JUXTAGLOMERULAR CELLS IN MICE AFTER LONG-TERM TREATMENT WITH CAPTOPRIL
— An Electron Microscopic Study —

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In mice, the juxtaglomerular cells were examined using qualitative and quantitative electron microscopy after an administration of captopril. After treatment with captopril for 2, 7 and 14 days, the specific granules were decreased in number, the Golgi apparatus was well developed, and the rough endoplasmic reticulum was dilated in profile. In the dilated rough endoplasmic reticulum, the intracisternal granules were occasionally seen after a 2-day treatment, were increased in size and number after a 7-day treatment and were hardly seen after a 14-day treatment. In a few cells associated with intracisternal granules on the 7th day, the Golgi apparatus was not well developed and some lysosomal bodies containing granules similar to the intracisternal granules were seen. In these cells, an excess of the intracisternal granules may be controlled by lysosomes. These findings suggest that the juxtaglomerular cells stimulate renin synthesis after long-term treatment with captopril, but a further increase in renin synthesis cannot be expected after the 7th day.

Captopril, an inhibitor of the angiotensin-converting enzyme or kininase II, is effective in decreasing blood pressure in the clinical field, although the mechanism of its hypotensive action has not entirely been clarified. Previously we found that in mice a single oral administration of captopril induced marked structural changes in the juxtaglomerular cells which are generally known to synthesize renin. Bengis et al. have reported that in normal or hypertensive rats, treatment with captopril for 7 days causes a decrease in blood pressure and an increase in plasma renin activity. As no information is available about the effects of the long-term treatment with captopril on the juxtaglomerular cells, we examined the cells after administration of captopril using qualitative and quantitative electron microscopy.

MATERIALS AND METHODS
A total of 20 male dd-mice, 3 months old, maintained by a commercial pellet diet (NMF, Oriental Enz. Co., Japan), were used in this study. Fourteen mice were given captopril (SQ 14,225) supplied from Sankyo Co., Ltd, Tokyo. Captopril was dissolved in their drinking water at a concentration of 10 mg/ml. Then, captopril was given in a dose of about 0.03 mg/g (body weight) per day for 2, 7 and 14 days. The remaining 6 untreated mice served as the normal controls. The mice were killed with chloroform, and their kidneys were removed quickly. Small pieces of the renal cortex were fixed in 2% glutaraldehyde, which was dissolved in a 0.05 M phosphate buffer (pH 7.5) with 1.5% sucrose, for 1.5 hours, postfixed in 2% OsO₄ for 2 hours and embedded.
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Fig. 1. Juxtaglomerular cell of a normal control. The cell contains numerous specific granules. The Golgi apparatus (G) is not well developed and the rough endoplasmic reticulum (rER) is short and flat in profile. × 15,000.

Figs. 2–6. Juxtaglomerular cells of male mice treated with captopril for 7 days. 2: The specific granules are decreased in number and the Golgi apparatus (G) is prominently developed. The rough endoplasmic reticulum (rER) which is distended is increased in number. × 15,000. 3: The Golgi apparatus (G) is prominently developed. The rough endoplasmic reticulum (rER) is dilated. Its membrane protrudes to form blebs towards the Golgi apparatus (arrow). Small cored vesicles are seen in the Golgi apparatus (white arrow). × 20,000. 4: The rough endoplasmic reticulum is dilated, and contains many dense granules, measuring below 0.25 μm in diameter. × 10,000. 5: The rough endoplasmic reticulum is swollen, and contains many dense granules, measuring up to 0.6 μm in diameter. × 10,000. 6: The rough endoplasmic reticulum is dilated and contains dense granules counting up to 25 (arrow). A lysosomal body (double arrows) contains dense granules which are similar to the intracisternal granules in size and density. × 20,000.

in Epon. The ultrathin sections were stained with uranyl acetate and lead citrate and examined using electron microscopy.

For quantitative analysis, the juxtaglomerular cells which were selected from the 4 juxtaglomerular apparatus for each mouse were photographed at a magnification of 5,000 times and enlarged to 10,000 times in prints. Then, a transparent plastic sheet with a regular lattice of points, 0.6 μm apart, was superimposed on the prints, and the volume ratios of the nucleus, specific granules, Golgi apparatus, rough endoplasmic reticulum, intracisternal granules and mitochondria in the juxtaglomerular cell were obtained using the point-counting method. The results obtained were statistically evaluated by Student’s t-test.

RESULTS

As reported in previous papers, the juxtaglomerular cells in normal mice were generally 5 to 20 μm in length and 3 to 10 μm in width. They contained numerous specific granules, ranging from 0.5 to 2.5 μm in diameter, which varied in density (Fig. 1). Small granules around the Golgi apparatus sometimes contained a crystalline structure with a periodicity of 8 to 10 nm. The Golgi apparatus was poorly developed (Fig. 1). The rough endoplasmic reticulum was scattered among the specific granules. It was generally flat and short in profile and contained moderately dense material (Fig. 1). The nucleus was round or oval, 3 to 7 μm in diameter.

In mice treated with captopril, the juxtaglomerular cells showed a decrease in the number of the specific granules (Fig. 2). The rough endoplasmic reticulum showed prominent changes. In many of the juxtaglomerular cells, the rough endoplasmic reticulum was dilated in shape and contained no dense granules in the cisterna (Fig. 2). Smooth-surfaced blebs were often seen in the membrane of the rough endoplasmic reticulum on the side facing the Golgi apparatus (Fig. 3). The Golgi apparatus was prominently developed with vesicular and lamellar components (Figs. 2 and 3). The Golgi cisternae and vacuoles occasionally contained crystalline material. The membrane-bound crystalline granules around the Golgi apparatus occurred more frequently as compared with the controls. They were sometimes fused together and appeared as irregularly-shaped granules. In addition, small cored vesicles, though few in number, were seen in the Golgi apparatus (Fig. 3).

In some of the juxtaglomerular cells, the rough endoplasmic reticulum was dilated to form a cisterna, and contained dense granules (Figs. 4–6). The intracisternal granules were rarely seen on the 2nd day after administration. They were round and homogeneous, though varying in density. The dense granules in a cisterna were few in number and small in size, being smaller than 0.25 μm in diameter. On the 7th day after administration, the intracisternal granules were often seen in the juxtaglomerular cells (about 30%), however, they varied in size and number from cell to cell. In some of the cells, dense granules in a cisterna were few in number and small in size (Fig. 4). The Golgi apparatus was well developed. In some other cells, the rough endoplasmic reticulum was distended more markedly and contained more numerous dense granules which were as many as 25 in a profile of the rough endoplasmic reticulum. The Golgi apparatus was poorly developed. In a few cells, the intracisternal granules were as large as 0.6 μm in diameter (Fig. 5). In these cells with very large intracisternal granules, the Golgi

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apparatus was poorly developed. The cells with poorly developed Golgi apparatus often had lysosomal bodies which contained a few dense granules that were similar in size and density to the intracisternal granules (Fig. 6). On the 14th day after administration, the intracisternal granules and the lysosomal bodies were little seen in the juxtагlomerular cells.

Figure 7 shows the quantitative results after administration of captopril for 7 and 14 days. The volume ratio of the specific granules showed a significant decrease (p < 0.05 and 0.02, respectively), and the ratios of the Golgi apparatus and the rough endoplasmic reticulum were significantly increased (p < 0.001). The intracisternal granules appeared only in the captopril-treated mice.

**DISCUSSION**

In secretory cells, secretory protein is synthesized in the rough endoplasmic reticulum, transferred to the Golgi apparatus and concentrated and matured into the secretory granules? In this manner, the juxtагlomerular cells may form the specific granules which contain renin.

It is generally known that renin, released from the juxtагlomerular cells into the blood, acts on angiotensinogen to produce angiotensin I, which is then converted to angiotensin II by a converting enzyme. Captopril inhibits the angiotensin-converting enzyme to cause a decrease in the level of angiotensin II and an increase in plasma renin activity due to the removal of a negative feedback mechanism.

As seen in the present results, many of the juxtагlomerular cells showed a decrease in the amount of the specific granules after treatment with captopril. At the same time, they showed a marked development of the Golgi apparatus and a dilation of the rough endoplasmic reticulum. In the juxtагlomerular cells, similar changes were observed after adrenalectomy or submaxillary sialoadenectomy, which are thought to suggest a marked release of renin followed by an enhanced synthesis.

Another noticeable change in the juxtагlomerular cells after treatment with captopril is the appearance of dense granules in the cisternae of the rough endoplasmic reticulum in 30% of the...
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The results also seem to be consistent with the clinical findings that in hypertensive patients treated with captopril the renin level is highest within 2 days and then remains unchanged or somewhat declined during the following 7 to 14 days!

REFERENCES


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