Inhibition of Renal Gluconeogenesis by Theophylline[1] (37669)

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(Introduced by A. Sellers)

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It has been shown that both parathyroid hormone (PTH) and β-adrenergic agents enhance renal gluconeogenesis in isolated tubules of the rat renal cortex (1–3). These stimulatory effects are likely to be mediated by the adenyl cyclase–cyclic adenosine 3′,5′-monophosphate (cyclic AMP) systems (1–3). Theophylline, an inhibitor of cyclic AMP phosphodiesterase, augments the effects of PTH and β-adrenergic agents on the concentration of cyclic AMP in isolated cortical tubules (3). Theoretically, theophylline may therefore cause further enhancement of renal gluconeogenesis in response to PTH and β-adrenergic agents. However, we have found that theophylline in a concentration of 10 m*M* suppressed renal gluconeogenesis both in the presence or absence of PTH and β-adrenergic agents (3). Theophylline in this concentration also caused a decrease in the concentration of adenosine triphosphate (ATP) in the cortical tubules (3); this fall in tissue ATP may be responsible for the suppression of renal gluconeogenesis by theophylline since certain gluconeogenic enzyme reactions are coupled with ATP. For example, the gluconeogenic reaction catalyzed by phosphoglycerate kinase is coupled with ATP. Also, the reaction catalyzed by a key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, is coupled with guanosine triphosphate, the level of which would change in parallel with that of ATP. The present investigation was undertaken to evaluate whether theophylline may also directly inhibit renal gluconeogenesis irrespective of its effect on ATP levels.

Methods. Male Wistar rats, weighing 180–200 g, were used. Rats were killed by decapitation and isolated cortical tubules were prepared by the methods described by Nagata and Rasmussen (1, 2, 4). The details of the incubation procedure for the evaluation of renal gluconeogenesis are described in a previous publication (3). In short, renal cortical tubules, containing 5–6 mg protein, were incubated at 37° for 20 min in 1.2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2% bovine serum albumin, 0.5 m*M* palmitate and various substrates (α-ketoglutarate, 5 m*M*; pyruvate, 10 m*M*; malate, 10 m*M*; or glycerol, 10 m*M*) with a mixture of 95% O_2 and 5% CO_2 as a gas phase. The concentration of calcium chloride in the buffer was 1.0 m*M* and that of bicarbonate 25 m*M*. The incubations were carried out in the absence or presence of theophylline (1, 4, or 10 m*M*). After the incubation, perchloric acid extracts were prepared. These extracts were neutralized with 3 *M* K_2CO_3 in 0.5 *M* triethanolamine to pH 5.5–6.5 and then analyzed for glucose and ATP by fluorometry (5).

Results. The results are presented in Tables I and II. Renal cortical tubules produced glucose from a variety of substrates. Theophylline significantly inhibited glucose production by the isolated cortical tubules from all substrates used (Table I); there was an inverse relationship between glucose production and the concentration of theophyl-

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TABLE I. Effects of Theophylline on Glucose Production by Isolated Renal Cortical Tubules from Various Substrates.[a]

	Substrates	Glucose production[a] (nmoles/mg protein/20 min) Theophylline, m*M* 0	1.0	10.0
I	α-Ketoglutarate, 5 m*M*	31.5 ± 0.5	24.9 ± 0.3	9.7 ± 0.5
	Pyruvate, 10 m*M*	86.8 ± 3.3	80.5 ± 1.7	27.3 ± 1.0
	Malate, 10 m*M*	56.8 ± 1.5	39.9 ± 1.2	23.1 ± 1.5
	Glycerol, 10 m*M*	57.5 ± 2.1	46.4 ± 2.0	24.5 ± 1.4
II	α-Ketoglutarate, 5 m*M*	35.0 ± 0.9	26.0 ± 1.4	10.1 ± 0.3
	Pyruvate, 10 m*M*	70.3 ± 2.2	60.5 ± 1.7	22.2 ± 1.0
	Malate, 10 m*M*	70.0 ± 2.3	56.2 ± 2.0	23.2 ± 1.2
	Glycerol, 10 m*M*	63.6 ± 3.9	50.2 ± 1.7	27.2 ± 0.7

[a] Isolated tubules containing 5–6 mg protein were incubated in 1.2 ml Krebs-Ringer-bicarbonate buffer (pH 7.4) at 37° with 95% O_2:5% CO_2 as gas phase. After 20 min incubation, perchloric acid extracts were prepared and analyzed for glucose by fluorometry. Values are the mean ± SD of triplicate incubations. All the values obtained with theophylline at a concentration of 1.0 m*M* and 10.0 m*M* are significantly ($p < 0.01$) lower than control (no theophylline) except for the value in Experiment I with pyruvate as substrate and theophylline in a concentration of 1.0 m*M* where p was < 0.025.

line in the incubation media (Table II). These data confirm our previous observations (3). However, the new information obtained in these studies is related to the effect of theophylline on ATP levels. Theophylline in a concentration of 1.0 m*M* did not decrease ATP levels in the cortical tubules but did inhibit significantly ($p < 0.01$) glucose production (Table II). With higher concentrations of theophylline (4.0 and 10.0 m*M*) both

TABLE II. Effects of Theophylline on Glucose Production and ATP Levels in the Isolated Tubules of Rat Kidney Cortex.

Substrate	Theophylline (m*M*)	Glucose production[a] (nmoles/mg protein/20 min)	ATP[a] (nmoles/mg protein)
α-Ketoglutarate, 5 m*M*	0	31.5 ± 0.5	3.50 ± 0.08
	1.0	24.9 ± 0.3[b]	3.60 ± 0.10
	4.0	16.7 ± 0.6[b]	3.27 ± 0.08[b]
	10.0	9.7 ± 0.5[b]	2.81 ± 0.04[b]
α-Ketoglutarate, 5 m*M*	0	33.5 ± 0.3	3.71 ± 0.05
	1.0	29.7 ± 0.3[b]	3.60 ± 0.06
	4.0	21.2 ± 0.3[b]	3.31 ± 0.12[b]
	10.0	16.5 ± 0.3[b]	2.86 ± 0.06[b]
Glycerol, 10 m*M*	0	60.4 ± 2.8	4.59 ± 0.09
	1.0	46.6 ± 1.5[b]	4.53 ± 0.09
	4.0	41.9 ± 2.4[b]	4.13 ± 0.06[b]
	10.0	34.3 ± 0.5[b]	3.93 ± 0.07[b]

[a] Isolated tubules containing 5–6 mg protein were incubated in 1.2 ml Krebs-Ringer-bicarbonate buffer (pH 7.4) at 37° with 95% O_2:5% CO_2 as gas phase. After 20 min incubation, perchloric acid extracts were prepared and analyzed for glucose and ATP by fluorometry. Values are presented as mean ± SD of triplicate incubations.

[b] Values are significantly different from control (no theophylline), $p < 0.01$.

glucose production and ATP levels were decreased.

Discussion. These data indicate that theophylline can suppress renal gluconeogenesis without any effect on ATP levels. Glucose production from pyruvate and α-ketoglutarate involves both mitrochondrial and cytosolic enzyme reactions while gluconeogenesis from malate and glycerol requires only cytosolic enzyme reactions (6). The finding in the present study that 1.0 m*M* theophylline inhibits gluconeogenesis from malate and glycerol indicates that theophylline exerts the direct suppressive effect on glucose production by inhibiting some cytosolic enzyme reaction(s). Thus, theophylline would suppress renal gluconeogenesis by a direct inhibition of certain cytosolic enzymes and/or by decreasing the level of ATP in the cell. However, the relative role of these two mechanisms in the inhibition of renal gluconeogenesis is at present difficult to assess. It has been reported that in rats fed chronically with theophylline the activities of certain gluconeogenic enzymes decreased in the liver including pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1, 6-diphosphatase (7). It is reasonable to suggest that theophylline may have similar effects on those enzymes in the renal cortex of the rats *in vitro*.

Summary. Theophylline, at a concentration from 1.0–10.0 m*M*, significantly inhibited glucose production from glycerol, malate, pyruvate, and α-ketoglutarate by the isolated tubules of rat renal cortex; the higher the theophylline concentration in the incubation media the smaller the glucose production rates were. Adenosine triphosphate (ATP) levels were decreased in the presence of theophylline at a concentration of 4 m*M* or higher. At a concentration of 1.0 m*M*, theophylline did inhibit glucose production from all these substrates but did not affect ATP levels in the renal tubule cell. These findings suggest that theophylline suppresses renal gluconeogenesis by a direct inhibition of certain cytosolic enzymes and/or by decreasing the levels of ATP in the cell.

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