in the tissue will reflect the balance between release from, synthesis and degradation within the tissue, measurements of tissue content of peptides therefore yield only limited information about the patterns of release. Further studies are obviously needed concerning 7B2 action and the pattern of release from MSG and gonadectomized rats and brain tissues from altered thyroid states.

Acknowledgement

We acknowledge our gratitude to Professor S. R. Bloom, Royal Postgraduate Medical School, London, for his generous gift of 7B2 antisera. We also wish to thank Dr. A. F. Parlow of the NIDDK for kindly supplying NIDDK immunoreagents.

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