SIMULTANEOUS STIMULATION OF URIC ACID SYNTHESIS AND GLUCONEOGENESIS IN CHICKEN HEPATOCYTES BY α-ADRENERGIC ACTION OF EPINEPHRINE AND CALCIUM

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1. Introduction

Synthesis of urate, the main nitrogen excretion product of birds, has been considered to be a cyclic process, where phosphoribosyl pyrophosphate acts as carrier onto which the purine ring is assembled [1]. Phosphoribosyl pyrophosphate is a rate-limiting metabolite in urate synthesis; variations in its concentration may alter the rate of synthesis, provided sufficient nitrogen is supplied. Ribose-5-phosphate, the precursor of phosphoribosyl pyrophosphate, is supposed to be mainly synthesized via the non-oxidative pentose phosphate shunt in tissues other than adipose tissue and lactating mammary glands [2–5]. Therefore the regulation of gluconeogenesis and glycolysis should be reflected in urate synthesis, as well. In [5] it was postulated that phosphorylation and inactivation of pyruvate kinase type M_2 in tumor cells might channel the carbon from glycolysis towards purine synthesis, which is in most steps identical to urate synthesis.

To test whether an inhibition of glycolysis by epinephrine or Ca^{2+} might enhance urate synthesis as well as gluconeogenesis, chicken hepatocytes were incubated with different glucogenic substrates and glutamine. Consistent with the assumption in [5], there was a strong correlationship between the decrease in lactate formation and the increase in gluconeogenesis and urate biosynthesis. Epinephrine enhanced gluconeogenesis and uric acid synthesis mainly via α-adrenergic mechanisms. Furthermore these actions of epinephrine were strongly inhibited by trifluoperazine.

* This publication is an essential part of the thesis of Peter Fister

2. Materials and methods

2.1. *Reagents*

Collagenase, uricase, lactate dehydrogenase and the test combination for the determination of glucose were obtained from Boehringer (Mannheim), epinephrine from Calbiochem (Giessen), bovine serum albumin from Biomol (Ilvesheim), and trifluoperazine as kind gift from Röhm Pharma (Darmstadt). Phentolamine and Penbutulol were gifts from Professor Glossmann (Giessen). All other reagents were of analytical grade and purchased from E. Merck (Darmstadt) and EGA (Steinbuch/Albach).

2.2. *Preparation of hepatocytes*

Male Lohmann-White Leghorn chicks (3–9 weeks old) were starved for 48 h or fed ad libitum with 13-B-Junghennenalleinfutter (Raiffeisen Hauptgenossenschaft, Frankfurt am Main). Hepatocytes were isolated by a modification of the method in [6]. Following a proposal in [1] the right branch of the vena hepatica was canniculated with a suitable polyethylene tube from the vena cava opening the right auricle of the heart as in [7]. For isolation and incubation Krebs–Henseleit bicarbonate medium without calcium was used which during incubation contained 2% bovine serum albumin additionally.

2.3. *Incubation of hepatocytes and experimental procedure*

The incubations were carried out in 25 ml Erlenmeyer flasks at 37°C, under gassing with 95% O_2/5% CO_2, and shaking in a Dubnoff shaker (100 osc./min). After preincubation for 45 min the experiments were started by mixing 1 ml cell suspension with 1 ml incubation medium containing the

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substrates and all other additions. The experiments were stopped after 60 min by addition of 0.1 ml 9.2 M $HClO_4$. After slight alkalization with 0.092 ml 9.2 M NaOH and 0.1 ml 1 M sodium phosphate buffer (pH 7.5) any insoluble material was centrifuged down. Enzymatic methods were used to determine the formation of urate [8], lactate [9] and glucose + glucose 6-phosphate [10] in the supernatant.

3. Results and discussion

Urate synthesis in chicken hepatocytes from carbon sources is low when only endogeneous nitrogen sources are available. A low urate synthesis is also found only in presence of glutamine and absence of exogeneous carbon sources [1]. Apparently in this case carbohydrates are lacking as precursors for the synthesis of phosphoribosyl pyrophosphate, onto which the urate molecule is assembled. To obtain considerably higher rates of urate production nitrogen and carbon sources must be combined. Table 1 shows the Ca^{2+}- and epinephrine-induced stimulation of urate synthesis from different substrate combinations of carbohydrate + glutamine in hepatocytes from fed and starved chicken.

As can be expected in the presence of an excess of substrates, there was no striking difference in the rates of urate synthesis between hepatocytes from fed and starved animals. The stimulation by epinephrine or Ca^{2+} varied, however, considerably with the nature of carbohydrate added. Whereas a 20% stimulation of urate synthesis was achieved from glucose+ glutamine, a 50% stimulation was reached from dihydroxyacetone + glutamine or lactate + glutamine. The findings that the extent of stimulation was greatest with lactate or dihydroxyacetone as carbon sources point to a control of urate synthesis by Ca^{2+} and epinephrine in the lower part of glycolysis.

To substantiate this assumption we studied whether there exists a correlation between urate formation, gluconeogenesis and lactate production in chicken hepatocytes incubated with dihydroxyacetone + glutamine. There was indeed a close positive correlation between the rates of gluconeogenesis and urate synthesis and a negative correlation between the rates of gluconeogenesis and lactate formation (fig.1 A). Also the stimulations of these syntheses by epinephrine, Ca^{2+}, or both were significantly correlated in the same directions, although the correlations were less close (fig.1B).

Metabolite profiles on the epinephrine-stimulated

Table 1

Ca^{2+}- and epinephrine-induced stimulation of urate synthesis from different substrate combinations in hepatocytes from fed and starved chicken

	Fed animals		48 h-starved animals	
	(nmol . h^{-1} 10^6 $cells^{-1}$)	(% of control)	(nmol . h^{-1} 10^6 $cells^{-1}$)	(% of control)
10 mM glucose + 2 mM Gln	7.6 ± 1.8	100	6.9 ± 1.1	100
+ 2.6 mM $CaCl_2$	9.3 ± 2.5	120 ± 5	8.4 ± 1.0	125 ± 10
+ 0.1 mM epinephrine	8.7 ± 1.8	117 ± 9	8.1 ± 1.4	118 ± 10
+ epinephrine + $CaCl_2$	9.2 ± 2.4	119 ± 20	8.2 ± 1.2	124 ± 16
10 mM dihydroxyacetone + 2 mM Gln	4.7 ± 1.0	100	6.3 ± 0.7	100
+ 2.6 mM $CaCl_2$	7.5 ± 1.7	159 ± 20	9.8 ± 1.8[b]	155 ± 17[c]
+ 0.1 mM epinephrine	8.5 ± 2.0[a]	175 ± 17[a]	9.7 ± 1.5[b]	153 ± 11[c]
+ epinephrine + $CaCl_2$	9.4 ± 2.5[b]	188 ± 31[b]	9.4 ± 1.6[b]	148 ± 14[c]
10 mM lactate + 2 mM Gln	5.9 ± 1.6	100	5.7 ± 0.7	100
+ 2.6 mM $CaCl_2$	9.0 ± 2.4	161 ± 21	9.1 ± 0.9[d]	164 ± 16[c]
+ 0.1 mM epinephrine	7.7 ± 2.4	128 ± 12	8.3 ± 0.9[c]	148 ± 7[b]
+ epinephrine + $CaCl_2$	8.8 ± 2.7	151 ± 31	8.4 ± 1.0[c]	152 ± 17[c]

Significance *vs* the corresponding control groups: [a] $p < 0.10$; [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$; Gln, glutamine

Values are $\bar{x}$ ± SEM from 5 different cell preparations. Incubations and measurements were carried out as in section 2. Significances were tested by one way analysis of variance for linked random samples and multiple comparison of means according to Scheffé

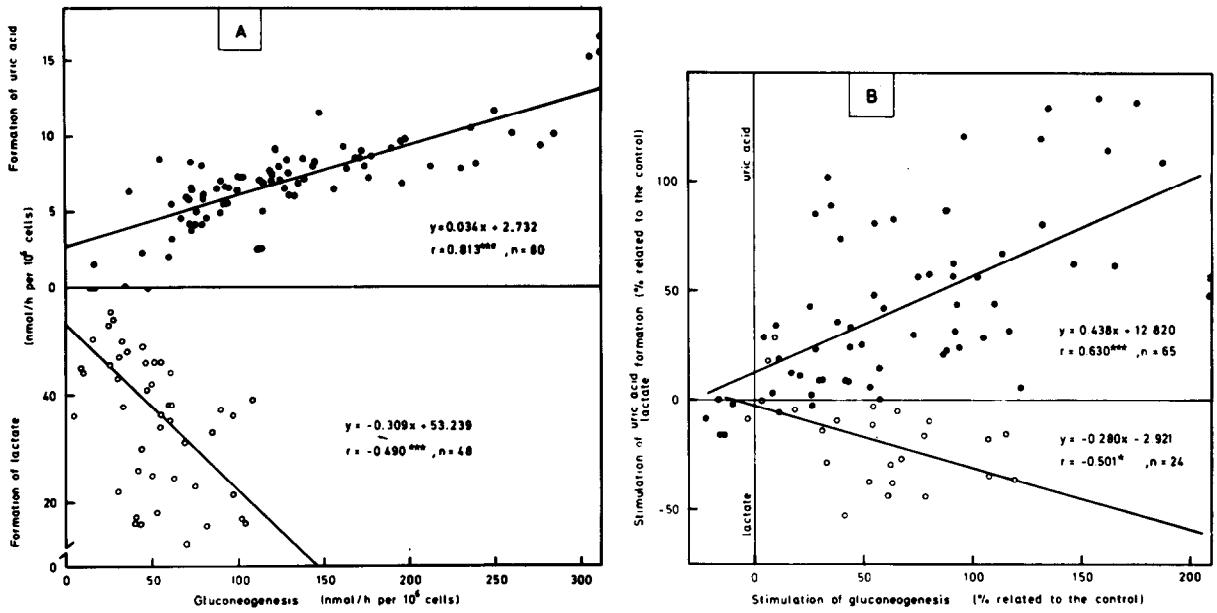

Fig.1. Correlation between urate synthesis, gluconeogenesis, and lactate formation (A) and between the corresponding alterations (B). Hepatocytes from 48 h-starved chicks were incubated with 10 mM dihydroxyacetone + 2 mM glutamine. Points are given from control incubations (A) and incubations in the additional presence of Ca^{2+}, epinephrine, both, antagonists, or epinephrine + antagonists (A,B). Calculations were done by linear regression analysis.

gluconeogenesis from lactate in rat liver show that there exists a crossover point between pyruvate and phosphoenol pyruvate and that there is no significant crossover at other steps of the lower part of the glycolytic sequence [11]. An inactivation of pyruvate kinase by adrenergic agonists has been observed in hepatocytes of different species [12–15]; in the rat the effect of epinephrine is α-adrenergic-related [13,14]. A blockade of pyruvate kinase by extracellular Ca^{2+} has also been proposed [16]. It has not been possible to demonstrate any alteration of pyruvate

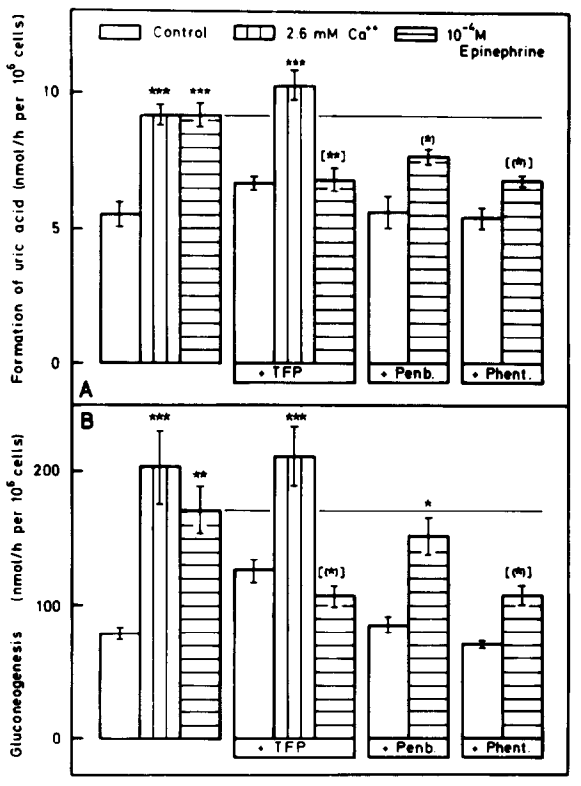

Fig.2. Effects of antagonists on Ca^{2+}- or epinephrine-induced stimulation of urate synthesis (A) and gluconeogenesis (B). Hepatocytes from 48 h-starved chicks were incubated with 10 mM dihydroxyacetone + 2 mM glutamine and the indicated additions: TFP (10^{-4} M trifluoperazine); Penb. (10^{-4} M penbutulol); Phent. (10^{-4} M phentolamine). Details are given in section 2. Values are $\bar{x}$ ± SEM from 5 different cell preparations. Statistical analysis was done by one-way analysis of variance for linked random samples and multiple comparison of means according to Scheffé. The stars without brackets show the significance *vs* the control group (first column), the stars in brackets those *vs* the epinephrine-treated group (third column): (*) $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

kinase activity in extracts of chicken hepatocytes [7]. This is probably due to pyruvate kinase type M_2, which is the major isoenzyme of chicken liver [18,19], whereas in other species it is the type L [20]. Contrary to this latter type [21] pyruvate kinase type M_2 is inactivated and phosphorylated by a cAMP-independent protein kinase [22,23], which is inhibited by phosphoribosyl pyrophosphate [24]. This renders possible a regulatory correlation between glycolysis and purine synthesis via pyruvate kinase type M_2 [5].

To specify the mechanism of action of epinephrine on urate synthesis and gluconeogenesis in presence of dihydroxyacetone + glutamine we used the adrenergic antagonists phentolamine and penbutulol and the phenothiazine drug trifluoperazine (fig.2). The stimulatory effect of epinephrine on both pathways was largely prevented by the α-blocker phentolamine and less by the β-blocker penbutulol. Trifluoperazine is used as inhibitor of calmodulin dependent activities [25] and of phospholipid-sensitive Ca^{2+}-dependent protein kinase [26]. In our experiments it suppressed epinephrine-dependent stimulation almost completely. From these findings epinephrine seems to act via an α-adrenergic mechanism involving Ca^{2+} as second messenger of the hormone action. But this conclusion is opposed by the result that the stimulatory effect of extracellular Ca^{2+} is not affected by trifluoperazine. Therefore it is more conclusive to attribute the inhibition of epinephrine action to the quality of trifluoperazine as α-adrenergic antagonist [27].

Epinephrine has been shown to increase the hepatic levels of phosphoribosyl pyrophosphate [28] and its availability for purine synthesis de novo in rats [29]. The effect of epinephrine on purine synthesis in rat hepatocytes may at least in part be explained via the effects of this hormone on carbohydrate metabolism [29]. Our data show that for chicken hepatocytes a strong correlation between both pathways can be demonstrated (fig.1). The data are thus consistent with an inhibition of pyruvate kinase as the consequence of the action of epinephrine and Ca^{2+}. But the hypothesis that an interconversion of pyruvate kinase might be responsible for the above demonstrated regulation of urate and glucose synthesis has to be proven more rigorously.

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