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Increase of urate formation by stimulation of sympathetic hepatic nerves, circulating noradrenaline and glucagon in the perfused rat liver

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In the isolated rat liver perfused in situ stimulation of the nerve bundles around the portal vein and the hepatic artery caused an increase of urate formation that was inhibited by the α_1-blocker prazosine and the xanthine oxidase inhibitor allopurinol. Moreover, nerve stimulation increased glucose and lactate output and decreased perfusion flow. Infusion of noradrenaline had similar effects. Compared to nerve stimulation infusion of glucagon led to a less pronounced increase of urate formation and a twice as large increase in glucose output but a decrease in lactate release without affecting the flow rate. Insulin had no effect on any of the parameters studied.

Urate; Allantoin; Hepatic nerve; Catecholamine; Glucagon

1. INTRODUCTION

The liver is innervated by sympathetic and parasympathetic nerves [1–4]. In the isolated rat liver perfused in situ it has been shown that electrical stimulation of the nerve bundles around the portal vein and the hepatic artery caused hemodynamic and a variety of metabolic changes: perfusion flow was reduced [5,6]. The flow reduction was accompanied by a redistribution of the hepatic microcirculation [7,8]. Glucose and lactate output increased [5,6], and ketone body formation [9], urea and glutamine production as well as ammonia uptake [10] and oxygen consumption decreased [7,8,11]; furthermore para-nitrophenol conjugation decreased [12] and glutathione efflux increased [13]. Stimulation of the nerve bundles also caused an overflow of noradrenaline into the hepatic vein [6,14], a transient release of Ca^{2+} followed by reuptake [13] and, conversely, a transient uptake of K^+ followed by release [13]. All changes could partly be mimicked by circulating noradrenaline [5,8–10] or phenylephrine [11,13], yet only at hyperphysiological concentrations. They were mediated predominantly via α-sympathetic receptors.

During the early phase of a study of a possible overflow into the hepatic vein of neuropeptides as putative cotransmitters of noradrenaline it was observed, that a substance absorbing at 280 to 290 nm was released upon nerve stimulation. This increase in absorbance was too large to be accounted for by the release of any putative peptide transmitter from the synaptic cleft. The output of a UV absorbing metabolite from the liver seemed more likely and among others purine derivatives were taken into consideration. A systematic search revealed that the increase in absorbance in the effluate from the liver was mainly due to uric acid (A. Nath, unpublished).

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Uric acid is an ubiquitous degradation product of purine nucleotides in mammalian cells [15]. Hyperuricemia is the major chemical symptom of gout. Since no direct information on the hormonal and nervous regulation of urate formation in mammalian liver was available, it seemed to be worthwhile to investigate the influence of hepatic sympathetic nerves, noradrenaline, glucagon and insulin on urate formation in the liver.

2. MATERIALS AND METHODS

2.1. *Materials*

All chemicals were reagent grade and from commercial sources. Enzymes, ATP and the Peridochrom test kit for urate determination were purchased from Boehringer (D-6800 Mannheim). Glucagon, insulin and noradrenalin were bought from Serva (D-6900 Heidelberg). Allopurinol was from Sigma (D-8028 Taufkirchen), the Merck Glucose System for glucose determination from Merck (D-6100 Darmstadt). Prazosine was kindly provided by Pfizer (D-7500 Karlsruhe).

2.2. *Animals*

Male Wistar rats (160–190 g, Winkelmann D-4791 Borchem) were kept on a 12 h day-night rhythm, 7 a.m. to 7 p.m. light, with free access to water and food (standard rat diet, Ssniff, D-4770 Soest). All experiments were started between 8.30 and 10.30 a.m.

2.3. *Liver perfusion*

Rat livers were perfused in situ without recirculation in a 37°C cabinet via the portal vein normally with a Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. The medium was equilibrated with 95% O_2 and 5% CO_2. The flow rate was 4 $ml \cdot min^{-1} \cdot g\ liver^{-1}$. In some experiments the perfusion buffer was fortified with 0.1% (w/v) bovine serum albumin and 30% (v/v) bovine erythrocytes, and then equilibrated with 13% O_2, 5% CO_2 and 82% N_2 mimicking arterial conditions; the flow rate was then 2 $ml \cdot min^{-1} \cdot g\ liver^{-1}$. After 30 min preperfusion the experiment was started; the perivascular nerves were stimulated with rectangular pulses (20 Hz, 2 ms, 20 V) using a bipolar platinum electrode [5]. Hormones and drugs were infused when indicated.

2.4. *Metabolite assays*

The perfusate was collected with a fraction collector at 1 min intervals and cooled on ice. Glucose and urate were determined with commercially available enzymatic test kits based on glucose dehydrogenase (EC 1.1.1.47) and urate oxidase (EC 1.7.3.3), respectively. Lactate was measured in a combined optical test using lactate dehydrogenase (EC 1.1.1.27) and glutamic pyruvic transaminase (EC 2.6.1.2) in a glutamate buffer system. Allantoin was determined according to Hornawsky and Müller [16]. Briefly, allantoin was hydrolyzed in 0.5 N NaOH to urea and glyoxylic acid which was reacted in HCl to its phenylhydrazone and subsequently in the presence of potassium hexacyanoferrate to 1,5-diphenylformazancarbonic acid, the absorption of which was measured at 520 nm. Hypoxanthine and xanthine were determined after conversion to urate by xanthine oxidase (EC 1.2.3.2) with the Peridochrom test kit for uric acid.

3. RESULTS

3.1. *Nerve stimulation- and hormone-dependent urate release*

Rat livers were perfused in a pressure-constant non-recirculating system with a Krebs-Henseleit bicarbonate buffer, pH 7.4, offering 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. After 45 min preperfusion the perivascular hepatic nerves were stimulated at a frequency of 20 Hz or either 1 μM noradrenaline, 1 nM glucagon or 100 nM insulin were infused over a 5 min period (fig.1).

Nerve stimulation caused a sevenfold increase of urate output (fig.1) and a twofold enhancement of allantoin (not shown; from 13 ± 0.5 to 26 ± 3.6 $nmol \cdot min^{-1} \cdot g^{-1}$; means ± SE, $n = 3$). Glucose and lactate output increased by about 2.5- and 2-fold, respectively. The flow rate was reduced by about one third. The peak values of urate, glucose and lactate output were reached in 3 min, the peak of flow reduction 2 min after the onset of stimulation. The metabolic and hemodynamic alterations already started to return to normal during the stimulation period thus showing escape phenomena.

Noradrenaline infusion caused similar metabolic

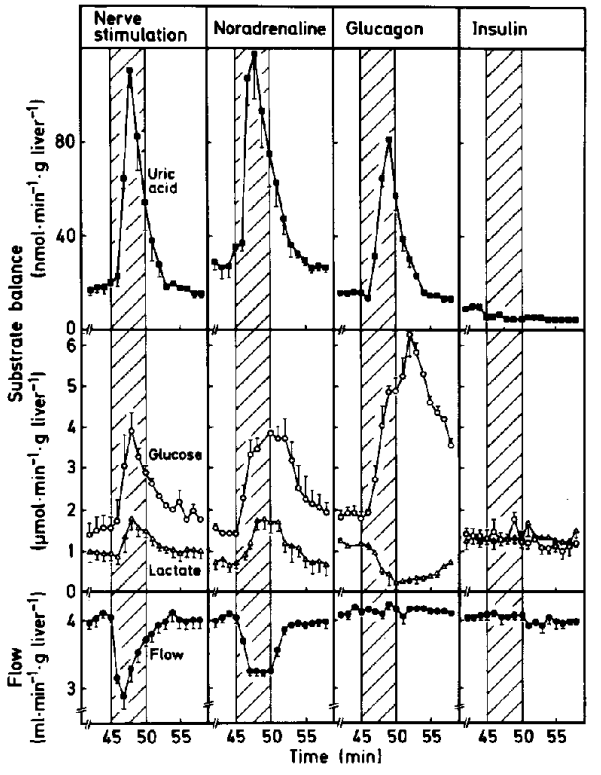

Fig.1. Urate, glucose and lactate output and perfusion flow in the perfused rat liver after nerve stimulation, noradrenaline, glucagon and insulin. Rat livers were perfused with a Krebs-Henseleit bicarbonate buffer containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. After 45 min either the nerves around the portal vein and hepatic artery were stimulated electrically (20 Hz, 20 V, 2 ms) or noradrenaline (10^{-6} M) or glucagon (10^{-9} M) or insulin (10^{-7} M) were infused for 5 min (hatched columns). Substrate balance is given by concentration in hepatic vein − concentration in portal vein in μmol·ml^{-1}·flow in ml·min^{-1}·g liver^{-1}. Values are means ± SE with $n = 3$.

changes as nerve stimulation; the reduction in flow rate, however, was lower. The escape phenomena of the alterations in glucose and lactate balance and in flow, that were observed after nerve stimulation, did not occur (fig.1).

Glucagon infusion led to a less pronounced increase in urate output than nerve stimulation. The peak value was about 25% lower and was reached only in the 4th min. Glucose output was increased by 3-fold, but lactate output was reduced to about 10%. No changes in flow rate were observed. Insulin had neither metabolic nor hemodynamic effects (fig.1).

The influence of oxygen input on urate and allantoin formation was studied by comparing metabolism during perfusion with erythrocyte-free and erythrocyte-containing buffer supplying oxygen at a rate of about 4 and 12 μmol·min^{-1}·g liver^{-1}, respectively (for calculation see [17]), which maintained the oxygen delivery clearly higher than the average oxygen uptake of 2–3 μmol·min^{-1}·g liver^{-1} [18,19]. With the higher oxygen input urate release was significantly lower (6 vs 16 nmol·min^{-1}·g^{-1}) and allantoin release higher (23 vs 12 nmol·min^{-1}·g^{-1}) than with the lower oxygen input. Similarly, after nerve stimulation the increase of urate release was smaller and of allantoin formation larger with the higher than with the lower oxygen delivery. The sum of urate and allantoin release remained similar.

3.2. *Mechanism of the nerve stimulation-dependent increase in urate output*

Urate release was totally blocked by the α_1-receptor blocker prazosine (table 1). Prazosine also blocked the increase of urate output after noradrenaline; however, it did not influence the increase of urate output after glucagon (not shown). An unspecific interference with urate formation could therefore be excluded. Thus, in rat liver all nerve stimulation-dependent metabolic and hemodynamic changes so far observed [5,6,9,10] appear to be mediated predominantly by α-receptors.

Nerve stimulation might either act via direct contacts of the liver nerves with the parenchymal cells or indirectly via noradrenaline overflow from the vessels or via hemodynamic changes causing hypoxia (cf. fig.5 in [5]). Sodium nitroprusside, a smooth muscle relaxant, was used to study the role of flow reduction in the mechanism of the nerve stimulation-dependent increase of urate output. When sodium nitroprusside was infused 5 min before and during the stimulus all nerve stimulation-dependent metabolic changes were reduced by 30% but the hemodynamic effects were reduced by 80% (table 1). Thus, reduction of perfusion flow cannot be a major step in the mechanism of increase in urate release after nerve stimulation.

Table 1

Alteration of the nerve stimulation-dependent increase of urate, glucose and lactate output and decrease in flow by allopurinol, prazosine and nitroprusside

Additions	Increase in formation of			Decrease in flow ($ml \cdot min^{-1} \cdot g\ liver^{-1}$)
	Urate ($nmol \cdot min^{-1} \cdot g\ liver^{-1}$)	Glucose ($\mu mol \cdot min^{-1} \cdot g\ liver^{-1}$)	Lactate ($\mu mol \cdot min^{-1} \cdot g\ liver^{-1}$)	
None	94 ± 16	2.5 ± 0.4	0.9 ± 0.1	−1.2 ± 0.2
Allopurinol	± 0[a]	1.8 ± 0.4	0.9 ± 0.1	−0.7 ± 0.1
Prazosine	6 ± 1	0.25 ± 0.17	0.2 ± 0.1	−0.2 ± 0.07
Nitroprusside	58 ± 10	1.6 ± 0.06	0.6 ± 0.03	−0.3 ± 0.2

[a] No statistically significant change

The perivascular nerves were stimulated for 5 min starting 45 min after the beginning of perfusion. Allopurinol, prazosine or sodium nitroprusside were infused 5 min before and during the stimulation period to reach a final concentration in the perfusate of 7 μM, 0.1 μM and 10 μM, respectively. The metabolic and hemodynamic values represent the difference between the basic and the peak values (cf. fig.1). The values are means ± SE with $n = 3$

The transient nature of the increase in urate output after nerve stimulation as well as after noradrenaline or glucagon infusion might indicate that urate was mobilized from a pre-existing pool. Alternatively, it might be newly formed by an increased purine degradation. One key enzyme of urate formation, xanthine oxidase, can be inhibited competitively by allopurinol. When allopurinol was infused 5 min before and during the stimulation period the increase in urate formation after nerve stimulation (table 1), noradrenaline or glucagon infusion (not shown) was completely abolished. It is therefore concluded that the increase in urate output was due to a de novo formation rather than to a release from a pre-existing pool.

Nerve stimulation in the presence of allopurinol was accompanied by an increase of the two immediate precursors of urate, xanthine and hypoxanthine, that was in the same range as the nerve stimulation-dependent increase in urate formation in the absence of allopurinol. This indicates that allopurinol acted as such by inhibiting the xanthine oxidase reaction (EC 1.2.3.2) and that it did not interfere, after conversion via a salvage pathway reaction to allopurinol ribose phosphate, with the glutamine phosphoribosylpyrophosphate amidotransferase reaction (EC 2.4.2.14), an early step in purine synthesis. The increase in glucose and lactate output as well as the reduction in perfusion flow were also slightly depressed by allopurinol (table 1). The mechanism of this allopurinol action is not understood.

4. DISCUSSION

In all mammals except primates the end products of purine degradation are urate formed from hypoxanthine and xanthine by the action of xanthine oxidase and allantoin formed from urate by the action of urate oxidase; in primates lacking urate oxidase urate is the sole end product [15]. In the present study it has been shown in the isolated perfused rat liver that electrical stimulation of nerves around the portal vein and the hepatic artery as well as noradrenaline and glucagon infusion caused a five to sevenfold increase in urate output (fig.1) and a twofold enhancement of allantoin release. This increase was not due to a release from pre-existing pools but to a de novo formation during the stimulation period (table 1).

Since both in the erythrocyte-free perfusion with the usual low, but 'sufficient' oxygen input and in the erythrocyte-containing perfusion with a high oxygen input urate release was increased several fold more than allantoin release, it can be conclud-

ed that the urate oxidase reaction was rate limiting in allantoin formation. Since oxygen is a substrate of both xanthine oxidase and urate oxidase, oxygen supply could have been a limiting factor, if the oxygen affinity of urate oxidase was lower than that of xanthine oxidase or if the latter enzyme operated in the dehydrogenase (type D) form [20,21]. A distinction between these possibilities may not be possible, because there is uncertainty as to the $K_{0.5}$ value for oxygen of the two oxidases in the cellular environment (in vitro the $K_{0.5}$ for oxygen was reported to be 25–250 μM with xanthine oxidase [22,23] and 80–100 μM with urate oxidase [18]) and because in the perfused liver in contrast to isolated hepatocytes reactions with widely different $K_{0.5}$ for oxygen such as cytochrome oxidase and urate oxidase can exhibit a very similar dependence on O_2 concentration [18].

The increase in urate formation must be due to an enhanced degradation of purine nucleotides, which in turn would require either an enhanced degradation of nucleic acids and/or an increased de novo or salvage pathway synthesis of purine nucleotides from 5-phosphoribosyl-1-pyrophosphate. The present findings do not allow to differentiate between these possibilities.

The increase in glucose output after nerve stimulation, noradrenaline or glucagon has been shown to be primarily due to an enhancement of glycogenolysis that is accompanied by an intracellular increase of glucose-6-phosphate [24]. Therefore, although there was no strict correlation between glucose output and urate formation, it seems to be possible that the elevated level of glucose-6-phosphate caused an enhanced flux into the pentose phosphate pathway replenishing the 5-phosphoribosyl-1-pyrophosphate pool and in turn increasing purine nucleotide synthesis. This hypothesis is in agreement with the finding that glucagon, cAMP or adrenaline (noradrenaline was not tested) increased the levels of 5-phosphoribosyl-1-pyrophosphate in rat hepatocytes by 1.5–2-fold although with considerably slower kinetics [25,26]. The finding that adrenaline (noradrenaline was not studied) increased urate formation slightly by 1.2–1.5-fold in chicken hepatocytes again with considerably slower kinetics [27] cannot be compared with the present observations since in birds urate is the end product not only of the degradation of purine nucleotides as in mammals but of all nitrogenous compounds including amino acids.

A physiological function of the increase in urate formation in the rat liver by sympathetic nerve action, circulating noradrenaline or glucagon is hard to envisage at the present stage.

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