Stimulation of glutamine metabolism by 3-aminopicolinate in isolated dog kidney-cortex tubules

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(Received 28 June 1982/Accepted 4 November 1982)

1. The effects of 3-aminopicolinate, a known hyperglycaemic agent in the rat, on glutamine metabolism were studied in isolated dog kidney tubules. 2. 3-Aminopicolinate greatly stimulated glutamine (but not glutamate) removal and glutamate accumulation from glutamine as well as formation of ammonia, aspartate, lactate, alanine and glucose. 3. The increased accumulation of aspartate from glutamine and glutamate, and the inhibition of glucose synthesis from various non-nitrogenous gluconeogenic substrates, as well as the increased accumulation of malate from succinate, support the proposal that 3-aminopicolinate is an inhibitor rather than a stimulator of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) in dog kidney tubules. 4. With glutamine as substrate, the increase in flux through glutamate dehydrogenase (EC 1.4.1.3) could not explain the large increase in glutamine removal caused by 3-aminopicolinate. 5. Inhibition by amino-oxyacetate of accumulation of aspartate and alanine from glutamine caused by 3-aminopicolinate did not prevent the acceleration of glutamine utilization. 6. These data are consistent with a direct stimulation of glutaminase (EC 3.5.1.2) by 3-aminopicolinate in dog kidney tubules.

In the rat, the hyperglycaemia induced by the administration of 3-aminopicolinate (Blank *et al.*, 1974; MacDonald *et al.*, 1978) has been attributed to the stimulation by this compound of liver phosphoenolpyruvate carboxykinase (MacDonald & Lardy, 1978*a,b*), a key gluconeogenic enzyme. More recently, Man *et al.* (1981) have shown that the effects of 3-aminopicolinate on the metabolite profile of the rat kidney were not consistent with a stimulation of the renal phosphoenolpyruvate carboxykinase. They also observed that 3-aminopicolinate caused *in vivo* an increase in both glutamine removal and ammonia formation by the kidney of rats that had recovered from metabolic acidosis, but they failed to demonstrate such an effect *in vitro* (Man *et al.*, 1981).

In the present paper, we show that, at least in dog kidney-cortex tubules, 3-aminopicolinate stimulates glutamine utilization and greatly alters the fate of both its carbon skeleton and amino nitrogen.

Methods and materials

Dogs

Kidneys were from mongrel dogs of either sex fed on a standard diet (U.A.R., Villemoisson-sur-Orge, France)

Preparation of kidney-cortex tubules and incubations

Kidney tubules were prepared by collagenase treatment as described previously (Baverel *et al.*, 1978). Incubations were performed at 37°C in a shaking water bath in 25 ml stoppered Erlenmeyer flasks in an atmosphere of O₂/CO₂ (19:1). Tubules were incubated in 4 ml of Krebs–Henseleit (1932) buffer (pH 7.40) with 5 mM-L-glutamine or -L-glutamate as substrate in the absence or presence of 3-aminopicolinate (sodium salt). The flasks were prepared in duplicate for all experimental conditions. Incubations were terminated by adding HClO₄ (final concn. 2%, w/v). In all experiments, zero-time flasks were prepared with and without substrate by adding HClO₄ before the tubules. After removal of the denatured protein by centrifugation (4000g for 10 min), the supernatant was neutralized with 20% (w/v) KOH for metabolite determination.

Analytical methods

Lactate, pyruvate, glucose, alanine, aspartate, citrate, 2-oxoglutarate, fumarate, malate, glutamate, glutamine, ammonia and also the dry weight of the amount of tubules added to the flasks were determined as previously described (Baverel *et al.*, 1978; Baverel & Lund, 1979).

Calculations

Net substrate utilization and product formation were calculated as the difference between the total contents of the flask (tissue + medium) at the start (zero-time flasks) and after 60 min of incubation. The metabolic rates are expressed in μmol of substance removed or produced/h per g dry wt. of tubule fragments. They are reported as means $\pm$ S.E.M. The results were analysed by Student's *t* test for paired data, comparing values obtained in the presence and the absence of 3-aminopicolinate.

The metabolic fate of the glutamine added as substrate was established by calculating carbon and nitrogen balance. Complete oxidation of glutamine was calculated as the difference between the removal of glutamine and the sum of glutamate, glucose (expressed in C_3 units), lactate, alanine and aspartate found. With glutamine as substrate, flux through glutamate dehydrogenase was calculated as the difference between flux through glutaminase (taken as the removal of glutamine) and the accumulation of glutamate, aspartate and alanine; flux through 2-oxoglutarate dehydrogenase was calculated as the removal of glutamine minus the accumulation of glutamate. With glutamate as substrate, flux through glutamate dehydrogenase was taken as the glutamate removed minus the alanine and aspartate found, and flux through 2-oxoglutarate dehydrogenase was taken as the glutamate removed.

Reagents

L-Glutamine, L-glutamate, glutaminase (grade V) and amino-oxyacetate were supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other enzymes and coenzymes were from Boehringer Corp. (Meylan, France). The other chemicals used were of analytical grade; 3-aminopicolinate was synthesized by the method of Sucharda (1925).

Results

In contrast with the finding by Man *et al.* (1981) in rat kidney-cortex slices, 3-aminopicolinate (0.05–5 mM) markedly stimulated glutamine removal by isolated dog kidney-cortex tubules (Table 1); 3-aminopicolinate also caused an increase in the accumulation of glutamate and the formation of ammonia, aspartate, lactate and alanine. This was also true for the formation of glucose, except with the highest concentration of 3-aminopicolinate, which inhibited glucose synthesis from glutamine (Table 1). No significant accumulation of intermediates of the tricarboxylic acid cycle was observed, which indicates that the glutamine removed and not accounted for by the carbon products found was completely oxidized. When tubules were incubated for 30 and 60 min in the presence of

Table 1. Effect of 3-aminopicolinate on the metabolism of 5 mM-L-glutamine in dog kidney tubules. Kidney tubules (14.4 ± 1.8 mg dry wt. per flask) were incubated for 60 min as described in the Methods and materials section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for five experiments performed in duplicate. Statistical difference was measured by the paired Student's *t* test against the control without 3-aminopicolinate: * $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$.

Experimental condition	Metabolite removal or production							Glutamine completely oxidized	Flux through glutamate dehydrogenase	Flux through 2-oxoglutarate dehydrogenase
	Glutamine	Glutamate	Ammonia	Aspartate	Alanine	Glucose	Lactate			
Glutamine (5 mM)	-524.7 ±16.9	+139.7 ±22.8	+915.7 ±34.7	+19.2 ±2.8	+25.2 ±4.9	+64.3 ±2.7	+8.0 ±3.3	204.0 ±15.7	340.6 ±20.1	385.0 ±18.4
Glutamine (5 mM) + 3-amino-picolinate (0.05 mM)	-672.1 ±23.0*	+157.5 ±21.2**	+1110.9 ±32.3*	+67.6 ±5.2*	+90.4 ±10.6**	+84.8 ±2.8*	+38.4 ±11.1***	148.6 ±9.6***	356.6 ±19.2	514.6 ±27.4*
Glutamine (5 mM) + 3-amino-picolinate (0.1 mM)	-757.1 ±22.3*	+162.1 ±23.0*	+1157.3 ±35.4*	+96.0 ±7.7*	+113.7 ±8.3*	+92.3 ±4.5*	+57.6 ±11.5**	143.1 ±16.4***	385.3 ±27.3	595.0 ±32.9*
Glutamine (5 mM) + 3-amino-picolinate (0.5 mM)	-817.9 ±25.7*	+174.4 ±22.7*	+1231.8 ±47.1*	+153.3 ±5.4*	+126.1 ±8.5*	+89.2 ±6.2**	+58.8 ±7.3*	126.9 ±11.2*	364.1 ±19.0	643.5 ±28.2*
Glutamine (5 mM) + 3-amino-picolinate (1 mM)	-856.7 ±25.1*	+176.9 ±21.5*	+1247.0 ±45.5*	+180.8 ±6.1*	+125.1 ±8.2*	+82.7 ±4.1*	+56.2 ±8.7**	152.1 ±7.9*	373.7 ±16.2***	679.6 ±28.7*
Glutamine (5 mM) + 3-amino-picolinate (5 mM)	-877.0 ±29.5*	+178.9 ±21.1**	+1185.2 ±40.0*	+333.3 ±25.3*	+74.6 ±11.3**	+47.7 ±6.9***	+45.2 ±2.5*	149.6 ±24.7	290.2 ±19.3***	698.1 ±37.0*
No added substrate	—	-14.4 ±1.4	+158.5 ±4.9	-17.4 ±1.6	-8.3 ±2.9	+10.2 ±0.9	+2.7 ±4.3	—	—	—

5 mM-glutamine plus 0.5 mM-3-aminopicolinate, the effects of 3-aminopicolinate on glutamine metabolism were roughly linear with time ($n = 4$, results not shown).

Carbon-balance calculations demonstrate that the carbon fragment of the glutamine used is accounted for mainly by complete oxidation (38.9%) and the formation of glutamate (26.6%) and glucose (24.5%) in the absence of 3-aminopicolinate; with increasing concentrations of 3-aminopicolinate, the proportion of the glutamine carbon removed that is accounted for by aspartate progressively increases. Similarly, nitrogen-balance calculations show that the proportion of the amino nitrogen of the glutamine removed found as aspartate progressively increases, whereas that found as ammonia progressively decreases, in the presence of increasing concentrations of 3-aminopicolinate.

A slight but significant increase in glutamate removal was also caused by the addition of 3-aminopicolinate (Table 2), but this increase was one order of magnitude less than that observed with glutamine as substrate (Table 1). With glutamate as substrate (Table 2), flux through glutamate dehydrogenase was not increased by 3-aminopicolinate, which indicates that the small increment in glutamate removal was due to increased transamination of glutamate with oxaloacetate and pyruvate. Formation of neither glucose nor ammonia from glutamate was stimulated by 3-aminopicolinate, and no significant accumulation of pyruvate, lactate or intermediates of the tricarboxylic acid cycle occurred.

When tubules were incubated with 5 mM-glutamine in the presence of 3-aminopicolinate plus amino-oxyacetate, an inhibitor of transaminases (Braunstein, 1964), the stimulation of flux through glutaminase was still observed despite a further increase in glutamate accumulation and a virtually complete inhibition of aspartate and alanine synthesis (Table 3); under this condition, the increase in

flux through glutaminase was also accompanied by an increase in flux through glutamate dehydrogenase, but 3-aminopicolinate did not reverse the inhibition of glucose synthesis from glutamine caused by the addition of amino-oxyacetate. Presumably owing to an inhibition of alanine synthesis by amino-oxyacetate, the 3-aminopicolinate-induced accumulation of lactate plus pyruvate further increased in the presence of 3-aminopicolinate plus amino-oxyacetate (see Table 3).

Discussion

In the present experiments, increased glutamine removal may have resulted from a direct stimulation of glutaminase by 3-aminopicolinate or/and from an increased removal of the glutamate (formed from glutamine), a well-known end-product inhibitor of renal glutaminase (EC 3.5.1.2) (Goldstein, 1966). In the presence of 3-aminopicolinate, increased accumulation of glutamate from glutamine, despite a very large increase in glutamate metabolism mainly by the reactions catalysed by aspartate aminotransferase and alanine aminotransferase and to a lesser extent by glutamate dehydrogenase (see Table 1), strongly suggests that this compound stimulates glutamine removal, not only by increasing glutamate metabolism, but also by exerting a direct stimulation of glutaminase. Additional evidence of the direct stimulation of glutaminase was obtained by the addition of amino-oxyacetate (1.0 mM), an inhibitor of transaminases (Braunstein, 1964), which completely suppressed the accumulation of alanine and aspartate induced by 3-aminopicolinate (0.5 mM) without suppressing the large stimulation of glutamine removal (Table 3). This observation also rules out the possible role of glutamine-pyruvate transaminase and glutamine-oxaloacetate transaminase in the stimulation of glutamine utilization caused by 3-aminopicolinate.

Flux through glutamate dehydrogenase was

Table 2. *Effect of 3-aminopicolinate on the metabolism of 5 mM-L-glutamate in dog kidney tubules*

Kidney tubules (19.8 ± 1.8 mg dry wt. per flask) were incubated for 60 min as described in the Methods and materials section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. Statistical difference was measured by the paired Student's t test against the control without 3-aminopicolinate: * $P < 0.002$; ** $P < 0.01$.

Experimental condition	Metabolite removal or production					Flux through glutamate dehydrogenase	Flux through 2-oxoglutarate dehydrogenase
	Glutamate	Ammonia	Aspartate	Alanine	Glucose		
Glutamate (5 mM)	–343.6 ± 42.0	+392.0 ± 25.3	+22.4 ± 7.7	+11.9 ± 2.4	+75.0 ± 6.3	309.3 ± 38.6	343.6 ± 42.0
Glutamate (5 mM) + 3-aminopicolinate (0.5 mM)	–367.0 $\pm 42.5^*$	+362.5 ± 21.5	+51.8 $\pm 6.2^{**}$	+31.7 $\pm 1.8^{**}$	+68.7 ± 4.3	283.5 $\pm 36.9^{**}$	367.0 $\pm 42.5^{**}$
No added substrate	–3.7 ± 5.9	+165.4 ± 4.8	–10.1 ± 5.2	–5.4 ± 2.2	+12.2 ± 1.2	—	—

Table 3. *Effect of amino-oxyacetate on the stimulation of the metabolism of 5 mM-L-glutamine by 3-aminopicolinate in dog kidney tubules*

Kidney tubules (16.4 ± 1.3 mg dry wt. per flask) were incubated for 60 min as described in the Methods and materials section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for six experiments performed in duplicate. Statistical difference was measured by the paired Students *t* test against the control without 3-aminopicolinate and/or amino-oxyacetate: **P* < 0.001; ***P* < 0.01; ****P* < 0.05.

Experimental condition	Metabolite removal or production							
	Glutamine	Glutamate	Ammonia	Aspartate	Alanine	Glucose	Lactate	Pyruvate
Glutamine (5 mM)	–472.8 ± 43.4	+119.5 ± 10.2	+843.0 ± 56.2	+20.3 ± 4.7	+33.9 ± 10.1	+65.0 ± 7.4	+10.7 ± 2.6	+10.2 ± 1.7
Glutamine (5 mM) + 3-aminopicolinate (0.5 mM)	–853.6 $\pm 44.6^*$	+152.5 $\pm 11.0^*$	+1287.4 $\pm 51.8^*$	+149.2 $\pm 22.1^*$	+145.4 $\pm 15.5^*$	+110.2 $\pm 20.8^*$	+42.6 $\pm 5.1^{**}$	+23.7 $\pm 3.5^{**}$
Glutamine (5 mM) + 3-aminopicolinate (0.5 mM) + amino-oxyacetate (0.5 mM)	–700.3 $\pm 31.2^*$	+180.1 $\pm 8.4^*$	+1214.8 $\pm 44.3^*$	+32.2 $\pm 4.5^{***}$	+10.9 ± 3.5	+51.5 $\pm 4.6^{***}$	+90.3 $\pm 5.5^*$	+56.4 $\pm 3.3^*$
Glutamine (5 mM) + 3-aminopicolinate (0.5 mM) + amino-oxyacetate (1 mM)	–693.7 $\pm 32.3^*$	+178.8 $\pm 5.9^*$	+1206.9 $\pm 51.8^*$	+22.9 ± 4.0	+10.6 ± 3.5	+50.0 $\pm 4.4^{***}$	+88.5 $\pm 6.1^*$	+25.0 $\pm 4.3^{***}$
Glutamine (5 mM) + amino-oxyacetate (0.5 mM)	–478.7 ± 33.7	+121.0 ± 9.6	+856.4 ± 47.7	+8.6 $\pm 2.9^{***}$	+8.8 $\pm 3.2^{***}$	+46.6 $\pm 5.1^*$	+36.2 $\pm 6.6^{**}$	+15.2 ± 2.3
Glutamine (5 mM) + amino-oxyacetate (1 mM)	–488.5 ± 40.1	+118.4 ± 8.5	+862.1 ± 49.5	+8.1 $\pm 2.6^{***}$	+8.8 $\pm 3.2^{***}$	+45.9 $\pm 5.1^*$	+38.4 $\pm 6.5^{**}$	+6.1 ± 0.5
No added substrate	—	–17.9 ± 1.0	+163.3 ± 12.2	–7.3 ± 3.9	–7.8 ± 2.7	+11.3 ± 1.2	–1.5 ± 0.8	+1.6 ± 0.6
No added substrate + amino-oxyacetate (1 mM)	—	–30.2 $\pm 1.1^*$	+131.2 $\pm 7.5^{***}$	–2.4 ± 2.3	+10.7 $\pm 3.0^*$	+7.5 $\pm 1.0^{**}$	+1.8 $\pm 1.0^{**}$	+0.6 ± 0.3
No added substrate + 3-aminopicolinate (0.5 mM)	—	–21.8 ± 1.3	+152.6 ± 8.7	–6.2 ± 2.7	–3.6 $\pm 3.0^{**}$	+5.3 $\pm 1.1^*$	–2.0 ± 0.8	+1.0 ± 0.3

increased by 3-aminopicolinate when glutamine, but not glutamate, was the substrate. This provides further evidence that, with glutamine as substrate, the increased flux through glutamate dehydrogenase (see Table 1) was secondary to the stimulation of glutaminase which caused an increased synthesis and accumulation of glutamate. Since the pathway for the conversion of glutamine into glucose necessarily involves glutamate dehydrogenase, the increase in glucose synthesis which paralleled the increase in flux through glutamate dehydrogenase (Table 1) was therefore also due to the stimulation of glutaminase by 3-aminopicolinate.

In the presence of 3-aminopicolinate, the stimulation of ammonia formation from glutamine was due in great part to the increased flux through glutaminase, because flux through glutamate dehydrogenase increased moderately or even decreased with the highest concentration of 3-aminopicolinate used (Table 1).

The fact that 3-aminopicolinate stimulated the formation of aspartate and alanine from both glutamine and glutamate (Tables 1 and 2) clearly indicates that this compound increased the availability of oxaloacetate and pyruvate respectively. Increased availability of oxaloacetate probably resulted, at least in part, from inhibition by 3-

aminopicolinate of the utilization by phosphoenolpyruvate carboxykinase of the oxaloacetate formed from glutamine or glutamate; consistent with such an inhibition is the close structural similarity of 3-aminopicolinate to quinolinate and 3-mercaptopicolinate, two well-known inhibitors of phosphoenolpyruvate carboxykinase (Veneziale *et al.*, 1967; DiTullio *et al.*, 1974), which cause accumulation of aspartate from glutamine in renal cortex (Watford *et al.*, 1980; Vinay *et al.*, 1980), as well as the demonstration that 3-aminopicolinate affects the activity of phosphoenolpyruvate carboxykinase isolated from rat liver (MacDonald & Lardy, 1978a,b) and renal cortex (Man *et al.*, 1981). The fact that aspartate and malate accumulate in rat kidney cortex *in vivo* after administration of 3-aminopicolinate has been observed (Man *et al.*, 1981) also supports the conclusion that this compound inhibits the renal phosphoenolpyruvate carboxykinase. Evidence that 3-aminopicolinate inhibits gluconeogenesis was obtained in experiments where tubules were incubated for 60 min with various non-nitrogenous glucose precursors used at a concentration of 5 mM in the presence of 0.5 mM-3-aminopicolinate. In these experiments, 3-aminopicolinate inhibited glucose synthesis by 44.3% (*P* < 0.01), 50.5% (*P* < 0.01), 36.3% (*P* < 0.001) and 34.1%

($P < 0.001$) from lactate, pyruvate, succinate and malate respectively; by contrast, glucose formation from glycerol was not decreased ($n = 4$; results not shown). 3-Aminopicolinate also stimulated the accumulation of malate from succinate ($P < 0.05$); all these results are consistent with an inhibition of phosphoenolpyruvate carboxykinase by 3-aminopicolinate.

It should be noted that, at a given concentration of 3-aminopicolinate (0.5 mM), aspartate synthesis from glutamine (Table 1) is much larger than that from glutamate (Table 2); and, since the degree of inhibition of phosphoenolpyruvate carboxykinase is the same with both substrates, this means that in the presence of 3-aminopicolinate the synthesis of oxaloacetate is much larger from glutamine than from glutamate. Calculations of Tables 1 and 2 indicate that flux through 2-oxoglutarate dehydrogenase (an estimate of the maximal capacity for synthesizing oxaloacetate from glutamine or glutamate) is of the same order with either glutamine or glutamate as substrate in the absence of 3-aminopicolinate, but this flux is doubled in the presence of 3-aminopicolinate (0.5 mM) only when glutamine is the substrate. Observation of this provides further evidence that 3-aminopicolinate, in addition to its inhibition of renal phosphoenolpyruvate carboxykinase, has a primary effect at an enzymic step specific to glutamine metabolism, namely glutaminase.

Inhibition of glucose synthesis from glutamine by 5 mM-3-aminopicolinate (Table 1) probably resulted from a potent inhibition of phosphoenolpyruvate carboxykinase by a high concentration of this compound, as suggested by the very large formation of aspartate observed under this condition. Increased availability of pyruvate, as revealed by the increased formation of pyruvate, alanine and lactate, especially with glutamine as substrate (Tables 1 and 3), is probably due to an increased synthesis of pyruvate (calculated as the sum of the glutamine completely oxidized and the pyruvate, lactate and alanine found), secondary to the stimulation of glutaminase.

Finally, inhibition of complete oxidation and therefore of flux of glutamine carbon through pyruvate dehydrogenase was probably due to diversion of the pyruvate formed from complete oxidation to synthesis of alanine and lactate (see Tables 1 and 3).

In summary, our results are in agreement with an inhibition of phosphoenolpyruvate carboxykinase and a stimulation of glutaminase by 3-aminopicolinate in dog renal tubules. To our knowledge, 3-aminopicolinate is the first synthetic compound capable of stimulating the renal glutaminase. The mechanism by which such a stimulation occurs is unknown; however, the known metal-chelating activity of 3-aminopicolinate (MacDonald & Lardy, 1978a) suggests that some metal (Fe^{2+} , Mn^{2+} , Co^{2+}) might have important regulatory influences on the activity of glutaminase, but this remains to be demonstrated.

This work was supported by grants from the INSERM (ATP 62.78.94, grant no. 002), the Université Lyon I (tranche C) and the U.E.R. de Biologie Humaine. We are grateful to Professor M. Pellet for his interest in the work, and to Dr. Ch. Michoudet, who performed some experiments for the revised version of this paper.

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