Spironolactone Bodies in Aldosteronomas and in the Attached Adrenals

Enzyme Histochemical Study of 19 Cases of Primary Aldosteronism and a Case of Aldosteronism Due to Bilateral Diffuse Hyperplasia of the Zona Glomerulosa

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The formation of spironolactone (S) bodies, eosinophilic laminated cytoplasmic inclusions, is induced in the aldosterone-producing cells of the human adrenal cortex after the administration of spironolactone. The aim of this study was to define the enzyme histochemical characteristics of S bodies, S-body-containing cells, and the apparently hyperplastic zona glomerulosa (zG) of adrenal tissues attached to aldosteronomas. S bodies were found in 14 of 19 aldosteronomas, in 10 of 19 adrenal tissues attached to aldosteronomas, and in the adrenal tissues in a patient with aldosteronism due to bilateral diffuse zG hyperplasia. The S bodies themselves exhibited most intense 3β-hydroxysteroid dehydrogenase (3βHSD) activity but did not exhibit glucose-6-phosphate dehydrogenase (G6PD), NADP-dependent isocitrate dehydrogenase (NADP-ICDH), or succinate dehydrogenase (SDH) activity, confirming histochemically the origin of S bodies in the smooth endoplasmic reticulum. In two adenomas, S bodies were found to be surrounded by reaction products of acid hydrolase but were not found in the other adenomas and the remaining adrenal tissues. S-body-containing cells, irrespective of being neoplastic or not, showed enhanced 3βHSD, G6PD, and NADP-ICDH activity and weak SDH activity (Type I pattern of enzyme activity). Though zG was hyperplastic in most of the adrenal tissues attached to the adenomas, zG cells that did not contain S bodies showed the opposite pattern (Type II pattern) of enzyme activity (ie, weak 3βHSD, G6PD, and NADP-ICDH activity and intense SDH activity), in contrast to those in the adrenal tissues in a patient with aldosteronism due to bilateral diffuse zG hyperplasia (which showed the Type I pattern). The results are consistent with the view that hyperplastic zG cells, except S-body-containing cells, in the case of aldosteronoma are not hyperfunctioning. The latter cells may have enhanced but possibly abortive steroidogenic activity. (Am J Pathol 1981, 103:404–410)

SPIRONOLACTONE (S) BODIES that are found in aldosterone-producing cells, are eosinophilic, round, laminated cytoplasmic inclusions surrounded by clear halos in preparations stained with hematoxylin and eosin.[1] Ultrastructurally, S bodies are composed of concentric whorls of tightly packed, agranular membranes[2–6] and have unique enzyme histochemical characteristics, which are described in this paper. The formation of S bodies is induced by spironolactone, which is a competitive antagonist of aldosterone and is used as a diuretic and antihypertensive agent or in the preoperative treatment of primary aldosteronism (PA). Both the direct and indirect effects of S on the zona glomerulosa (zG) of the adrenal have been considered to play a role in the induction of the formation of S bodies. The indirect effects are thought to be mediated through stimulation of zG cells by a reactivated

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renin-angiotensin system and/or by sodium deficiency due to sodium diuresis after the administration of S. The direct effects of S have been hypothesized based on the turnover of endoplasmic reticulum of zG cells.[2,4,6] While S bodies were found in the adrenal cortices of a high percentage of patients who had various disorders other than Conn's syndrome and who were treated with S,[1-3] these bodies have been found relatively rarely in cases of PA. In the single case studies of PA it has been reported that S bodies were found only in the aldosteronoma,[5] only in the adrenal cortex attached to the adenoma,[4,7] or in both tissues.[6,8] Conn et al reported that only 1 of 25 patients with PA had a few S bodies in the zG of the nontumorous portion of the gland.[9] In our present series, however, S bodies in the adrenal tissues attached to adenomas were found in 10 out of 19 patients with PA.

The principal goal of this study was to define the enzyme histochemical characteristics of S bodies and S-body-containing cells and to assess the enzyme histochemical activity of the apparently hyperplastic zG of the adrenal tissues attached to aldosteronomas, in comparison with the activity of the adrenal tissues in a patient with aldosteronism due to bilateral diffuse zG hyperplasia.

Materials and Methods

Specimens

Surgical specimens of adrenocortical adenomas and the adrenal cortices attached to the adenomas were obtained from 19 Japanese patients with primary aldosteronism (PA) at Keio University Hospital (14 cases) and at five neighboring hospitals. The average age of the patients was 44 years (range 25 to 60 years) for 9 male patients and 40 years (range 21 to 52 years) for 10 female patients. All of the patients had received spironolactone (S) in differing amounts (more than 800 mg per patient) during various periods of time (more than 8 days), with or without cessation of the drug before the adrenalectomy. The bilateral adrenal cortex of a 3-year-old female with hyperaldosteronism due to diffuse hyperplasia of bilateral adrenal zona glomerulosa (zG) was also examined. This last patient had also taken S (25–250 mg/day) for 5 months until 10 months prior to adrenalectomy at the National Children's Hospital in Japan.

Histochemistry

For assay of dehydrogenases 2–5-mm-thick tissues were promptly frozen on dry ice and sectioned at 8 μ in a cryostat at − 20 C. The sections were stained for the following enzymes by previously described methods: glucose-6-phosphate dehydrogenase (G6PD),[10] NADP-linked isocitrate dehydrogenase (NADP-ICDH),[10] 3β-hydroxysteroid dehydrogenase (3βHSD; substrate: dehydroepiandrosterone and pregnenolone),[11] succinate dehydrogenase (SDH),[12] and lactate dehydrogenase.[13] For hydrolases, 2–5-mm-thick tissues were fixed in 4.5% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.4, for 8 hours, and washed overnight in cold Holt's hypertonic gum sucrose solution.[14] Sections (6 μ) were prepared in a cryostat and stained for the hydrolases by previously described methods: alkaline phosphatase,[15] acid phosphatase,[16] β-glucuronidase,[17] and N-acetyl-β-glucosaminidase.[18] Nuclear counterstaining was produced with methyl green after the enzyme reaction. Sections serial to those for dehydrogenases or hydrolases were stained with Sudan black B and hematoxylin and eosin and impregnated with silver for reticulin staining.

For histology and phospholipid histochemistry, the tissues were fixed in 4.5% paraformaldehyde solution, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin, Luxol fast blue, or Sudan black B, and impregnated with silver for reticulin staining.

Results

Enzyme Histochemistry of the S Body and S-Body-Containing Cells

Cells that contained S bodies showed a characteristic pattern of enzyme activity irrespective of whether they were neoplastic or nonneoplastic or whether they appeared in the adrenals of cases of PA or of a case of aldosteronism due to diffuse hyperplasia of the zG. S-body-containing cells had markedly increased 3βHSD (with either dehydroepiandrosterone or pregnenolone being used as the substrate), G6PD, and NADP-ICDH activity, and diminished SDH activity (Figures 1 and 2). Significantly enhanced or diminished lactate dehydrogenase activity could not be detected histochemically in S-body-containing cells. S-body-containing cells did not show alkaline phosphatase activity, irrespective of their cell type being zG, zona fasciculata or zona reticularis (Figure 1). The S body itself showed far more intense activity of 3βHSD than did the surrounding cytoplasm, but it did not exhibit G6PD, NADP-ICDH, or SDH activity (Figure 1).

Acid hydrolases, ie, acid phosphatase, β-glucuronidase, and N-acetyl-β-glucosaminidase, could not be detected histochemically in either the S body itself or in the cytoplasm surrounding the S body in paraformaldehyde-fixed tissues of 11 of 13 tumors and in all of 9 attached adrenals examined that contained S bod-

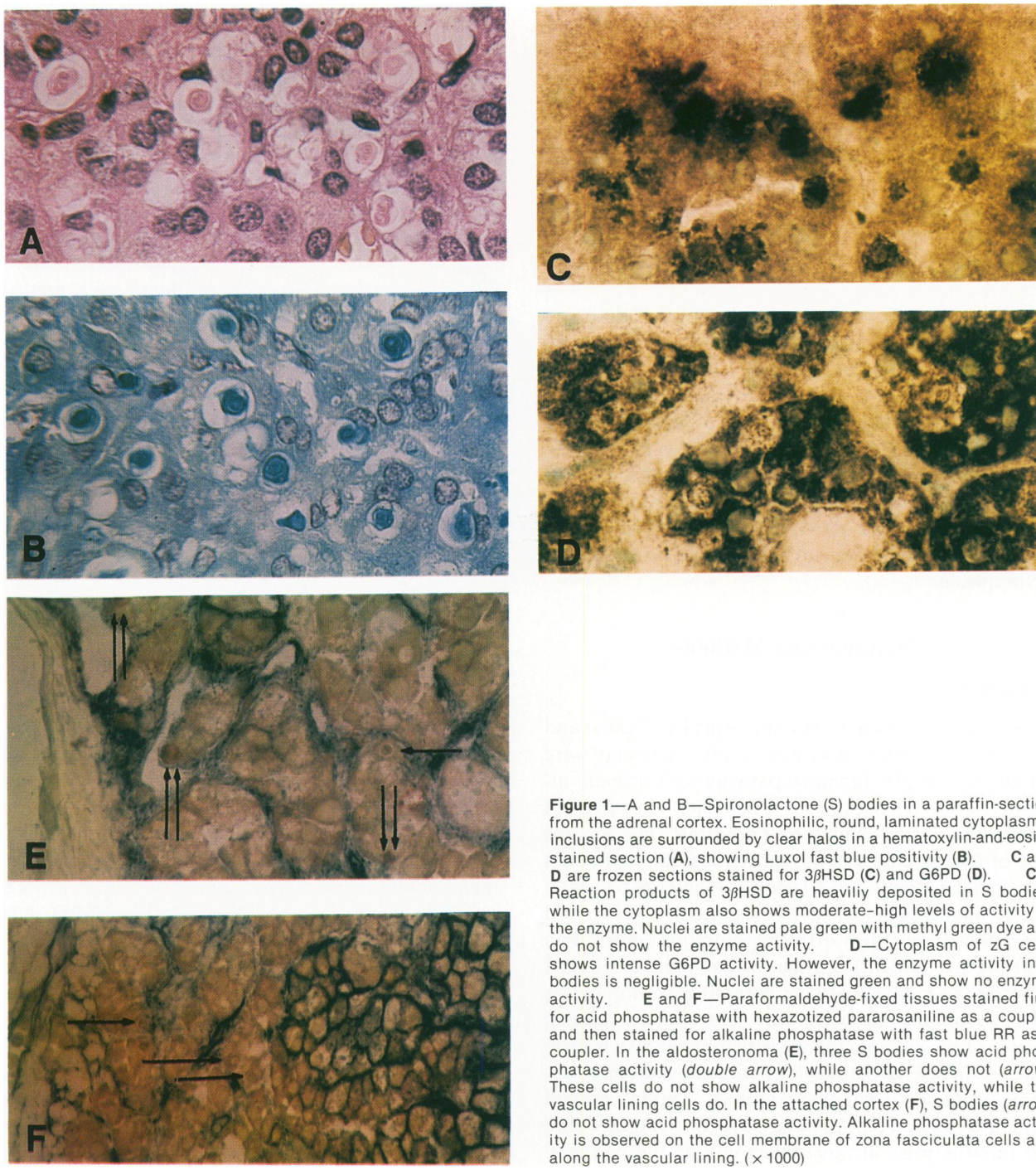

Figure 1—A and B—Spironolactone (S) bodies in a paraffin-section from the adrenal cortex. Eosinophilic, round, laminated cytoplasmic inclusions are surrounded by clear halos in a hematoxylin-and-eosin-stained section (**A**), showing Luxol fast blue positivity (**B**). **C** and **D** are frozen sections stained for 3βHSD (**C**) and G6PD (**D**). **C**—Reaction products of 3βHSD are heaviliy deposited in S bodies, while the cytoplasm also shows moderate-high levels of activity of the enzyme. Nuclei are stained pale green with methyl green dye and do not show the enzyme activity. **D**—Cytoplasm of zG cells shows intense G6PD activity. However, the enzyme activity in S bodies is negligible. Nuclei are stained green and show no enzyme activity. **E** and **F**—Paraformaldehyde-fixed tissues stained first for acid phosphatase with hexazotized pararosaniline as a coupler and then stained for alkaline phosphatase with fast blue RR as a coupler. In the aldosteronoma (**E**), three S bodies show acid phosphatase activity (*double arrow*), while another does not (*arrow*). These cells do not show alkaline phosphatase activity, while the vascular lining cells do. In the attached cortex (**F**), S bodies (*arrow*) do not show acid phosphatase activity. Alkaline phosphatase activity is observed on the cell membrane of zona fasciculata cells and along the vascular lining. (× 1000)

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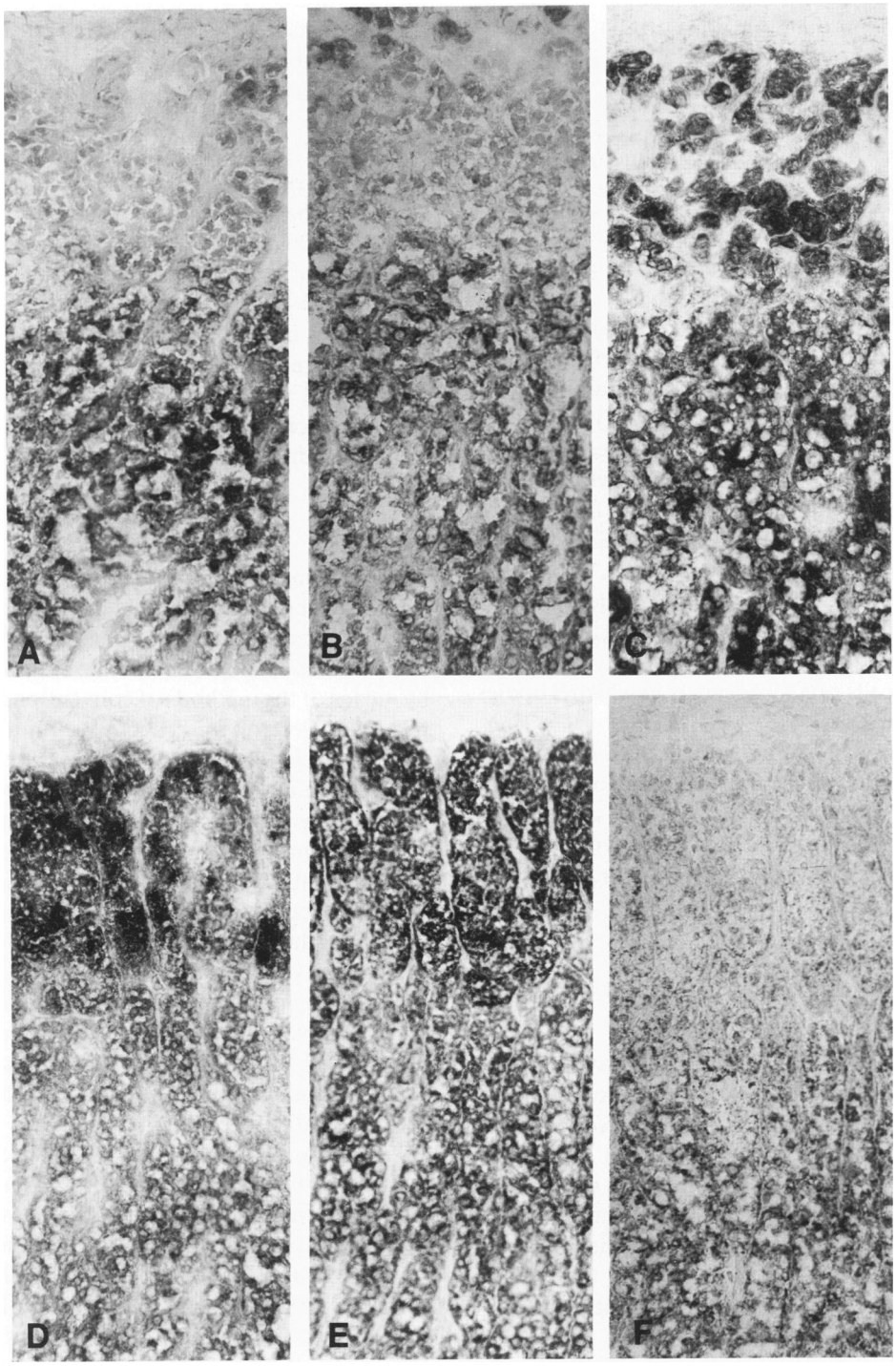

Figure 2—A, B, and **C**—Type II pattern enzyme activity of 3βHSD (**A**), G6PD (**B**), and SDH (**C**) in frozen sections from the adrenal cortex attached to aldosteronoma. ZG cells in the upper part of the figure show much weaker 3βHSD and G6PD activity in comparison with zona fasciculata cells in the lower part of the figure. These cells show, however, intense SDH activity. **D, E,** and **F**—Type I pattern enzyme activity of 3βHSD (**D**), G6PD (**E**), and SDH (**F**) of the adrenal cortex in a patient with aldosteronism due to bilateral diffuse zG hyperplasia. The activity of 3βHSD and G6PD is more intense in the zG (upper part) than in the zona fasciculata. In addition, it is obvious that S bodies have by far the more intense 3βHSD activity, forming almost black dots at low-power magnification. SDH activity is, however, weak in zG. (×200)

ies (Figure 1). However, in two adenomas, reaction products of acid hydrolases were heavily deposited around the S bodies and were occasionally seen within the inclusion bodies themselves (Figure 1).

Enzyme Histochemical Studies of the zG Cells of the Nontumorous Part of the Adrenal Tissues From Cases of PA and of the Adrenal From a Case of Bilateral Diffuse zG Hyperplasia

The zG of the adrenal cortices attached to aldosterone-producing tumors was focally or diffusely hyperplastic in all of the cases examined in this study. But zG cells could be divided into two groups according to the patterns of activity of cellular dehydrogenases as follows: Type I: Intense 3βHSD, G6PD, and NADP-ICDH activity and weak SDH activity; Type II: a reverse pattern of activity of these enzymes, ie, weak 3βHSD, G6PD, and NADP-ICDH activity and intense SDH activity (Figure 2). The Type I pattern of enzyme activity was observed only in S-body–containing cells and in their neighboring cells, which constitute a minor part of this zone. The Type II pattern was observed in the remaining cells, that is, in 1) the area of zG other than at clusters of S-body–containing cells and 2) zG cells of all 9 adrenals in which S bodies were not detected.

The zG of the adrenal in a patient with aldosteronism due to bilateral diffuse hyperplasia of this zone was markedly hyperplastic in a regular cordlike arrangement, and all the zG cells showed Type I enzyme activity, irrespective of the presence or absence of S bodies (Figure 2).

Incidence of S Bodies According to Cell Type, Sex, and Amount of S Administered

In sections stained with hematoxylin and eosin, S bodies were observed as eosinophilic round, laminated cytoplasmic inclusions surrounded by clear halos (Figure 1). They were positive for Luxol fast blue stain (Figure 1B) and Sudan black B stain in sections of both frozen and paraffin-embedded tissues. These bodies could also be identified by the characteristic pattern of dehydrogenase activity, that is, the presence of intense 3βHSD activity and the absence of G6PD, NADP-ICDH, and SDH activity.

In adenomas, S bodies were found chiefly in compact cells, and relatively rarely in lipid-rich clear cells, zG type cells, and so-called hybrid cells. They could not be found in the cells that simulate compact cells but that showed the Type II pattern of enzyme activity. Inclusion bodies were present in all of 10 adenomas of female patients, in contrast to only 4 of 9 tumors of male patients. In the adrenal cortices attached to the tumors, S bodies were found in zG cells and rarely in the cells of the zona fasciculata just beneath the zG and could not be found in the cells throughout almost all of the zona fasciculata and zona reticularis. S bodies were present in 6 of 10 adrenals from female patients and in 4 of 9 adrenals from male patients. More S bodies were more often found in the tissues of female patients than in those of male patients among those who had S bodies in their tumor tissues and/or in the attached cortices.

The amount of S administered to a patient did not correlate with the presence of S bodies. For example, one patient had taken a total of 3000 mg of the S but did not have S bodies either in his adenoma or in the adrenal zG, while another patient who had been administered only 800 mg had ample numbers of S bodies in her adrenal zG.

Discussion

Since Janigan reported the formation of Spironolactone (S) bodies in the zona glomerulosa (zG) of the adrenal tissues in patients treated with S,[1] morphologic studies of S bodies have been performed chiefly by means of the methods of conventional histochemistry[1,2] and electron microscopy.[2-4,6] In the present paper, we would like to discuss the enzyme histochemical characteristics of S bodies and S-body–containing cells and the distinctive pattern of enzyme activity differentiating the apparently hyperplastic zG of the adrenals in patients with primary aldosteronism due to cortical adenoma from that in a patient with aldosteronism due to bilateral diffuse zG hyperplasia.

In the first part of the study, it was clarified that S bodies have most intense 3β-hydroxysteroid dehydrogenase activity in neoplastic and nonneoplastic adrenal cortical tissues (Figure 1C). One can easily find this structure upon microscopic examination of 3β HSD-stained tissues at low to medium magnification (Figure 2D). The finding of the presence of 3βHSD activity and the absence of glucose-6-phosphate dehydrogenase and succinate dehydrogenase activity in S bodies confirms the origin of the inclusion in the smooth endoplasmic reticulum, because these enzymes are chiefly located in the smooth endoplasmic reticulum,[19] the cytoplasm, and the mitochondria, respectively. It was found that an intense relationship existed between S bodies and lysosomal enzymes in the two aldosteronomas (Figure 1E). This finding is consistent with the view that S bodies are exposed to lysosomal hydrolase in certain circumstances. Nevertheless, S bodies in most of our patients showed no relationship to lysosomes, as Kovacs et al have pointed

out in their ultrastructural study of a case.[4] The rarity of finding S bodies exposed to lysosomal hydrolase seems to be one reason why S bodies are stable in the cytoplasm and are often found in the adrenals of patients who have not recently taken S.

S-body-containing cells showed the Type I pattern of enzyme activity (ie, enhanced 3βHSD, G6PD, and NADP-ICDH activity and weak SDH activity), appearing to have enhanced but possibly abortive[9,20,21] steroidogenic activity. In addition, zG cells other than S-body-containing cells also showed the same Type I pattern in the adrenal tissues in a patient with aldosteronism due to bilateral diffuse zG hyperplasia (Figure 2D, E, and F). This enzyme histochemical finding might be identical in its meaning with a finding by Jenis and Hertzog[2] in an ultrastructural study on the adrenals in non-PA patients treated with S. They reported that the zG was hypertrophied in all the patients with S body inclusions.

Hyperplasia of the zG is also a common finding in the adrenals attached to aldosteronomas.[22] However, in this study most zG cells which did not have S bodies exhibited the Type II pattern of enzyme activity (ie, weak 3βHSD, G6PD, and NADP-ICDH activity and intense SDH activity, Figure 2A, B, and C), in contrast to those in a patient with aldosteronism due to bilateral diffuse zG hyperplasia. This enzyme pattern has also been observed, in our previous study,[23,24] in the zona fasciculata of the adrenal cortices attached to cortisol-producing tumors but not in the zona reticularis of the adrenal attached to a virilizing tumor, and is regarded as being the enzyme histochemical expression of long-unstimulated cells. The findings in the present study show that apparently hyperplastic zG cells in PA are not stimulated much by angiotensin II and/or sodium deficiency, the stimulation of which can occur after the administration of S.[9] If this is the case, it might be somewhat difficult to prove that such stimulation plays the primary role in the formation of S bodies, and alternatively the primary and direct role of S itself might be hypothesized. Such stimulation, however, is probably necessary in order for zG cells of the adrenals attached to aldosteronomas to regain the features of aldosterone-producing cells in PA. It is generally accepted that S bodies appear exclusively in aldosterone-producing cells.[2,9] A relatively low incidence of S bodies in the adrenal gland in cases of PA seems to be because the unstimulated zG cells of the adrenal tissues have lost such features.

In this study S bodies were characteristically found chiefly in the compact cells of the aldosteronomas and infrequently in the clear cells, zG-type cells, and so-called hybrid cells, in contrast to nonneoplastic counterparts in which S bodies were found fairly exclusively in zG cells. These findings are consistent with the view that compact cells in adenomas also play a part in producing aldosterone.

Finally, inclusions ultrastructurally similar to S bodies were observed in a previous study of virilizing adrenal adenoma in a female patient who was not administered S.[24] The inclusions, though smaller in size, histochemically showed positivity for both Luxol fast blue and Sudan black B but showed no relationship to lysosomal enzymes. In addition, the compact cells of this adenoma showed only weak lysosomal hydrolase activity, in contrast to nonneoplastic zona reticularis cells. The inclusion, however, was different from the S bodies in that it lacked 3βHSD activity. The reason for this difference is that in contrast to aldosterone-producing adrenal cortical cells, this type of virilizing adrenocortical tumor, as well as normal zona reticularis, characteristically lacks 3βHSD activity in the smooth endoplasmic reticulum.

In summary, S bodies are equipped with 3βHSD and can be, though rarely, exposed to lysosomal enzymes at least in some aldosteronomas. S-body-containing cells seem to have an enhanced but possibly abortive steroidogenic activity because they show the Type I pattern of enzyme activity. Most zG cells other than S-body-containing cells in the adrenals attached to aldosteronomas show a Type II pattern of enzyme activity consistent with decreased steroidogenic activity. This histochemical finding is characteristic of PA caused by cortical adenoma and can be helpful in differentiating PA from aldosteronism caused by bilateral diffuse zG hyperplasia, which diffusely shows a Type I pattern of enzyme activity.

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