α_{2u} -Globulin is present in the rat anterior pituitary

(α_{2u} -globulin mRNA)

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Communicated by Seymour Lieberman, March 21, 1983

ABSTRACT The possibility that the pituitary gland may contain as yet undiscovered regulatory factors is intriguing. Recent reports have suggested the presence, in the anterior pituitary, of a number of proteins of extrapituitary origin. α_{2u} -Globulin, a rat serum and urinary protein, previously shown to be synthesized in the submaxillary gland and in the liver under anterior pituitary control, has now been localized by immunocytochemistry in the cytoplasm of some cells of the anterior pituitary. No α_{2u} -globulin could be detected in either the intermediate or posterior pituitary. The presence of α_{2u} -globulin was confirmed and quantitated by radioimmunoassay. Using RNA blot analysis and cloned α_{2u} -globulin cDNA probes, we could not detect α_{2u} -globulin mRNA sequences in pituitary RNA, indicating that α_{2u} -globulin is not synthesized therein. The presence of α_{2u} -globulin, presumably of circulatory origin, in certain anterior pituitary cells suggests that it may play a role in anterior pituitary function.

The role of neurohormones and peripheral hormones (e.g., sex steroids, thyroid hormones, glucocorticoids) as pituitary hormone modulators is well established. Recent reports have described numerous other factors not of hypophyseal origin in this gland and suggested that these factors may play modulatory roles therein. Examples are angiotensin II (1–3) and epidermal growth factor (4–7), which have specific receptors in the rat anterior pituitary and modulate pituitary hormone secretion. Also, substance P, known to be synthesized in the central nervous system and gut, was recently localized by immunocytochemistry within specific cells of the anterior pituitary of the guinea pig (8). It is evident that the pituitary gland may contain as yet undiscovered regulatory factors. Pituitary hormones directly or indirectly regulate the function of many “nonendocrine” organs. It is therefore reasonable to consider whether secretory products from these organs, released into the serum, may in turn regulate pituitary hormone function by a feedback mechanism.

α_{2u} -Globulin is a rat urinary and serum protein (9, 10) of unknown function. Only liver (10, 11) and the submaxillary salivary gland (12, 13) have been reported to synthesize α_{2u} -globulin. α_{2u} -Globulin provides an interesting system to study hormonal regulation of gene expression because its hepatic biosynthesis is multihormonally controlled, involving the pituitary as a prime modulator (9–14). Despite numerous studies, the physiological role of α_{2u} -globulin remains obscure. To elucidate its function, we have used a sensitive immunocytochemical technique (11, 12) to examine numerous tissues. In this report, we present evidence that (i) α_{2u} -globulin is immunochemically localized in some cells of the rat anterior pituitary, but not in any cells of the intermediate or posterior pituitary, and (ii) these cells are devoid of detectable levels of α_{2u} -globulin mRNA se-

quences. The presence of α_{2u} -globulin in anterior pituitary cells suggests a possible role of this polypeptide in anterior pituitary function.

MATERIALS AND METHODS

Animals and Immunocytochemical Procedure. We examined male and female rats 22, 30, 35, and 65–100 days old. Eighty-five rats were used. The animals were decapitated, and the pituitaries and wafers from the liver were immediately removed and either immersed in Bouin's fixative (for immunocytochemistry) or frozen in liquid nitrogen (for subsequent RNA extraction and α_{2u} -globulin radioimmunoassay). α_{2u} -Globulin was localized in histological sections of the Bouin-fixed paraffin-embedded tissues by using an indirect immunoperoxidase procedure described earlier (11, 12).

To confirm the specificity of the immunocytochemical reaction, we first absorbed the antibody with purified α_{2u} -globulin at various concentrations as determined in our study on the liver and submaxillary gland (11, 12). Control sections in which preimmune serum or the diluting buffer was used in place of the α_{2u} -globulin antibody were devoid of specific staining.

To verify that the α_{2u} -globulin antibodies do not crossreact with either prolactin, growth hormone, follicle-stimulating hormone, luteinizing hormone, thyrotropic hormone, or adrenocorticotrophic hormone (ACTH), we treated the α_{2u} -globulin antibodies (under the conditions described in Fig. 1C) with each of these pituitary hormones (0.1 mg of hormone per 0.2 mg of IgG). Rat prolactin, growth hormone, follicle-stimulating hormone, luteinizing hormone, and thyrotropic hormone were provided by the National Pituitary Agency of the National Institute of Arthritis, Metabolic, and Digestive Diseases. Porcine ACTH was obtained from Sigma.

Radioimmunoassay of α_{2u} -Globulin. Each of 18 adult male pituitaries was homogenized in 1 ml of phosphate-buffered saline (0.01 M phosphate buffer, pH 7.5/0.9% NaCl)/1% Triton X-100/1% deoxycholate in a Potter-Elvehjem homogenizer, the homogenates were centrifuged at $5,000 \times g$ for 30 min, and the individual supernates were immediately frozen and stored at -20°C until assayed. For comparative purposes, similar homogenates were prepared from liver wafers cut from 10 of the 18 animals. To measure plasma α_{2u} -globulin level, trunk blood was collected, heparin was added at 50 units/ml, and the blood was centrifuged at low speed to separate the plasma. Double-antibody radioimmunoassays were done in duplicate on samples from the 18 pituitaries, 10 livers, and four sera. α_{2u} -Globulin was iodinated with ^{125}I by using chloramine-T and radioimmunoassays were carried out by a protocol similar to that of the radioimmunoassay of pituitary hormones (15). The sensitivity is 0.01 $\mu\text{g/ml}$. Data reported are mean $\pm$ SEM. Protein mea-

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surements were carried out according to Lowry (16).

Detection of α_{2u} -Globulin mRNA by RNA Blot Analysis. To investigate whether the α_{2u} -globulin found in the anterior pituitary was synthesized therein, we carried out, in three separate experiments, RNA blot analyses of RNA extracted from 10–15 pooled pituitary glands of either male or female rats using the procedures described in our previous reports (11–13). The nick-translated clonal α_{2u} -cDNA probe used has been fully characterized and its sequence has been determined (17).

RESULTS AND DISCUSSION

Fig. 1 illustrates the α_{2u} -globulin-containing cells in the rat anterior pituitary. No cells are reactive either in the intermediate

or in the posterior lobes. α_{2u} -Globulin was detected in most but not all of the anterior pituitaries of both male and female rats of all ages examined. The specificity of the immunocytochemical reaction was shown by the complete absence of immunoperoxidase staining by antibody previously adsorbed with pure α_{2u} -globulin (Fig. 1C). The possibility that the anti- α_{2u} -globulin, which had been prepared against α_{2u} -globulin purified from urine, might be interacting with a known anterior pituitary hormone was precluded by absorption experiments showing that prior incubation of the antibody with prolactin, growth hormone, follicle-stimulating hormone, luteinizing hormone, adrenocorticotrophic hormone, or thyrotrophic hormone did not alter the ability of the antibody to react with these anterior pi-

FIG. 1. (A) Immunocytochemical localization of α_{2u} -globulin in adult male rat pituitary. Numerous immunoreactive cells are found throughout the anterior lobe (AL) but no immunoreactive cells are observed in the intermediate (IL) or posterior (PL) lobes. (B) The boxed area in A is shown at higher magnification. Note the strong cytoplasmic staining in the immunoreactive cells (arrow). (C) Immunocytochemical control. This section, which is consecutive to that shown in A, was incubated with anti- α_{2u} -globulin previously absorbed overnight with pure α_{2u} -globulin (90 μ g of α_{2u} -globulin per 200 μ g of antiserum IgG). Note the complete absence of immunocytochemical reaction. Double-headed arrows indicate landmarks common to both fields.

FIG. 2. RNA blot analysis of pituitary and hepatic RNA. These experiments, which were carried out exactly as described in refs. 3–5, 7, show the absence of detectable levels of α_{2u} -globulin mRNA sequences in male pituitary total RNA (lane 1). However, mature male hepatic α_{2u} -globulin mRNA sequences are readily observed (lane 2). Each lane contains 30 μ g of RNA and RNAs were prepared and analyzed concurrently.

tuitary cells. Additional evidence for α_{2u} -globulin specificity in our immunocytochemical reaction is the absence of amino acid homology between α_{2u} -globulin (17) and polypeptide hormones of known sequence. Immunoreactive α_{2u} -globulin was also quantitated by radioimmunoassay. The average pituitary α_{2u} -globulin content in the adult rat was 89 ± 9 ng per pituitary or 0.006% of total pituitary soluble proteins. The adult male liver contains 0.6% of its soluble protein as α_{2u} -globulin, which is 100-fold that of the pituitary. The plasma level of α_{2u} -globulin was 54 ± 1 μ g/ml.

Sensitive RNA blot studies using nick-translated cloned α_{2u} -globulin cDNA (17) were unable to detect α_{2u} -globulin mRNA sequences in pituitary RNA (Fig. 2). Thus, although α_{2u} -globulin is present in the anterior pituitary (Fig. 1), it seems not to be synthesized therein. Presumably α_{2u} -globulin is taken up from the circulation as are other pituitary regulatory polypeptides. The selective presence of α_{2u} -globulin in the anterior but neither the intermediate nor the posterior pituitary suggests that this substance may play a role within the anterior pituitary.

α_{2u} -Globulin is the major urinary protein of the male rat, which excretes as much as 20–30 mg/day (9). A homologous protein family called MUP (mouse urinary protein) has been described in the mouse (18, 19). We have recently localized this urinary protein in mouse liver (11) and anterior pituitary (unpublished data). The biological role of these rodent urinary proteins is unknown. The presence of α_{2u} -globulin and mouse urinary protein in the rodent anterior pituitary may be of import in that rodent urinary substances have been shown to be pheromones. Responses to unidentified rodent pheromones include changes in sexual behavior, aggressiveness, and precocious onset of sexual maturity in females (20–25). These behavioral and reproductive changes after exposure of the rodent

to urine are accompanied by altered circulating levels of follicle-stimulating hormone, luteinizing hormone, and prolactin (20, 21, 23). Such changes in anterior pituitary hormone levels have been proposed to mediate the biological effects of these urinary pheromones (23).

We thank Ms. Rosheen Walsh for secretarial assistance. This work was supported by National Cancer Institute Grant CA22376.

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