Succinylacetone Effects on Renal Tubular Phosphate Metabolism: A Model for Experimental Renal Fanconi Syndrome (43211)

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Abstract. **Phosphaturia is a prominent component of the renal Fanconi syndrome associated with the autosomal recessive disease, hereditary tyrosinemia. Succinylacetone (SA), the metabolic by-product of the enzyme deficiency, can be shown to produce multiple adverse effects on rat renal epithelial cell function *in vitro*. With the use of this compound, we have examined its interaction with P_i handling by the renal tubule cell in order to form a basis for understanding the effects of endogenously generated SA in causing phosphaturia in the genetically affected kidney. In this report we have shown complete inhibition of sodium-dependent phosphate uptake by renal brush border membrane vesicles, decreased ATP production by the SA-exposed renal tubule, and reversible inhibition of State 3 oxidation of glutamate by isolated renal mitochondria. We conclude that the phosphaturia observed in hereditary tyrosinemia results from multiple metabolic effects of SA on the renal tubule which are additive and lead to intracellular P_i depletion and diminished ATP production.** [P.S.E.B.M. 1991, Vol 196]

The renal Fanconi syndrome is frequently associated with several different inborn errors of metabolism, among which is hereditary tyrosinemia (1). Affected patients excrete increased amounts of urinary amino acids, protein, glucose, phosphate, and a biochemical marker compound, succinylacetone (SA), which is a metabolic by-product of the defective enzyme step in tyrosine catabolism. Since it is known that the gene defect is expressed in kidney, as well as liver, the persistence of a renal Fanconi syndrome in diminished severity following orthoptic liver transplantation (2) suggests the possibility of renal autotoxicity.

Since no naturally occurring animal model for hereditary tyrosinemia exists, we have used SA *in vitro* to investigate its role in altering renal tubular function and production of membrane transport dysfunction leading to the abnormalities observed in the renal Fanconi syndrome (3–5). Thus, we have demonstrated SA-induced inhibition of D-glucose and amino acid uptake in isolated renal tubules and brush border membrane vesicles which is readily reversible. Oxygen consumption is inhibited in isolated renal tubules and membrane fluidity is enhanced in the vesicle. In addition, sodium-entry is significantly impaired by SA in the brush border membrane vesicle.

Since the uptake of sugar and amino acids by isolated renal tubules is active, and therefore energy dependent, an examination of the effects of SA on P_i disposition within the renal tubular epithelial cell is of great importance to understanding the membrane transport dysfunction induced by SA. Accordingly, we have investigated the action of SA on uptake of P_i by brush border membrane vesicles and the effects on tubule ATP content and substrate oxidation in isolated mitochondria. The results of these studies comprise the basis for this report.

Materials and Methods

Renal brush border and plasma membrane vesicles were made from the kidneys of adult male Sprague-Dawley rats, weighing 150–200 g (Charles River Breeding Laboratories, Wilmington, MA) using methods described previously (6–8). Final preparations were suspended in THM buffer, pH 7.4 (2 m*M* Tris/Hepes + 100 m*M* mannitol), and contained 0.3–0.4 mg of membrane protein/ml as determined by the method of

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Table I. Effect of 4 m*M* Succinylacetone on ATP in Renal Tubules[a]

Incubation time (min)	Control	Experimental
0	2.11 ± 0.09 (84.4)[c]	1.61 ± 0.07[b] (64.4)
30	2.35 ± 0.11 (94.0)	2.07 ± 0.08[b] (82.8)
60	2.04 ± 0.06 (81.6)	1.81 ± 0.07[b] (72.4)

[a] Tubules were incubated at 37°C with and without added 4 m*M* SA (final concentration) and with continuous gassing, as detailed in Materials and Methods. Aliquots were removed at the times indicated and immediately placed into a perchlorate-EDTA solution. Data are expressed as mean values in nmol ATP/mg protein ± SE, $n = 5$.
[b] Experimental values were different from controls at a level of $P < 0.05$ or less as determined by Student's *t* test.
[c] Numbers in parentheses are the calculated mean/mg tubules.

I). In both cases, the tubules maintained consistent ATP levels throughout the period of incubation, although the decrement caused by SA was seen immediately upon contact. Restoration to control levels could be achieved by transfer of SA-exposed tubules to fresh buffer without added SA. The time required for this transfer and subsequent immediate sampling was approximately 5 min.

Mitochondrial oxidation of representative substrates is shown in Table II. Oxidation of glutamate by renal mitochondria in metabolic State 3 (ADP-stimulated) was depressed immediately by 4 m*M* SA. The probability of a similar respiratory depression with pyruvate/malate was less than 5%, however. By contrast, oxidation of both glutamate and pyruvate/malate was decreased significantly ($P < 0.001$ and <0.005, respectively) by 4 m*M* SA in liver mitochondria (data not shown). In no instance was succinate oxidation depressed.

Discussion

Increased urinary phosphate excretion is a prominent component of the renal Fanconi syndrome associated with hereditary tyrosinemia. In the latter genetically transmitted disorder, the kidney is known to express the gene defect and, thus, to produce succinylacetone endogenously. We have used SA to examine its relationship to phosphate metabolism in normal rat kidney, studying its effects on membrane uptake, mitochondrial oxidation, and generation of cellular ATP. Although the concentration of SA used (4 m*M*) appears to be high, generation of SA within the cell may expose the cellular interior to levels this high or higher; intracellular and/or tissue levels in genetically affected humans are not reported. Blood and urine levels of SA reported from affected patients do not reflect intracellular SA concentrations. Moreover, studies in isolated tubules indicate that 4 m*M* is an ideal concentration to use for *in vitro* membrane transport experiments (3).

Our membrane uptake studies indicate that P_i entry is sodium-gradient dependent, consistent with several previous reports of phosphate uptake in renal brush border membranes (17–19). Inhibition of this process by 4 m*M* SA was significant and immediate, as we have previously demonstrated for other physiologic substrates (3–5). Moreover, the decreased rate of sodium entry into the vesicle induced by SA, which we have reported elsewhere (5), would help to explain the diminished uptake of phosphate which we observed in these studies. Although we have reported increased membrane fluidity in these vesicles induced by SA (5), a direct effect on membrane transport proteins cannot be excluded as a basis for our observations. Neither alkaline phosphatase (in the brush border vesicles)- nor sodium-potassium ATPase (in plasma membranes)-specific activities are changed by SA, thus eliminating any postulate that these membrane-bound enzymes play a role in SA-induced phosphate transport inhibition.

An immediate and persistent reduction in cellular ATP content was observed in the presence of SA, suggesting either diminished synthesis or increased catabolism. The demonstration of diminished entry of P_i into the vesicle with SA exposure is consistent with a diminished intracellular P_i pool for ATP synthesis, although the immediacy of the reduction in ATP content would suggest a more proximal mechanism, since a diminished P_i pool would require a finite time over which to develop. This is especially relevant, in that the

Table II. Respiratory Inhibition by SA in Renal Mitochondria[a]

	Glutamate		Pyruvate/malate		Succinate	
	State 3	State 4	State 3	State 4	State 3	State 4
Control	130.8 ± 5.7	23.5 ± 2.2	102.7 ± 5.5	28.8 ± 1.5	222.4 ± 9.8	70.0 ± 3.6
SA (4 m*M*)	108.4 ± 3.6	19.3 ± 1.1	91.5 ± 3.0	24.0 ± 1.4	201.0 ± 6.2	65.0 ± 3.4
P[b]	<0.02	NS	NS	NS	NS	NS

[a] Mitochondria were isolated and assayed as described in Materials and Methods. Substrate concentrations were 1.4 m*M*, except pyruvate which also contained 0.3 m*M* malate. Both State 3 (ADP-stimulated) and State 4 (ADP-exhausted) respiratory velocities were measured, and the results calculated as ng atoms oxygen/min/mg mitochondrial protein. Data are shown as the mean ± SE from determination in four separate animals. Student's *t* test was used to determine significance.
[b] *P* values > 0.05 were considered to be not significant (NS).

preparative buffer (Krebs-Ringer buffer) contains a significant amount of P_i (11), which would prevent depletion of the P_i pool during preparation. Thus, impaired phosphate uptake may contribute to, but cannot likely cause, the reduction in cellular ATP that we observed in the presence of SA.

Our observation that SA depresses pyridine nucleotide-linked mitochondrial respiration provides a possible explanation for immediate and persistently depressed ATP levels in the intact cell. Since there was no concomitant increase in State 4 (ADP-depleted) respiration in the presence of SA, the latter can be said to have no uncoupling effect on oxidative phosphorylation. Thus, the mitochondrion is rendered partially deficient in ATP-generating ability from NAD-linked substrates, but remains normally efficient in producing ATP from other, flavin-linked pathways, such as succinate and fatty acyl CoA. It should also be noted that this deficiency, although significant, is not total; hence, total ATP production in the SA exposed mitochondrion probably results from a net of reduced electron-flow through the pyridine nucleotide-linked steps, plus normal flow through the subsequent steps of the electron transport chain. That there is reduced electron flow overall follows from the lack of increase in State 4 respiration in the presence of SA. The persistent and constant reduction of ATP in the SA-exposed tubule cells suggests that the mitochondrial inhibitory effect is not one which results in increased damage with time of contact. Earlier fine structural examination of the mitochondria of SA-exposed tubule cells (3) support this hypothesis. Additional support is provided by our earlier observations of diminished oxygen consumption by isolated renal tubules in the presence of SA. In this case, inhibition compared with control could not be reversed, but substrate supplementation was shown to increase oxygen consumption in both experimental and control tubules, raising the former to the original control level and the latter above initial levels (4). Thus, while the decrement caused by SA remained, increased oxygen consumption could still be achieved, consistent with mechanistic inhibition, rather than structural damage.

The present findings may have great relevance to an understanding of the renal tubular dysfunction seen in patients with hereditary tyrosinemia. Since the defect in gene expression results in the production of SA within the tubule cell itself, the mitochondria may be exposed to very high concentrations of this compound. Although oxidative phosphorylation continues, it does so at a reduced rate, lowering cellular ATP levels. Although SA has no direct inhibitory effect on renal tubular ATPase, by reducing available ATP it exerts an indirect inhibitory effect on cation transport. As a consequence, cation-dependent entry of compounds such as amino acids and glucose is reduced, as well as the entry of P_i for replenishment of the P_i pool in the cell. Confirmation of this hypothesis will require studies in whole animals, including measurements of phosphate clearance, as well as intracellular P_i and sodium pools, and ATP levels in kidneys from SA-treated animals.

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