Relationship Between Gluconeogenesis and Glutathione Redox State in Rabbit Kidney-Cortex Tubules

Katarzyna Winiarska, Jakub Drożak, Michał Węgrzynowicz, Adam K. Jagielski, and Jadwiga Bryła

The intracellular glutathione redox state and the rate of glucose formation were studied in rabbit kidney-cortex tubules. In the presence of substrates effectively utilized for glucose formation, ie, aspartate + glycerol + octanoate, alanine + glycerol + octanoate, malate, or pyruvate, the intracellular reduced glutathione/oxidized glutathione (GSH/GSSG) ratios were significantly higher than those under conditions of negligible glucose production. Changes in the intracellular GSH/GSSG ratio corresponded to those in glucose-6-phosphate content and reduced nicotinamide adenine dinucleotide phosphate/oxidized nicotinamide adenine dinucleotide phosphate (NADPH/$NADP^+$) ratio obtained from malate/pyruvate measurements. Gluconeogenesis stimulation by extracellular adenosine triphosphate (ATP) or inosine caused an elevation of the intracellular GSH/GSSG and NADPH/$NADP^+$ ratios, as well as glucose-6-phosphate level. Surprisingly, in the presence of 5 mmol/L glucose, both the intracellular GSH/GSSG and NADPH/$NADP^+$ ratios and glucose-6-phosphate content were almost as low as under conditions of negligible glucose synthesis. L-buthionine sulfoximine (BSO)-induced decline in both the intracellular glutathione level and redox state resulted in inhibition of gluconeogenesis accompanied by accumulation of phosphotrioses and a decrease in fructose-1,6-bisphosphate content, while cysteine precursors altered neither GSH redox state nor the rate of glucose formation. In view of the data, it seems likely that: (1) intensive gluconeogenesis rather than extracellular glucose is responsible for maintaining a high intracellular GSH/GSSG ratio due to effective glucose-6-phosphate delivery for NADPH generation via the pentose phosphate pathway; (2) a decline in the intracellular glutathione level and/or redox state causes a decrease in glucose synthesis resulting from a diminished flux through aldolase; (3) induced by cysteine precursors, elevation of the intracellular GSH level does not affect the rate of glucose formation, probably due to no changes in the intracellular GSH/GSSG ratio.

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GLUTATHIONE (L-γ-glutamyl-L-cysteinylglycine) is the predominant nonprotein thiol in mammalian cells. Its intracellular concentrations are in the range of 0.5 to 10 mmol/L (see Hammond et al[1] for review). Under physiologic conditions, more than 98% of intracellular glutathione exists as the reduced thiol form (GSH), while the rest is present mainly as the oxidized disulfide form (GSSG) or mixed disulfides. Reduction of GSSG to GSH is catalyzed by GSH reductase utilizing nicotinamide adenine dinucleotide phosphate (NADPH) as reducing equivalent. Glutathione plays several vital roles: it scavenges free radicals, regulates gene expression and enzyme activities, controls the process of cell death, detoxifies xenobiotics, and maintains other antioxidants in their reduced forms. Disturbed glutathione status has been reported to accompany many diseases, eg, diabetes,[2,3] neurodegenerative diseases,[4] viral infections,[5] cirrhosis, and alcoholic disease.[6]

It is commonly accepted that exposition to high glucose concentrations can induce oxidative stress, which is considered to be the main cause of diabetic complications.[7,8] Moreover, following hyperglycemia, the susceptibility of healthy humans to oxidative stress is increased.[9] To elucidate the mechanisms of glucose-induced oxidative stress, several hypotheses have been proposed, including glucose auto-oxidation, glycation of proteins, and formation of advanced glycation end products (AGEs) (see Bonnefont-Rousselot et al[7] for review). High glucose has been reported to diminish GSH levels as a result of decreased expression of γ-glutamylcysteine synthetase, the key enzyme of glutathione synthesis,[10,11] and inhibition of glucose-6-phosphate dehydrogenase, an enzyme responsible for NADPH delivery for GSSG reduction.[12] On the other hand, it is necessary to point out that NADPH generation via the oxidative phase of pentose phosphate pathway is a glucose-consuming process.[13]

In view of these observations, the aim of this study was to investigate the intracellular glutathione status with respect to glucose formation. As the kidney, in addition to liver, makes a significant contribution to glucose whole body metabolism[14] and the intracellular localization of gluconeogenic enzymes in rabbit kidney is similar to that in humans,[15] rabbit kidney-cortex tubules were chosen to be the subject of this investigation.

MATERIALS AND METHODS

Isolation and Incubation of Kidney-Cortex Tubules

Male California strain rabbits (2 to 3 kg body weight) were used throughout the experiments. All animal use procedures were approved by the First Warsaw Local Commission for the Ethics of Experimentation on Animals. Animals were fed ad libitum with standard rabbit chow and had free access to water. Rabbits were anesthetized with pentobarbital (30 mg/kg body weight). Kidney-cortex tubules were isolated according to the method described by Guder at al[16] and modified by Zabłocki et al.[17] Freshly isolated renal tubules (about 10 mg dry weight) were incubated for 1 hour at 37°C, under the atmosphere of 95% O_2 + 5% CO_2, in 2 mL Krebs-Ringer bicarbonate buffer in 25-mL plastic Erlenmeyer flasks (Nalgene, Rochester, NY) sealed with rubber stoppers. Amino acids and glycerol were added to the incubation medium at 2 mmol/L concentrations, malate and pyruvate at

From the Institute of Biochemistry, Warsaw University, Warsaw, Poland.

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Address reprint requests to Jadwiga Bryla, PhD, Ul. I. Miecznikowa 1, 02-089 Warsaw, Poland.

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Table 1. Glucose Formation, Intracellular GSH and GSSG Contents and GSH/GSSG Ratios in Rabbit Kidney-Cortex Tubules Incubated With Various Substrates

Substrates	Glucose Formation (μmol $\times$ g^{-1} dw $\times$ h^{-1})	GSH (μmol $\times$ g^{-1} dw)	GSSG (μmol $\times$ g^{-1} dw)	GSH/GSSG
Aspartate	3.7 ± 0.2	2.34 ± 0.22	0.101 ± 0.015	23.7 ± 3.8
Glycerol	4.3 ± 0.3	1.82 ± 0.20	0.050 ± 0.008	36.9 ± 2.5
Octanoate	2.8 ± 0.1	2.02 ± 0.09	0.096 ± 0.010	24.4 ± 4.1
Glycerol + octanoate	4.1 ± 0.3	1.68 ± 0.18	0.054 ± 0.009	31.8 ± 6.5
Aspartate + octanoate	2.9 ± 0.2	2.06 ± 0.24	0.060 ± 0.008	35.3 ± 6.0
Aspartate + glycerol	15.4 ± 1.1*	2.30 ± 0.16	0.047 ± 0.008	48.9 ± 5.4*
Aspartate + glycerol + octanoate	94.0 ± 5.6*	2.78 ± 0.32	0.046 ± 0.005	60.2 ± 8.1*
Alanine + glycerol + octanoate	53.5 ± 5.9*	2.50 ± 0.20	0.048 ± 0.006	47.8 ± 6.0*
Malate	85.0 ± 7.4*	2.06 ± 0.34	0.040 ± 0.007	55.3 ± 7.3*
Pyruvate	48.2 ± 6.0*	1.85 ± 0.21	0.037 ± 0.005	49.3 ± 6.4*

NOTE. Renal tubules were incubated for 60 minutes under conditions described in Materials and Methods. Values are means ± SD for 5 to 7 experiments.

Abbreviation: dw, dry weight.

*$P < .01$ *v* values for renal tubules incubated in the presence of aspartate, glycerol, octanoate, glycerol + octanoate or aspartate + octanoate, ie, exhibiting negligible glucose synthesis.

5 mmol/L, while octanoate (as emulsion in bovine serum albumin[18]) was present at 0.5 mmol/L concentration.

Analytical Methods

Intracellular metabolite contents were estimated in tubules separated from the incubation medium following centrifugation through the silicone oil layer into 12% perchloric acid (PCA).[19] To establish the rate of gluconeogenesis, glucose was measured in supernatants obtained after the centrifugation of incubation mixture. To avoid nonenzymatic GSH oxidation, samples used for GSSG determinations were centrifuged into 50 mmol/L N-ethylmaleimide (NEM) in 12% PCA.[20] Excess NEM was removed by hexane extraction. Samples used for GSH determination were stored as PCA extracts, while the others were neutralized immediately after deproteinization. GSH levels were determined by high-performance liquid chromatography (HPLC) after derivatization with N-(1-pyrenyl)maleimide (NPM).[21] Glucose, GSSG, and other intracellular metabolites were measured either spectrophotometrically or fluorimetrically by standard enzymatic techniques.[22]

Measurement of Enzyme Activities

Aldolase, fructose-1,6-bisphosphatase, and glucose-6-phosphate dehydrogenase activities were measured in the cytosolic fraction obtained after the homogenization of kidney cortex in 0.15 mol/L KCl, pH 7.4, (1 g/5 mL) and centrifugation for 30 minutes at 70,000 $\times$ *g*. Aldolase activity was determined as described by Yeltman and Harris.[23] Fructose-1,6-bisphosphatase activity was measured according to Ozaki et al.[24] Glucose-6-phosphate dehydrogenase activity was determined by the method of Lohr and Wahler.[25]

For malic enzyme activity determination, freshly isolated renal tubules (about 6 mg dry weight) were sonicated (2 $\times$ 10 seconds) in 0.2 mL 50 mmol/L triethanolamine, pH 7.4, containing 3 mmol/L $MnCl_2$ and 0.02 % bovine serum albumin. The enzyme activity was measured fluorimetrically according to Liu et al.[26]

Enzymes and Chemicals

Glutathione reductase, lactate dehydrogenase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase were purchased from Roche Diagnostics GmbH (Mannheim, Germany). Collagenase (type IV) and all other chemicals were obtained from Sigma Chemical (St Louis, MO).

Statistical Methods

STATISTICA Version 5 (StatSoft, Tulsa, OK) was used for all calculations. Significance of the observed differences was estimated using analysis of variance (ANOVA). When data for more than 2 groups were compared, Tukey's post hoc test was applied after ANOVA. Values are expressed as means ± SD for 3 to 7 experiments.

RESULTS

The Rate of Glucose Formation and the Intracellular Glutathione Redox State

Rabbit kidney-cortex tubules were incubated in the presence of substrates used with various rates for glucose synthesis (Table1). In agreement with our previous observations,[27] aspartate, glycerol and octanoate were not used for glucose formation in kidney-cortex tubules when applied as sole substrates. Glucose synthesis from glycerol + octanoate or aspartate + octanoate was also negligible, while aspartate + glycerol seemed to be a rather poor glucose precursor. However, the rates of gluconeogenesis from aspartate + glycerol + octanoate and malate were similar and twice higher than those determined in the presence of either alanine + glycerol + octanoate or pyruvate.

The intracellular level of GSH varied depending on the substrate added to the incubation medium, but not on the rate of glucose formation. The highest GSH content was determined in renal tubules effectively producing glucose in the presence of either aspartate + glycerol + octanoate or alanine + glycerol + octanoate. On the other hand, with malate or pyruvate as glucose precursors, the intracellular GSH level was similar to that in tubules incubated either with glycerol, octanoate, and aspartate as sole substrates or in the presence of glycerol + octanoate, ie, under conditions of negligible glucose production. Similarly, the intracellular GSSG content was also substrate-dependent. Renal tubules incubated with either aspartate or octanoate as sole substrates exhibited significantly higher intracellular GSSG levels in comparison to those in the presence of other tested substrates. However, in rabbit renal tu-

Table 2. Intracellular Malate and Pyruvate Contents and Malate/Pyruvate Ratios in Rabbit Kidney-Cortex Tubules Incubated With Various Substrates

Substrates	Malate (μmol $\times$ g^{-1} dw)	Pyruvate (μmol $\times$ g^{-1} dw)	Malate/Pyruvate
Aspartate	0.18 ± 0.04	0.22 ± 0.03	0.77 ± 0.18
Glycerol	0.14 ± 0.04	0.18 ± 0.04	0.78 ± 0.18
Glycerol + octanoate	0.29 ± 0.05	0.30 ± 0.05	0.93 ± 0.20
Aspartate + glycerol	0.50 ± 0.09*	0.35 ± 0.08	1.44 ± 0.37*
Aspartate + glycerol + octanoate	0.85 ± 0.14*	0.32 ± 0.04	2.56 ± 0.39*
Alanine + glycerol + octanoate	0.52 ± 0.09*	0.31 ± 0.05	1.75 ± 0.14*
Malate	ND	0.33 ± 0.02	ND

NOTE. Renal tubules were incubated for 60 minutes under conditions described in Materials and Methods. Values are means ± SD for 3 to 5 experiments.

Abbreviation: ND, not determined.

*$P < .01$ *v* values for renal tubules incubated in the presence of either glycerol, aspartate or glycerol + octanoate, ie, exhibiting negligible glucose synthesis (Table 1).

bules, an intensive glucose synthesis was always accompanied by relatively high values of intracellular GSH/GSSG ratio (range, 47.8 ± 6.0 to 60.2 ± 8.1), independently on both GSH and GSSG intracellular levels. In renal tubules incubated with either aspartate + glycerol + octanoate or malate, both the rates of gluconeogenesis and the intracellular GSH/GSSG ratios were the highest, while in tubules exhibiting negligible glucose formation in the presence of glycerol + octanoate, aspartate + octanoate, glycerol, aspartate or octanoate, the intracellular GSH/GSSG ratios were significantly diminished (range, 23.7 ± 3.8 to 36.9 ± 2.5). It is also worth noting that in tubules incubated with alanine + glycerol + octanoate, aspartate + glycerol or pyruvate, the intracellular GSH/GSSG ratios were slightly lower than those measured under conditions of the most effective glucose production and significantly higher than those under conditions of negligible glucose synthesis.

Because glutathione reductase, the enzyme responsible for maintaining intracellular glutathione in its reduced form, is NADPH-dependent,[28] we have determined the intracellular malate/pyruvate ratios in renal tubules to estimate intracellular $NADPH/NADP^+$.[29] As shown in Table 2, the highest malate/pyruvate ratio was observed in tubules exhibiting the high rate of glucose synthesis (with aspartate + glycerol + octanoate) and the high value of intracellular GSH/GSSG ratio (Table 1). On the other hand, under conditions of negligible glucose formation, the intracellular malate/pyruvate ratio was 3-fold lower than that in the presence of aspartate + glycerol + octanoate. Moreover, in renal tubules incubated with alanine + glycerol + octanoate or aspartate + glycerol, values of intracellular malate/pyruvate ratios were about 40% lower than those in the presence of aspartate + glycerol + octanoate. Thus, changes in the intracellular $NADPH/NADP^+$ ratio were matching those in the GSH/GSSG one (Table 1). Therefore, it is likely that under conditions of effective glucose formation, an increased availability of NADPH for GSSG reduction may contribute to the elevation of the intracellular GSH/GSSG ratio.

Because glucose-6-phosphate dehydrogenase is considered the key enzyme of the oxidative phase of NADPH-delivering pentose phosphate cycle,[13] we have measured the intracellular glucose 6-phosphate level in renal tubules incubated under conditions of both intensive and negligible glucose synthesis. In renal tubules intensively synthesizing glucose from aspartate + glycerol + octanoate, the intracellular glucose-6-phosphate content was for about 4-fold higher than that in tubules exhibiting negligible glucose synthesis (0.147 ± 0.030, 0.030 ± 0.005, and 0.040 ± 0.009 μmol $\times$ g^{-1} dry weight (dw) with aspartate + glycerol + octanoate, glycerol + octanoate and aspartate, respectively; $P < .01$ for aspartate + glycerol + octanoate *v* both glycerol + octanoate and aspartate). In view of these data, it is likely that in tubules intensively synthesizing glucose, the elevated intracellular glucose-6-phosphate content might be responsible for an accelerated NADPH generation via enzymes of the oxidative phase of the pentose phosphate pathway.

Effective gluconeogenesis was accompanied by accumulation of intracellular malate (compare Tables 1 and 2), so one could suggest that the increased availability of the substrate for NADP-specific malic enzyme may also be responsible for the elevation of intracellular $NADPH/NADP^+$ ratio in renal tubules. To examine this possibility, we have compared activities of both glucose-6-phosphate dehydrogenase and malic enzyme in rabbit kidney-cortex. The activity of malic enzyme (0.87 ± 0.08 μmol $\times$ min^{-1} $\times$ g^{-1} total cellular protein at 30°C) turned out to be almost 30 times lower than that of glucose-6-phosphate dehydrogenase (23.5 ± 2.5 μmol $\times$ min^{-1} $\times$ g^{-1} total cellular protein at 30°C), suggesting that the reaction catalyzed by the former enzyme is not an important source of NADPH in rabbit kidney.

Because under conditions of high rates of gluconeogenesis, rabbit renal tubules exhibited increased GSH/GSSG ratios (Table 1), we have determined the intracellular glutathione redox state in the presence of exogenous glucose applied at a physiologic concentration. Surprisingly, the addition of 5 mmol/L glucose failed to maintain high values of both intracellular GSH/GSSG and malate/pyruvate ratios, as well as glucose-6-phosphate content (30.2 ± 6.2, 0.90 ± 0.10, and 0.065 ± 0.009 μmol $\times$ g^{-1} dw, respectively; $P < .01$ *v* corresponding values for renal tubules effectively synthesizing glucose). Moreover, an increase in glucose concentration in incubation medium up to 20 mmol/L did not result in significant changes in either GSH/GSSG and malate/pyruvate ratios (32.3 ± 5.4 and 0.79 ± 0.10, respectively) or glucose-6-phosphate level (0.069 ± 0.009 μmol $\times$ g^{-1} dw). Thus, it might be suggested that

Table 3. Effect of Extracellular ATP and Inosine on Glucose Formation, Intracellular GSH and GSSG Levels, Malate and Pyruvate Contents, as Well as GSH/GSSG and Malate/Pyruvate Ratios in Rabbit Kidney-Cortex Tubules Incubated With Alanine + Glycerol + Octanoate

Additions	Glucose Formation (μmol $\times$ g^{-1} dw $\times$ h^{-1})	GSH (μmol $\times$ g^{-1} dw)	GSSG (μmol $\times$ g^{-1} dw)	Malate (μmol $\times$ g^{-1} dw)	Pyruvate (μmol $\times$ g^{-1} dw)	GSH/GSSG	Malate/Pyruvate
None	63.3 ± 6.2	3.10 ± 0.10	0.063 ± 0.005	0.52 ± 0.08	0.32 ± 0.06	49.6 ± 4.0	1.61 ± 0.18
ATP	91.0 ± 8.1*	3.17 ± 0.37	0.050 ± 0.010†	0.51 ± 0.07	0.20 ± 0.03†	65.0 ± 8.8†	2.52 ± 0.34†
Inosine	80.0 ± 9.1*	2.91 ± 0.28	0.042 ± 0.008†	0.51 ± 0.07	0.21 ± 0.04†	69.3 ± 9.0†	2.49 ± 0.32†

NOTE. Renal tubules were incubated for 60 minutes under conditions described in Materials and Methods. Both ATP and inosine were added at 200 μmol/L concentrations. Values are means ± SD for 3 to 7 experiments.

*$P < .01$; †$P < .05$ *v* corresponding control value with no ATP and no inosine.

exogenous glucose is a poor source of glucose-6-phosphate limiting NADPH generation via glucose 6-phosphate dehydrogenase.

To prove the hypothesis that intensive glucose formation is of importance for maintenance of a high renal glutathione redox state, we have measured both GSH/GSSG and NADPH/NADP$^+$ ratios, as well as glucose-6-phosphate levels after stimulation of gluconeogenesis by extracellular adenosine triphosphate (ATP) and inosine.[30] As shown in Table 3, upon the addition of either ATP or inosine at a concentration of 200 μmol/L, glucose synthesis in the presence of alanine + glycerol + octanoate was accelerated approximately 40%. Both ATP and inosine increased the intracellular GSH/GSSG and NADPH/NADP$^+$ ratios approximately 35% and 60%, respectively, while the intracellular glucose-6-phosphate content was elevated from 0.110 ± 0.012 μmol $\times$ g^{-1} d.w. with no ATP and inosine up to 0.137 ± 0.010 and 0.148 ± 0.016 μmol $\times$ g^{-1} d.w. in the presence of ATP and inosine, respectively ($P < .05$ *v* control value with no ATP and no inosine).

Effect of L-Buthioninesulfoximine, N-Acetylcysteine, and 2-oxo-4-Thiazolidinecarboxylic Acid on the Intracellular Glutathione Redox State and the Rate of Gluconeogenesis

In view of the observed relationship between the rate of gluconeogenesis and glutathione redox state, the aim of further investigations was to study the influence of intracellular glutathione changes on the rate of glucose formation. To decrease the intracellular glutathione level, renal tubules were incubated in the presence of L-buthioninesulfoximine (BSO), a potent inhibitor of γ-glutamylcysteine synthetase,[31] while cysteine precursors, N-acetylcysteine (NAC) and 2-oxo-4-thiazolidinecarboxylic acid (OTZ), were added to elevate glutathione content.[32]

As shown in Table 4, 5 mmol/L BSO diminished the intracellular level of both GSH (approximately 80% and 70% in the presence of aspartate + glycerol and aspartate + glycerol + octanoate, respectively) and GSSG (approximately 70% and 30% in the presence of aspartate + glycerol and aspartate + glycerol + octanoate, respectively), resulting in a decline in the intracellular GSH/GSSG ratios of approximately 50% under both conditions. BSO-induced decreases in both the intracellular glutathione level and redox state were accompanied by decreased rates of glucose formation (approximately 50% and 30% with aspartate + glycerol and aspartate + glycerol + octanoate as glucose precursors, respectively). In the presence of alanine + glycerol + octanoate, pyruvate, or malate, the rates of glucose synthesis were also diminished approximately 40% (data not shown). An elevation of BSO concentration up to 10 mmol/L did not result in a further decrease in glucose formation (data not shown). It should also be pointed out that the addition of BSO did not change the rate of glycolysis in renal tubules incubated with aspartate + glycerol + octanoate, as concluded from lactate formation measurements (52.4 ± 3.0 and 47.0 ± 4.4 μmol $\times$ g^{-1} dw $\times$ h^{-1} in the absence and presence of BSO, respectively).

To identify the steps of gluconeogenesis inhibited in the presence of BSO, we have measured the intracellular contents of gluconeogenic intermediates in renal tubules. As shown in Fig 1, the addition of 5 mmol/L BSO into the incubation medium containing aspartate + glycerol + octanoate resulted

Table 4. Effect of BSO, NAC, and OTZ on Intracellular GSH and GSSG Contents, GSH/GSSG Ratios, and Glucose Formation in Rabbit Kidney-Cortex Tubules

Substrates	Additions	GSH (μmol $\times$ g^{-1} dw)	GSSG (μmol $\times$ g^{-1} dw)	GSH/GSSG	Glucose Formation (μmol $\times$ g^{-1} dw $\times$ h^{-1})
Aspartate + glycerol	None	2.78 ± 0.21	0.054 ± 0.006	50.2 ± 4.8	18.4 ± 3.1
	BSO	0.46 ± 0.03*	0.017 ± 0.003*	26.9 ± 2.9*	9.1 ± 2.0*
	NAC	3.58 ± 0.32*	0.089 ± 0.007†	44.1 ± 5.6	44.2 ± 5.0*
	OTZ	4.04 ± 0.41*	0.084 ± 0.010†	50.7 ± 6.2	18.2 ± 2.8
Aspartate + glycerol + octanoate	None	3.37 ± 0.28	0.055 ± 0.006	62.2 ± 5.4	91.0 ± 5.8
	BSO	0.97 ± 0.07*	0.037 ± 0.003†	25.5 ± 2.4*	63.2 ± 7.2*
	NAC	4.04 ± 0.34*	0.070 ± 0.009†	60.6 ± 6.5	90.4 ± 4.9
	OTZ	4.50 ± 0.47*	0.080 ± 0.012†	54.1 ± 6.8	88.3 ± 7.7

NOTE. Renal tubules were incubated for 60 minutes under conditions described in Materials and Methods. BSO was present at 5 mmol/L concentration, while NAC and OTZ were added at 2 mmol/L concentrations. Values are means ± SD for 3 to 7 experiments.

*$P < .01$; †$P < .05$ *v* corresponding control value with no additions.

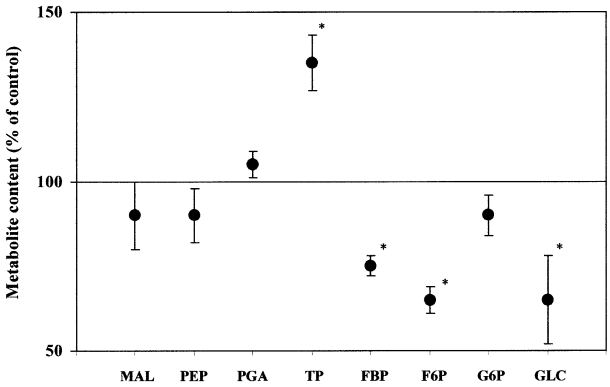

Fig 1. BSO-induced changes in the intracellular levels of gluconeogenic intermediates in rabbit renal tubules incubated with aspartate + glycerol + octanoate. The concentrations of intracellular metabolites in the presence of 5 mmol/L BSO are expressed as percentage of control values measured with no BSO. The control values (expressed in μmol × g^{-1} dw) for metabolites listed from left to right are: malate (MAL) 0.76 ± 0.08; phosphoenolpyruvate (PEP) 0.25 ± 0.03; 3-phosphoglycerate and 1,3-bisphosphoglycerate (PGA) 0.83 ± 0.10; 3-phosphoglyceraldehyde and phosphodihydroxyacetone (TP) 0.32 ± 0.04; fructose-1,6-bisphosphate (FBP) 0.16 ± 0.01; fructose-6-phosphate (F6P) 0.06 ± 0.01; glucose-6-phosphate (G6P) 0.20 ± 0.03; glucose (GLC) 4.74 ± 0.52. *$P < .05$ *v* corresponding control value with no BSO.

in an accumulation of phosphotrioses accompanied by a significant decline in fructose-1,6-bisphosphate level, indicating the inhibition of flux through aldolase. However, neither aldolase activity (233 ± 12 and 250 ± 14 μmol × min^{-1} × g^{-1} protein at 30°C in the absence and presence of BSO, respectively) nor fructose-1,6-bisphosphatase activity (84 ± 4 and 85 ± 5 μmol × min^{-1} × g^{-1} protein at 30°C in the absence and presence of BSO, respectively) were directly inhibited by BSO, as concluded from the measurements of effects of BSO on these enzyme activities in the cytosolic fraction of kidney cortex. Thus, it seems likely that the observed decline in aldolase activity might be due to a diminished level and/or redox state of the intracellular glutathione.

At 2 mmol/L concentrations, cysteine precursors (NAC and OTZ) resulted in approximately 40% increase in intracellular GSH and GSSG contents and did not change GSH/GSSG ratios in renal tubules incubated in with aspartate + glycerol or aspartate + glycerol + octanoate (Table 4). The elevation of NAC and OTZ concentrations up to 5 mmol/L did not cause a further increase in the intracellular glutathione content (data not shown). OTZ did not alter the rate of glucose formation, whereas NAC did not affect the rate of gluconeogenesis in the presence of aspartate + glycerol + octanoate and stimulated this process in the presence of aspartate + glycerol as glucose precursor. As the substitution of NAC by sodium acetate at the same concentration also resulted in an increase in glucose production from aspartate + glycerol (up to 50.2 ± 6.1 μmol × g^{-1} dw × h^{-1} and accompanied by the increase in GSH/GSSG ratio up to 54.2 μmol × g^{-1} dw; $P < .01$ *v* control value with no acetate and NAC), the NAC stimulatory effect on gluconeogenesis might be due to acetyl units released during NAC breakdown. Both NAC and OTZ did not change the rates of gluconeogenesis in renal tubules incubated with alanine + glycerol + octanoate, malate, or pyruvate as glucose precursors (data not shown), confirming our suggestions. Thus, the elevation of the intracellular glutathione content in rabbit renal tubules incubated with either NAC or OTZ did not affect the rate of glucose formation, as these compounds did not induce changes in GSH/GSSG ratios.

DISCUSSION

Current knowledge about the relationship between glucose and glutathione metabolism is generally limited to reports concerning the oxidative action of hyperglycemia.[7-12] In this investigation, we have tried to study the relationship between renal glutathione redox state and the rate of gluconeogenesis.

In agreement with our previous reports,[27,30,33] the present data indicate that in renal tubules effective gluconeogenesis from aspartate and alanine occurs only in the presence of glycerol and octanoate (Table 1) and is stimulated by extracellular ATP or inosine (Table 3). The rates of glucose synthesis from alanine + glycerol + octanoate in the presence of extracellular ATP or inosine or aspartate + glycerol + octanoate are as high as in the presence of malate, considered as a good renal glucose precursor,[17] and accompanied by the highest intracellular GSH/GSSG ratios. Renal tubules synthesizing glucose at lower rates than those measured with aspartate + glycerol + octanoate, or malate, ie, in the presence of alanine + glycerol + octanoate, pyruvate or aspartate + glycerol exhibit slightly diminished intracellular GSH/GSSG ratios, while negligible glucose formation is always accompanied by relatively low values of GSH/GSSG ratio. The relationship between the rate of glucose synthesis and the intracellular GSH/GSSG ratio is shown in Fig 2 summarizing the results of separate experiments for renal tubules incubated under both nongluconeogenic and gluconeogenic conditions. The data for renal tubules incu-

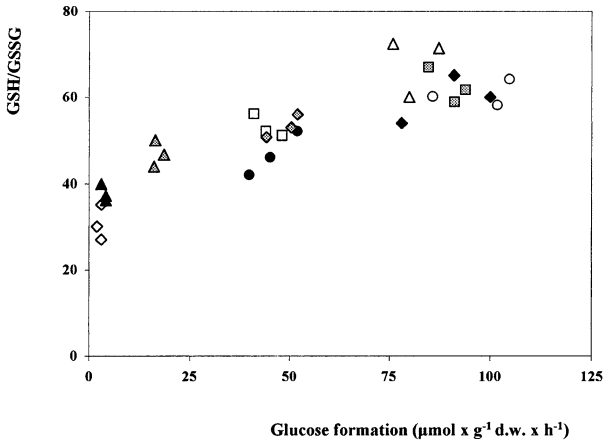

Fig 2. Relationship between the rate of glucose synthesis and the intracellular GSH/GSSG ratio. Each symbol represents data of a separate experiment with the use of kidney-cortex tubules incubated in the presence of the following compounds: aspartate (◇), glycerol (▲), aspartate + glycerol (△), aspartate + glycerol + acetate (◈), aspartate + glycerol + octanoate (◆), alanine + glycerol + octanoate (□), alanine + glycerol + octanoate + ATP (▩), alanine + glycerol + octanoate + inosine (△), pyruvate (●), and malate (○).

bated in the presence of either cysteine precursors or BSO are not included, as these compounds themselves change the intracellular glutathione levels (Table 4), so the side effects are not excluded. As shown in Fig 2, changes in the rates of glucose formation under various conditions studied differ by approximately 50-fold (range, 2.0 to 106.2 μmol $\times$ g^{-1} dw $\times$ h^{-1}), while GSH/GSSG ratios under nongluconeogenic and gluconeogenic conditions vary approximately 2.5-fold (range,27.9 to 73.6 μmol $\times$ g^{-1} dw). Thus, the manifold changes in the rate of glucose formation correspond to relatively small alterations in the intracellular GSH/GSSG ratio. However, in view of the data presented, the relationship between the rate of glucose synthesis and the intraclellular GSH/GSSG ratio seems to be unquestionable.

Because glutathione reductase, the enzyme responsible for GSSG reduction, is NADPH-dependent[28] and NADPH is generated via enzymes of the oxidative phase of pentose phosphate pathway,[13] intensive gluconeogenesis might be a source of glucose-6-phosphate used subsequently for NADPH production. An increase in both glucose-6-phosphate level and NADPH/$NADP^+$ ratio in renal tubules intensively synthesizing glucose (Table 2) seems to confirm this suggestion. The elevation of both GSH/GSSG and NADPH/$NADP^+$ ratios accompanied by increased glucose-6-phosphate levels in renal tubules incubated under conditions of gluconeogenesis stimulation by extracellular ATP or inosine (Table 3) additionally supports our hypothesis. An increase in intracellular GSH concentration after activation of glucose-6-phosphate dehydrogenase expression[34] is also in agreement with this possibility. Moreover, the stimulatory effect of dehydroascorbate on glutathione levels has also been attributed to an enhancement of the pentose phosphate pathway activity.[35]

Because under conditions of effective gluconeogenesis, the accumulation of intracellular malate, the substrate for NADPH-delivering malic enzyme reaction, is observed (Tables 1 and 2), it is likely that this enzyme may also contribute to the elevation of intracellular NADPH/$NADP^+$ ratio. However, measurements of both glucose-6-phosphate dehydrogenase and malic enzyme activities indicate that, in contrast to other species (including rat, dog, and guinea pig),[36,37] the activity of malic enzyme in rabbit kidney-cortex is almost 30 times lower than that of glucose-6-phosphate dehydrogenase. Thus, it seems likely that in rabbit kidney-cortex tubules participation of malic enzyme in NADPH generation is of minor importance in comparison to that of pentose phosphate pathway.

Surprisingly, exogenous glucose at physiologic concentrations fails to maintain the intracellular GSH/GSSG ratio as high as that under conditions of intensive gluconeogenesis, because it turned out to be a poor source of glucose-6-phosphate for NADPH generation. This phenomenon resembles the so called "glucose paradox", an indirect way of glucose utilization for glycogen synthesis in liver.[38,39]

In reports from various laboratories, both unchanged[40,41] and decreased[42,43] GSH/GSSG ratios have been demonstrated after treatment with BSO, an inhibitor of γ-glutamylcysteine synthetase.[31] BSO administration in vivo differentiates GSH/GSSG ratio in various mouse tissues.[44] Similarly to rat hepatocytes,[45] BSO-induced decline in the intracellular glutathione level in rabbit renal tubules is accompanied by diminished rates of glucose synthesis (Table 4). However, in contrast to rat hepatocytes, BSO markedly decreases the intracellular GSH/GSSG ratio in renal tubules. Our findings do not support the hypothesis by Saez et al[45] that a diminished rate of gluconeogenesis after glutathione depletion is the result of a decline in phosphoenolpyruvate carboxykinase and glycerol-3-phosphate dehydrogenase activities. In view of the changes in intracellular gluconeogenic intermediate contents in rabbit renal tubules (Fig 1), a diminished flux through aldolase seems to be responsible for decreased rates of glucose formation in the presence of BSO. As BSO does not affect aldolase activity by itself, the inhibition of this enzyme may result from a decline in glutathione level and/or redox state. An inactivation of purified mammalian muscle aldolase by GSSG has been reported to be fully reversible by incubation of the enzyme in the presence of dithiotreitol or glutathione reductase.[46] Thus, it is likely that renal aldolase activity may be diminished under conditions of the significantly lowered intracellular GSH/GSSG ratio.

In agreement with the observations that acylase[47] and oxoprolinase[48,49] activities in kidney are very high, cysteine precursors, NAC and OTZ,[32] effectively increased GSH level in rabbit kidney-cortex tubules (Table 4) with no changes in both the GSH/GSSG ratio (Table 4) and the rate of gluconeogenesis, confirming our suggestion about the relationship between the rate of glucose formation and glutathione redox state. No effects of NAC on renal GSH redox state are, however, in contrast to NAC-induced increases in the intracellular glutathione/GSSG ratio in human endothelial cells,[50] human alveolar macrophages,[51] and rabbit carotid body chemoreceptor cells,[52] indicating discrepancies in the action of NAC on glutathione redox state in various cells. Different effects of the extracellular ATP on cyclic adenosine monophosphate (cAMP) levels in hepatocytes[53] and kidney-cortex tubules[30] confirm this suggestion.

In summary, results presented in this investigation indicate that: (1) in the presence of substrates effectively used for glucose formation, the intracellular GSH/GSSG ratio is higher than that determined under conditions of negligible glucose synthesis; (2) changes in the intracellular GSH/GSSG ratio reflect those in NADPH/$NADP^+$; (3) in contrast to exogenous glucose, intensive gluconeogenesis is of importance for maintaining high intracellular GSH/GSSG ratios due to glucose-6-phosphate generation and in a consequence effective NADPH formation for GSSG reduction; (4) BSO-induced decline in the intracellular glutathione content and/or redox state causes a decrease in the rates of glucose formation, probably due a diminished flux through aldolase; and (5) an elevation of the intracellular GSH content on the addition of cysteine precursors (NAC and OTZ) is accompanied by changes in neither glutathione redox state nor the rates of glucose synthesis. These data provide new insights into present knowledge about the mutual regulation of glutathione and glucose metabolism in renal tubules.

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