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INTRACELLULAR CBG-LIKE MOLECULES IN THE RAT PITUITARY

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Summary—The localization of transcortin (CBG) in pituitary cells of the rat was investigated using the peroxydase–antiperoxydase (PAP) technique. A rabbit antiserum against purified rat plasma transcortin was used as the primary antiserum. Transcortin-like (CBG-like) immunoreactive products were found in the cytoplasma of certain cells in the anterior pituitary, but not in the intermediate lobe and weakly in the posterior pituitary. It is postulated that the CBG-like molecules participate in the cellular uptake process of corticosterone, thereby modulating the feedback signal of this steroid on pituitary function.

INTRODUCTION

Receptor sites for glucocorticoids and mineralocorticoids have been identified in the anterior pituitary [1–5]. Accordingly, when such labelled adrenal steroids or their analogs are given intravenously in tracer amounts to adrenalectomized rats, the radioactive steroid is bound to its receptor and retained in cell nuclei of the gland. Thus, extensive cell nuclear retention was noted for [^{3}H]aldosterone [6] or the potent synthetic glucocorticoid [^{3}H]dexamethasone [7–9]. The latter steroid is a potent suppressor of ACTH release [10]. Principal localization of the labelled dexamethasone–receptor complex was found in the corticotrophs, and to a lesser extent in gonadotrophs, somatotrophs and cells stained with antisera to prolactin [11].

The posterior pituitary also accumulated [^{3}H]dexamethasone, but the intermediate lobe appeared virtually devoid of adrenal steroid receptor containing cells [11]. However, if the naturally occuring glucocoticoid of the rat, corticosterone, was infused there was only very low uptake and retention of this steroid in cell nuclei of the pituitary [7,8]. Moreover, pretreatment with a 100 fold excess of unlabelled corticosterone did not interfere with [^{3}H]dexamethasone cell nuclear localization, while the same dose of unlabelled dexamethasone reduced uptake to below 10% [11–13]. Note that [^{3}H]corticosterone accumulated preferentially in neurons of the hippocampus of rat brain [7].

In 1976 it was postulated independently by two groups [12,13] that the presence of transcortin-like molecules (corticosteroid binding globulin, CBG-like) in the pituitary could explain the low uptake and poor competition of corticosterone in the pituitary (CBG-like molecules: K_D: 14.6 nM; B_{max}: 163 fmol/mg protein). These CBG-like molecules resemble plasma transcortin in binding specificity towards corticosterone, in molecular weight, isoelectric focusing profile, ammonium sulphate precipitability and immunologic properties [12–14]. The proteins lack the ability to transport corticosterone to cell nuclei [13].

The CBG-like binding system is present in pituitary cytosol after extensive perfusion [13] and remains associated with isolated pituitary cells [15]. Besides cytosol also membrane preparations of the pituitary displayed binding specificity similar to that displayed by the CBG-like binding system [16]. Immunocytochemical observations reported from this laboratory [17] and from elsewhere [18] indicated intracellular localization of the CBG-like binding system in the pituitary. Specificity and sensitivity of the used peroxydase–antiperoxydase (PAP) technique has been improved recently and we now report that localization of CBG-like immunoreactive products is restricted to certain cell types of the anterior pituitary.

MATERIALS AND METHODS

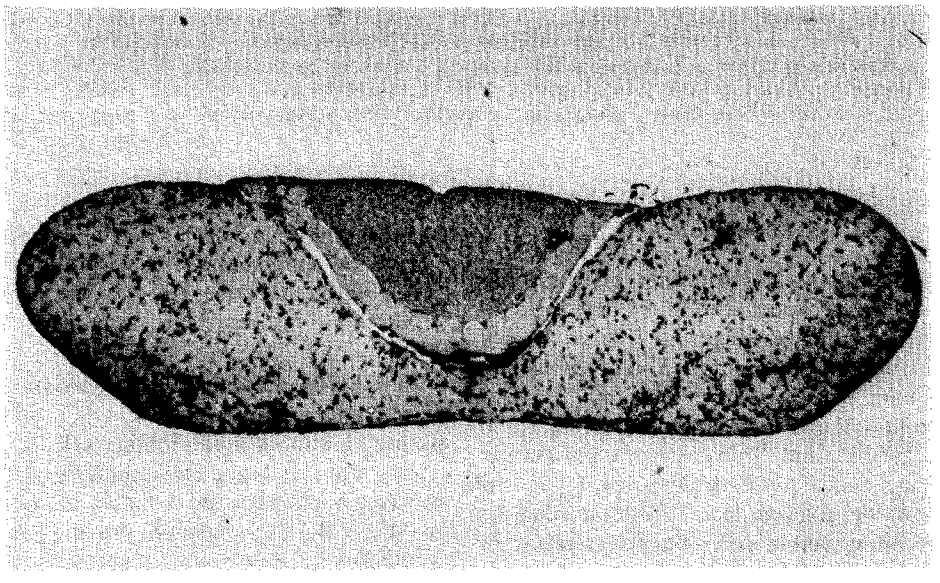
Male Wistar rats of our own breeding (200–250 gram bodyweight) were extensively perfused through the heart with saline under Nembutal anesthesia. The whole pituitary was excised and fixed in 4% picric acid for 24 h at room temperature.

After fixation the pituitaries are dehydrated in a graded series of ethanol and amylacetate and subsequently embedded in paraffin. Section of 5 μm thickness were cut and mounted on glass slides coated with 0.5% bovine serum albumin and prepared for the staining procedure in 0.01 M phosphate buffered saline via routine histological procedures. Peroxydase–antiperoxydase technique (PAP) was applied according to Sternberger [19]. Transcortin was purified from rat serum by affinity chromatography and antibodies were raised in rabbits [14]. The sec-

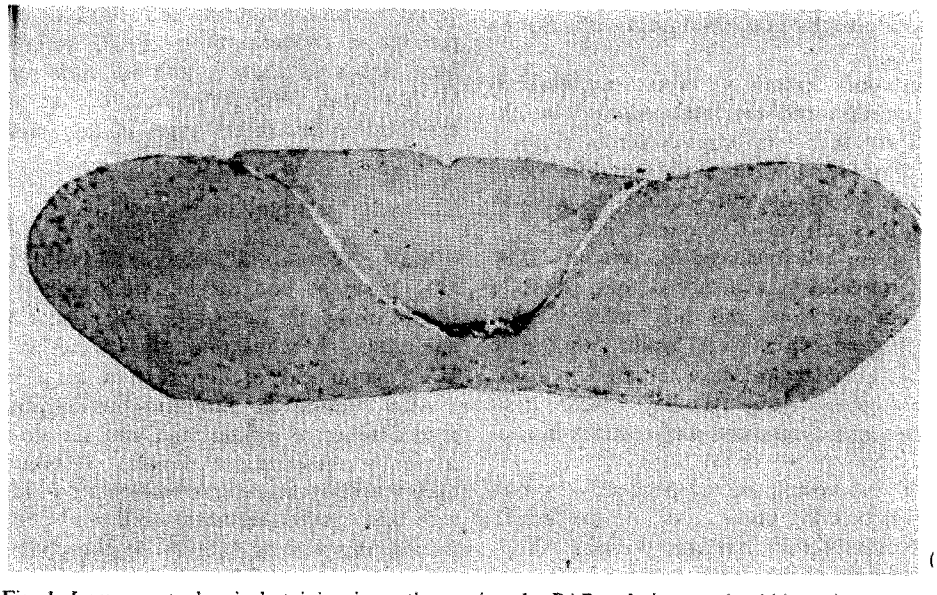
tions were incubated with the immunocytochemical reagents in the following sequence: (1) rabbit antiserum to rat plasma transcortin dilution of 1:1600 for 72 h at 4°C. (2) swine antiserum to rabbit immunoglobulin (Nordic Immunological Laboratories, Tilburg, The Netherlands, 1:40) for 60 min at room temperature. (3) rabbit PAP complex (Sternberger, Jarretsville, Maryland, U.S.A.) dilution 1:200 for 60 min at room temperature.

After each incubation the sections were washed in 0.01 M phosphate buffered saline. Finally, the sections were incubated for 1–10 min in a solution of 0.05% 3,3′ diaminobenzidine tetrahydrochloride (DAB, Sigma, Amsterdam) and 0.01% H_2O_2 in 0.05 M Tris buffer pH = 7.4. Finally, the glass slides with the sections were washed with distilled water, dehydrated and embedded in Depex.

For demonstration of the specificity of the staining the following controls were used: (1) omission of the first antiserum and substitution with non-immune swine serum. (2) absorption of primary antiserum with purified rat transcortin or with rat serum.



(A)



(B)

Fig. 1. Immunocytochemical staining in sections using the PAP technique and rabbit antiserum to rat plasma transcortin (CBG). A: Transversal section through the pituitary showing PAP reaction product deposited in cells of the anterior pituitary. Posterior lobe shows weak staining and intermediate lobe is not stained. Magnification: × 50. B: Control section with primary antiserum absorbed by transcortin prior to staining reaction

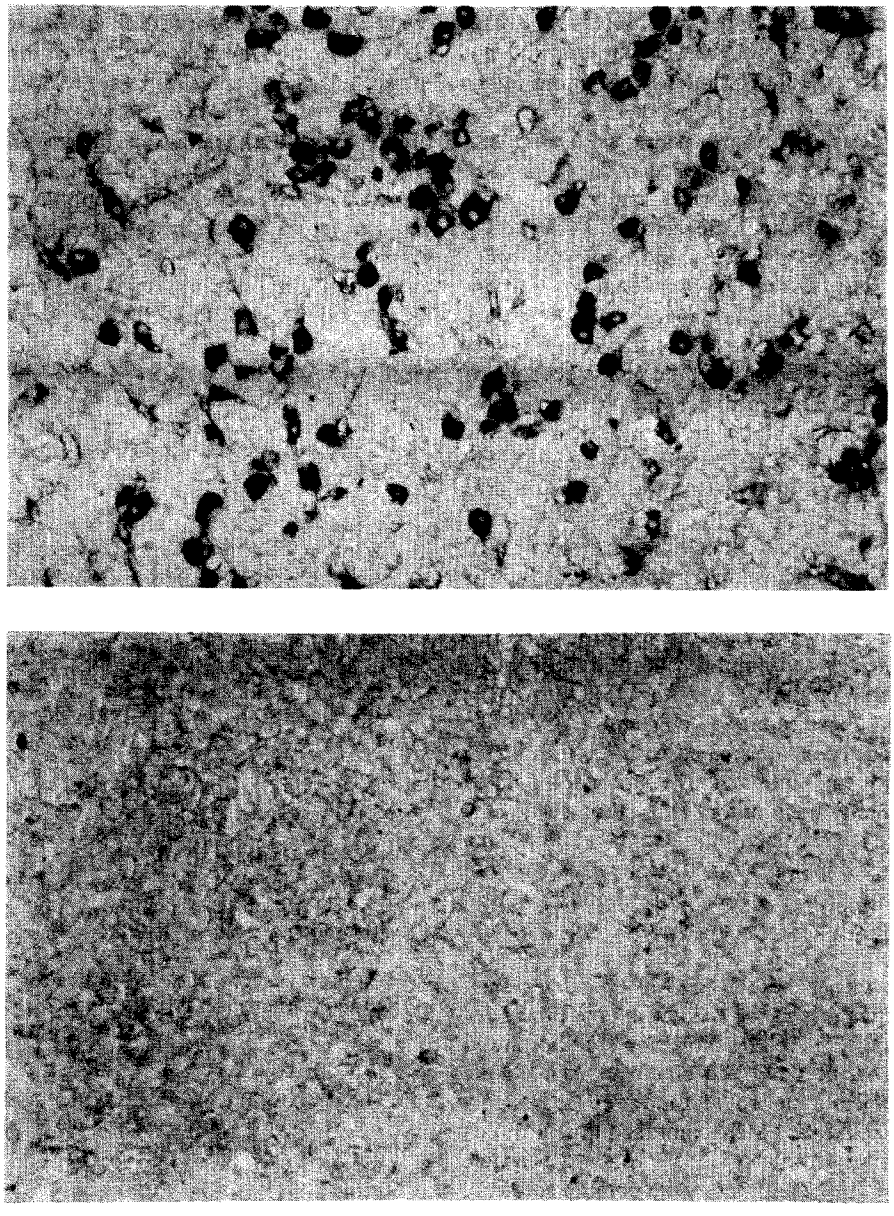


Fig. 2. Immunocytochemical staining of cells of anterior pituitary. Magnification: × 320.

RESULTS

Immunoreactive products were stained in cells of the anterior pituitary, which is indicative of the presence of CBG. The stained cells were randomly present in the anterior pituitary. There were no stained reaction products in the intermediate lobe, and cells of the posterior lobe were weakly stained (Fig. 1A). At higher magnification immunoreactive products were visible predominantly in the cytoplasm (Fig. 2). The type of cells stained was not defined. However, in a parallel series of sections anti-ACTH as primary antiserum did not seem to stain many of the cells containing the CBG-like molecules. Absorbtion of primary antiserum with purified CBG abolished specific binding (Fig. 1B), indicating that staining demonstrated sites of attachment of CBG antibodies to antigenic CBG present in anterior pituitary cells. Control sections incubated without the primary antiserum were consistently unstained.

DISCUSSION

The immunocytochemical staining of particular cells in the anterior pituitary suggests the intracellular localization of protein is antigenically indistinguishable from rat plasma CBG. This finding extends previous observations in which another fixative (Bouin-Hollande) was used, that resulted in a somewhat weaker and more uniformly distributed staining of the cytoplasm of anterior pituitary cells [17].

Intracellular localization of rat CBG was also reported by another group, but the authors did not specify cell types [18]. The intensive staining of the posterior pituitary reported by these authors was noted by us as well using Bouin as fixative but appeared however not to be specific. The staining of the posterior pituitary was weak under the present experimental conditions with picric acid fixative.

The present study provides a morphological proof of a series of biochemical and functional observations on the presumptive intracellular localization of a CBG-like binding system in the anterior pituitary [5,12,13,15]. Such a CBG-like binding system appears absent from the intermediate, but present in low amounts in the posterior lobe. Intracellular binding systems resembling CBG have been noted as well in other cells such as human lymphocytes and uterus [20–23].

The origin of the CBG-like binding system might well be the plasma. Hormonal manipulations such as adrenalectomy, thyroid hormone or estrogen treatment that are known to affect the plasma transcortin level are reflected and amplified in magnitude in the capacity of the pituitary TL system [15,24].

Moreover, the pituitary is depleted of CBG-like molecules when rats are treated with the transcortin antiserum [5] and intravenously infused iodinated CBG could enter the pituitary [18]. During ontogeny there is rapid decline in pituitary CBG and rat plasma CBG and the binding system is undetectable with labelled corticosterone between day 6 and day 10 of post-natal age [25]. Thereafter, the levels show a rapid rise and reach adult levels at 4 weeks of age [25].

There is evidence that the CBG-like binding system actively participates in the cellular uptake process of corticosterone [26]. Cell fractionation studies showed association of the CBG-like binding system at the plasma cell membrane [16] and the protein probably can be internalized via the membrane in tissues, such as the pituitary, which has a protein permeable vascular bed [18]. That membrane components may be involved in glucocorticoid uptake became evident in work with a pituitary tumor cell line AtT-20/01-1 [27]. The membrane components of these cells, however, differ from the CBG-like binding system in the present study in that they also displayed high affinity towards dexamethasone.

While the entry of corticosterone is facilitated *in vitro* [26] possibly by mediation of CBG-like binding system, the ultimate extent of cell nuclear localization is however much lower *in vivo* [7] after a pulse injection than *in vitro* in pituitary tissue [7] or pituitary tumor cells [1]. The difference is not only due to an apparent lower intrinsic ability of the corticosterone–receptor complex to promote cell nuclear translocation, but also seems a consequence of participation of the CBG-like binding system. The CBG-like sites are thought to compete with the glucocorticoid receptor sites for binding of corticosterone and as a consequence less steroid is made available to the receptor for cell nuclear translocation [7,28]. Certainly, this role is accounted for by both the intracellular CBG and the extracellular CBG binding system in the plasma, since both regulate the amount of free steroid. Dexamethasone does not bind to CBG and bypasses this mechanism, which contributes to the extensive cell nuclear localization of the synthetic glucocorticoid. Although we did not identify in the present study the cells that contain the largest amount of CBG-like molecules as corticotrophs, it might well be that this CBG-like binding system modulates the feedback signal of corticosterone on pituitary function.

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