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THE LOCALIZATION OF RENAL GLUTATHIONE OXIDASE ACTIVITY STUDIED IN THE ISOLATED, PERFUSED RAT KIDNEY

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SUMMARY: The metabolism of extracellular glutathione was studied in the isolated, perfused rat kidney. Both recirculating and single-pass perfusions were associated with rapid conversion of reduced glutathione to glutathione disulfide in the perfusate. Only a minor fraction of perfusate glutathione was recovered in urine; however, this fraction was markedly increased in the presence of the inhibitor of γ-glutamyltransferase, serine·borate. In contrast, serine·borate had no effect on either oxidation or disappearance of perfusate glutathione. The results indicate that renal glutathione oxidase activity is restricted to glutathione present in plasma, while γ-glutamyltransferase acts on glutathione in the glomerular filtrate.

It is now well established that the kidney plays an important role in the metabolism of plasma glutathione (1). Recent studies have shown that renal metabolism of extracellular reduced glutathione (GSH) most probably involves a series of reactions catalyzed by glutathione oxidase (2), γ-glutamyltransferase and cysteinylglycine dipeptidase (3). All of these enzymes have been found to be present in the plasma membrane of tubular epithelial cells (2).

Previous studies have revealed that renal γ-glutamyltransferase is located exclusively in the brush border of the tubular epithelium (4) and thus presumably is restricted in its action on extracellular substrates to those present in the tubular fluid. A similar distribution has recently been suggested for renal glutathione oxidase activity (5) which, in fact, has been implied to be a catalytic property of γ-glutamyltransferase (6).

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In the present study we have used the isolated, perfused rat kidney to investigate the localization of renal glutathione oxidase activity. Our results indicate that this activity is distributed differently from that of γ-glutamyltransferase, and suggest that renal glutathione oxidase activity is restricted to glutathione present in plasma. It thus appears that renal glutathione oxidase and γ-glutamyltransferase activities are catalyzed by separate enzymes located on opposite sides of the tubular epithelium.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200-250 g) were fed water and pelleted rat food ad libitum. With the animals in ether anaesthesia, the ureters and major blood vessels on the posterior abdominal wall were exposed and cannulated. The isolated preparation consisted of the aorta and inferior caval vein with both kidneys attached via their vessels and with the ureters hanging free. Perfusion was performed in a thermostated, carbogen-gassed system at a pump pressure of 60-80 cm H_2O and a flow rate of 2-3 ml/g kidney/min. Samples were taken from the perfusate at regular intervals, while urine was collected in 3 min-batches. The perfusate was either recirculated in a closed system (recirculating perfusion) or passed only once through the kidneys (single-pass perfusion) with samples taken simultaneously from the inflowing (arterial) and outflowing (venous) sides.

GSH and glutathione disulfide (GSSG) were assayed fluorometrically (7) in samples deproteinated with 5% HPO_3. GSH and GSSG were purchased from Sigma Chemical Co., St. Louis, Mo. All other reagents were of analytical grade and obtained from local commercial sources.

RESULTS

As shown in the Table there was a rapid disappearance of GSH from the perfusate during recirculating perfusion as well as single-pass perfusion of isolated rat kidneys. In both cases GSSG accumulation in the perfusate accounted for more than 90% of the GSH loss. Like previously found with isolated renal plasma membrane fractions (5,8), glutathione oxidase activity in the perfused kidney was strongly inhibi-

TABLE

Metabolism of extracellular glutathione by the isolated, perfused rat kidney

	GSH decrease in perfusate µmol/min	GSSG increase in perfusate µmol/min	GSH recovery in urine nmol/min	GSSG recovery in urine nmol/min
Recirculating perfusion				
No addition (n=3)	5.60	5.32	4.6	2.3
EDTA, 2 mM (n=3)	1.82	1.40	3.9	not detectable
Serine·borate, 20 mM (n=3)	5.45	5.10	68.2	40.4
Single-pass perfusion				
No addition (n=3)	5.78	5.38	4.8	not detectable
EDTA, 2 mM (n=2)	1.50	1.30	4.0	not detectable
Serine·borate, 20 mM (n=3)	5.38	4.79	53.5	not detectable

One pair of kidneys were perfused with Krebs-Henseleit buffer, pH 7.4, supplemented with 25 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 1 mM GSH. The table presents the results, given in GSH-equivalents, of one experiment typical of the number stated within parentheses.

ted by EDTA (ethylenediamine-tetraacetic acid). In contrast, perfusion in presence of serine·borate did not markedly affect either GSH disappearance or GSSG accumulation in the perfusate.

Renal perfusion with GSH was associated with the appearance of low concentrations of glutathione in urine. However, the urinary recovery of glutathione was dramatically increased when a combination of serine and borate was added to the perfusate suggesting that, in the absence of this inhibitor, most of the glutathione in the glomerular filtrate was subjected to γ-glutamyltransferase-mediated degradation. During recirculation of a perfusate containing serine·borate as well as GSH, both GSH and GSSG contributed to the urinary fraction of glutathione, whereas only GSH was recovered in urine during single-pass perfusion. This observation, which was made independently of whether or not serine·borate was present in the perfusate, strongly suggests that GSH filtered in the glomeruli escapes glutathione oxidase activity.

DISCUSSION

Recent studies in our laboratory have demonstrated the presence of glutathione oxidase activity in the plasma membrane of renal epithelial cells (2). The activity appears to be restricted to extracellular GSH, which is rapidly oxidized to GSSG, and is inhibited by KCN and by various metal chelating agents (2,8). Tate and coworkers have subsequently studied the subcellular localization of renal glutathione oxidase activity in detail (5) and concluded on the basis of this investigation, and on results of copurification experiments (6), that renal glutathione oxidase activity is probably a catalytic property of γ-glutamyltransferase.

This hypothesis is not supported by the results of the present investigation. In accordance with several previous studies (1,9,10), γ-glutamyltransferase activity was observed only with glutathione present in the glomerular filtrate, and no evidence was obtained for γ-glutamyltransferase-mediated degradation of glutathione present in perfusate. In contrast, glutathione oxidase activity was restricted to GSH present in perfusate; GSSG was not detected in urine during single-pass perfusion with GSH. Moreover, in recent experiments ureter ligation has been shown to result in stoichiometric conversion of GSH to GSSG in perfusate during renal perfusion with GSH; during perfusion with GSSG under the same conditions no loss of GSSG from perfusate was observed (11).

Thus, the results of the present investigation strongly suggest that renal glutathione oxidase and γ-glutamyltransferase activities are catalytic functions of separate enzymes located on opposite sides of the tubular epithelium. A possible contribution of γ-glutamyltransferase to the observed glutathione oxidase activity is difficult to exclude entirely, but appears to be of little probability.

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