Glucocorticoids induce glutamine synthetase in folliculostellate cells of rat pituitary glands in vivo and in vitro

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ABSTRACT

Glutamine synthetase (GS) is a glucocorticoid-inducible enzyme that has a key role for glutamate metabolism in the central and peripheral nervous system. In this study GS activity was measured and the amount of immunoreactive GS (ir-GS) cells in the rat anterior pituitary gland was quantified as a function of age. In addition, the effects of GS inhibitors, glucocorticoid administration, and adrenalectomy on GS activity were examined. Some of the ir-GS cells were also immunoreactive for S100 protein (ir-S100) which is a known marker for folliculostellate cells (FS) in the anterior pituitary. FS cells expressing GS were first detected in 3-d-old rats, and this cell population, expressed as the immunostained cell area divided by a standard unit area, increased as a function of age. The percentages of FS cells also expressing GS were 0.2, 6.4, 25 and 74% at 3 d, 30 d, 60 d and 2 y of age, respectively. GS enzyme activity also increased in parallel with the increase of ir-GS cell population maturation. The subcutaneous injection of methionine sulfoximine, a GS and \( \gamma \)-glutamylcysteine synthetase inhibitor, reduced pituitary GS activity by 83%, but increased the population of ir-GS cells 3.5-fold in 30-d-old rats. Buthionine sulfoximine, a specific inhibitor of \( \gamma \)-glutamylcysteine synthetase, had little effect on GS activity or the ir-GS cell population. Neither methionine sulfoximine nor buthionine sulfoximine changed the population of ir-S100 protein cells (FS cells). Dexamethasone and hydrocortisone increased the population of ir-GS cells by 3.1 and 4.2-fold, respectively, within 12 h after administration. A significant increase of GS activity due to the injection of glucocorticoids was observed in the anterior pituitary, but not in the brain, retina or liver of immature rats. Adrenalectomy did not cause decrease of pituitary GS activity, and dexamethasone administration increased GS activity in both adrenalectomised and intact rats. In the monolayer culture of anterior pituitary cells, glucocorticoids increased GS activity by \( \times 1.5 \), and methionine sulfoximine reduced the activity by over 94%. These results demonstrate that GS in folliculostellate cells is a glucocorticoid-inducible enzyme in vivo and in vitro, and that the age-dependent increase of GS activity is independent of endogenous adrenal glucocorticoids.

Key words: Adrenalectomy; methionine sulfoximine; S100 protein.

INTRODUCTION

Agranular cells dispersed among glandular cells of the adenohypophysis have been named folliculostellate (FS) cells. They were first recognised as a distinct sixth cell type in the rat due to their unique ultrastructural characteristics by Farquhar (1957). She considered these FS cells or agranular stellate cells to be a subtype of corticotrophs. It is now known that this is not the case and that the FS cells are a unique cell type forming follicles or ductules into which their apical cell surfaces project microvilli and cilia. During the development of the rat, the FS cells appear first in the cluster of adenohypophysial cells at 16 d of gestation (Svalander, 1974). They form cellular networks connected to each other by junctional complexes and appear to be satellites of the granulated secretory cells (Vila-Porcile, 1972; Soji et al. 1992). This association of FS cells with granulated secretory cells is especially evident in the close association of FS cells with...
gonadotrophs under conditions such as castration (Shirasawa et al. 1983). While many investigators have postulated roles for the pituitary FS cells in the normal functioning of the glandular cells, little information about their physiological or metabolic function is currently available.

Two studies have suggested that FS cells synthesise glutamine, a molecule with a number of important functions. An early study demonstrated that the incubation of anterior pituitaries with radiolabelled glucose rapidly resulted in the incorporation of the radioactivity into glutamine, and the incorporated value was much higher than that in most brain areas (Andersson et al. 1961). Immunohistochemical studies utilising a specific antibody to glutamine demonstrated that the cells with high concentrations of glutamine were FS cells (Pow, 1993). Although glutamine has long been known to play a role in such crucial functions as nitrogen, neurotransmitter and energy metabolism (Yu et al. 1982; Ward et al. 1983), recent studies demonstrating glutamine synthetase (GS, L-glutamate ammonia ligase, EC 6.3.1.2), an enzyme that catalyses the ATP-dependent condensation of glutamate and ammonia to form glutamine in many diverse cell types, suggest an important role for this amine. High concentrations of GS are found in astrocytes in the brain (D’Amelio et al. 1990), hepatocytes in the liver (Bennett et al. 1987), pigment and Müller cells in the retina (Riepe & Norenburg, 1977), and the cytotrophoblast and mesenchyme layers of placental villi (DeMarco et al. 1997). These data all suggested to us that there should be GS in the FS cells, and that the metabolism of this amino acid in FS cells may have an important role in the anterior pituitary gland. In this study we first demonstrate the presence of ir-GS in rat FS cells, and then focus our attention on the GS induction by glucocorticoids and GS inhibitors in vivo and in vitro.

MATERIALS AND METHODS

Reagents

L-methionine sulfoximine (MSO, an irreversible inhibitor of both GS and γ-glutamylcysteine synthetase), L-buthionine-[S,R]-sulfoximine (BSO, an inhibitor specific to γ-glutamylcysteine synthetase), dexamethasone and hydrocortisone were obtained from Sigma (St Louis, MO, USA), and others were from Wako Pure Chemicals (Osaka, Japan). Dexamethasone and hydrocortisone were dissolved as a 1 mg/ml stock in olive oil, and other reagents were freshly prepared as 10 mg/ml solutions in phosphate buffered saline (PBS).

Animals and tissue preparation

Male Sprague-Dawley rats were maintained under conditions of controlled temperature (22–24 °C) and illumination (8:00–20:00 h daily). In the first experiment, males ranging in age from 3 d to 2 y were killed and the anterior pituitary, retina, brain and liver were removed and processed either for immunohistochemistry or for GS activity. For light microscopy and immunohistochemical staining, the pituitaries from each group were quickly removed and fixed overnight in Bouin’s solution without acetic acid at 4 °C. They were dehydrated in an ethanol series, and embedded in Paraplast embedding medium (Sigma, St Louis, MO, USA). Serial sagittal sections, 2 μm in thickness, were mounted on poly-l-lysine coated slides for immunohistochemistry. For GS activity, the anterior lobes were separated from the intermediate and neural lobes under a stereoscopic microscope, and quickly frozen in liquid nitrogen. Brain, eyes and liver were also frozen in liquid nitrogen at the same time and stored at −80 °C until GS activity assay. In a second experiment, 30-d-old rats were injected subcutaneously with glucocorticoids, one of the two enzyme inhibitors (MSO or BSO) or PBS at a dose of 10 μg/g body weight, and killed at a number of time intervals after injection. Again, the anterior pituitary, retina, brain and liver were removed and processed either for immunohistochemistry or for GS activity as described above. In addition, control pituitaries from 30-d-old rats were prepared for cell culture and treated in vitro with the enzyme inhibitors or glucocorticoids (see below). In a third experiment, 30, 60, and 120-d-old rats were adrenalectomised bilaterally or sham operated under pentobarbital sodium (Abbott, North Chicago, IL, USA) anaesthesia (20–25 mg/kg bw, i.p.). They were maintained for 30 d on laboratory chow (Clea Japan Inc., Tokyo, Japan) and tap water, or 0.9% saline ad libitum. Again, the anterior pituitary, retina, brain and liver were removed and processed either for immunohistochemistry or for GS activity as described above. In a fourth experiment, 5-d-old rats were adrenalectomised under the same conditions and returned to their mothers. Ten days later the animals were killed and the tissues processed as above. Finally, in a fifth experiment, 15-d-old rats were adrenalectomised, and maintained on 0.9% saline in their drinking water for 2 wk. These rats were then injected subcutaneously with a freshly prepared glucocorticoid solution, or buffer and killed 12 h later, and the tissues processed as above. The absence of adrenal glands in the adrenalectomised rats in all experiments was
carefully confirmed under a stereoscopic microscope after killing.

**Immunohistochemistry**

Serial pituitary sections were immunostained by the avidin-biotin-peroxidase complex method (Hsu et al. 1981). Antiserum to bovine S-100 protein α subunit (Life Science Laboratories, Tokyo, at a dilution of 1:6000) was used as a marker for FS cells, and a mouse monoclonal antibody to ovine GS (at a concentration of 0.5 µg/ml (Transduction Laboratories, Lexington, Kentucky, USA) was also employed for immunohistochemical procedures. To check the specificity of the antibodies, bovine S-100 protein α subunit and ovine GS (Sigma, St Louis, MO, USA) were used for the preabsorption test, and staining with both antibodies was completely abolished by the respective antigens.

**Cell culture**

Pituitary glands from 30-d-old male rats were used for monolayer culture. After eliminating the neural and intermediate lobes from pituitary glands, anterior lobes were incubated with 0.05% trypsin at 37°C for 10 min. They were gently pipetted to disperse the cells, and kept staining for a few minutes to precipitate large clumps of cells. The supernatant was transferred to a centrifuge tube, and the precipitate used for another cycle of trypsin treatment. The supernatant was centrifuged at 500 g for 10 min, and precipitated pituitary cells were suspended at a concentration of 3 × 10⁸ cells/ml in Ham’s F-12 medium containing 10% fetal bovine serum and antibiotics. The pituitary cells were cultured in a 24-well multidish (Nunc, Roskilde, Denmark) under 95% and 5% CO₂. After 3 d the culture medium was changed to a fresh one containing 0.55 µM of MSO, 0.45 µM BSO, 0.3 µM dexamethasone or 0.28 µM hydrocortisone, and pituitary cells were incubated for an additional 16 h. The cultured cells were washed 3 times with PBS and then lysed by freeze-thawing in 100 µl of 10 mM sodium phosphate buffer, pH 7.2, containing 0.1% Nonident P-40 (Sigma, St Louis, MO, USA). The lysate was stored at −80°C until assaying GS activity.

**GS assay**

Thawed samples were homogenised in 10 mM sodium phosphate buffer, pH 7.2, containing 0.1% Nonident P-40, and then centrifuged at 10000 g for 15 min at 4°C. GS activity in the supernatants was measured spectrophotometrically using the glutamine-γ-glutamyltransfer assay of Miller et al. (1978) with minor modifications. The assay mixture consisted of 1 ml, and final reagent concentrations were 100 mM L-glutamine, 50 mM imidazole-HCl (pH 6.8), 0.5 mM manganese chloride, 50 mM hydroxylamine-HCl, 25 mM potassium arsenate, and 0.2 mM disodium ADP. The reaction was initiated with 50 µl supernatant at 37°C for 15–60 min and terminated by the addition of 1.0 ml of 0.37% FeCl₃, 0.3 M trichloroacetic acid, and 0.6 M HCl at 4°C. The precipitate was removed by centrifugation at 1000 g for 15 min, and γ-glutamyl hydroxamate was determined by comparing the absorbance at 505 nm to blanks including all reagents except ADP and potassium arsenate. One unit of GS activity is defined as the amount of activity catalysing the formation of 1 µmol of γ-glutamyl hydroxamate in 15 min under the assay conditions.

**Cell area and statistical analysis**

The population of FS cells immunostained with antibodies to GS or S100 protein was measured in the study. As named after their shape, FS cells are stellate, their slender cytoplasm penetrating between oval or spherical-shaped glandular cells. Because they have a tendency to make clusters, in any given field, cell cytoplasm may be found with and without a nucleus. This makes it extremely difficult to measure the cell area exactly for the immunoreactive cells with nuclei. To overcome this problem, the sum of the immunostained cell area occupied per unit area was employed to express the population of immunoreactive FS cells with and without nuclei. Over 20 pictures of immunostained sections in each group were taken at ×50 magnification by a 3CCD-camera (HV-C20S, Nikon, Tokyo, Japan) equipped with a light microscope, and stored in a Macintosh computer connected to the 3CCD-camera. The pictures were analysed by measuring the sum of immunostained cell area in a defined area (3 × 10⁵ pixel² equivalents to 0.224 mm²) using the NIH Image software. The population was expressed in terms of mm²/mm². Data were analysed using Bonferroni’s procedure for multiple comparison procedure. The P value was set at 0.05.

**RESULTS**

**Age-dependent change of GS in FS cells**

Positive immunohistochemical staining with the antibody to GS was found in pituicytes in the neural lobe, cells in the intermediate lobe in the tissue surrounding
the lobules of endocrine cells, some of marginal layer cells facing the pituitary cleft and stellate-shaped cells in the anterior lobe (Fig. 1) of the rat pituitary gland. For the immunoreactive stellate-shaped cells in the anterior lobe the immunoreaction product was limited to their cytoplasm and they had long, slender cell processes penetrating between ovals or spherical-shaped glandular cells. To further characterise the ir-GS stellate cells, adjacent sections were immunostained with antibodies to S100 protein, a marker protein of rat FS cells. Although the population of ir-S100 cells was larger than the population of ir-GS cells, there were many cells which were reactive to both antibodies (Fig. 2).

A few ir-GS cells were first detected in 3-d-old rats and the ratio of stained area to defined area (see Methods) in these pituitaries was quite small (0.002 mm²/mm²). This ratio increased slowly over the first 2 mo, 0.01 and 0.03 mm²/mm² by 25 and 60-d-olds, respectively, and then rapidly increased to the adult level of 0.15 mm²/mm² in 150-d-old rats. In contrast to GS immunoreactivity, the ratio for the immunoreactive area to S100 to the standard area was relatively high at all time periods examined. The ratio was 0.19 mm²/mm² in anterior pituitary sections of 30-d-old rats. This value temporarily dropped in 40 and 60-d-old animals, but recovered back to adult levels, 0.2 mm²/mm² in 70-d-olds (Fig. 3a). In the neonatal rats less than 1% of the total ir-S100 cells were reactive for GS. However, as the animals matured, the percentage of ir-S100 cells which were reactive for GS increased to 7, 25, 55 and 78% in 30, 60, 90 and 150-d-old rats, respectively. The GS activity detected in the adenohypophyseal homogenate of 2-d-old rats was small (0.02 units/pituitary), and then increased slowly to 0.17 units/pituitary by 15 d of age (Fig. 3b). From this point on GS activity increased rapidly to 2.2 units/pituitary in 60-d-old rats, and then resumed a slow increase to 3.2 units/pituitary by 2 y of age. Taken together, the above results suggest

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**Fig. 1.** Distribution of ir-GS cells in the pituitary gland. (a) Pituicytes in the neural lobe (N), mesenchymal cells in the intermediate lobe (IM), and stellate cells in the anterior lobe (A) showing immunoreactivity to GS antibody × 140. (b) Higher magnification of demarcated area in a. Some of marginal layer cells facing to the pituitary cleft (PC) and stellate cells in the anterior lobe are immunoreactive to GS antibody. × 570.

**Fig. 2.** Immunohistochemistry of FS cells in 90-d-old male rats. Adjacent sections were stained with GS and S-100 protein α subunit antibody (a, b, respectively). Numbers correspond to the same cells, and ir-GS cells are identical to ir-S100 protein cells. × 570.
Fig. 3. Age-dependent changes of the population of ir-GS cells and ir-S100 protein cells, and GS activity. (a) The population of ir-GS cells (closed circles) is higher than that of ir-S100 protein cells (closed squares) at each age. (b) Adenohypophysial GS activity increase with age. Values show the mean and s.e. for 4–6 rats.

Table. GS activities of anterior pituitary, brain, retina, and liver after subcutaneous injection of MSO and BSO to 30-d-old rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vehicle</th>
<th>MSO</th>
<th>BSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.66 ± 0.04</td>
<td>0.11 ± 0.03**</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>Brain</td>
<td>2.61 ± 0.10</td>
<td>0.63 ± 0.05**</td>
<td>1.96 ± 0.11*</td>
</tr>
<tr>
<td>Retina</td>
<td>52.7 ± 3.2</td>
<td>3.46 ± 1.09**</td>
<td>41.9 ± 1.7</td>
</tr>
<tr>
<td>Liver</td>
<td>1.35 ± 0.05</td>
<td>0.02 ± 0.01**</td>
<td>1.13 ± 0.06</td>
</tr>
</tbody>
</table>

Data express the mean and s.e. for 5 rats. GS activity of pituitary, brain, retina, and liver are expressed in terms of units/pituitary, units/mg tissue, units/retina, and units/mg tissue, respectively. Asterisks indicate statistical differences between drug-treated and untreated rats: *P ≤ 0.05; **P ≤ 0.01.

that the number of FS cells immunoreactive for cytoplasmic GS increases with age and that this increase is paralleled by an increase in GS activity.

Effect of MSO and BSO on GS

GS activities of anterior pituitary, brain, retina and liver were measured in 30-d-old rats at 12 h after the subcutaneous injection of the enzyme inhibitors. As summarised in the Table, the treatment of MSO, a GS and γ-glutamylcysteine synthetase inhibitor, caused a significant reduction of GS activity in the anterior pituitary as well as in other tissues. The percentage reduction in adenohypophysial GS activity (83% inhibition) was higher than in the brain (76%) and lower than in the retina and liver (93 and 98%, respectively). On the other hand, treatment with BSO, a γ-glutamylcysteine synthetase inhibitor, did not significantly affect GS activities in the anterior pituitary, retina and liver, but caused a slight inhibition of GS activity in the brain (P < 0.04).

The effect of MSO on pituitary ir-GS cells of 30-d-old rats is shown in Figure 4. The photomicrographs
Fig. 5. Time-dependent change in the population of ir-GS cells after the administration of MSO to 30-d-old rats. Values show the mean and s.e. for 5 rats.

seemed to indicate that clusters of FS cells become immunoreactive to GS antibody in MSO-treated rats and that the population of ir-GS cells in MSO-treated rats was higher than in the controls. To confirm that the suspected increase in the population of ir-GS cells due to MSO-treatment, the immunostained area in anterior pituitary sections was measured after MSO-injection (Fig. 5). In contrast to the reduction of GS activity by MSO treatment, the population of ir-GS cells increased significantly at 6 h, and reached 5 times the control at 12 h after the injection of MSO. The population of ir-GS cells then gradually decreased to the control level of 80 h. Since the population of ir-S100 protein cells remained at a constant level (0.19 mm²/mm²) at all times after the injection of MSO, the percentage of ir-S100 protein cells immunoreactive for GS increased to 89% at 12 h, a value that is higher than that in 2-y-old rats. These results demonstrate that the population of cells immunoreactive for S100 protein (FS cells) is not affected by MSO treatment, but that additional FS cells become immunoreactive for the GS protein due to MSO treatment. The data suggest that a number of FS cells, which are immunonegative or slightly immunopositive to the GS antibody under normal conditions, have the capacity to synthesise and/or accumulate high amounts of GS molecules when GS activity is inhibited.

**Induction of pituitary GS by dexamethasone**

The injection of glucocorticoids into 30-d-old rats caused the increase of the population of pituitary ir-GS cells similar to that seen in MSO-treated rats. Dexamethasone increased the population of ir-GS cells by a factor of 5 at 12 h. This was followed by a gradual decrease to a 2.5 fold increase over controls at 80 h (Fig. 6). Hydrocortisone also increased the ir-GS cell population by a factor of 4 at 12 h after the injection. Mitotic figures were rarely observed in the pituitary glands of either control or dexamethasone-injected rats. In contrast, the population of ir-S100 protein cells was not affected by the injection of dexamethasone. The population of ir-S100 protein cells was 0.20 ± 0.02 (mean ± s.e.) in dexamethasone-treated rats and 0.19 ± 0.01 mm²/mm² in control rats. Thus glucocorticoids have the capacity to stimulate GS synthesis, but not S100 protein, in FS cells. The effects of dexamethasone on the GS activities were also investigated in the anterior pituitary gland, retina, brain, and liver of neonatal rats (Fig. 7). Adenohypophysial GS activity was very low in control neonatal rats, and increased slowly over the next few weeks in comparison with the other tissues. Dexamethasone significantly increased the adenohypophysial GS activity by a factor of 2. On the other hand, the administration of dexamethasone had negligible effects on GS activities was in retina, brain, and liver. Therefore, at least for the first 30 d after birth, the ability of dexamethasone to stimulate GS activity is restricted to the anterior pituitary gland.

**Effect of adrenalectomy on GS cells**

The effects of endogenous adrenal glucocorticoids and exogenously administered glucocorticoids on the adenohypophysial GS activity was determined at various stages of development. GS activities from 4 groups of rats, namely, sham-operated control rats, controls with dexamethasone-administration, adrenalectomised rats, and adrenalectomised rats with...
dexamethasone administration (Fig. 8) were compared at various ages. Adenohypophysial GS activity increased with age, and the enzyme activity in adrenalectomised rats was comparable with that in controls of the corresponding age. In contrast to the results in untreated groups, dexamethasone administration significantly increased the GS activities in both control and adrenalectomised rats at all ages studied, up to and including 150 d of age. Therefore the age-dependent increase of pituitary GS activity is independent of endogenous adrenal glucocorticoids, but a small increase of GS activity in the anterior pituitary gland can be induced by exogenous glucocorticoids.

Effect of GS inhibitors and glucocorticoids on pituitary cell culture

Figure 9 shows the direct effects of MSO, BSO, dexamethasone, and hydrocortisone on the GS ac-
tivity in anterior pituitary cells in vitro. The MSO-treatment reduced the GS activity to 6% of the control, and the value was lower than that observed in 30-d-old rats in vivo. BSO-treatment, however, did not affect any GS activity in the primary cell culture as observed in vivo. Adding either dexamethasone or hydrocortisone to the culture medium significantly increased the GS activity by 1.5 fold of that seen in controls. The in vitro studies demonstrated that MSO and glucocorticoids can act directly on anterior pituitary cells to change GS activity.

DISCUSSION

FS cells have been studied as a unique cell type in the adenohypophysis after the insightful report by Farquhar (1957). Because of their localisation and appearance, some have speculated that the function of these cells is to regulate hormone secretion and/or cell metabolism of granulated pituitary cells (Soji et al. 1992). Proteins such as S100, glial fibrillary acidic protein, vimentin, cytokeratin, and nonneuronal enolase (Shirasawa et al. 1988; Marin et al. 1989; Tsuchida et al. 1993) have been found in FS cells and have been used as marker proteins for FS cells. From a number of these immunocytochemical studies, others have speculated that FS cells are glial, mesenchymal or lymphoid dendritic-like cells. While the marker proteins are probably related to FS cell function to some extent, the physiological and/or metabolic functions of these cells have yet to be determined.

The present study demonstrates that GS activity is present in the anterior pituitary gland, and that GS is highly concentrated in FS cells. GS activity has also been reported in various tissues including brain, retina, liver, kidney, spleen, fat, epididymis, skeletal muscle and stomach (Iqbal & Ottaway, 1970; Miller, 1975; Kvidera & Carey, 1994) and many potential functions have been attributed to the GS activity related to the cell type in which it has been found. GS is thought to be important in ammonium detoxification in brain and liver (Kanamori et al. 1996; Sugimoto et al. 1997). GS has another important role to produce glutamine in the glutamate-glutamine cycle to reduce the capacity of neurons to replenish their neurotransmitter glutamate (Gibbs et al. 1996). GS has been found to utilise a large variety of decarboxylase amino acids as substrates such as L and D-glutamate, Nα-methyl-D-glutamate, β-amino-δ-glutarate, and α-aminoadipate. As an excitatory amino acid neurotransmitter, glutamate stimulates the release of luteinising hormone (Olney et al. 1976) and prolactin (Login, 1990), and ed the glutamate analogues, D,L-α-aminoadipate and N-methyl-D-aspartate, also stimulate the release of luteinising hormone and thyroid stimulating hormone (Price et al. 1978; Bruni & Vriend, 1984) from the pituitary gland. Glutamate receptors are present in anterior pituitary cells (Yoneda & Ogita, 1986; Villalobos et al. 1996), and the glutamate receptor subunit GLuR1 is expressed in gonadotrophs (Petrusz, 1994). On the other hand, high-level exposure to glutamate and D,L-α-aminoadipate leads to degradation of neurons and to severe metabolic and endocrine disorders in neonatal animals (Olney, 1971; Bruni & Vriend, 1984). These reports suggest that there should be a control system in the pituitary to maintain optimal levels of glutamate in the microenvironment and a system to repair the damage caused by an excess amount of ammonia and/or glutamates for optimal regulation of hormone synthesis and secretion. The presence of GS in the FS cells may be one of the systems to reduce any imbalances in the amount of glutamates in the clusters of endocrine cells.

During postnatal development, a temporal decrease of the ir-S100 protein cell population was observed in mature rats (40–60 d of age) which seemed to be due to a decrease in the number of FS cells in the pituitary gland (Fig. 3a). Although this seems unusual at first, it can be explained by 2 characteristics of the parameter being measured. First, the denominator in the ratio defining the population parameter is a fixed area. The second characteristic is that of the cell population being sampled. A number of reports have demonstrated differential growth rates among the different types of pituitary cells. Growth hormone cells and prolactin cells have a high growth rate while other hormone-producing cells have low growth rates during maturation (Shirasawa & Yoshimura, 1982; Takahashi et al. 1984). Thus if the mitotic rate of growth hormone cells and prolactin cells is greater than the mitotic rate of FS cells during certain periods of maturation, these cells will occupy a greater proportion of the fixed area being measured and thus reduce the immunostained area in the fixed area. Thus even though the FS cells are growing at given rate, the population parameter being measured will reflect a decrease.

In the rat brain, GS activity and the level of S100 protein have been reported to increase relatively slowly for the first 8–12 d of life, and then rise abruptly to adult values by 25–30 d of postnatal age (Herscheman et al. 1971; Weir et al. 1984). Glucocorticoids have the ability to induce brain GS in 11-d-old rats, and then they subsequently lose this ability in
the forebrain and olfactory bulbs (Patel et al. 1983b). In contrast, GS activity in the cerebellum gradually increases to adult levels by 90 d of life, and glucocorticoids still have an ability to induce cerebellar GS by 90 d (Patel et al. 1983a, b). These authors suggested that the induction of GS was related to the differentiation rather than proliferation of brain cells. In the pituitary, GS activity increases slowly during the life of rats, and dexamethasone has an ability to induce GS activity even in 150-d-old rats (Fig. 8). Thus FS cells in the pituitary may not reach a plateau in GS synthesis until very late in the life of the rat and the induction by glucocorticoids may be associated with the differentiation and/or maturation of the cells.

MSO, a GS inhibitor, is also an irreversible inhibitor of $\gamma$-glutamylcysteine synthetase peripherally (Palekar et al. 1975). Administration of MSO is associated with alterations in amino acid metabolism (van den Berg & van den Velden, 1970), gluconeogenesis and astrocytic glycogen content (Hever et al. 1986; Swanson et al. 1989). BSO is a specific inhibitor of $\gamma$-glutamylcysteine synthetase and does not inhibit GS activity (Ronzio & Meister, 1968). The present results demonstrate that BSO affects neither pituitary GS activity nor the population of GS cells, and that MSO specifically interacts with GS molecules to decrease GS activity. In order to explain the increase in the population of ir-GS cells in response to MSO treatment, it is postulated that there is a feedback mechanism between glutamine levels and the synthesis of the GS protein in FS cells. That is, the absence of glutamine (reduction or elimination of GS activity due to the presence of MSO) causes a stimulation in the amount of GS protein produced which in turn increases the immunoreactive GS protein even though the newly synthesised GS activity is also inhibited by MSO. The net effect is the synthesis and accumulation of GS molecules in the cell cytoplasm that changes immunonegative or slightly immunopositive FS cells into ir-GS cells which results in an increase of the ir-GS cell population.

The effects of glucocorticoids on gene expression in general and in particular pituitary hormone expression have been well established (Suter & Schwartz, 1985; Subramaniam et al. 1997). The presence of glucocorticoid receptors in the pituitary gland has also been demonstrated by means of immunocytochemistry using a specific antibody to glucocorticoid receptor (Antakly & Eisen, 1984; Kononen et al. 1993). Kononen et al. (1993) showed that the majority of adrenocorticotropic hormone cells and growth hormone cells in the anterior lobe had glucocorticoid receptors while the adrenocorticotropic hormone cells of the intermediate lobe did not express glucocorticoid receptor. The glycoprotein hormone cells also had glucocorticoid receptors, but to a lesser degree, and only a minority of the prolactin cells expressed glucocorticoid receptor. There was no mention of the glucocorticoid receptor status of FS cells. The present study is the first to demonstrate that pituitary FS cells respond to glucocorticoids in vitro by increasing the synthesis of GS and suggest that the glucocorticoid receptor is present in FS cells.

Finally, our findings that the age dependent increase in GS activity in the rat pituitary is not affected by removal of endogenous glucocorticoids (adrenalectomy), yet exogenously administered glucocorticoids (dexamethasone and hydrocortisone) stimulate GS activity in the pituitary at all ages is not without precedent. Adrenalectomy did not alter anterior pituitary glucocorticoid receptor type II mRNA expression, and yet exogenous glucocorticoid treatment led to an increase in glucocorticoid receptor mRNA levels (Sheppard et al. 1990). Possible explanations for such phenomena include differential steroid specificity of the different types of glucocorticoid-mineralocorticoid receptors, differential metabolism of glucocorticoids, or a combination of both (Sheppard & Funder, 1987). Although our results provide some interesting clues as to a possible role of glucocorticoid-inducible GS in FS cells, further analyses will be required to elucidate the involvement of GS in the physiological control of hormone secretion and cell differentiation in the anterior pituitary.

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REFERENCES


MILLER RE, HACKENBERG R, GERSHMAN H (1978) Regulation of glutamine synthetase in cultured 3T3-L1 cells by insulin, hydrocortisone, and dibutyryl cyclic AMP. *Proceedings of the National Academy of Sciences of the USA* 75, 1418–1422.


VAN DEN VERG CJ, VAN DEN VELDEN J (1970) The effect of methionine sulfoximine on the incorporation of labelled glucose,


