

Effects of Several Enzyme-Inhibitors on Gluconeogenesis in the Isolated Liver Cells of Eel, *Anguilla japonica*

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Abstract

Effects of several enzyme-inhibitors on gluconeogenesis in the eel liver were investigated. 3-Mercaptopicolinic acid, an inhibitor of cytosolic phosphoenolpyruvate carboxykinase, inhibited gluconeogenesis from lactate and pyruvate by 60 and 76 %, respectively. These inhibitions by 3-mercaptopicolinic acid were consistent with the intracellular distribution of phosphoenolpyruvate carboxykinase, of which distribution in the liver cells was about 60 % in cytosole and about 40 % in mitochondria. Although it is known that 3-aminopicolinic acid is an activator of phosphoenolpyruvate carboxykinase and increases gluconeogenesis in mammalian liver, 3-aminopicolinic acid inhibited phosphoenolpyruvate carboxykinase and gluconeogenesis in the eel liver.

Cycloserine, an inhibitor of transaminases, inhibited gluconeogenesis from lactate and pyruvate by 40 and 60 %, respectively. These results were coincident with those obtained by using amino-oxyacetate, which is an inhibitor of transaminases.

Malonate, an inhibitor of succinate dehydrogenase, had no effect on gluconeogenesis. Fluoro acetate, an inhibitor of malate synthetase, and maleic acid, an inhibitor of transaminase, inhibited slightly gluconeogenesis, but not significantly.

It has been demonstrated by the investigation of the effect of some inhibitors and the intracellular distribution of some enzymes that the pathway of gluconeogenesis in the liver of the eel, *Anguilla japonica*, is different from that in mammalian liver as described previously¹⁾. D-Malate, an inhibitor of malate dehydrogenase, did not inhibit gluconeogenesis from pyruvate. Amino-oxyacetate, an inhibitor of transaminases, inhibited gluconeogenesis from lactate and pyruvate. These inhibitors are useful to determine the pathway.

This paper reports the effect of 3-aminopicolinic acid, malonate, fluoro acetate, maleic acid, cycloserine on gluconeogenesis. These compounds are the inhibitors of phosphoenolpyruvate carboxykinase²⁻⁴⁾, succinate dehydrogenase⁵⁾, malate synthetase⁶⁾, and transaminases⁷⁻⁸⁾, respectively. The effect of 3-aminopicolinic acid, an activator of phosphoenolpyruvate carboxykinase⁹⁾, on gluconeogenesis was also investigated. The purpose of this investigation is whether the effects of the above compounds on gluconeogenesis

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genesis are consistent with the proposed pathway of gluconeogenesis in the eel liver reported previously¹⁾.

Materials and Methods

Materials [2-¹⁴C]-Pyruvate (9.67 mCi/mmole) and DL-[2-¹⁴C]-lactate (29 mCi/mmole) were purchased from the Radiochemical Center, Amersham. Collagenase and albumin (Bovine serum, Fraction V) were purchased from P-L Biochemical, Inc. and Armour Pharmaceutical Co., respectively. 3-Mercaptopicolinic acid and 3-aminopicolinic acid were kindly gifted by Dr. N. W. DiTullio, Smith Kline and French Inc. Lactate dehydrogenase and pyruvate kinase were obtained from Oriental Yeast Co. Other chemicals were obtained from Wako Pure Chemical Ind.

Animal Cultured eels were obtained from Sueyoshi Co. They were kept in freshwater aquaria at 20 to 25 °C and fasted for several days before experiments.

Preparation of isolated liver cells Eel was anaesthetized in 0.5 % urethane and the isolated liver was perfused with modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing collagenase (15 mg/100 ml buffer) as described previously^{10,11)}. After the digestion of the liver by collagenase, the liver cells were gathered by centrifugation at 50 ×g for 1.5 min and washed by 3 times with Krebs-Ringer bicarbonate buffer. Washed cells were suspended in 20 ml of 0.1 % albumin-bicarbonate buffer.

Measurement of the incorporation of ¹⁴C-labelled substrates into glucose [2-¹⁴C]-Pyruvate (50 μCi) and DL-[2-¹⁴C]-lactate (50 μCi) were dissolved in 4 ml of 0.5 M unlabelled substrate. The reaction mixture contained, in a final volume of 2 ml, one ml of cell suspension, 20 μl of ¹⁴C-labelled substrate, and Krebs-Ringer bicarbonate buffer solution gassed with 95 % O₂-5 % CO₂. The reaction was initiated by the addition of cell suspension and stopped by the addition of 0.2 ml of 60 % HClO₄. After centrifugation at 3,000 rpm for 10 min, the supernatant was neutralized with 2 M K₂CO₃. The neutralized solution was applied on columns of Dowex 1×8 (formate type) and Dowex 50×8 (H⁺ type) as described previously¹²⁾. ¹⁴C-Glucose was eluted with 4 ml of water and the radioactivity of the eluate was counted by liquid scintillation counter (Beckman LS-230).

The assay of phosphoenolpyruvate carboxykinase The incubation mixture contained, in a final volume of 0.4 ml, 50 μl of homogenate of the eel liver, 6 mM ITP, 6 mM oxaloacetate, 10 mM KF, 9 mM MgSO₄, 1.35 mM KCl, 0.625 mM dithiothreitol, and 37.5 mM Tris-Cl (pH 8.2). The reaction mixture was incubated at 30 °C for 5 min and stopped by the addition of 1 mg of KBH₄ and 0.2 ml of 0.2 % HClO₄. After centrifugation at 3,000 rpm for 10 min, the supernatant was neutralized with 0.2 M Tris base. The content of phosphoenolpyruvate produced by phosphoenolpyruvate carboxykinase in the homogenate was determined by enzymatic method using lactate dehydrogenase and

pyruvate kinase¹³⁾.

Results and Discussion

Effects of 3-mercaptopicolinic acid and 3-aminopicolinic acid on gluconeogenesis and phosphoenolpyruvate carboxykinase activity 3-Mercaptopicolinic acid at 5 mM inhibited gluconeogenesis from pyruvate and lactate by 60 and 76 %, respectively. Inhibitory effect of 3-mercaptopicolinic acid on gluconeogenesis was observed in rat and guinea pig liver^{2,3)}. It was reported that gluconeogenesis from lactate in those mammalian liver was completely inhibited by 100 μ M of 3-mercaptopicolinic acid³⁾.

Table 1. Effects of 3-mercaptopicolinic acid (3-MP) and 3-aminopicolinic acid (3-AP) on gluconeogenesis in isolated eel liver cells. The initial concentration and radioactivity of substrates were 5 mM and 0.25 μ Ci, respectively. Incubation was performed at 30 °C. The data are the means \pm S. E. of three observations.

Addition	(mM)	Gluconeogenesis (μ moles glucose/g wet weight-h)			
		Substrate Pyruvate		Lactate	
		(%)		(%)	
None	(5)	11.9 \pm 1.3	(100)	22.1 \pm 2.9	(100)
3 - MP	(5)	4.7 \pm 0.5	(40) *	5.1 \pm 0.6	(23) *
3 - AP	(5)	5.2 \pm 0.5	(44) *	17.2 \pm 1.9	(78)

* Significantly different from inhibitor-free controls at $P < 0.01$

3-Aminopicolinic acid at 5 mM also inhibited gluconeogenesis in the eel liver as shown in Table 1. Both of 5 mM of 3-mercaptopicolinic acid and 3-aminopicolinic acid inhibited phosphoenolpyruvate carboxykinase activity by 60 and 75 %, respectively (Table 2). However, 3-aminopicolinic acid stimulated phosphoenolpyruvate carboxykinase activity and gluconeogenesis in rat liver⁹⁾. Although it is assumed that the properties of phosphoenolpyruvate carboxykinase in the eel liver is different from those in rat liver, we have no explanation for this difference at the present time.

Table 2. Effects of 3-mercaptopicolinic acid (3-MP) and 3-aminopicolinic acid (3-AP) on phosphoenolpyruvate carboxykinase (PEPCK) activity

Addition	(mM)	PEPCK activity	
		μ mole PEP/mg protein-min	%
None		7.61×10^{-2}	100
3-MP	(5)	1.97×10^{-2}	26
3-AP	(5)	3.31×10^{-2}	43

As described previously, intracellular distribution of phosphoenolpyruvate carbox-kinase in the eel liver is about 60 % in cytosol and about 40 % in mitochondria¹⁾. If it is also the case for the eel liver that 3-mercaptopycolinic acid inhibits only the enzyme in cytosol as reported by Robinson and Oei⁴⁾, it is assumed that the inhibitory effect of 60 to 76 % on gluconeogenesis is due to the inhibition of the cytosolic enzyme and consistent with the proposed pathway of gluconeogenesis reported previously¹⁾.

Effect of malonate, fluoro acetate, maleic acid, and cycloserine on gluconeogenesis

As shown in Table 3, malonate had no effect on gluconeogenesis. It is known that malonate inhibits succinate dehydrogenase in rat liver. Succinate dehydrogenase is a mitochondrial enzyme bounding to inner membrane of mitochondria. It is assumed that malonate did not permeate into the mitochondria. Fluoro acetate, which is known to be an inhibitor of malate synthetase in yeast, inhibited gluconeogenesis slightly, but not significantly. Maleic acid also inhibited gluconeogenesis slightly. Maleic acid is an inhibitor of transaminase. The inhibitory effect of maleic acid on transaminases seems to be lower than that of cycloserine or amino-oxyacetate.

Table 3. Effects of malonate, fluoro acetate, and maleic acid on gluconeogenesis in isolated eel liver cells. The initial concentration and radioactivity of substrate were 5 mM and 0.24 μ Ci, respectively. Incubation was performed at 30 °C. The data are the means \pm S. E. of three observations

Addition	(mM)	Gluconeogenesis (μ moles glucose/g wet weight·h)	
		Pyruvate	%
None		53.8 \pm 6.9	100
Malonate	(5)	51.0 \pm 5.4	95
Fluoro acetate	(5)	41.8 \pm 5.7	78
Maleic acid	(5)	40.9 \pm 4.5	76

Table 4. Effects of cycloserine and amino-oxyacetate on gluconeogenesis in isolated eel liver cells. The initial concentration and radioactivity of substrates were 5 mM and 0.25 μ Ci, respectively. Incubation was performed at 30 °C. The data are the means \pm S. E. of three observations

Addition	(mM)	Gluconeogenesis (μ moles glucose/g wet weight·h)			
		Substrate		Lactate (%)	
		Pyruvate	(%)		(%)
None		64.1 \pm 9.9	(100)	37.3 \pm 4.4	(100)
Cycloserine	(5)	25.4 \pm 5.9	(40) *	23.4 \pm 3.7	(63) **
Amino - oxyacetate	(5)	14.8 \pm 4.2	(23) *	10.9 \pm 1.6	(29) *

*. ** Significantly different from inhibitor-free control at $P < 0.01$ and $P < 0.05$, respectively

Cycloserine at 5 mM inhibited gluconeogenesis from lactate and pyruvate by 40 and 60 %, respectively (Table 4). Because the proposed pathway of gluconeogenesis from lactate and pyruvate is the same pathway, it is uncertain why the inhibition of gluconeogenesis from lactate was lower than that from pyruvate. The inhibitory effect of amino-oxyacetate was almost same extent on gluconeogenesis from lactate and pyruvate¹⁾. Among inhibitors of transaminases examined, amino-oxyacetate inhibited most greatly gluconeogenesis.

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