Ultrastructural Changes in Rat Adrenocortical Cells Produced by a 4-Aminopyrazolopyrimidine (4-APP) Dosage

Masanori Murakoshi, Yoshiyuki Osamura and Keiichi Watanabe
Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara-city, Kanagawa, 259-11, Japan

ABSTRACT. The relation between adrenal cholesterol synthesis and ultrastructural changes was studied in rat adrenal glands stimulated by 4-aminopyrazolopyrimidine (4-APP). Ultrastructural observations were focused on the inner fasciculata-reticularis (Zone I) and the outer fasciculata (Zone O) of the adrenal glands. Zone I in 4-APP-treated rat adrenals had increased tubules and vesicles in the smooth endoplasmic reticulum, mitochondrial enlargement and the accumulated osmiophilic lipid droplets. In contrast, Zone O of the 4-APP-treated rat adrenals showed a decrease in osmiophilic lipid droplets and no increase in any of the elements of the smooth endoplasmic reticulum.

These results are strong evidence that under 4-APP treatment cholesterol synthesis takes place in the increased smooth endoplasmic reticulum in Zone I cells. The functional status of the cells of the inner and outer zones of the adrenal cortex also is discussed.

Intraperitoneal administration of 4-aminopyrazolopyrimidine (4-APP) has been reported to inhibit the hepatic release of lipoprotein and to decrease serum cholesterol levels remarkably (1). In these 4-APP-treated animals, lowered serum cholesterol levels are known to result in extrahepatic production of cholesterol including the adrenal cortex (2).

Adrenocortical steroid hormones are formed within the cortex primarily from cholesterol and de novo cholesterol formation from acetate is of minor significance for normal steroidogenesis (3). In the rat, administration of ACTH increases the adrenal uptake of cholesterol from plasma (5). Ultrastructural changes in adrenocortical cells stimulated by ACTH have also been well established (7, 9) but no ultrastructural studies of adrenal-cortical changes caused by 4-APP treatment have been reported.

This report mainly deals with the ultrastructural changes in rat adrenocortical cells induced by 4-APP administration as they are seen show to ultrastructural changes corresponding to cholesterol synthesis and subsequent steroid metabolism.

MATERIALS AND METHODS

Male Wistar rats weighing 180 to 200 g were injected intraperitoneally with 2 mg of 4-aminopyrazolopyrimidine (4-APP, Sigma Co.) daily for 3 days. Control rats were injected

Abbreviations used: 4-APP, 4-aminopyrazolopyrimidine; ACTH, adrenocorticotropic hormone; sER, smooth endoplasmic reticulum.
M. Murakoshi, Y. Osamura and K. Watanabe

with saline solution instead of 4-APP. When the rats were killed, they were perfused with 0.01 M phosphate buffered saline (pH 7.2) through the left ventricle of the heart. The adrenal glands were removed immediately and fixed in 2.5% glutaraldehyde containing $5 \times 10^{-4}$ M digitonin in 0.1 M phosphate buffer (pH 7.2) for 3 h at 4°C. After post-fixation for 90 min at 4°C in 1% OsO$_4$ in 0.1 M phosphate buffer (pH 7.2), the tissue blocks were dehydrated in graded alcohols then embedded in Epon 812. Sections 1-μm thick were cut with a Porter Blum microtome then stained with toluidine blue to identify the two cortical zones, the inner fasciculata-reticularis (Zone I) and outer fasciculata (Zone O). When these zones had been identified on the 1-μm sections, ultrathin sections from each zone were cut then stained with uranyl acetate and lead citrate and examined in a JEOL 100-C electron microscope.

RESULTS

In 4-APP-treated rats, Zone I had more lipids than the same zone in normal untreated rats (Figs. 1A, 1B), but in Zone O of 4-APP-treated rats, lipid accumulation was less than in the same zone in normal untreated rats (Figs. 1C, 1D). The detailed ultrastructural features of Zone I and Zone O were as follows:

_Ultrastructural features of the cells in Zone I (inner fasciculata-reticularis)._ Representative ultrastructural features of adrenocortical cells in Zone I from a normal untreated rat are shown in Fig. 2. Many vesicular profiles of the smooth endoplasmic reticulum were observed in the cytoplasm, and mitochondria with tubulo-vesicular cristae were distributed evenly throughout the cytoplasm. Occasional lipid droplets were present in the cytoplasm. The number and sizes of these droplets were much smaller than for droplets of Zone O cells (Figs. 2, 7).

On 4-APP administration, the smooth endoplasmic reticulum (sER) increased remarkably both vesicularly and tubularly (Figs. 3, 4). Some of the increased sER was grouped very compactly in the form of minute vesicles or irregularly folded fine tubules that occupied certain cytoplasmic areas (Fig. 3). Much of the vesicular sER was dilated markedly, and some parts contained osmiophilic materials (Fig. 3). Some fine tubular sER and mitochondrial outer membranes were closely related (Fig. 3, inset). Golgi lamellae were moderately dilated (Figs. 3, 4). Enlarged mitochondria increased in number under 4-APP treatment (Fig. 10). Mitochondrial vesicular cristae also increased (Fig. 11). Highly osmiophilic materials of varying size present in the mitochondria of 4-APP-treated rat adrenocortical cells (Figs. 4, 6).

Intracytoplasmic lipid droplets increased markedly in number and size as compared to the droplets of the untreated rat (Figs. 2, 4, 9). Some fine tubular sERs were closely related to the lipid droplets (Fig. 5).

_Ultrastructural features of the cells in Zone O (outer fasciculata)._ A representative ultrastructural view of the cells of Zone O in an untreated rat is given in Fig. 7. The numerous vesicular profiles of the smooth endoplasmic reticulum (sER) were dilated moderately (Fig. 7). Mitochondria of varying size that contained vesicular cristae predominated (Fig. 7). As in the Zone I cells from the 4-APP-treated rat, numerous

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Fig. 1A, 1B. Light microscopy of Zone I in 4-APP-treated and untreated rats. In the 4-APP-treated rat (1A) many lipid droplets are present in the cytoplasm (arrows). (1A) 4-APP-treated (1B) untreated IF: Inner fasciculata, R: Zona reticularis, M: Medulla, ×150 (1-μm section).

Fig. 1C, 1D. Light microscopy of Zone O in 4-APP-treated and untreated rats. In the untreated rat (1D) many lipid droplets are present in the cytoplasm (arrows). (1C) 4-APP-treated (1D) untreated OF: Outer fasciculata, ×150 (1-μm section).
enlarged lipid droplets were present in the Zone O cells of the untreated rat (Fig. 7).

On 4-APP administration, the vesicular sER slightly dilated. Occasional aggregates of glycogen granules were present in the cytoplasm (Fig. 8, inset). Mitochondria were enlarged slightly (Figs. 12, 13). The most characteristic change produced by 4-APP administration was the depletion of lipid droplets (Fig. 8).

**DISCUSSION**

Our ultrastructural studies of the adrenal cortex of rat that had received 4-APP clearly demonstrated two identifiable zones, Zone I (inner fasciculata-reticularis) and Zone O (outer fasciculata) as seen from the number and morphology of the characteristic osmiophilic lipid droplets present. A 2.5% glutaraldehyde solution containing $5 \times 10^{-4}$ M digitonin was much superior to a simple glutaraldehyde solution when used as a fixative for the preservation of intracytoplasmic lipids that include cholesterol, and of the membrane and contents of the mitochondria and smooth endoplasmic reticulum.

Remarkable ultrastructural changes were observed in Zone I cells treated with 4-APP. An increased endoplasmic reticulum, that was prominent, tubular and smooth, enlarged mitochondria with numerous intramitochondrial vesicular cristae and accumulated osmiophilic lipid droplets were characteristic. Biochemically, adrenocortical cholesterol synthesis increases 42-fold in 4-APP-treated as compared to untreated rats (2). As the smooth endoplasmic reticulum reportedly contains the enzymes involved in the synthesis of cholesterol (4), it may be the site of cholesterol
synthesis and of the accumulation of this synthesized cholesterol and perhaps of its final metabolites, steroid hormones, as well (6). On the bases of our observations and these facts, the increased tubular smooth endoplasmic reticulum in 4-APP-treated adrenocortical cells probably corresponds to highly activated cholesterol synthesis.

It has been reported elsewhere that ACTH stimulates the uptake of cholesterol into adrenocortical cells as well as stimulating steroid hormone synthesis (5). Under this ultrastructural condition the depletion of lipid droplets has been observed (8, 10). In this regard, the 4-APP-treated cells of Zone I obviously differ from ACTH-stimulated adrenocortical cells because the marked accumulation of large amounts of lipid droplets. This evidence suggests that there is an imbalance between the increased cholesterol and subsequent steroid synthesis and steroid hormone secretion in Zone I cells treated with 4-APP. That is, 4-APP might work on Zone I cells not only to increase cholesterol synthesis, but to some extent to impair the secretion of steroids,
the final metabolite of the synthesized cholesterol. If this is the case, the increased and enlarged lipid droplets would represent accumulated steroids or their metabolites.

In mitochondria, electron dense, lipid-like inclusions, myelin fingers or both were present in the matrix. The tubular and smooth endoplasmic reticulum showed some direct transition to the mitochondrial outer membrane. These features strongly

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**Fig. 9.** Diameter of lipid droplets in Zone I cells of 4-APP-treated and control rat adrenocortical cells.

**Fig. 10.** Diameters of mitochondria in Zone I cells in 4-APP-treated and control rat adrenocortical cells.
Changes in Adrenocortical Cells by 4-APP

suggest that the synthesized cholesterol is transferred to the mitochondria through this transition structure.

When 4-APP was administered, the cells of Zone 0 showed neither the prominent, smooth endoplasmic reticulum nor the lipid droplet accumulation characteristic of Zone I cells treated with 4-APP. The conspicuous decrease in lipid droplets was the most characteristic feature of the Zone O cells treated with 4-APP. Because the adrenocortical cells in the outer fasciculata zone are known to be the most active in

Fig. 11. Numbers of vesicular cristae in mitochondria 1–1.5 μm in diameter in Zone I cells in 4-APP-treated and control rat adrenocortical cells.

Fig. 12. Diameters of mitochondria in Zone O cells in 4-APP-treated and control rat adrenocortical cells.
M. Murakoshi, Y. Osamura and K. Watanabe

cholesterol uptake from the plasma and in subsequent steroidogenesis, the decrease in lipid droplets in the Zone O cells may be caused by serum cholesterol levels being markedly lowered by 4-APP treatment. Thus, the adrenocortical cells of Zone O may utilize serum cholesterol more effectively than the cells of Zone I, but they may lack the ability to synthesize cholesterol de novo which is characteristic of the cells of Zone I.

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REFERENCES


Fig. 13. Numbers of vesicular cristae in mitochondria 1–1.2 μm in diameter in Zone O cells in 4-APP-treated and control rat adrenocortical cells.


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