Chapter 5
5. STUDIES ON ISOLATED BRUSH BORDER MEMBRANE

5.1 INTRODUCTION

The kidney maintains body fluids and electrolyte balance by regulating excretion of ions, salts, metabolic waste materials and water into the urine. Progressive deterioration in renal function often occurs in the absence of the original cause of injury (Brenner, 1985). Histologically, the end result of progressive glomerular injury is frequently focal and global glomerulosclerosis. Kidney, a target organ for the toxic actions of various drugs and chemicals (Roxe, 1975) leads to proximal tubular necrosis, a frequent manifestation of toxic nephropathy.

A number of extra-renal effects make it difficult to study purely nephrogenic changes in the whole animal in response to toxic insult. The complex function and highly organized structure of the kidney renders certain limitations to in vivo systems. Isolated perfused kidney is used for the investigation of separate function at the cellular and subcellular level. The isolated kidney may however be studied by several techniques. These include renal tissue slices, the isolated perfused kidney, ex vivo perfusion and in vivo infusion. The functions of the lower part of the nephron can be studied by microperfusion after puncture in situ or after dissection of single tubular units in vitro. Each of these techniques has inherent limitations and none are likely to be artefact free (Ormstad, 1981).

Much of the renal cortical mass consists of proximal tubules. The proximal tubule is probably the most important part of the nephron as far as nephrotoxicity is concerned. Its essential function is the osmotic reabsorption of
salt and water, along with other solutes and at most of the protein that escapes
the glomerular filtering process. The structure of the cells are modified at its end
and in particular, the surface area of both apical and basal surfaces are increased
by the presence of brush border and basal infoldings respectively, while the
enzymatic make up of the two surfaces are different (Maunsbach, 1966).

Brush border from renal tissues are derived exclusively from plasma
membrane fragments of the proximal convoluted tubule. Brush borders which
occur on the apical side of the cell, histologically are a parallel array of microvilli,
which number approximately 6500 per cell in the first part of the tubule and
increases the effective surface area by forty - fold (Wilfong and Neville, 1970). The
microvilli are covered by a negatively charged cell coat and between their bases
are invaginations of the cell membrane into the cytoplasm, indicating that
endocytosis is taking place in this region. Smaller peptides are probably degraded
by brush border hydrolytic enzymes before absorption (Welling and Welling, 1975;
Carone et al., 1980). The hydrolytic membrane bound enzymes of the microvilli in
the proximal tubules are mainly intrinsic proteins (Booth and Kenny, 1980).

Toxic damage to the kidney caused by drugs occurs in the proximal
tubule (Whelton and Walker, 1974). Several investigators have noted that brush
border degeneration occurs early in toxicity (Andrews, 1975). It appears logical,
therefore, to look for an index of this part of the nephron which would permit
early recognition of alterations in brush border membrane. ADR is known to cause
proximal tubular damage leading to decrease in enzyme activities of the brush
border membrane. Thus the purpose of this investigation was to test the efficacy
of LA as a nephroprotectant against ADR induced damage to brush border
membrane of the proximal tubular cell.
5.2 MATERIALS AND METHODS

5.2.1 Materials, animal model, line elements and experimental setup are as in sections 2.2.1, 2.2.2, 2.2.3 and 3.2.2 respectively. Collection of rat tissues and preparation of tissue homogenate are as in section 2.2.6 and 2.2.7 respectively.

5.2.2 Isolation of brush border membrane from kidney

The method of Malathi et al. (1979) was followed for isolation of brush border membrane from kidney cortex.

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Fresh or frozen kidney cortex
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Homogenize in 50 mM mannitol/2 mM Tris-HCl pH 7.0 for 5 min
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Add 1 M CaCl₂ to final concentration
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Stir 10 min at 4°C
Discard pellet ↓ 3000 x g for 15 min
Supernatant
Discard supernatant ↓ 43,000 x g for 20 min
Pellet resuspended in mannitol/Tris HCl buffer
Discard supernatant ↓ 43,000 x g for 20 min
Pellets of brush border membrane.
Suspended in buffer and used for the assay of enzymes
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5.2.3 ALP Activities in Rat Kidney: ALP was assayed in total kidney homogenate and in brush border membranes as described in sections 2281, 2281, 22191, 2285, 22142 respectively.

5.3 RESULTS AND DISCUSSION

The kidney is a target organ for the toxic actions of various drugs and chemicals (Roxe, 1975). The maintenance of normal renal function necessitates a dynamic biochemical status which is reflected by the high renal blood and oxygen uptake (Guder and Ross, 1980). Reabsorption, transport and/or degradation of proteins, peptides or aminoacids are functions of proximal tubule and there is little evidence that other segments of nephron have the mechanisms for the luminal uptake or transport of these substances (Coudrier et al., 1988). Early change in the kidney after chemical insult is an alteration of the brush border enzymes. Quantification of the brush border enzymes may therefore provide a sensitive specific index of renal damage (Stroo and Hook, 1977).

Figures 5.1a,b,c,d and e represent the alterations in the activities of marker enzymes in total kidney homogenate and isolated brush border membranes. Rat renal brush borders are rich in ALP (Glossman and Gips, 1974), which has often been used as a marker enzyme for brush border plasma membrane preparations. High activity of this enzyme is localised in the cortex than the outer medulla (Kempson and Price, 1979). Brush border ALP is involved in the transport of hexoses by a mechanism involving their sequential phosphorylation and dephosphorylation (Miller and Crane, 1961).
Fig 5.1a Effect of ADR and LA on the activity of ALP in total kidney homogenate and brush border membrane.

Bars represent the mean ± SD for six rats.

ALP: One unit of enzyme activity is expressed as u molar, and comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV, and d-groups I and II. Values are statistically significant at *p < 0.05.
Fig 5.1b Effect of ADR and LA on the activity of Y-GT in total kidney homogenate and brush border membrane

Bars represent the mean ± SD for six rats.
Y-GT-One unit of enzyme activity is expressed as μ moles of p-nitroaniline liberated
Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and II, and d-groups II and III.
Values are statistically significant at *P < 0.05.
ADR is reported to bind to the acidic phosphatases (Gowanda et al., 1980a,b) present on the brush border membrane. Hence, its binding may inhibit the activity of the neighbouring ALP molecule. The above report tends to support the present observations where a decrease (p<0.05) in ALP activity was observed in group II rats administered with ADR.

γ-GT, which degrades GSH is located on the luminal side of the brush border of the straight proximal tubule (Curthoys et al., 1980). Biosynthesis and utilization of GSH is balanced, producing steady state levels in tissues and is facilitated by γ-glutamyl cycle. The activities of the various enzymes involved in the breakdown of the tripeptide are higher in renal tissues than in liver and correspondingly there is greater turnover of GSH in the kidney (Wellner et al., 1974).

The first stage in GSH breakdown is catalysed by γ-glutamyl moiety to an acceptor aminoacid, thus forming a γ-glutamyl amino acid. Sodium dodecyl sulphate electrophoresis reveals the subunit nature of γ-GT, which consists of two subunits, a smaller subunit, unaltered by proteolytic solubilisation and a large subunit with proteolytic sensitive hydrophobic domain that serves as an anchor in the membrane (Tsao and Curthoys, 1982).

The present study revealed that the activity of γ-GT was found to be decreased (p<0.05) during ADR administration in the total homogenate and in the brush border membrane fractions of the kidney. Decreased activity in the tissues can be correlated to its increased urinary excretion as discussed elsewhere in this thesis. A decrease in γ-GT activity of the renal tissue was observed in lead intoxicated rats (Sivaprasad et al., 2002) which was reverted to near normal on LA treatment.
Fig 5.1c Effect of ADR and LA on the activity of Na+, K+-ATPase in total kidney homogenate and brush border membrane.

Bars represent the mean ± SD for six rats.

Na+, K+-ATPase—One unit of enzyme activity is expressed as μ moles of Pi liberated.

Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV and d-groups II and IV.

Values are statistically significant at *P < 0.05.
Fig 5.1d Effect of ADR and LA on the activity of ADH in total kidney homogenate and brush border membranes.

Bars represent the mean ± SD for six rats.

ACP-One unit of enzyme activity is expressed as μ moles.

Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV, and d-groups II and IV.

Values are statistically significant at *P < 0.05.
Proximal and distal tubules in the cortex express the high activity of Na⁺, K⁺-ATPase. Na⁺, K⁺ - ATPase appears to be regulated by aldosterone and serves as a marker enzyme with other plasma membrane markers during subfractionation studies (Schmidt et al., 1975). Na⁺, K⁺-ATPase plays a major role in renal absorption and changes in its activity reflect alterations in transcellular sodium transport (Stokes, 1990). It functions as the primary active transport pump for Na⁺ and K⁺ in renal tubule epithelia. Measurement of Na⁺, K⁺-ATPase levels in renal tissue have been employed extensively in studies of renal electrolyte transport and its regulation (Terada and Knepper, 1989).

Microvilli of the brush border increases the luminal cell surface of the tubule cells approximately forty times. Hence it appears that a significant proportion of total plasma membrane Na⁺, K⁺-ATPase may be found in the brush border segment (Liang and Sacktor, 1977). It was found that the activity of Na⁺, K⁺ - ATPase was decreased (p<0.05) in the kidney of rats with nephrosis induced by ADR. In view of the finding, it seems reasonable to suggest that ADR may act directly on the tubular cells, thereby decreasing Na⁺, K⁺-ATPase activity.

Lysosomal enzyme levels are altered significantly in ADR treated rats. Singal et al. (1988) have also shown that the administration of ADR to rats was associated with changes in lysosomal enzyme activities in myocardium and in serum.

Renal β-Glu is localised in the lysosomes of tubular epithelial cells. Its excretion pattern in health and disease was found to be most similar to that of another lysosomal enzyme in urine, β-Gal. Thus, the lysosomal enzymes (ACP and
Fig 5.1e Effect of ADR and LA on the activity of B-Glu in total kidney homogenate and brush border membrane

Bars represent the mean ± SD for six rats
B-Glu-One unit of enzyme activity is expressed as n moles of p-nitrophenol liberated
Comparisons were made between a-groups I and II, b-groups I and III, c-groups I and IV and d-groups II and IV
Values are statistically significant at *P < 0.05
β-Glu showed a significant (p<0.05) decline in the brush border membrane of ADR administered rats (group II).

Apart from ADR administration, pretreatment with LA maintained the activities of ALP, γ-GT, Na⁺, K⁺-ATPase, ACP and β-Glu (p<0.05) to near normal levels in group IV animals (ADR + LA) suggesting the membrane stabilizing effect of LA.