Distribution of Angiotensinogen Immunoreactivity in Rat Anterior Pituitary Glands (43260)

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Abstract. **Angiotensin II (AII) has been previously shown to be localized in the gonadotropes of the rat anterior pituitary gland. Renin and angiotensin-converting enzyme, two enzymes that participate in the generation of AII, also have been shown to be present in gonadotropes. To determine whether angiotensinogen, the precursor to AII, is present in the same cells, we have stained rat anterior pituitary sections with an antirat angiotensinogen antiserum. Angiotensinogen staining was observed in cells that had a distinctive distribution at the periphery of the gland; the number of these cells and the intensity of the staining were increased in the pituitaries of rats that had been nephrectomized 24 hr before sacrifice. When double staining was performed, we never observed colocalization of angiotensinogen with any of the known pituitary hormones or with S100 protein. The results show that in the rat anterior pituitary gland, angiotensinogen is present, at least for the most part, in cells that are different from those containing renin, angiotensin-converting enzyme, and AII.** [P.S.E.B.M. 1991, Vol 197]

Angiotensinogen, the glycoprotein precursor of angiotensin II (AII), plays a key role in the regulation of blood pressure. The limiting step in the production of circulating AII appears to be the cleavage of hepatic angiotensinogen by renin from the juxtaglomerular apparatus of the kidney. The released decapeptide angiotensin I is subsequently processed by angiotensin-converting enzyme (ACE) into the bioactive octapeptide AII.

Renin mRNA has been identified in a number of tissues other than kidney, including the anterior pituitary gland, the adrenal gland, the reproductive tissues, and the brain (1–5). The colocalization of AII in these tissues (6–9) has led to the proposal that there are tissue renin-angiotensin systems whose role might be to produce AII for local action (10). In the rat anterior pituitary gland, renin, ACE, and AII have been localized in gonadotropes (8, 11, 12). The authenticity of AII immunoreactivity in the anterior pituitary gland has been supported by high-pressure liquid chromatography (8). In addition, AII immunoreactivity has been shown to persist in pituitary cells or explants maintained for up to 2 weeks in serum-free medium, suggesting that pituitary AII is synthesized locally (8).

Angiotensinogen has been found in anterior pituitary extracts (13). In humans, angiotensinogen immunoreactivity has been reported to coexist with renin and ACE immunoreactivity in the same prolactin-containing cells (14). In contrast to humans, recent studies indicate that angiotensinogen immunoreactivity may be present in various types of cells of the rat anterior pituitary gland (15). The purpose of the present study was to investigate further the distribution of angiotensinogen immunoreactivity within the rat anterior pituitary gland, and compare it with the staining pattern of other pituitary cell markers.

Material and Methods

Immune Reagents. The characteristics of the antirat angiotensinogen antiserum (ORNI, gift from J. Bouhnik, Paris) have been published previously (16). The antiserum was raised in rabbits by injecting highly purified, homogeneous rat angiotensinogen. It displayed 100% cross-reactivity with rat angiotensinogen and negligible cross-reactivity with angiotensin I and II, tetradecapeptide substrate, and mouse, dog, hog, bovine, rabbit, monkey, and human angiotensinogen. Purified rat angiotensinogen was also obtained from J.

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Bouhnik. The antiserum to bovine S100 protein was a gift from H. Herschman (UCLA, Los Angeles). The anti-ACTH antiserum (raised against 17–39 synthetic ACTH) was developed and characterized by M. S. Brownfield (17). Rabbit antisera against pituitary hormones (rat luteinizing hormone-β, human follicle-stimulating hormone-β, rat prolactin, human growth hormone, human and thyroid-stimulating hormone-β) were developed by Dr. A. F. Parlow and obtained from the NIADDK. Reagents for the avidin-biotin staining were obtained from Vector Laboratories (Burlingame, CA).

Tissues and Staining. Pituitaries were collected from adult male Wistar rats (Simonsen Laboratories, Gilroy, CA), either intact or bilaterally nephrectomized 24 hr before sacrifice. Four rats were used in each group. The pituitaries were fixed by immersion in Bouin Holland sublimate and embedded in paraffin as described previously (18). Five-micrometer sections were cut coronally and collected on gelatin-coated slides. Immunoperoxidase staining was performed by the avidin-biotin peroxidase complex technique, as previously described (18). Angiotensinogen was detected with the ORNI antiserum at a 1/500 dilution, using diaminobenzidine as a chromogen. This procedure identified labeled cells by means of brown deposits. When double staining was performed, staining for the pituitary cell markers was carried out first. The unreacted antibodies were then removed according to the method of Tramu *et al.* (19) by immersion in a solution containing 0.15 *M* $KMnO_4$ and 0.01 H_2SO_4. After careful washing with Tris-buffered saline, the ORNI antiserum was applied, incubated, and detected using the method described above, except that 4-chloronaphthol was used as the chromogen. This second step labeled angiotensinogen-containing cells with blue deposits.

Results

Some angiotensinogen staining was present in the pituitaries of intact animals, but it was not abundant: few cells were stained, and the intensity of the staining was faint. When rats were nephrectomized 24 hr before sacrifice, both the intensity of the staining and the number of positive cells were enhanced (Fig. 1). Although the difference was dramatic and observed consistently in all of the nephrectomized rats, it was difficult to quantitate the extent of the increase because of the weakness of the staining in the pituitaries of intact rats. The distribution of the angiotensinogen-positive cells was not random: they formed a distinct rim at the periphery of the gland (Fig. 1). These cells were small, polygonal, and had sparse cytoplasm. When the antiserum (final dilution 1/500) was preincubated for 2 hr at room temperature with purified rat angiotensinogen (final concentration 10 μg/ml), the staining was completely abolished (data not shown).

Staining with other pituitary cell markers revealed discrete populations of cells. The growth hormone-containing cells were most abundant: they were small and ovoid and were distributed throughout the gland, constituting approximately 40% of all anterior pituitary cells. The prolactin-containing cells were small, had a polygonal shape, and were present throughout the pituitary. They constituted approximately 15% of all cells. The luteinizing hormone (LH)-containing cells were large, ovoid, and distributed throughout the gland but were more concentrated in the dorsal zone; they constituted approximately 10% of all pituitary cells. The appearance and distribution of the follicle-stimulating hormone-containing cells were similar. The thyroid-stimulating hormone cells were medium-sized, ovoid cells that were much less abundant (approximately 2–4% of all cells), and they were more frequently seen in the medial part of the gland. The ACTH-containing cells were polygonal to stellate, were distributed throughout the gland, and constituted approximately 5% of total cells. The S100 protein-containing cells had a distinctive stellate shape with many processes, were distributed throughout the gland, and constituted approximately 15% of total cells.

When double staining was performed for pituitary cell markers and angiotensinogen in sections of pituitaries from nephrectomized rats, the blue stain for angiotensinogen always appeared in different cells than the brown stain for other pituitary cell markers (Fig. 2). Since essentially all follicle-stimulating hormone-containing cells also contain LH (20), only the LH staining is shown in Figure 2 for the purpose of identifying gonadotropes. Although we must consider the possibility that some faint blue staining was masked in cells containing dark brown deposits, this is unlikely to have occurred for two reasons. First, when we performed double staining using chloronaphthol (blue) for the pituitary cell markers and diaminobenzidine (brown) for angiotensinogen, no brown immunoreactivity was observed in the cells stained in blue for pituitary markers. Second, the morphologic appearance and distribution of the angiotensinogen-containing cells was different from any of the types of cells identified with the other pituitary cell markers. Our data are therefore strong evidence that the cells containing angiotensinogen immunoreactivity are different from the ones stained with the other pituitary cell markers.

Discussion

In this article, we show that certain pituitary cells contain angiotensinogen immunoreactivity. These cells have a distinctive morphology and distribution. The peripheral distribution of the cells is not likely to be an artifact caused by poor penetration of fixatives, since other antibodies readily identified other cell types distributed throughout the gland.

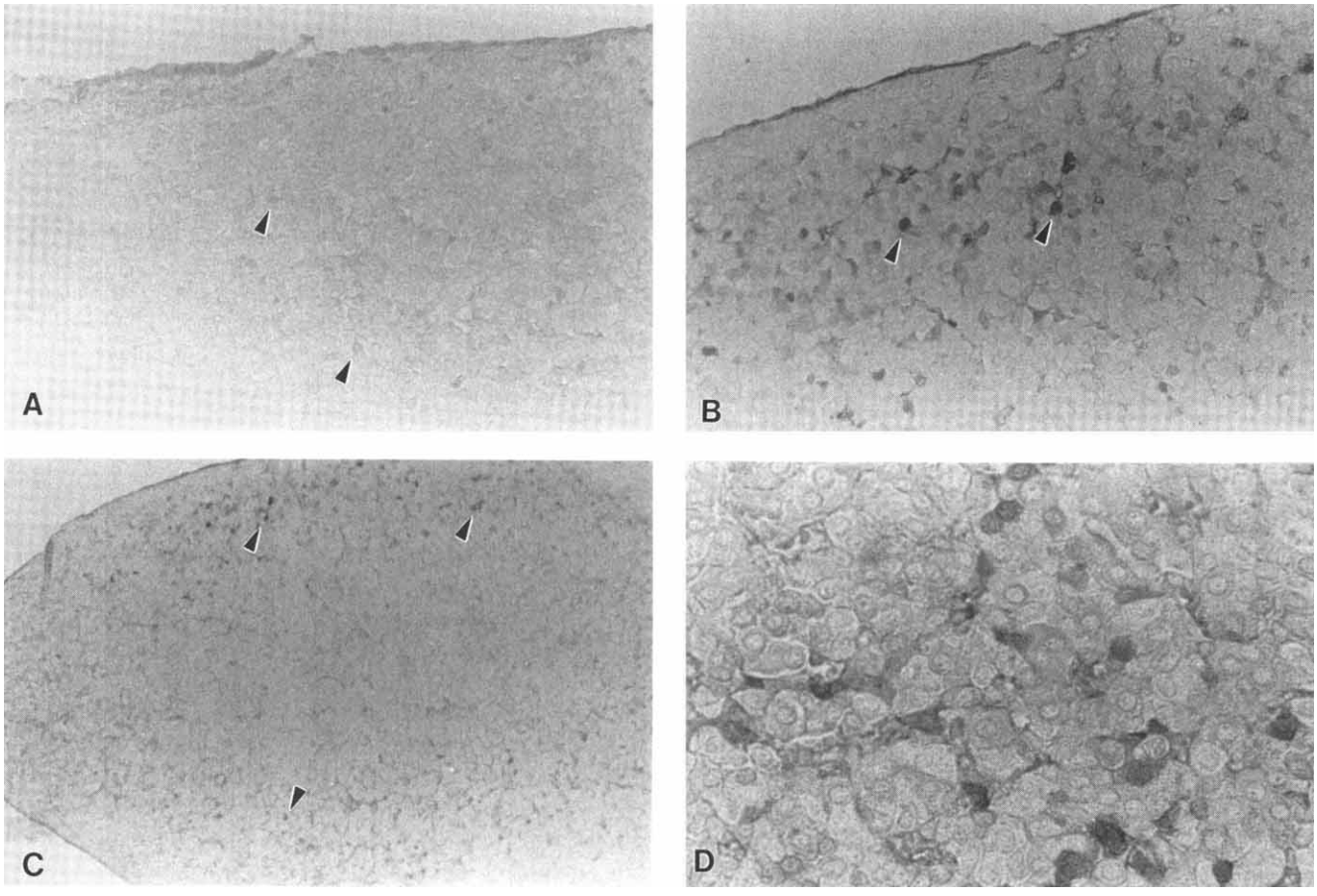

Figure 1. Single staining of rat anterior pituitary sections with the ORNI antiangiotensinogen antiserum. (A) Pituitary section from an intact rat (original magnification ×63). The arrowheads point to some cells containing weak staining. (B) Pituitary section from a nephrectomized rat (original magnification ×63). The photograph shows a pituitary region similar to the one presented in A. The arrowheads point to some of the cells containing angiotensinogen immunoreactivity. (C) Pituitary section from a nephrectomized rat (original magnification ×25). The arrowheads point to some of the cells containing angiotensinogen immunoreactivity. At this lower magnification, it is apparent that the positive cells have a distinct distribution at the periphery of the gland. (D) Pituitary section from a nephrectomized rat (original magnification ×160). This is a higher magnification of some of the cells presented in B.

There is good evidence that angiotensin II is synthesized locally in the rat anterior pituitary gland (8). Gonadotropes are the likely site of synthesis of AII since renin, ACE, and AII have been localized in these cells (8, 11, 12). However, we did not find angiotensinogen in these cells. In addition, we never observed colocalization of angiotensinogen with any of the other known pituitary hormones. The distribution and appearance of the various pituitary cell types was in good agreement with previous reports (21, 22). Furthermore, we never observed colocalization of angiotensinogen with S100 protein, a protein that in rat is a marker for the agranular nonsecretory folliculostellate cells (23). Therefore, the exact identity of the pituitary cells that stained with our antiangiotensinogen antiserum remains undetermined. In human pituitary glands, other cell types, including null cells and oncocytes, have been described (22). However, those cells have no histologic or immunochemical markers. Macrophages have also been described in rodent pituitaries, as in other endocrine tissues (24). Macrophages in other tissues have been reported to contain ACE (25). However, there is no evidence that they contain angiotensinogen, and their distribution in the gland appears to be more random than the angiotensinogen-containing cells (24).

Using an antiserum raised in their own laboratory, Thomas and Sernia (15) have recently found angiotensinogen immunoreactivity in endothelial cells and in small cells surrounding follicle-like structures in the rat anterior pituitary gland. The latter cells did not appear to contain S100 or prolactin. They appeared to be located mainly at the periphery of the gland, although it is difficult to determine whether these cells are the same as the ones we describe in the study presented here. In addition, when Thomas and Sernia (15) increased the sensitivity of detection by silver intensification, they reported colocalization of angiotensinogen with LH and AII in some gonadotropes. Since the amount of immunoreactivity in gonadotropes was much lower than that in the small perifollicular cells,

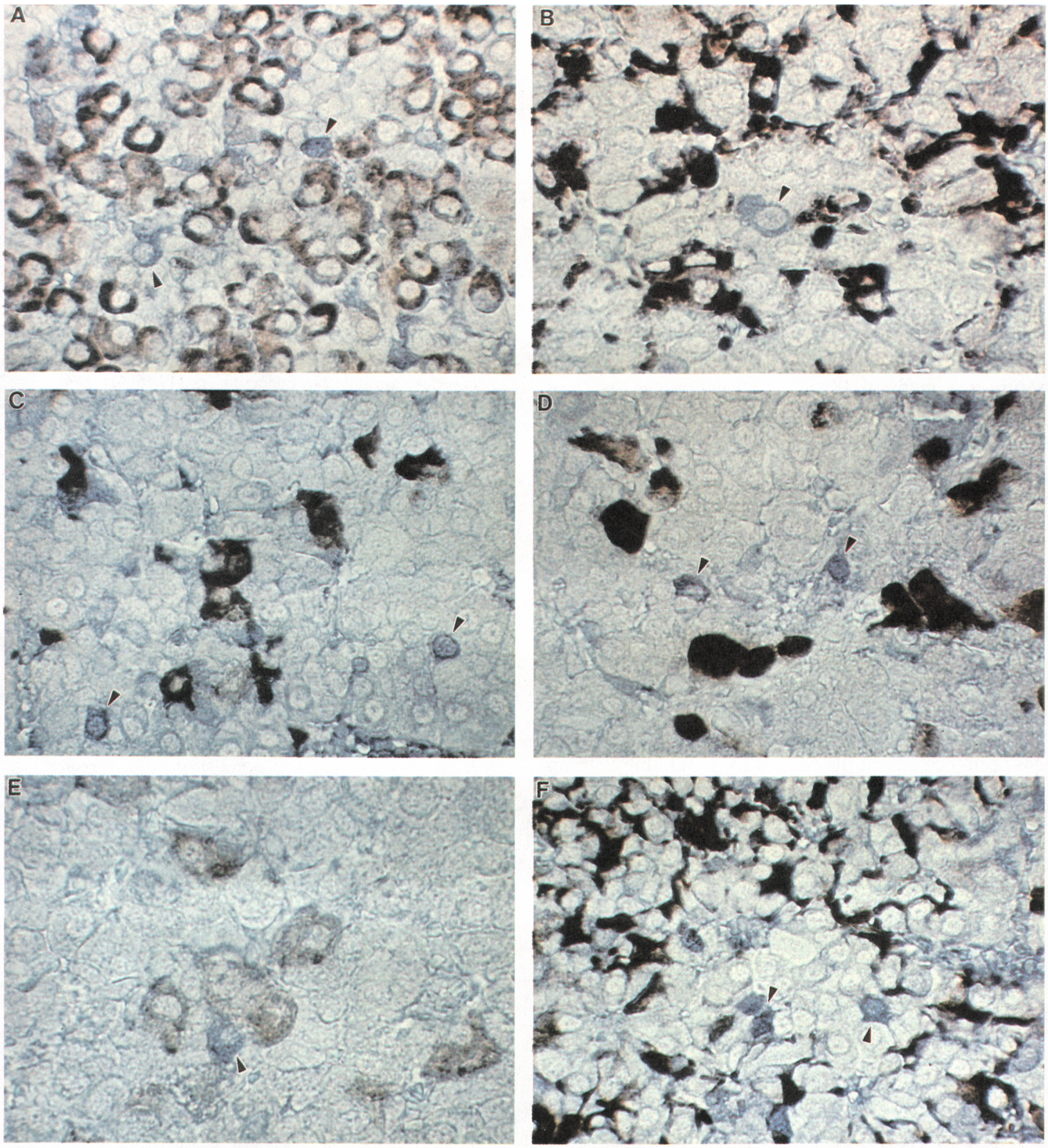

Figure 2. Double staining of angiotensinogen (blue) and pituitary cell markers (brown) on pituitary sections from nephrectomized rats. The arrowheads point to blue angiotensinogen-containing cells that can be clearly distinguished from the cells containing brown immunoreactivity. The antisera revealed with the brown staining in each panel were as follows. (A) Antirat growth hormone (original magnification ×200). (B) Antirat prolactin (original magnification ×250). (C) Anti-ACTH 17–39 (original magnification ×200). (D) Antirat LH-β (original magnification ×200). (E) Antihuman thyroid-stimulating hormone-β (original magnification ×250). (F) Antibovine S100 protein (original magnification ×200).

they hypothesized that the latter cells may be the actual site of production, while the immunoreactivity present in gonadotropes may be internalized material.

Thomas and Sernia (15) did not investigate the effect of nephrectomy, a condition that increases the concentration of angiotensinogen mRNA in the liver (26) and adipose tissues (27). We found that this condition greatly increased the amount of angiotensinogen immunoreactivity in the pituitary gland. It also increases circulating angiotensinogen (28, 29), and this raises the question of whether the increased angiotensinogen detected by the ORNI antiserum is produced locally or internalized by certain pituitary cells from the circulation. Although we cannot answer this question on the basis of immunocytochemistry alone, our previous studies indicated that AII was still present in anterior pituitary cells after 14 days of organ culture in serum-free medium (8). This strongly suggests local synthesis of angiotensinogen. In addition, the recent report that small amounts of angiotensinogen mRNA are detectable in rat anterior pituitary extracts (30) makes it likely that there is local synthesis of angiotensinogen.

Taken together, the available data indicate that most, if not all, of the angiotensinogen immunoreactivity is present in different cells than the gonadotropes containing renin, ACE, and AII. This separation of components is not unique to the anterior pituitary. In rat brain, angiotensinogen is made by astrocytes (31–33) whereas AII is found in neurons (34–36). In the rat adrenal gland, renin and AII are found in the zona glomerulosa (1, 9), whereas angiotensinogen mRNA has been localized in the capsule (37). In kidneys, renin and AII colocalize in the secretory granules of juxtaglomerular cells, whereas angiotensinogen has been detected in the proximal tubules (38, 39). These data indicate that in these tissues, synthesis of AII may occur through some kind of shuttling of angiotensinogen from one cell type to another. This hypothesis is consistent with recent data indicating that angiotensinogen is not stored in the same secretory granules where renin and AII are packaged (40). Further experiments are needed to determine whether such shuttling of angiotensinogen actually occurs in the pituitary gland.

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