Biochemical Pharmacology, Vol. 30, No. 14, pp. 1895–1900, 1981.
Printed in Great Britain.

0006-2952/81/141895-06 $02.00/0

EFFECT OF 3-AMINOPICOLINIC ACID ON RENAL AMMONIAGENESIS IN THE RAT

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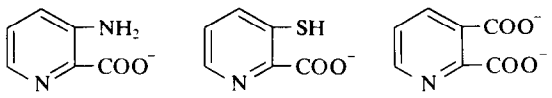
(*Received* 9 *July* 1980; *accepted* 21 *November* 1980)

Abstract—The effects of 3-aminopicolinate, a known activator of phosphoenolpyruvate carboxykinase (PEPCK), on renal ammoniagenesis were studied in normal rats and in rats allowed to recover for 2 days from ammonium chloride induced metabolic acidosis. The administration of 3-aminopicolinate (5 mg/100 g body wt) did not affect glomerular filtration rate, renal blood flow, arterial blood pH, pO_2 or pCO_2 in either normal or "recovered" rats. A significant increase in renal glutamine extraction and total ammonia production was observed in recovered rats, but not in normal rats, after 3-aminopicolinate treatment. Although this compound activated partially purified renal PEPCK, the profile of metabolites from freeze-clamped kidneys was not consistent with an activation of the enzyme. Thus, the enhancement of renal ammoniagenesis *in vivo* by 3-aminopicolinate was probably due to an effect other than activation of PEPCK.

It is well established that during metabolic acidosis the mammalian kidney excretes strong acids largely as the ammonium salts [1]. Stone and Pitts [2] have demonstrated that glutamine is the major precursor of urinary ammonia. Despite intensive studies in recent years, the mechanism underlying the adaptive increase in ammonia production during metabolic acidosis is still a matter of debate. Nevertheless, several effects of acidosis have been considered to be of regulatory significance. They include (i) enhanced glutamine transport into mitochondria [3]; (ii) increased activity of phosphate-dependent glutaminase (EC 3.5.1.2) [4]; (iii) increased activity of phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) [5], which facilitates the removal of glutamate, an inhibitor of phosphate-dependent glutaminase [6]; (iv) activation of glutamate dehydrogenase (EC 1.4.1.3) [7]; and (v) activation of 2-oxoglutarate dehydrogenase (EC 1.2.4.2) [8].

Some doubt was cast on the phosphate-dependent glutaminase hypothesis when Rector and Orloff [9] showed that the activity of the enzyme does not increase in the acidotic dog. In addition, Goldstein [10] and Bignal *et al.* [11] found that the production of urinary ammonia is enhanced in acidotic rats although enzyme induction is inhibited by administration of actinomycin D. Furthermore, a recent report from this laboratory [12] has shown that, during recovery from metabolic acidosis in the rat, a rapid fall in the activity of PEPCK is associated with a decrease in ammonia excretion, whereas the activity of phosphate-dependent glutaminase and mitochondrial glutamine metabolism *in vitro* remain elevated. Parry and Brosnan [12] attributed the regulation of renal metabolism of glutamine during metabolic acidosis to extra-mitochondrial factors. Among suggested regulatory factors is the localization of PEPCK in the cytosol. It is known that the conversion of oxaloacetate to phosphoenolpyruvate (catalyzed by PEPCK) is a rate-limiting step in the gluconeogenic pathway. Therefore, an increase in the activity of PEPCK could facilitate the disposal of the carbon skeleton of glutamine and, thereby, the metabolism of ammonia.

3-Aminopicolinate is a metal-chelating agent that has a pronounced hyperglycemic effect in the rat [13, 14]. MacDonald and Lardy [14] have shown that the compound causes hyperglycemia by activating PEPCK. Structurally, 3-aminopicolinate is similar to 3-mercaptopicolinate and quinolinate, which are known inhibitors of the enzyme [15–18].



3-Aminopicolinate 3-Mercaptopicolinate Quinolinate

The purpose of the present work was to employ 3-aminopicolinate *in vivo* so as to activate PEPCK and thus to study the effects of PEPCK activity on renal ammonia production.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats weighing 350–480 g were used in all experiments. Induction of, and recovery from, metabolic acidosis in these animals were carried out as described previously [12].

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In vivo *clearance studies*. The effect of 3-aminopicolinate on glutamine extraction and total ammonia production by the right kidney was studied in normal and 2-day "recovered" rats. Following pentobarbital anesthesia (60 mg/kg body wt, i.p.), the abdominal cavity was opened and the right ureter was catheterized. The left jugular vein was also catheterized and, after injecting a priming dose of [^{14}C]inulin (2 μCi), a solution containing 5% mannitol with a maintenance dose of [^{14}C]inulin was infused at 0.03 ml/min (0.02 μCi/min). The rat was then transferred to a temperature-controlled cabinet (38°). After 40 min of inulin infusion, 3-aminopicolinate (5 mg/100 g body wt, pH 7.4) was injected into the left sephanous vein and the infusion was continued for an additional 20 min. Control rats received the same volume of saline. At the end of the experiment, a renal venous sample was drawn slowly into a heparinized syringe and then an arterial sample was taken. One part of each blood sample was immediately deproteinized with an equal volume of ice-cold 6% perchloric acid. The perchloric acid extract was centrifuged at 600 *g* for 20 min at 4°; the supernatant fraction was removed and neutralized with 20% K_2CO_3. The precipitated $KClO_4$ was removed by centrifugation and the neutralized extract was used for assay of metabolites. Blood pH, pO_2 and pCO_2 were measured in an IL-213 blood-pH gas analyzer (Instrumentation Laboratories, Lexington, MA, U.S.A.) using a small (0.2 ml) arterial sample. The remaining arterial and venous blood was centrifuged and 0.1 ml of plasma was digested overnight with NCS tissue solubilizer (Amersham/Searle, Oakville, Ontario). Plasma [^{14}C]inulin was subsequently determined using a Beckman model LS-330 liquid scintillation counter.

Freeze-clamp experiments. In these studies, normal rats and rats allowed to recover from metabolic acidosis for 2 days were anethetized with pentobarbital, and then 3-aminopicolinate (5 mg/100 g body wt) was injected i.v. The abdominal cavity was opened 20 min later and the right kidney was quickly freeze-clamped [19]. After grinding the frozen tissue in a cooled mortar under liquid nitrogen, the frozen powder was deproteinized with 6% perchloric acid and prepared for metabolite assays as described above.

Gel-filtration of crude phosphoenolpyruvate carboxykinase. A cytosolic fraction was prepared from rat kidney cortex by homogenizing 1 g of cortex in 3 ml of an ice-cold medium consisting of 0.25 M sucrose, 3 mM Tris (pH 7.4) and 1 mM EGTA* and centrifuging the mixture at 100,000 *g* for 90 min. The resulting cytosol was dialyzed in the cold for 24 hr against 10 mM Tris–HCl, 2 mM dithiothreitol (pH 7.5) and a 2-ml fraction applied to a Sephadex G-100 column (1.5 × 60 cm) equilibrated with 50 mM Tris–HCl (pH 7.0). Fractions were collected (6 ml), made 2 mM in dithiothreitol, and assayed for PEPCK activity in the presence and absence of 1 mM 3-aminopicolinate. The fractions were preincubated for 10 min at 0° with either 0.2 mM $FeCl_2$, or 0.2 mM $FeCl_2$ plus 1 mM 3-aminopicolinate, and were then assayed for PEPCK according to the method of Seubert and Huth [20].

Metabolite measurements. Enzymic assays were performed for glucose [21], pyruvate [22], lactate [23], phosphoenolpyruvate, 2-phosphoglycerate, and 3-phosphoglycerate [24], citrate [25], 2-oxoglutarate [26], malate [27], L-glutamate [28], L-aspartate [29], glucose-6-phosphate and ATP [30], ADP and AMP [31], and ammonia [32]. L-Glutamine was measured on a Beckman amino acid analyzer.

Materials. Enzymes, coenzymes, and substrates were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. [^{14}C]Inulin was obtained from the New England Nuclear Corp., Lachine, Quebec, and NCS tissue solubilizer was supplied by Amersham/Searle, Oakville, Ontario. 3-Aminopicolinic acid was a gift from Mr. N. diTullio, Smith Kline & French, Philadelphia, PA, U.S.A.

Statistical analysis. Results are presented as means ± S.E.M.; significant differences were determined using Student's unpaired *t*-test. $P < 0.05$ was taken to indicate a statistically significant difference.

RESULTS

Effect of 3-aminopicolinate on renal PEPCK. It was first necessary to establish whether, or not, the activity of renal PEPCK in the presence of Fe^{2+} was augmented by 3-aminopicolinate. Demonstration of this effect in liver requires removal of the ferroactivator protein. This was accomplished by gel-filtration as described by Bentle *et al.* [33]. Figure 1 shows a pronounced activation of PEPCK when assayed in the presence of 3-aminopicolinate.

In vivo *clearance studies*. As shown in Table 1, the administration of 3-aminopicolinate to normal and recovered rats did not affect renal function. The glomerular filtration rate and renal blood flow were similar in the control and 3-aminopicolinate-treated animals. There were no significant differences in the arterial blood pH, pO_2 and pCO_2 between control and 3-aminopicolinate-treated rats.

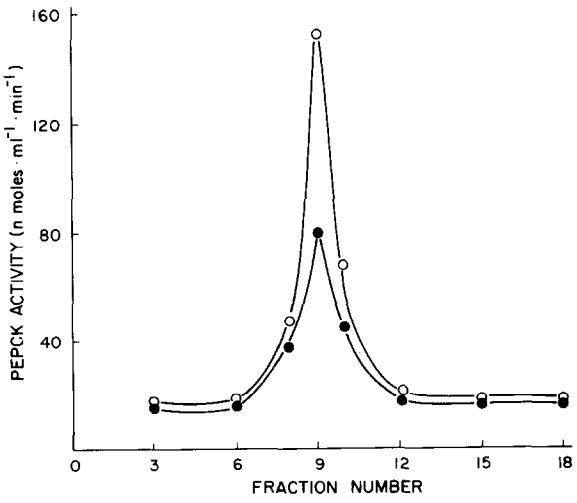

Fig. 1. Effect of 3-aminopicolinate on the activity of renal phosphoenolpyruvate carboxykinase (PEPCK) from normal rats. For details see text. Key: (● — ●) 0.1 mM $FeCl_2$; and (○ — ○) 0.1 mM $FeCl_2$ + 1 mM 3-aminopicolinate.

* EGTA, ethyleneglycolbis(amino-ethylether)tetraacetate.

Table 1. Effect of 3-aminopicolinate on renal function and arterial blood acid–base parameters in normal rats and in rats allowed to recover from ammonium chloride induced acidosis for 2 days*

	Glomerular filtration rate [ml·min^{-1}·(100 g body wt)$^{-1}$]	Renal blood flow [ml·min^{-1}·(100 g body wt)$^{-1}$]	pH	Arterial pCO_2	pO_2
Normal					
(A) Control (5)	0.46 ± 0.02	3.40 ± 0.45	7.48 ± 0.01	31.60 ± 2.03	111.94 ± 8.00
(B) 3-Aminopicolinate (4)	0.47 ± 0.03	3.60 ± 0.45	7.49 ± 0.02	27.23 ± 1.19	105.45 ± 6.64
2-Day recovered					
(C) Control (4)	0.45 ± 0.04	2.77 ± 0.12	7.51 ± 0.01	32.30 ± 0.90	103.10 ± 6.90
(D) 3-Aminopicolinate (4)	0.54 ± 0.05	3.20 ± 0.14	7.52 ± 0.02	31.3 ± 1.00	107.67 ± 4.57

* Results are means ± S.E.M.; numbers of experiments are given in parentheses. For other experimental details see the text.

Table 2. Effect of 3-aminopicolinate on renal arteriovenous differences for blood metabolites in the kidneys of normal and recovered rats*

	Renal arteriovenous difference (mmoles/liter)		
	Lactate	Ammonia	Glutamine
Normal rats			
(A) Control (5)	−0.25 ± 0.03	+0.04 ± 0.01	−0.07 ± 0.01
(B) 3-Aminopicolinate (4)	−0.25 ± 0.12	+0.07 ± 0.01	−0.09 ± 0.02
Recovered rats			
(C) Control (4)	−0.30 ± 0.05	+0.04 ± 0.01	−0.07 ± 0.01
(D) 3-Aminopicolinate (4)	−0.26 ± 0.06	+0.11 ± 0.02†	−0.14 ± 0.01†

* Results are means ± S.E.M.; the numbers of experiments are given in parentheses. A minus sign indicates an uptake and a plus sign indicates a release of a metabolite. For other experimental details see text.

† Significantly different from own control ($P < 0.05$).

Table 3. Effect of 3-aminopicolinate on rates of glutamine extraction and ammonia production by kidneys of normal and recovered rats*

	Glutamine extraction [μmoles · min^{-1}. (100 g body wt)$^{-1}$]	Renal venous ammonia production [μmoles · min^{-1}. (100 g body wt)$^{-1}$]	Urinary ammonia production [μmoles · min^{-1}. (100 g body wt)$^{-1}$]	Total renal ammonia production [μmoles · min^{-1}. (100 g body wt)$^{-1}$]
Normal				
(A) Control (5)	0.24 ± 0.05	0.13 ± 0.03	0.21 ± 0.03	0.34 ± 0.04
(B) 3-Aminopicolinate (4)	0.35 ± 0.04	0.28 ± 0.05†	0.19 ± 0.02	0.47 ± 0.04
2-Day recovered				
(C) Control (4)	0.23 ± 0.05	0.09 ± 0.02	0.20 ± 0.03	0.30 ± 0.04
(D) 3-Aminopicolinate (4)	0.44 ± 0.05†	0.36 ± 0.07†	0.24 ± 0.04	0.59 ± 0.10†

* Results are means ± S.E.M.; numbers of experiments are given in parentheses. For other experimental details see text.
† Significantly different from own control ($P < 0.05$).

Effect of 3-aminopicolinate on renal arteriovenous concentrations of metabolites in normal and recovered rats. No significant changes in arteriovenous differences for blood lactate, ammonia, and glutamine were evident in normal rats after 3-aminopicolinate treatment (Table 2). Marked increases in the output of ammonia and in the uptake of glutamine were observed in recovered rats treated with 3-aminopicolinate.

Effect of 3-aminopicolinate on glutamine extraction and ammonia production by kidneys of normal and recovered rats. The administration of 3-aminopicolinate did not significantly influence either the rate of glutamine extraction or the rate of total ammonia production by kidneys of normal, fed rats (Table 3). In contrast, the compound caused a 2-fold increase in the rates of glutamine extraction and total ammonia production by the kidneys of recovered rats.

Effect of 3-aminopicolinate on the renal content of intermediary metabolites. The concentrations of the measured renal metabolites were similar in normal and recovered rats except that in the recovered group pyruvate, lactate, 3-phosphoglycerate; 2-oxoglutarate and glutamate were increased significantly and citrate and glutamine were decreased significantly (Table 4). Administration of 3-aminopicolinate changed the concentrations of metabolites in the normal and recovered rats in the same direction: lactate, malate and aspartate concentrations rose while glutamine and 2-oxoglutarate fell. An increased level of glutamate was evident on administration of 3-aminopicolinate to the recovered animals only. No significant differences in the renal contents of the adenine nucleotides were evident between the two experimental groups.

DISCUSSION

Phosphoenolpyruvate carboxykinase is affected by transition metal ions in a complex fashion. The enzyme from rat cytosol is stimulated by Fe^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} [34], although preincubation of the purified enzyme with Fe^{2+} causes irreversible loss of activity [35]. The enzyme may also be regulated by a specific protein, named ferroactivator, which has been purified from rat liver [33]. The mechanism by which ferroactivator activates PEPCK is uncertain. It has been suggested that ferroactivator permits Fe^{2+} to activate PEPCK [36]. Reynolds [35] has shown, however, that ferroactivator protects purified PEPCK from inhibition when preincubated with Fe^{2+}. 3-Aminopicolinic acid appears to mimic ferroactivator, and Reynolds [37] has shown that it, too, protects purified PEPCK against inhibition by Fe^{2+}. There are, thus, two proposals as to how 3-aminopicolinic acid activates PEPCK *in vivo*. According to MacDonald *et al.* [38], 3-aminopicolinate binds Fe^{2+} and the resulting complex activates PEPCK. A more recent proposal by Reynolds [35] suggests that PEPCK may be in a partially inactivated state *in vivo* due to a balance between inactivation by O_2 and Fe^{2+} and reactivation by thiols. The effect of 3-aminopicolinate, then, would be to lower the concentration of Fe^{2+} by chelation and to permit reactivation to predominate.

Table 4. Effect of 3-aminopicolinate on metabolite concentrations in the kidneys of normal and recovered rats*

Metabolite	Metabolite concentrations (nmoles/g wet kidney)			
	Normal		2-Day recovered	
	A (4) Control	B (4) 3-Aminopicolinate	C (4) Control	D (4) 3-Aminopicolinate
Glucose	4282.0 ± 73.2	3996.3 ± 76.8	5603.0 ± 269.2	5556.8 ± 304.1
Glucose-6-phosphate	84.5 ± 2.6	60.7 ± 2.1†	91.5 ± 5.7	90.8 ± 5.2
Fructose-6-phosphate	20.0 ± 2.8	19.3 ± 2.5	19.3 ± 0.6	22.3 ± 4.8
Fructose 1,6-diphosphate	16.0 ± 2.4	19.0 ± 2.5	20.0 ± 1.9	22.0 ± 0.7
Triose phosphate	20.0 ± 2.8	15.0 ± 3.1	21.5 ± 2.7	26.8 ± 3.0
3-Phosphoglycerate	156.0 ± 12.2	168.5 ± 23.5	241.5 ± 6.9‡	212.3 ± 19.8
2-Phosphoglycerate	23.0 ± 1.6	17.8 ± 3.4	19.5 ± 1.3	17.5 ± 2.5
Phosphoenolpyruvate	88.3 ± 3.5	88.0 ± 9.6	67.0 ± 11.2	52.5 ± 5.3
Pyruvate	103.5 ± 5.5	109.0 ± 15.3	218.5 ± 16.8‡	219.3 ± 28.7
Lactate	271.5 ± 17.2	422.3 ± 27.8†	765.0 ± 46.8‡	977.3 ± 53.7†
Citrate	206.5 ± 21.2	252.0 ± 15.7	116.0 ± 5.1‡	120.7 ± 8.1
2-Oxoglutarate	148.0 ± 12.5	95.0 ± 16.3	445.0 ± 31.2‡	229.7 ± 53.4†
Malate	322.2 ± 46.3	610.5 ± 47.8†	246.0 ± 110.9	447.8 ± 30.1†
Glutamine	943.8 ± 50.8	742.8 ± 45.6†	547.0 ± 28.7‡	394.0 ± 52.7
Glutamate	1847.0 ± 73.9	2019.8 ± 93.0	2318.8 ± 64.9‡	2686.5 ± 111.9†
Aspartate	753.0 ± 51.6	1124.8 ± 56.2†	908.3 ± 92.6	2949 ± 443.4†
ATP	1593.5 ± 78.6	1660.0 ± 91.1	1630.0 ± 70.0	1780 ± 70.0
ADP	482.5 ± 29.0	414.8 ± 9.0	409.5 ± 39.8	364.3 ± 29.3
AMP	221.3 ± 2.9	229.0 ± 14.5	232.0 ± 29.1	205.0 ± 9.5
Total adenine nucleotides	2322.3 ± 118.8	2303.8 ± 96.2	2323.8 ± 62.0	2350.0 ± 91.2
$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	2.60 ± 0.03	4.10 ± 0.64	3.63 ± 0.54	4.70 ± 0.70

* Kidneys were freeze-clamped 20 min after i.v. injection of aminopicolinate (5 mg/100 g body wt) or saline. Results are means ± S.E.M.; numbers of experiments are given in parentheses.

† Significant difference of the 3-aminopicolinate-injected animals when compared to their own controls ($P < 0.05$).

‡ Significant difference between the normal animals (group A) and the recovered animals (group C).

Regardless of the details of the effect of 3-aminopicolinate on PEPCK activity, the fact that hepatic PEPCK is activated *in vivo* after its administration suggested to us that it may prove useful as a tool to study the possible role of this enzyme in determining the rate of renal ammoniagenesis. Our initial studies (Fig. 1) showed that renal PEPCK activity in the presence of 3-aminopicolinate and Fe^{2+} was greater than when assayed in the presence of Fe^{2+} alone. This is not surprising in view of the similarity between the cytosolic PEPCK from rat liver and that from rat kidney. For example, rat kidney PEPCK is precipitated by antibody raised against rat liver PEPCK [39].

Aminopicolinate injection did increase renal glutamine extraction and ammonia production in rats that had been allowed to recover from acidosis, but no significant increase was found in normal rats. The difference between the responses of these two groups of rats may be related to the elevated activities of glutaminase in the kidneys of rats allowed to recover from acidosis [12]. In preliminary experiments (data not shown), intraperitoneal injection of 3-aminopicolinate was rapidly followed by enhanced renal extractions of glutamine as measured by arteriovenous differences in both normal and recovered rats. In the experiments reported in Table 3, a significant increase in renal venous ammonia production was evident in normal animals treated with 3-aminopicolinate. In addition, the data in Table 4 show that 3-aminopicolinate decreased kidney glutamine levels in normal and recovered rats. Decreased kidney glutamine levels generally accompany increased rates of ammoniagenesis in the rat [8, 40]. It is possible, therefore, that 3-aminopicolinate stimulated ammoniagenesis in normal rats but that the effect was not large enough to be observed in the present experiment.

The freeze-clamp data are not consistent with an activation of PEPCK. The renal contents of phosphoenolpyruvate, 3-phosphoglycerate, and 2-phosphoglycerate did not increase after injection of 3-aminopicolinate. This is in sharp contrast to its effect in liver. In fact, the elevated renal contents of malate and aspartate are more consistent with inhibition of PEPCK than with activation. Inhibition of renal PEPCK by administration of 3-mercaptopicolinic acid *in vivo*, however, results in markedly decreased levels of phosphoenolpyruvate and increased levels of 2-oxoglutarate [8]. In the present experiments, the renal contents of phosphoenolpyruvate was relatively constant, while the content of

2-oxoglutarate decreased, after administration of 3-aminopicolinic acid. Therefore, PEPCK was probably not inhibited in our experiments.

The mechanism by which 3-aminopicolinate stimulated ammoniagenesis is not clear. Increased tissue concentrations of malate and aspartate have been found in liver after 3-aminopicolinate and have been tentatively attributed to an action of this compound separate from its effect on PEPCK [38]. This appears to be the case in kidney also, although we have not been able to identify the additional site of action. Experiments with slices of renal cortex showed no effect of 50 μM 3-aminopicolinate on ammonia production or gluconeogenesis from glutamine. Gluconeogenesis from lactate, glutamate, 2-oxoglutarate, or glutamine was unaffected by 50 μM 3-aminopicolinic acid but it was markedly inhibited by 1 mM 3-aminopicolinic acid (data not shown). Similarly, ammonia production from glutamine by isolated mitochondria was unaffected by 50 μM 3-aminopicolinate (data not shown). In view of its effect in chelating divalent metal ions, it is not surprising that 3-aminopicolinate may exert an effect other than on PEPCK. The fact that its administration resulted in decreased 2-oxoglutarate levels may explain, in part, the effects of renal ammonia production in view of the inhibitory effect of this metabolite on glutamine metabolism by mitochondria [41].

Acknowledgements—We thank Mr. N. diTullio for the gift of 3-aminopicolinic acid. This work was supported by the Medical Research Council of Canada.

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