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Effects of Quinolinic Acid and Glucagon on Gluconeogenesis in the Perfused Rat Liver *

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Previous findings that 2.5 mM quinolinic acid inhibits gluconeogenesis more strongly from alanine than from lactate have been confirmed. 15 mM quinolinic acid completely inhibited gluconeogenesis from lactate as well as from alanine whereas the formation of glucose from fructose and the production of urea from ammonia and lactose were not affected. The pattern of the gluconeogenic intermediates was the same in the presence of 15 mM quinolinic acid as with 2.5 mM of the inhibitor. It is concluded that high as well as low concentrations of quinolinic acid inhibit gluconeogenesis at the step between oxaloacetate and phosphoenolpyruvate. Furthermore, 5-methoxyindole-2-carboxylic acid, an inhibitor of mitochondrial pyruvate metabolism, also completely blocked gluconeogenesis from lactate whereas glycerol conversion to glucose was only weakly inhibited. All these results do not support the concept of an alternate pathway of gluconeogenesis from lactate proposed by others.

2.5 mM quinolinic acid also partially blocked the formation of urea from alanine. It is suggested that quinolinic acid may have a second site of action causing an inhibition of the glutamate-pyruvate transamination owing to lack of 2-oxoglutarate in the cytosol.

In the presence of quinolinic acid, glucagon caused about the same increase in aspartate and malate tissue levels in the absence of added substrates as in the presence of added lactate or alanine. Therefore, no additional effect of glucagon on gluconeogenesis from lactate or alanine prior to the block by quinolinic acid could be demonstrated.

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Tryptophan was first shown by RAY *et al.* (14) in *in vivo* experiments and later by VENEZIALE *et al.* (24) in the isolated perfused rat liver to be an inhibitor of gluconeogenesis. Of the degradation products of tryptophan, quinolinic acid was found to be the last metabolite with inhibitory properties and this compound was therefore proposed to be the active one (24). Measurements of intermediates of the gluconeogenic pathway in the presence of tryptophan or quinolinic acid showed a crossover between oxaloacetate and phosphoenolpyruvate indicating that the inhibition occurs at the step catalysed by phosphoenolpyruvate carboxykinase (24) (EC 4.1.1.32).

The original liver perfusion work was performed with alanine as substrate (24). With 2.4 mM quinolinic acid, an inhibition of more than 90 % was observed (24), and this result was confirmed by SÖLING *et al.* (17) in a similar system. However, later work by VENEZIALE *et al.* (23) showed that in the presence of this same quinolinic acid concentration gluconeogenesis from pyruvate was much less inhibited than that from alanine. These authors also reported in the same work that the specific radioactivity of 3-phosphoglyceric acid was about twice as high as that of phosphoenolpyruvate when labelled pyruvate was used as substrate in the absence of quinolinic acid. They concluded that there may exist an alternate pathway for gluconeogenesis in which the phosphoenolpyruvate carboxykinase is not an obligatory step for the conversion of pyruvate to glucose.

The stronger inhibition of the conversion of alanine than that of lactate into glucose in the presence of low concentrations of quinolinic acid could be confirmed; however, with higher concentrations of the inhibitor, gluconeogenesis from both substrates was equally effected. Further results presented here are compatible with the conclusion that also high concentrations of quinolinic acid inhibit gluconeogenesis from lactate by specifically blocking phosphoenolpyruvate carboxykinase, and that the existence of another pathway of gluconeogenesis is therefore unlikely. An alternate explanation for the unequal inhibitory pattern of low quinolinic acid concentrations will be discussed.

Results will be presented on the use of quinolinic acid as a tool in an attempt to elucidate a site at which glucagon acts on gluconeogenesis.

Materials and Methods

Animals. Male albino rats (CFN COBS) from the Tierzuchtinstitut of the University of Zürich were used. Food was withheld 24 hours prior to the experiments. The average weight was 170-230 g.

Technique of liver perfusion. The operative technique, methodology and apparatus used were in principle those described by VENEZIALE *et al.* (24). Perfusate flow rates were maintained at 5-6 ml per min per g liver for the first 15 min and at 2 ml per min per g liver afterwards.

The isolated livers were perfused by recycling 100 ml of perfusate, pH 7.4, consisting of human erythrocytes (3-4 weeks old, containing 4.5 g hemoglobin), 2 g bovine serum albumin (fraction V), 50 mg glucose, 2 mg Aureomycin and Krebs-Ringer bicarbonate buffer (5) oxygenated with a mixture of 95 % O_2 and 5 % CO_2.

Analytical methods. For the glucose determination, 0.2 ml of 0.33 M perchloric acid, neutralized with KOH, and the glucose was determined in the supernatant by the glucose-oxydase and peroxidase method (method and reagents from Hoffman-La Roche Diagnostica, Basel), urea was determined in 0.4 ml of perfusate in 1 ml samples of deproteinized perfusate according to RABINOVITZ *et al.* (13). Tissue samples were taken at the end of per-

fusion by the freeze clamp method and extracted according to HOHORST *et al.* (9). Levels of hepatic metabolites were assayed in these extracts according to standard enzymatic methods (3). For the determination of glutamate dehydrogenase (3), liver samples of rats were frozen, thawn, homogenized in 0.1 M phosphate buffer pH 7.0, sonicated, centrifuged at 100,000 *g* for 30 min, and the enzymatic activity was determined in the resulting supernatant. Glutamate-pyruvate transaminase was determined in liver samples of the perfused livers, homogenized in 10 ml per g liver of 0.9 % NaCl containing 6.6×10^{-4} M EDTA pH 7.4 (16).

When the incorporation of labelled carbon into glucose was measured, a sample of the perfusate was deproteinized with perchloric acid, neutralized with KOH and the supernatant was passed through an Amberlite IRA-410-chloride (20-50 mesh) column to remove labelled anions. The glucose in the eluate was measured, diluted with unlabelled glucose and converted into glucose-phenylosazone (6). The osazone was recrystallized at least 3 times and counted according to VENEZIALE *et al.* (23) in a scintillation counter.

Materials. Bovine serum albumin (Cohn fraction V) and quinolinic acid (purissimum) were obtained from Fluka (Buchs, Switzerland), labelled alanine and lactate from New Englands Nuclear (Boston, US), and enzymes for the analytical determinations from Boehringer (Mannheim, Germany). Human blood was provided by the Swiss Red Cross in Bern to whom we are especially thankful. All other chemicals were of reagent grade.

Expression of results. Unless otherwise specified, results are expressed as means ± S.D. The significance of differences between means was established by the Student's *t* test.

Results

The gluconeogenesis from alanine was much more inhibited (76 %) than that

Table I. *Effect of 2.5 mM and 15 mM quinolinic acid on gluconeogenesis from lactate or alanine in the perfused liver.*

Substrates were added at 45 min and, when indicated, quinolinic acid (Q.A.) at 15 min after start of the perfusion. Glucose formation was measured in the perfusate and the intermediates in the liver tissue at 125 min. Numbers in brackets refer to number of experiments. ± values are standard deviations except in the experiment with alanine and 15 mM quinolinic acid where maximal deviations are given. p values express statistical significance (t-Student test) against control perfusions without the inhibitor. n.m. = not measured.

System	Δ Glucose 45-125 min	Aspartate	Malate	Phosphoenol-pyruvate	3-Phospho-glycerate
			μmoles/g liver		
L-Alanine (20 mM)	24.5±9.2 (7)	1.61±0.33	0.16±0.02	0.21±0.07	0.17±0.10
L-Ala. + 2.5 mM Q.A.	5.8±6.7 (8) $p < 0.001$	5.17±1.73 $p < 0.001$	0.36±0.17 $p < 0.05$	0.09±0.05 $p < 0.005$	0.15±0.10 $p < 0.60$
L-Ala. + 15 mM Q.A.	1.5±1.2 (2)	7.58±1.10	0.35±0.07	n.m.	n.m.
L-Lactate (20 mM)	31.7±5.8 (5)	0.57±0.19	0.27±0.16	0.11±0.04	0.075±0.086
L-Lac. + 2.5 mM Q.A.	21.6±4.5 (8) $p < 0.01$	0.99±0.16 $p < 0.005$	0.59±0.16 $p < 0.001$	0.07±0.03 $p < 0.10$	0.042±0.031 $p < 0.40$
L-Lac. + 15 mM Q.A.	1.0±3.8 (6) $p < 0.001$	0.99±0.49 $p < 0.20$	1.75±0.84 $p < 0.005$	0.04±0.03 $p < 0.01$	< 0.005

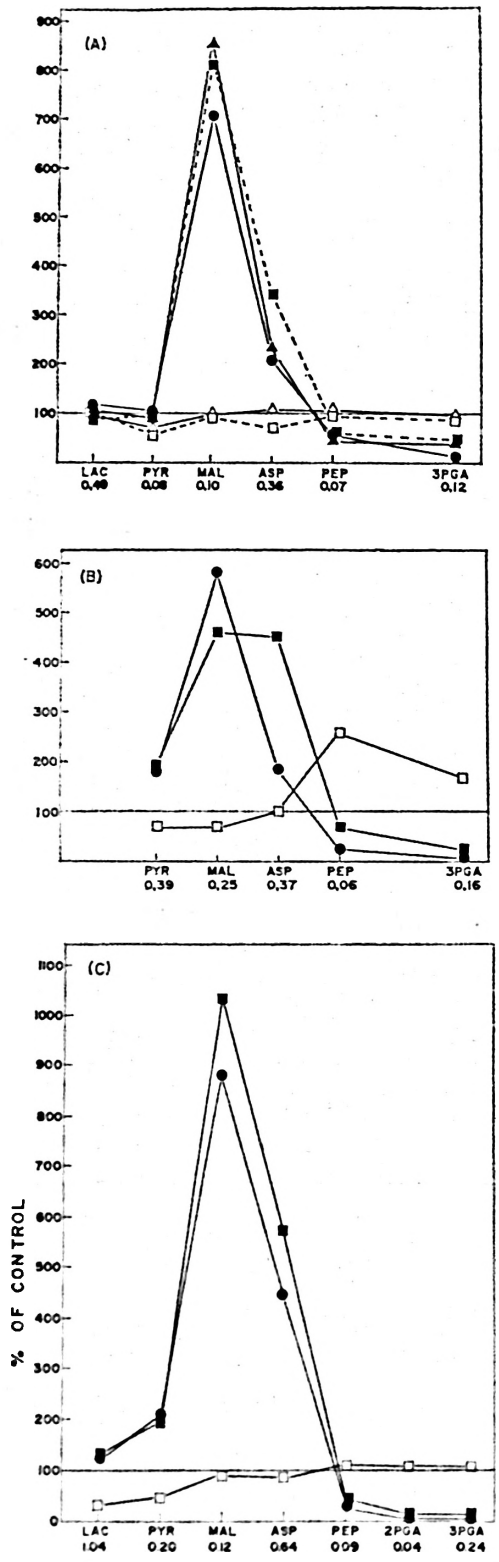

Fig. 1. *Effects of glucagon and quinolinic acid on tissue levels of intermediates in perfusions without added substrate (A), with 20 mM lactate (B) or with 5 mM alanine (C).* Values are plotted as percentages of the levels in the control experiments (100 %) indicated on the abszissa in μmoles/g liver. The metabolites listed are LAC: lactate; PYR: pyruvate; MAL: malate; ASP: aspartate; PEP: Phosphoenolpyruvate; 2PGA: 2-phosphoglycerate; 3PGA: 3-phosphoglycerate. When indicated, 15 mM quinolinic acid was added at 15 min, 10^{-7} M glucagon at 45 min and 3×10^{-8} M at 65, 85 and 105 min. 10^{-7} M dibutyryl-c-AMP was added at 45 min and 5×10^{-7} M at 85 min after start of the perfusion; 10 times less glucagon was added in the perfusions with alanine. Each point represents the average value of at least 7 perfusions after 125 min of perfusion. All the levels of MAL, ASP, PEP, 2PGA and 3PGA in the perfusions containing quinolinic acid with or without glucagon were significantly different ($p < 0.05$) from the controls. For the exact values and statistical differences between perfusions with and without glucagon sea table VII. Open symbols: no quinolinic acid added, closed symbols: with quinolinic acid; ■, □: with glucagon; ▲, △: with dibutyryl-c-AMP; ●: with quinolinic acid only.

from lactate (29 %) in the presence of 2.5 mM quinolinic acid whereas equal and nearly complete inhibition from both substrates was observed with 15 mM of the inhibitor (table I). In the presence of either substrate and low concentrations of quinolinic acid, the levels of the intermediates malate and aspartate were higher and those of phosphoenolpyruvate and 3-phosphoglycerate somewhat lower than in the controls without inhibitor. This crossover between the tetracarbon-intermediates and phosphoenolpyruvate became even more pronounced when high concentrations of the inhibitor were used (fig. 1).

The determination of the inhibition of gluconeogenesis above about 80 % becomes increasingly inaccurate because of the contribution by endogenous substrates such as glycerol and because the erythrocytes in the perfusion medium and also the non-parenchymal tissue (e.g. Kuffer cells) utilize some glucose, thereby obscuring the glucose balance. The initial glucose content in the perfusion amounted to 3 mM, and in several experiments with 15 mM quinolinic acid a net glucose deficit was observed. Therefore, in order to more accurately determine the extent of inhibition by 15 mM quinolinic acid, experiments with labelled alanine and lactate were performed. The results in table II show that the inhibition from both substrates amounted to more than 86 % with both substrates.

In order to test a possible general toxic effect of quinolinic acid, two other biosynthetic pathways were examined in the presence of 15 mM quinolinic acid. Neither the conversion of fructose to glucose nor the urea production from ammonium carbonate was significantly altered whereas gluconeogenesis from lactate was again strongly inhibited in the same livers (tables III and IV).

Table II. *Effect of 15 mM quinolinic acid on the incorporation of labelled substrates into glucose.*

The substrates were added at 45 min and, when indicated, quinolinic acid at 15 min after the start of the perfusion. The specific radioactivity of glucose was measured after 125 min of perfusion (see Methods). The specific radioactivity of added alanine and lactate was 15×10^3 dpm/μmole. The absolute amounts incorporated into glucose in the presence of quinolinic acid were an average of 1.5 μmole of alanine and 1.2 μmole for lactate.

Additions	Incorp. into glucose % of added ^{14}C
20 mM (U-^{14}C) L-Alanine	16.0±1.5 (4)
20 mM (U-^{14}C) L-Alanine + 15 mM quinolinic acid	0.6±0.2 (4)
20 mM (U^{14}C) L-Lactate	20.8±6.4 (5)
20 mM (U^{14}C) L-Lactate + 15 mM quinolinic acid	0.5±0.1 (3)

Table III. *Effect of 15 mM quinolinic acid on glucose formation from lactate and fructose.*

To all perfusions 20 mM L-lactate was added at 45 min, 10 mM fructose at 125 min, and when indicated quinolinic acid at 15 min after start of the perfusion.

Additions	Glucose change in perfusate 45-125 min	125-205 min
	μmoles/g liver	
None	30.7±7.7 (4)	40.1±14.1 (4)
Quinolinic acid (15 mM)	3.7±3.4 (9)	37.2±10.8 (9)

Table IV. *Effect of 15 mM quinolinic acid on the formation of urea and glucose in the presence of lactate, pyruvate, ammonium carbonate and ornithine.*

To all perfusions 18 mM L-lactate, 2 mM pyruvate, 5 mM ammonium carbonate and 2.5 mM ornithine were added at 45 min. 2.5 mM ammonium carbonate and 1.25 mM ornithine were again added at 85 min. Urea and glucose changes were measured in the perfusate.

Additions	Δ urea	Δ glucose 45-105 min
	μmoles/g liver	
None	95.7±12.3 (4)	35.4±7.9 (4)
Quinolinic acid (15 mM)	93.2±11.8 (6)	0.8±2.8 (6)

Table V. *Effect of 5-methoxyindol-2-carboxylic acid (Mica) on gluconeogenesis from lactate and glycerol.*
To all perfusions 20 mM L-lactate was added at 45 min, 10 mM glycerol at 85 min and, when indicated, Mica at 15 min after start of the perfusions.

Additions	Glucose change in perfusate 45-85 min	85-125 min
	μmoles/g liver	
None	13.11±3.80 (6)	35.6±6.6
0.8 mM Mica	0.58±1.05 (6)	28.8±5.7

In the presence of 5-methoxyindole-2-carboxylic acid, an inhibitor of pyruvate carboxylase (2, 8), gluconeogenesis from lactate was almost completely inhibited whereas glycerol conversion to glucose changed only little (table V). Experiments with labelled lactate (not shown) also showed a more than 95 % inhibition of incorporation of label into glucose. It should however be mentioned that this inhibitor has more toxic side effects than quinolinic acid because experiments analogous to those of table IV yielded very variable results showing mostly an inhibition of urea formation.

The possibility of a faster degradation of quinolinic acid in the presence of alanine than of lactate was considered. Perfusions with 2.5 mM quinolinic acid showed, in agreement with the results of HAGINO *et al.* (7), only a small quinolinic acid uptake in the presence of either substrate, as more than 2 mM of the inhibitor was still present in the perfusate after 125 min of perfusion.

As a further explanation for the differing inhibitory pattern of 2.5 mM quinolinic acid the possibility was considered that quinolinic acid could have an additional inhibitory effect on alanine transamination, thus lowering the pyruvate production and as a consequence also that of glucose. Measurements of glutamate-pyruvate transaminase activities in supernatants of livers perfused with 2.5 mM quinolinic acid showed no differences as compared to enzyme activities in control experiments (result not shown), indicating that there was no inactivation of the enzyme.

KLAHR and SCHOOLWERTH (10) reported that 0.5 mM quinolinic acid inhibit bovine liver glutamate dehydrogenase *in vitro*. This would diminish the 2-oxoglutarate available for alanine transamination and thereby the pyruvate production. However, no inhibition of bovine liver glutamate dehydrogenase could be observed in this work with up to 2 mM quinolinic acid in either direction and with either NAD(H) or NADP(H). Rat liver supernatants also showed no decrease in enzyme activity with the same concentrations of the inhibitor.

After the calculations made by SPYDEVOLD *et al.* (18), quinolinic acid lowers cytosolic 2-oxoglutarate level. Hence, the production of urea from alanine should be inhibited by small concentrations of quinolinic acid due to lack of the amino group acceptor 2-oxoglutarate. The perfusion experiments summarized in table VI show, consistently with SPYDEVOLD's *et al.* (18) report, that this is indeed the case.

Quinolinic acid was used in an attempt to elucidate at which step or steps glucagon or its probable messenger cyclic AMP exerts its stimulatory action on gluconeogenesis. Figure 1 shows crossover plots of experiments with and without added substrates, in which the effects of quinolinic acid in the presence and absence of glucagon were tested. It can be seen that the addition of the inhibitor alone always led to the expected sharp crossover between the four-carboxylic acids and phosphoenolpyruvate. In the absence of quinolinic acid, glucagon induced an increase of glucose production of a mean of 24 μmoles per g liver in the presence of lactate or alanine and of 6 μmoles when no substrate was added. The increase of tissue malate plus aspartate levels were of 0.56 in the absence of added substrates, of 1.32

Table VI. *Effect of 2.5 mM quinolinic acid on the formation of urea from alanine and ornithine.*
To all perfusions 10 mM L-alanine and 1 mM ornithine were added at 45 min and when indicated 2.5 mM quinolinic acid at 15 min after start of the perfusions. Urea was measured in the perfusate and the intermediates in the liver tissue at 125 min.

System	Δ Urea 45-125 min	Glutamate	2-Oxoglutarate	Aspartate	Malate
			μmoles/g liver		
10 mM L-Alanine	48.3±9.3 (5)	4.50±0.66	0.22±0.14	0.64±0.15	0.13±0.05
10 mM L-Ala. + 2.5 mM Q.A.	34.1±6.1 (7) $p < 0.01$	6.71±0.78 $p < 0.005$	0.30±0.22 $p < 0.50$	1.55±0.32 $p < 0.005$	0.38±0.25 $p < 0.05$

in the presence of lactate and of 1.02 μmoles per g liver when alanine was added (table VII). It should also be noted that glucagon always caused a decrease in pyruvate levels. Furthermore, with lactate as substrate, a higher value of phosphoenolpyruvate was observed in the presence of the hormone than in its absence.

The addition of dibutyryl-cyclic AMP to the system without added substrates caused similar effects as glucagon (figure 1 *A*) supporting the concept that the effects of glucagon are mediated via the cyclic nucleotide.

Table VII. *Effect of glucagon and quinolinic acid on gluconeogenesis in perfused rat liver.*
Values are taken from experiments shown in figure I. The glucose values refer to the change of glucose in the perfusate betwen 45 and 125 min of perfusion. n.s. = difference not statistically significant ($n > 0.05$).

Substrate	Additions	Glucose	Aspartate	Malate
			μmoles/g liver	
20 mM Lactate	Control	29.6±8.7 (10)	0.37±0.11	0.25±0.08
	Glucagon	54.1±8.3 (8) $p < 0.001$	0.41±0.12 n.s.	0.18±0.07 n.s.
	Q.A.	1.8±3.6 (9)	0.70±0.31	1.44±0.58
	Q.A., Glucagon	5.3±2.2 (7) $p < 0.05$	1.69±0.58 $p < 0.005$	1.11±0.48 n.s.
5 mM Alanine	Control	5.4±4.5 (11)	0.64±0.09	0.12±0.02
	Glucagon	28.3±4.1 (9) $p < 0.001$	0.54±0.03 $p < 0.025$	0.11±0.03 n.s.
	Q.A.	—3.5±4.4 (10)	2.86±0.92	1.06±0.38
	Q.A., Glucagon	0.76±4.1 (11) $p < 0.05$	3.69±1.07 n.s.	1.25±0.40 n.s.
None	Control	2.3±2.1 (9)	0.36±0.09	0.10±0.03
	Glucagon	8.1±4.0 (7) $p < 0.005$	0.25±0.06 $p < 0.005$	0.10±0.02 n.s.
	Q.A.	1.9±2.0 (8)	0.77±0.23	0.73±0.17
	Q.A., Glucagon	5.1±2.2 (9) $p < 0.005$	1.23±0.19 $p < 0.005$	0.83±0.18 n.s.

It should furthermore be noted that, even in the presence of quinolinic acid, glucagon caused a small but significant increase in glucose production in the absence as well as in the presence of added substrates. Pulse label experiments (not shown) with ^{14}C-lactate showed no corresponding increases of label in glucose indicating that the substrate did not originate from compounds below the phosphoenolpyruvate carboxykinase step.

Discussion

The perfusions with 15 mM quinolinic acid show that gluconeogenesis from *lactate* is inhibited more than 90 %. The measured intracellular amounts of intermediates of the gluconeogenetic pathway are in complete agreement with the conclusions made earlier (24) that quinolinic acid caused a block of the phosphoenolpyruvate carboxykinase step. The crossover plots gave no evidence of any further inhibitory site of quinolinic acid between pyruvate and 3-phosphoglycerate. Also no effect of the inhibitor was found on the formation of glucose from fructose and on the production of urea from ammonia and lactate. Both of the pathways require considerable amounts of ATP, and the production of ATP therefore appears not to be affected by quinolinic acid. A general toxic effect of 15 mM quinolinic acid should then be discarded. These results, together with the finding that low as well as high concentrations of quinolinic acid yield similar crossover patterns of the intermediates do not support the concept of another pathway of gluconeogenesis bypassing the phosphoenolpyruvate carboxykinase step (23). This conclusion is also supported by the demonstrated action of the somewhat less specific inhibitor 5-methoxyindole-2-carboxylic acid which blocked the conversion of lactate to glucose by more than 95 %.

The effect of quinolinic acid on gluconeogenesis from *alanine* is more complex because this pathway is more inhibited by 2.5 mM quinolinic acid than the conversion of lactate to glucose. It was furthermore observed that 2.5 mM quinolinic acid inhibited the formation of urea from alanine whereas the much faster urea synthesis from ammonia and lactate was uneffected by 15 mM of the inhibitor (tables IV and VI). The exact site of this additional effect of quinolinic acid could not be elucidated; however, a possible explanation should be considered. As already outlined in the «Results» section, SPYDEVOLD *et al.* (18) have calculated that the cytosolic 2-oxoglutarate concentration decreases in liver by more than 80 % as a result of treatment with quinolinic acid. If this hypothesis is true, the transamination of alanine to pyruvate would be inhibited because most of the glutamate-pyruvate transaminase activity is located in the cytosol (19). As a result both gluconeogenesis and ureogenesis from alanine would be inhibited. Supporting this latter proposal is the fact that the percent changes in 2-oxoglutarate, aspartate, glutamate and malate in the livers perfused in the presence and absence of quinolinic acid were very similar (table VI) to those observed by SPYDEVOLD *et al.* (18) in *in vivo* experiments. This finding, together with the fact that the inhibitor had no effect on the urea production from added ammonium ions (table IV), supports the conclusion that transamination from alanine may become limiting for gluconeogenesis in the presence of 2.5 mM quinolinic acid.

When glucagon was added to perfusions containing lactate or alanine, an increase of about 24 μmoles of glucose per g of liver was observed as compared to only 6 μmoles in the absence of substrates. It is obvious that one or several, so far unknown, additional mechanisms have to be operative for this large stimulation by glucagon in the presence of substrates. If such an additional site of action is located between pyruvate and oxaloacetate a large increase in malate and aspartate

levels would be expected as a result of glucagon addition to perfusions with quinolinic acid. The observed increases in malate and aspartate were, however, in the same order of magnitude as those without added substrate indicating that glucagon acts beyond oxaloacetate . These results are in agreement with those from UI *et al.* (20) who showed in livers perfused with tryptophan that in the presence of labelled lactate and pyruvate the extra malate and aspartate accumulated was derived from unlabelled sources, e.g. from endogenous protein.

Furthermore, it is known that, in the absence of added substrates, the addition of glucagon or dibutyryl-cyclic AMP causes increases in glucose formation, ketogenesis and ureogenesis (13). It has been concluded that the hormone and the nucleotide stimulate lipolysis and proteolysis and thereby provide increased amounts of gluconeogenic precursors. In the presence of quinolinic acid, a more than twofold stimulation of gluconeogenesis (table VII) and a more than fourfold increase in urea production (not shown) upon addition of glucagon, as well as a 60% increase in aspartate (table VII) and a fivefold increase in urea production (not shown) was observed, supporting the concept of an increased proteolysis.

MALLETTE *et al.* (11) have demonstrated that glucagon stimulates the entry of alanine into the hepatic cell. In the presence of quinolinic acid, an increased accumulation of pyruvate and lactate due to the increased entry of alanine into the cell would be expected upon addition of glucagon. As shown in figure 1 *C*, such an increase was not obtained. MALLETTE *et al.* (11), with 9 mM alanine, observed an accumulation of intracellular alanine indicating that the glutamate-pyruvate transamination had become limiting. Since here 5 mM alanine was used, it is possible that also the transamination became limiting and furthermore, as outlined earlier, the addition of quinolinic acid may have caused a lowering of 2-oxoglutarate in the cytosol resulting in an inhibition of the transamination.

Even though the experiments with quinolinic acid make the existence of a major site of action of glucagon on gluconeogenesis between lactate and oxaloacetate unlikely, such an action cannot be ruled out by these results. As also found out by others (20, 27), the addition of glucagon to livers perfused with lactate or alanine consistently lowered the pyruvate concentration (fig. 1). Because the flow of pyruvate through the pyruvate carboxylase step must be stimulated in the presence of glucagon, an activation of this enzyme by the hormone has been proposed, such as lowering of the K_m value for pyruvate or facilitation of the transport of pyruvate through the mitochondrial membrane (1). Such a stimulation should lead to a corresponding increase in accumulation of aspartate and malate. When glucagon was added in the presence of quinolinic acid, however, such an effect was not found. Some feedback mechanism may therefore exist which reduces the activity of pyruvate carboxylase when gluconeogenesis is blocked beyond oxaloacetate. This idea is supported by the fact that addition of glucagon only lowers pyruvate in the absence but not in the presence of quinolinic acid. In conclusion it should be noted that glucagon is known to have at least one site of action beyond oxaloacetate because the conversion of propionate, dihydroxyacetone, and of fructose to glucose have also been shown to be stimulated by glucagon (4, 21, 22).

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Resumen

Se confirma que el ácido quinolínico 2,5 mM inhibe la gluconeogénesis a partir de alanina

más fuertemente que a partir de lactato. A 15 mM inhibe totalmente la gluconeogénesis tanto a partir de lactato como de alanina, sin que se vean afectadas la formación de glucosa a partir de fructosa ni la producción de urea a partir de amonio y lactato. Los niveles de los productos intermediarios son iguales en presencia de ácido quinolínico 15 mM y 2,5 mM. Se deduce que tanto a concentraciones de ácido quinolínico altas como bajas la gluconeogénesis es inhibida entre el oxalacetato y el fosfoenolpiruvato. Además, el ácido 5-metoxiindol-2-carboxilico, inhibidor del metabolismo mitocondrial del piruvato, bloquea también completamente la gluconeogénesis a partir de lactato, siendo sólo débilmente inhibida la conversión de glicerol en glucosa. Estos resultados van en contra del concepto de una segunda vía para la gluconeogénesis a partir de lactato propuesta por otros investigadores.

El ácido quinolínico 2,5 mM bloquea parcialmente la formación de urea a partir de alanina. Se sugiere que el ácido quinolínico pueda tener un segundo lugar de acción que ocasione la inhibición de la transaminación de la alanina debido a falta de 2-oxoglutarato en el citosol.

En presencia de ácido quinolínico, el glucagón causa aumentos similares en los niveles hepáticos de aspartato y malato tanto al añadir lactato o alanina como sin adición de sustratos. Así pues, no se ha podido demostrar ningún efecto adicional del glucagón en la gluconeogénesis a partir de lactato o alanina anterior al bloqueo por ácido quinolínico.

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