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Histochemical and ultrastructural study of renal cortical necrosis in rats treated with oestrone + vasopressin, and its prevention with a vasopressin antagonist

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Summary. Renal cortical necrosis was induced by the administration of vasopressin to oestrogen-pretreated rats. Histochemical (succinic dehydrogenase, trichrome, perjod acid Schiff) and electronmicroscopic methods were applied to examine how the vasopressin antagonist $d(CH_2)_5Tyr(Met)AVP$ influences the development of this renal cortical necrosis. The experiments revealed that vasopressin did not induce hypoxia or necrosis in the renal tubules if the antagonist was administered simultaneously, even after oestrogen pretreatment. The conclusion is drawn that this pressor antagonist may be of value for the prevention of renal cortical necrosis in rats or in human beings.

Keywords: oestrone, vasopressin, renal cortical necrosis, vasopressin antagonist

Human bilateral renal necrosis is most common in pregnancy (Duff & More 1941; Sheehan & Moore 1953; Deutsch *et al.* 1971; Matlin & Gary 1974; Di Paolo & Facchini 1982; Hiault *et al.* 1982). At the present stage of our knowledge, the development of bilateral renal cortical necrosis cannot be prevented with certainty in clinical practice. We have constructed a renal cortical necrosis model with the aim of studying the prevention of renal cortical necrosis (Kovács *et al.* 1964; László 1981). After the administration of vasopressin to rats pretreated with oestrone, bilateral renal cortical necrosis occurred in all these animals. The question was studied of how the vasopressin antagonist (1-(β-mercapto-β,β-cyclopentamethylene-propionic acid)-2-*o*-methyltyrosine) arginine-vasopressin, ($d(CH_2)_5Tyr(Met)$-AVP), influences the development of renal cortical necrosis in response to vasopressin in rats pretreated with oestrogen.

Materials and methods

Experiments were performed on 60 male Wistar rats weighing 180–220 g, maintained on a standard diet. The animals were divided into four groups, each of 15 rats.

Group 1. For 10 days a daily dose of 1.0 mg oestrone acetate (Hogival, Chinoin) was administered s.c.

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Group 2. A dose of 10 iu synthetic lysine-vasopressin (Sandoz) was administered s.c.

Group 3. For 10 days a daily dose of 1.0 mg oestrone acetate was administered s.c., with a single dose of 10 iu synthetic lysine-vasopressin s.c. on the 10th day.

Group 4. For 10 days the rats were treated with a daily dose of 1.0 mg oestrone acetate. On the 10th day a 10 μg dose of the antagonist, $d(CH_2)_5Tyr(Met)AVP$ was administered s.c., with 10 iu lysine-vasopressin s.c. immediately afterwards.

The animals treated only with oestrone acetate were killed by bulbar crushing on the tenth day of treatment, and the other animals 24 h after the vasopressin administration (10 animals in each group), and the kidneys were examined histologically. For light microscopic investigations two pieces of tissue were prepared. One half of the kidney was sectioned with a cryostat. Succinic dehydrogenase (SDH) activity was detected according to the method of Pearse (1972). The other half of the kidney was fixed in buffered formaldehyde and embedded in paraffin. Slides 4–6 μm in thickness were stained with haematoxylin and eosin, and also with the perjod acid Schiff (PAS) technique, and by Masson's trichrome method (Putt 1972).

For electronmicroscopy, the five animals remaining in each group were perfused through the left ventricle with a fixative containing 4% formaldehyde (freshly prepared from paraformaldehyde) and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.4). After perfusion for 30 min, pieces of tissue excised from the kidney were post-fixed in the same solution for 6 h, washed overnight in 0.1 M sodium cacodylate buffer (pH = 7.0) and post-fixed in 1% buffered OsO_4 for 2 h. Subsequently, the blocks were dehydrated in graded alcohols and were finally embedded in Araldite. Ultrathin sections were cut on a Reichert Ultrotome, contrasted with uranyl acetate and lead citrate, and examined under a Tesla BS 500 electron-microscope.

Results

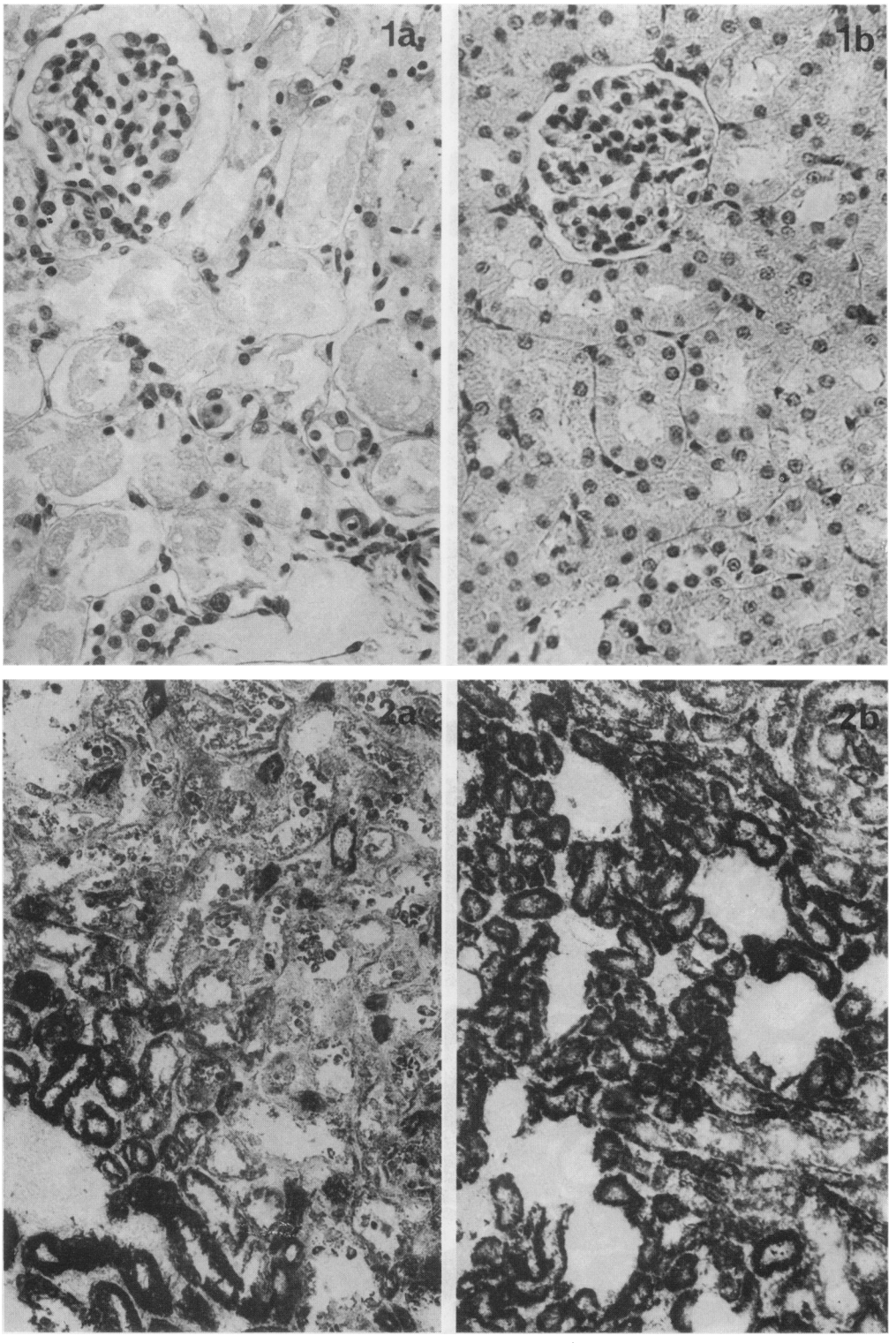
Vasopressin administered after oestrogen pretreatment gave rise to characteristic morphological changes. Both kidneys were slightly enlarged. On gross inspection the outer surface showed a reddish-yellow, irregular, patchy mottling. On the cut surface there were yellowish, infarct-like necrotic areas of various sizes, and irregular outline, fairly sharply defined, and partially coalesced. This cortical necrosis was detected in every rat, but the extent varied from animal to animal.

Histologically, large areas of the cortex were necrotic, the proximal convoluted tubules had no nuclei and their cytoplasm was swollen, homogeneous and eosinophilic (Fig. 1*a*). The glomeruli had no such SDH activity.

In other places there were small areas of less severe damage, where the convoluted tubules were relatively intact (Fig. 2*a*), as shown by purple staining with the trichrome method (Fig. 3*a*) and by only slight degenerative changes and mild disintegration of the epithelium of the tubules. However, by the PAS technique there was a complete disappearance of the brush border of these proxi-

Fig. 1. *a*, Damaged tubular epithelia of the kidney with partial or complete necrosis. After oestrone acetate + LVP administration. H & E, × 320. *b*, Picture of normal renal cortex. Oestrone acetate + LVP + $d(CH_2)_5Tyr(Met)AVP$. H & E, × 320.

Fig. 2. *a*, Disappearance of SDH activity from the necrotic tubular epithelia. Oestrone acetate + LVP. SDH. × 120. *b*, Preserved SDH activity in the renal tubular epithelia. Oestrone acetate + LVP + $d(CH_2)_5Tyr(Met)AVP$. SDH. × 120.


1a
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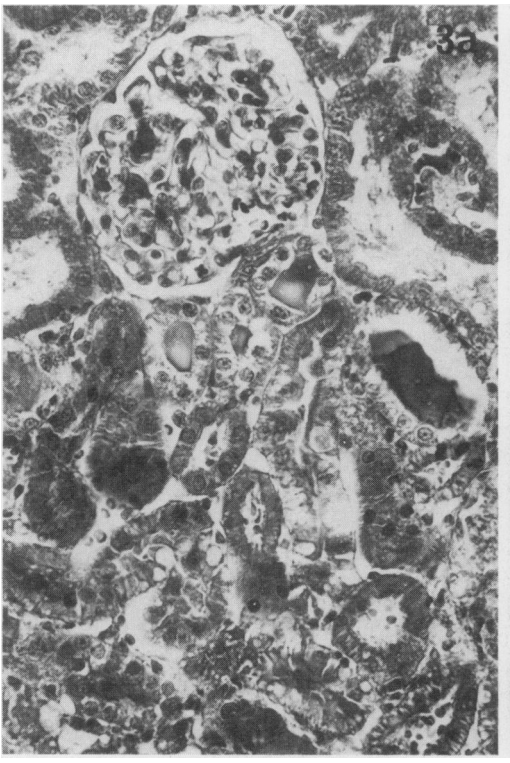
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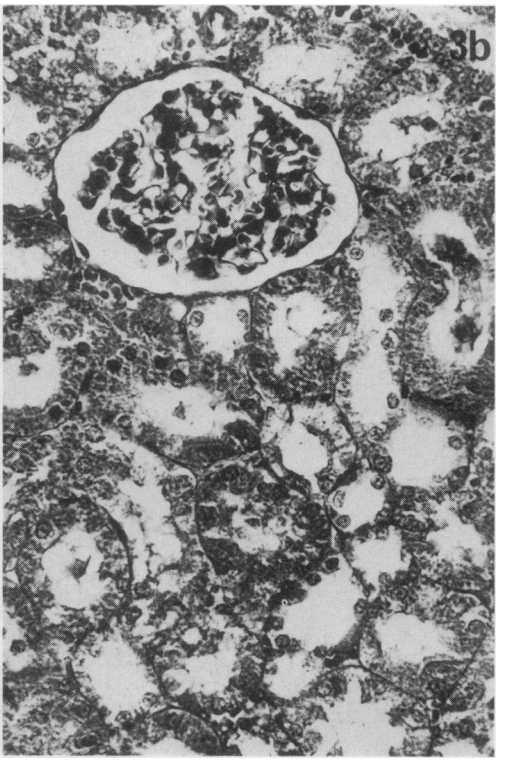
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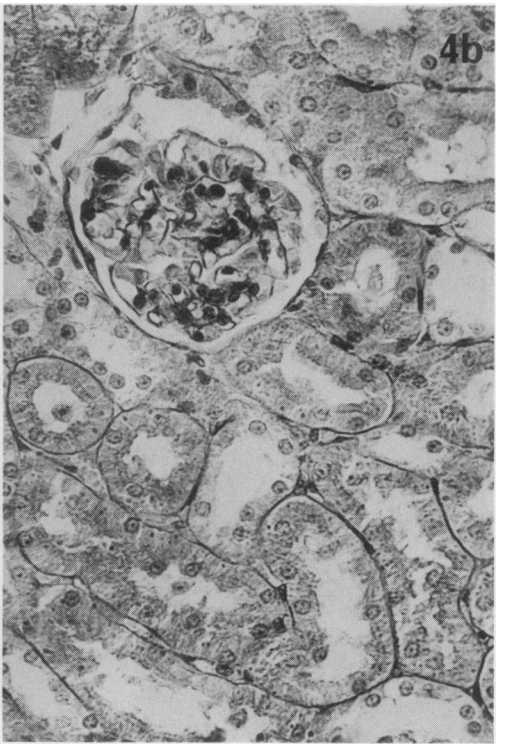
4a


4b

mal tubules (Fig. 4*a*) and hyaline casts were present in the lumens.

In contrast to this histological picture of large areas of severe necrosis of cortex with a few smaller areas of lesser damage, the administration of the vasopressin antagonist in the rats of Group 4 had almost completely prevented the serious damage of the epithelial cells of the tubules (Fig. 1*b*). This can be seen in (Fig. 2*b*) which shows a well-maintained SDH activity. Figure 3*b* where the trichrome method was used, illustrates the preserved structure of the tubules. The same histological picture was observed after PAS staining, which demonstrated normal epithelial cells with a well-distinguished line of brush border (Fig. 4*b*). Corresponding changes could be seen in the electronmicroscopic pictures.

Figure 5*a* presents the electronmicroscopic picture of a normal renal cortex. At 24 h after the administration of LVP, extensive necrosis of the epithelial cells could be observed in the region of the proximal convoluted tubules of the renal cortex (Fig. 5*b*). The brush border was disorganized, and intracellular organelles, e.g. mitochondria, were markedly swollen. At more advanced stages of this degenerative process, the rupture of the cell membrane and the complete disorganization of the structure of the epithelial cells could be detected (Fig. 5*c*). In sharp contrast, electronmicroscopy did not indicate any structural damage of the epithelial cells in tissue specimens obtained from the rats of Group 4; i.e. the oestrogen-pretreated animals that had been treated with the vasopressin antagonist before the administration of LVP (Fig. 5*d*).

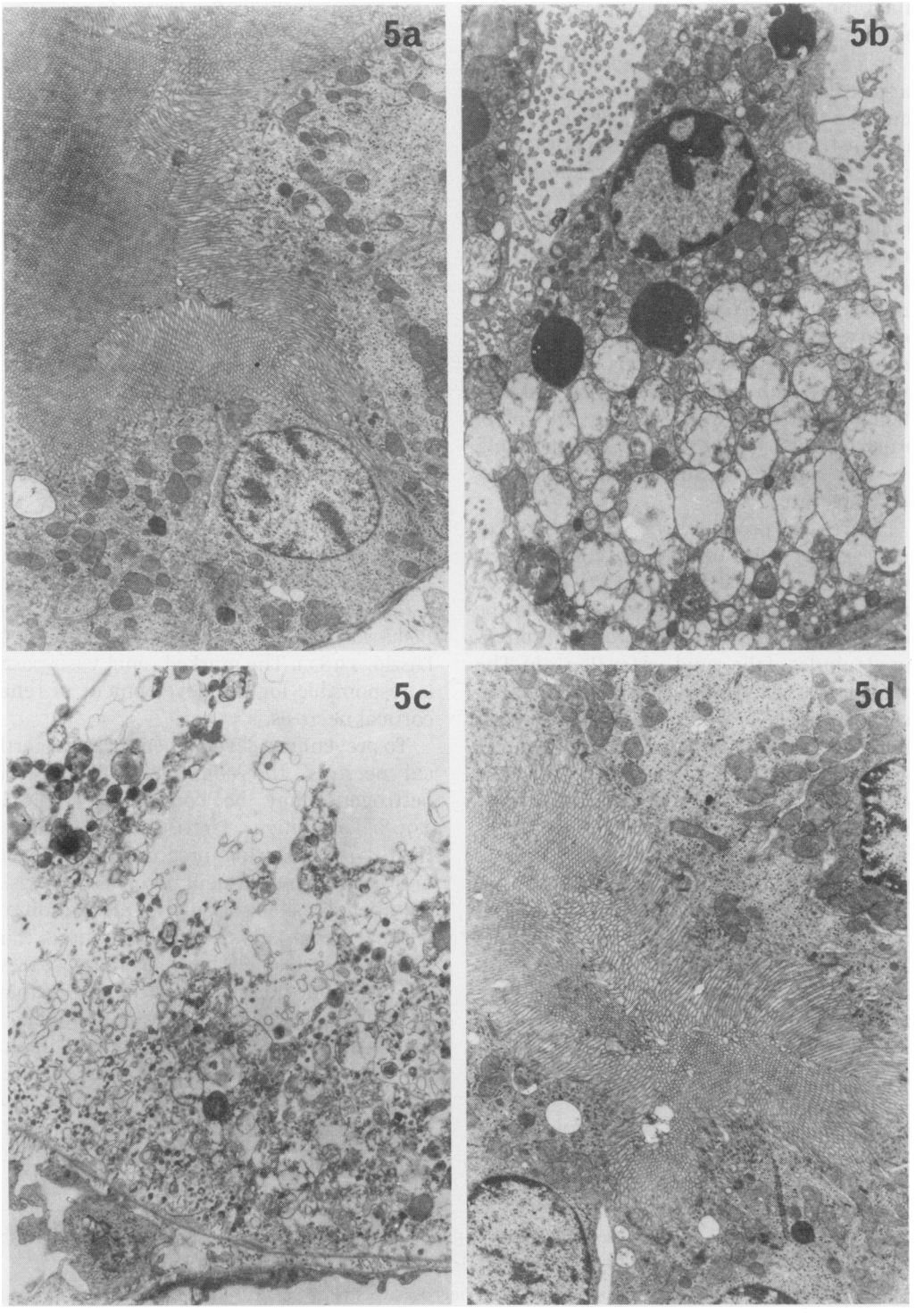
Discussion

Vasopressin is known to play a role in the maintenance of the blood pressure (Cowley *et al.* 1974; 1980; 1981; Laycock *et al.* 1979; Malayan *et al.* 1980; Schwartz & Reid 1981; 1983; Zerbe *et al.* 1982). After severe bleeding or dehydration, the secretion of vasopressin is increased (Ginsburg & Brown 1956; Sachs *et al.* 1967; Chien & Usami 1974; Andrews & Brenner 1981; Schwartz & Reid 1981; 1983; Aisenbrey *et al.* 1982) this is of importance in the correction of the hypotension or the restoration of the blood pressure (Laycock *et al.* 1979; Altura 1980; Cowley *et al.* 1980; Schwartz & Reid 1981; 1983; Andrews & Brenner 1981; Aisenbrey *et al.* 1982; Rundgren *et al.* 1982; Zerbe *et al.* 1982; 1983; Al–Omar Azzawi & Shirley 1984). A similar mechanism can be presumed to be concerned in the bleeding that may occur as a complication of child-bearing in human beings. During pregnancy, the oestrogen level is raised, and this increases the sensitivity of the renal arteries to the vasoconstrictive effect of vasopressin (Byrom 1937; 1938; Byrom & Pratt 1959; Lloyd & Pickford 1961; 1962; Honoré 1962*a*, *b*; László, 1981). The marked renal vasospasm is responsible for the development of renal cortical necrosis.

To prevent the development of renal cortical necrosis, the administration of anti-oestrogens may be considered (Walpole 1968; Labhsetwar 1970; Billard & McDonald 1973). During pregnancy, however, anti-oestrogen treatment involves some risk. Another possibility for the prevention of cortical necrosis is to avoid situations that induce an extremely high level of vasopressin secretion (haemorrhage, hypovolaemia, shock, etc.) (Schrier *et al.* 1968; Rocha E Silva & Rosenberg 1969; Errington & Rocha E Silva 1974). Experiments have been made with the local infusion of urokinase and heparin into the renal artery (Jones *et al.* 1975).

Fig. 3. *a*, Renal cortical necrosis. Oestrone acetate + LVP. Trichrome. × 320. *b*, Mild degeneration in the tubular epithelia. Oestrone acetate + LVP + d(CH_2)$_5$Tyr(Met)AVP. Trichrome. × 320.

Fig. 4. *a*, Damaged tubular epithelia with partial necrosis. Oestrone acetate + LVP. PAS. × 320. *b*, Mild denegeration in the renal tubular epithelia. Oestrone acetate + LVP + d(CH_2)$_5$Tyr(Met)AVP. PAS. × 320.


5a
5b
5c
5d

Antagonist compounds have recently been synthesized which block the biological effects of vasopressin. It appeared logical to test such compounds for their ability to prevent renal cortical necrosis.

The pressor antagonist, $d(CH_2)_5Tyr(Met)$-AVP, was prepared in the Department of Biochemistry at the University of Ohio (Kruszynski *et al.* 1980; Manning & Sawyer 1982). Even in small amounts, it reduces the elevation of blood pressure that is caused by vasopressin. Its 'effective dose' is 0.16 nmol/kg; this is the single dose necessary for the effect of 2 iu vasopressin agonist to be modified to that of 1 iu. The decreasing effect on the peripheral vascular resistance is considered to be responsible for the occurrence of this phenomenon (Zerbe *et al.* 1982; Rose *et al.* 1983).

Our investigations clearly demonstrate that the pressor antagonist, $d(CH_2)_5$-Tyr(Met)AVP completely prevents the development of the renal cortical necrosis that otherwise is produced by vasopressin administration in oestrogen-pretreated rats. A change indicative of significant hypoxia cannot be detected either histochemically or electron-microscopically. This compound presumably blocks the enhancing effect of vasopressin on the renal vascular resistance through a competitive mechanism (Butlen *et al.* 1978); as a consequence, the renal blood flow is increased (Al–Omar Azzawi & Shirley 1984), and subsequently renal necrosis does not develop. Our experimental observations permit the conclusion that administration of the pressor antagonist may in the future be an important means of preventing human renal cortical necrosis.

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Fig. 5. *a*, Electronmicroscopic picture of a normal renal cortex. The epithelial cells of the proximal convoluted tubules are covered by microvilli. × 5200. *b*, Serious structural changes can be seen 24 h after LVP administration in the kidney of rats pretreated with oestrogen. The mitochondria are swollen; the cell nucleus moves towards the lumen. × 5200. *c*, In the most seriously damaged areas, only remnants of necrotized epithelial cells are visible. The capillary endothelium and basal membrane are intact. × 5200. *d*, The vasopressin antagonist $d(CH_2)_5Tyr(Met)AVP$ inhibits LVP-induced renal cortical necrosis in rats pretreated with oestrogen. The ultrastructural picture coincides fully with that for the control. × 5200

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