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Possible involvement of eicosanoids in the actions of sympathetic hepatic nerves on carbohydrate metabolism and hemodynamics in perfused rat liver

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In isolated rat liver perfused at constant pressure with Krebs-Henseleit buffer containing 5 mM glucose, 2 mM lactate, 0.2 mM pyruvate and 0.1% bovine serum albumin, perivascular nerve stimulation (20 V, 20 Hz, 2 ms) and infusion of ATP (100 μM), noradrenaline (1 μM) or arachidonic acid (100 μM) caused an increase in glucose and lactate output and a reduction of perfusion flow. The metabolic effects of nerve stimulation but not those of ATP and noradrenaline were inhibited strongly by the phospholipase A_2 inhibitor bromophenacyl bromide (BPB, 20 μM) and the cyclooxygenase inhibitor indomethacin (Indo, 20 μM) and only slightly by the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 20 μM). In contrast, the hemodynamic effects not only of nerve stimulation but also of ATP and noradrenaline were inhibited strongly by BPB and Indo and slightly by NDGA. The metabolic and hemodynamic actions of arachidonate were inhibited specifically by Indo. These results suggest that the effects of nerve stimulation were at least partially mediated or modulated by eicosanoids, especially by prostanoids.

Noradrenaline; extracellular ATP; Icosanoid; Arachidonic acid; (Perfused rat liver, Sympathetic hepatic nerve)

1. INTRODUCTION

The liver is innervated by sympathetic and parasympathetic, afferent and efferent nerves [1-4]. In isolated rat liver perfused in situ stimulation of perivascular nerve bundles was shown to cause an increase in glucose and lactate output [5] as well as urate and allantoin formation [6], reduction of ketogenesis [7], urea release and ammonia uptake [8] as well as oxygen utilization [9,10], decrease as well in intrahepatic redistribution of flow [5,9,22], overflow of noradrenaline into the hepatic vein [11], and transient increase of calcium efflux and potassium uptake [12]. The nerve effects could be caused indirectly via hemodynamic changes or noradrenaline overflow from the vasculature into the sinusoids or directly by neurotransmitter release within nerve contacts with (almost) each hepatocyte or with only some periportal hepatocytes and signal propagation via gap junctions (cf. fig.5 in [5]). Evidence for a direct mode of nerve action has recently been presented [13]. Since in rat liver the parenchymal and non-parenchymal cells were found to be innervated [14], the possibility was considered that the nerve action might be mediated at least partially or modulated by eicosanoids, which are produced only in the sinusoidal cells, such as Kupffer and endothelial cells [15,16].

Here, the metabolic and hemodynamic effects caused by nerve stimulation, ATP, noradrenaline and arachidonic acid were studied in the presence of bromophenacyl bromide (BPB), an inhibitor of arachidonate formation from phospholipids [17], (Indo), an inhibitor of prostanoid synthesis [18], and nordihydroguaiaretic acid (NDGA), an inhibitor of leukotriene production [19]. The results

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allow one to conclude that in rat liver non-parenchymal cells play a mediator or modulator role in the metabolic actions of hepatic nerves.

2. MATERIALS AND METHODS

2.1. *Materials*

All chemicals were of reagent grade and from commercial sources. Enzymes, bovine serum albumin (BSA) and adenosine 5'-triphosphate (ATP) were purchased from Boehringer (Mannheim). Noradrenaline bitartrate was from Serva (Heidelberg). Indo, NDGA and dimethyl sulfoxide (DMSO) were from Sigma (Deisenhofen).

2.2. *Animals*

Male Wistar rats (150–200 g) were obtained from Winkelmann (Borchen). At least 1 week before experiments, they were subjected to a 12 h day-night rhythm with free access to food (standard diet 1320 of Altromin, Lage). All experiments were started between 9 and 12 a.m. Animals were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body wt).

2.3. *Liver perfusion*

The liver was perfused in situ without recirculation via the portal vein in a 37°C cabinet as in [5]. Perfusion was started with erythrocyte-free Krebs-Henseleit bicarbonate buffer containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. At 20 min before the first stimulus, the perfusion medium was enriched with 0.1% BSA and, where indicated, with inhibitors of eicosanoid synthesis. BPB, Indo and NDGA were dissolved first in DMSO (concentration approx. 33 mM for each inhibitor) and then added slowly to the perfusion medium containing 0.1% BSA, reaching final concentrations as indicated in the figures. Perfusion pressure was constant at about 10 cmH_2O with a flow rate of 3.9–4.1 $ml \cdot min^{-1} \cdot g^{-1}$ liver under basal conditions.

2.4 *Nerve stimulation and infusion of signal compounds*

The hepatic nerves were stimulated with a bipolar platinum wire electrode placed perivascularly around both the portal vein and hepatic artery which was not perfused but was still joined to the portal vein (20 V, 20 Hz, 2 ms). ATP, noradrenaline and arachidonic acid were infused, reaching final concentrations as indicated in the figures. Arachidonic acid was dissolved in the perfusion medium enriched with 10% BSA; this mixture was sonicated for 2–3 min before infusion.

2.5. *Determination of metabolites and noradrenaline*

Metabolites were measured with standard enzymatic techniques [20]. Noradrenaline was quantitated radiochemically after enzymatic methylation with *S*-[^{3}H]adenosylmethionine, conversion to [^{3}H]normethanephrine and separation by thin-layer chromatography [11].

3. RESULTS

3.1. *Influence of nerve stimulation, ATP, noradrenaline and arachidonate on carbohydrate metabolism and hemodynamics*

Electrical nerve stimulation (20 V, 20 Hz, 2 ms; which was sufficient to cause maximal effect [21]) resulted in an increase in glucose and lactate output, and a decrease of flow, reaching a maximum at about 3 and 2 min, respectively; these changes of substrate balance and flow then began to return to the pre-stimulation level in spite of continued stimulation indicating an 'escape' phenomenon [22] (fig.1).

Infusion of ATP (100 μM), which is a constituent of noradrenaline-containing vesicles of sympathetic nerve terminals [23], as well as infusion of noradrenaline (1 μM), which is the typical sympathetic neurotransmitter, mimicked the nerve actions; they also caused a remarkable increase in glucose and lactate output and a reduction of flow. With ATP the peak values of both metabolic and hemodynamic responses were higher yet also more retarded than those of nerve stimulation. Flow reduction showed a recovery during continued ATP infusion, i.e. an escape phenomenon (fig.1). With noradrenaline the peak value of the metabolic response was similar to and those of the hemodynamic changes smaller than the effects of nerve stimulation (fig.1). Arachidonic acid (100 μM), the precursor of eicosanoid synthesis, caused a qualitatively similar, yet clearly smaller response than the other stimuli (fig.1).

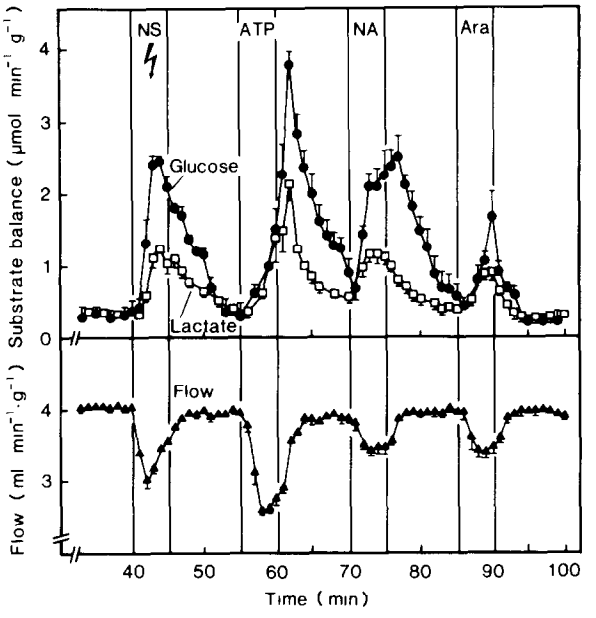

Fig.1. Glucose and lactate balance and perfusion flow in perfused rat liver following perivascular nerve stimulation and ATP, noradrenaline and arachidonate infusion. Livers were perfused in situ without recirculation via the portal vein with erythrocyte-free media containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. The nerve plexus around the portal vein and hepatic artery was stimulated (20 V, 20 Hz, 2 ms; NS), then ATP (100 μM), noradrenaline (1 μM, NA) and arachidonate (100 μM, Ara) were infused. Values are means ± SE of 3 separate determinations each.

3.2. *Effect of inhibitors of eicosanoid synthesis on the metabolic changes caused by the various stimuli*

The liver was perfused with inhibitors of eicosanoid synthesis 20 min prior to the first stimulation. BPB, a phospholipase A_2 inhibitor [17], suppressed the increase in glucose output caused by nerve stimulation, measured as the area under the curve, remarkably by almost 80%. Indo, a cyclooxygenase inhibitor [18], and NDGA, a lipoxygenase inhibitor [19], also inhibited glucose output after nerve stimulation by about 70 and 40%, respectively. Only BPB and not Indo or NDGA reduced lactate output significantly by about 60% (fig.2). The effects of ATP on glucose output were partially suppressed by the inhibitors, whereas those on lactate balance remained unaltered (fig.2). The metabolic effects of noradrenaline were not significantly influenced by the inhibitors (fig.2). On the other hand, arachidonic acid-induced metabolic changes were inhibited specifically and almost completely by Indo but not by BPB or NDGA (fig.2).

3.3. *Effect of inhibitors of eicosanoid synthesis on the hemodynamic changes caused by the various stimuli*

The decrease of flow caused by nerve stimulation, measured as the area under the curve, was suppressed significantly with BPB by about 85% and with Indo by about 45%. The ATP-induced flow change was reduced with the inhibitors partially by about 50%. The hemodynamic effect of noradrenaline was also inhibited by the inhibitors, yet only the inhibition with BPB by about 65% was statistically significant. The hemodynamic effects of arachidonic acid were inhibited specifically by Indo, but not by BPB or NDGA.

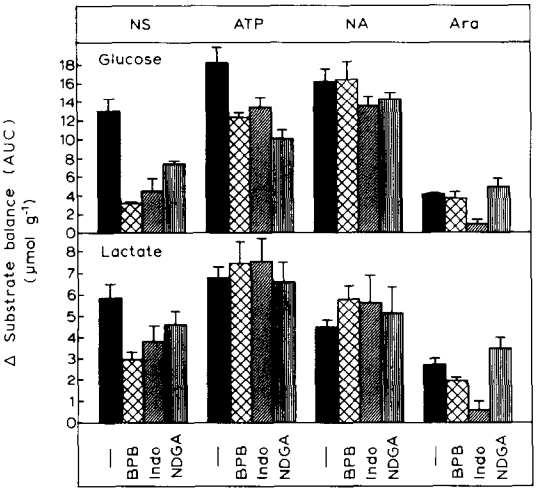

Fig.2. Effect of inhibitors of eicosanoid synthesis on the alterations of glucose and lactate balance in perfused rat liver induced by perivascular nerve stimulation and ATP, noradrenaline and arachidonate infusion. Livers were perfused as in fig.1. BPB, Indo and NDGA were added to the media at final concentrations of 20 μM each 20 min before the onset of nerve stimulation (NS), which preceded the infusions of ATP, then noradrenaline (NA) and finally arachidonate (Ara). Alterations are expressed as the areas under the curve (AUC) in μmol·g^{-1}. Values are means ± SE of 3 perfusions each.

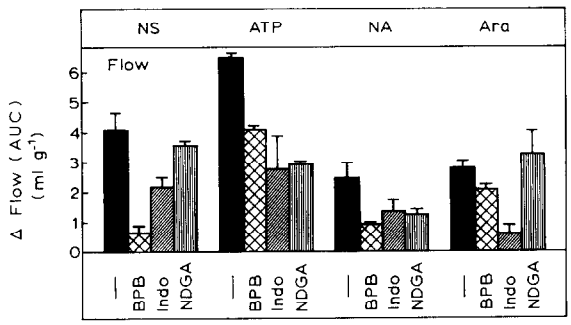

Fig.3. Effect of inhibitors of eicosanoid synthesis on the decrease of perfusion flow in perfused rat liver induced by perivascular nerve stimulation and ATP, noradrenaline and arachidonate infusion. Livers were perfused as in fig.1; the inhibitors BPB, Indo and NDGA were added and the stimuli applied as in fig.2. Alterations are expressed as the areas under the curve (AUC) in ml · g^{-1}. Values are means ± SE of 3 perfusions each.

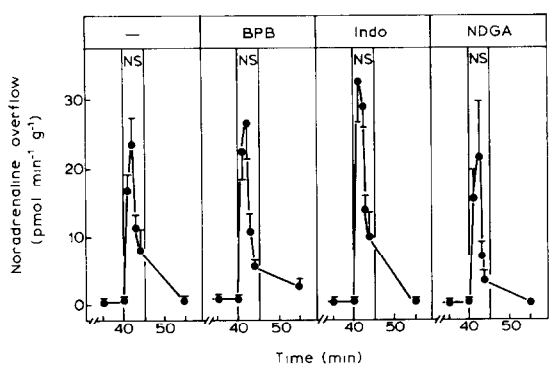

Fig.4. Noradrenaline overflow into the hepatic vein induced by perivascular nerve stimulation with or without inhibitors of eicosanoid synthesis. Livers were perfused as in fig.1; the inhibitors BPB, Indo and NDGA were added and the nerves stimulated as in fig.2. Noradrenaline was determined in the effluent before, during and after nerve stimulation. Values are means ± SE of 3 perfusions each.

3.4. *Noradrenaline overflow during nerve stimulation*

In order to determine whether the inhibitors of eicosanoid synthesis suppressed the nerve action by blocking noradrenaline release from the nerve terminals, noradrenaline overflow into the effluent was measured during nerve stimulation with or without inhibitors, Noradrenaline overflow was not significantly influenced by any of the inhibitors (fig.4).

4. DISCUSSION

It has been shown here with perfused rat liver that: (i) inhibitors of eicosanoid biosynthesis strongly inhibit the increase in glucose and lactate release caused by hepatic nerve stimulation, but only slightly so or not at all that caused by extracellular ATP and noradrenaline; and (ii) the inhibitors reduced the decrease of perfusion flow elicited by nerve stimulation, ATP and noradrenaline (figs 2,3).

4.1. *Comparison of the effects of nerve stimulation with those of ATP and noradrenaline*

Stimulation of the hepatic nerve plexus in perfused rat liver was previously shown to cause predominantly sympathetic effects mediated via α-receptors [4,5]. Therefore, the nerve effects were compared to the actions of noradrenaline, the accepted sympathetic neurotransmitter, and of ATP, a possible sympathetic 'cotransmitter' [23]. In perfused liver noradrenaline has been shown previously to increase glucose and lactate output and to decrease perfusion flow under an enforced constant pressure [5–7] or to increase portal pressure at constant flow [10]; similarly, ATP has been reported to increase glucose output and portal pressure [24,25].

In the present study, ATP and noradrenaline infusion caused qualitatively similar changes to nerve stimulation; however, the kinetics and extent of the changes were different (fig.1). Apparently, infusion of the accepted neurotransmitter noradrenaline and of the putative cotransmitter ATP could not mimick the nerve actions exactly.

4.2. *Eicosanoids as possible mediators or modulators of nerve action*

In isolated rat liver both the metabolic and hemodynamic effects caused by nerve stimulation were inhibited by BPB, Indo and NDGA, inhibitors of eicosanoid synthesis (figs 2,3). The finding that noradrenaline overflow after nerve stimulation was not influenced by these inhibitors (fig.4) indicated that they did not inhibit the nerve function unspecifically but that they acted primari-

ly as inhibitors of eicosanoid synthesis. Since the inhibitory effects of the cyclooxygenase inhibitor Indo were greater than those of the lipoxygenase inhibitor NDGA, and arachidonic acid-induced changes were specifically inhibited by Indo, prostaglandins and/or thromboxanes might be more important than leukotrienes as mediators or modulators of nerve action. If, as appears to be accepted [15,16], eicosanoids can be formed in the liver only by non-parenchymal rather than by parenchymal cells, the results of the present study indicate that the endothelial and Kupffer cells of the sinusoids play an important role as mediator and/or modulator cells for sympathetic nerve actions on liver metabolism and hemodynamics. Since noradrenaline overflow from the nerve endings was not altered by the inhibitors (fig.4), it appears that eicosanoids did not act on the nerve cells [26] but on parenchymal liver cells.

Since the metabolic effects of ATP were slightly inhibited and the hemodynamic effects clearly inhibited by the inhibitors (figs 2,3), it may be concluded that eicosanoids could also be involved as mediators or modulators of ATP action. With respect to metabolic changes, such a function can be expected to be less important, since ATP has been reported to act on parenchymal cells directly [27–30].

The finding that the metabolic effects of noradrenaline were not affected by the inhibitors, while the hemodynamic actions were clearly reduced (figs 2,3), indicates that eicosanoids may play a role in the control of liver blood flow but not of metabolism by circulating noradrenaline.

A role of eicosanoids in the regulation of liver functions was suggested previously for the action in isolated perfused liver of platelet-activating factor on glycogenolysis [31], of zymosan on portal pressure [32] and of heat-aggregated immunoglobulin G on glycogenolysis and portal pressure [33]. A possible involvement of eicosanoids in nerve action was also indicated by the finding in anaesthetized rabbits in vivo that glycogen phosphorylase activation by preganglionic stimulation of the splanchnic, rather than by postganglionic stimulation of the hepatic nerves, was inhibited by intraperiportal infusion of Indo [34]. Since prostaglandins of the E series were shown to inhibit glucagon-stimulated glycogenolysis and cAMP formation [35,36], the possibility has to be considered that prostaglandins of the F series or thromboxanes may be involved in mediating metabolic nerve action.

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