Activation of glycogenolysis and norepinephrine overflow in the perfused rat liver during repetitive perivascular nerve stimulation

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During in situ perfusion of rat liver stimulation of nerve bundles around hepatic artery and portal vein resulted in an increase of glucose output, a switch from lactate uptake to output and in a decrease of portal flow. These effects remained essentially the same during 3 stimulation periods at 20 min intervals; norepinephrine overflow, however, was strongly decreased during the second and third period. The metabolic and hemodynamic effects were not correlated to norepinephrine overflow during repetitive stimulations and during stimulations in the presence of norepinephrine, phentolamine, propranolol or desipramine.

1. INTRODUCTION

In intact animals pre-ganglionic stimulation of the splanchnic nerve [1–5] and post-ganglionic stimulation of the hepatic nerves [6–10] were found to increase glucose output by the liver in rabbit [1–4], cat [5–8], dog [5,9], pig [5], sheep [5] and man [10]. In perfused liver preparations pre-ganglionic [11] and post-ganglionic [12–15] nerve stimulation was also shown to enhance glucose output in toad [11], mouse [12] and rat [13–15]. The effects of hepatic nerves have been reviewed in [16,17]. In all of these studies the nerves were stimulated applying ~20 V for 0.5–2 ms at 20 Hz during a single period only ranging from 1–30 min; the effectiveness of repetitive nerve stimulation is unknown.

The nerve-dependent increase of glucose output was due predominantly to an α-adrenergic activation of glycogenolysis in the fed animal [12,13] and to an increase of gluconeogenesis in the fasted animal [15]. During peripheral adrenergic neurotransmission norepinephrine released into the synaptic cleft appears in part in the venous blood of the innervated organ; this transmitter overflow has been studied mainly in the spleen, heart and colon [18]. Norepinephrine overflow from the liver after stimulation of hepatic nerves has been studied so far only in the anesthetized dog [19]. In this investigation norepinephrine overflow was measured only at one time point (i.e., in a cumulative manner); the kinetics of the process were not reported.

It was the object of this study to investigate in the perfused liver the metabolic effectiveness of repetitive nerve stimulations, the kinetics of norepinephrine overflow and the possible correlation between metabolic effects and overflow. It was found that the change of glucose and lactate output from livers of fed rats and the reduction of blood flow remained essentially the same during three 5 min periods of stimulation at 20 min intervals, while the overflow of norepinephrine was strongly decreased during the second and third stimulation period. Apparently the metabolic and hemodynamic effects of the hepatic nerves were not correlated to norepinephrine overflow.

2. MATERIALS AND METHODS

All chemicals were reagent grade and from commercial sources. Enzymes were purchased from Boehringer (D-6800 Mannheim); bovine serum
albumin from Serva (D-6900 Heidelberg); and S-[\[^3\]H]adenosylmethionine (SAM) from Amersham-Buchler (D-3300 Braunschweig). Norepinephrine was supplied by Fluka (CH-9470 Buchs); propranolol by Rhein-Pharma (D-6831 Planckstadt); phenolamine and desipramine were a gift from Ciba-Geigy (D-7867 Wehr).

Male Wistar rats (130–160 g) were kept on a 12 h day–night rhythm, 7–19 h, with free access to food, standard diet 1320 of Altromin (D-4937 Lage). Experiments were started at 9.30 h. The technique of the in situ perfusion with media containing 5 mM glucose, 2 mM lactate, erythrocytes, bovine serum albumin and Krebs-Ringer bicarbonate buffer and of the perivascular nerve stimulation (20 V, 2 ms, 20 Hz) has been described in [13].

Glucose and lactate concentrations were measured photometrically with standard enzymatic methods using hexokinase (EC 2.7.1.1) plus glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and lactate dehydrogenase (EC 1.1.1.27), respectively. Norepinephrine was quantitated radiochemically [20] after enzymatic methylation with [\[^3\]H]SAM, conversion to [\[^3\]H]normetanephrine and separation by thin-layer chromatography.

3. RESULTS

Rat liver was perfused without recirculation offering 5 mM glucose and 2 mM lactate for 40 min. Perivascular nerve stimulation for the next 5 min resulted in an increase of glucose output, in a switch from lactate uptake to output, in a decrease of portal flow and in the overflow of norepinephrine (fig. 1). Epinephrine or dopamine were not found in the perfusate. Peak values were reached for glucose and lactate release after 4 min, for the flow change after 2 min and for norepinephrine release after 45 s. Thus, neurotransmitter overflow

Fig. 1. Substrate exchange, portal flow and norepinephrine overflow during repetitive electrical nerve stimulation in the rat liver perfused in situ. Hatched columns represent periods of stimulation. Values are means ± SEM of 4 expt.
was by far the fastest effect caused by nerve stimulation.

During a second and third stimulation period after 65 min and 90 min of perfusion, respectively, the same alterations of substrate exchange and of portal flow, slightly diminished to 70–85%, were observed; yet norepinephrine overflow was clearly reduced to ∼20% (fig. 1). Apparently, the metabolic and hemodynamic nerve effects were not correlated to neurotransmitter overflow.

The diminished norepinephrine overflow during the repetitive stimulations may be caused by a partial depletion of the neurotransmitter in the hepatic nerve endings, where its level is the resultant of release into and uptake, respectively reuptake from the synaptic cleft, of de novo synthesis and degradation. There could have been no uptake in the periods before and after stimulations since norepinephrine was absent from the synthetic perfusion medium. Therefore, norepinephrine in a physiological concentration (5 nM) [21], which by itself was ineffective, was infused to allow uptake. However, norepinephrine overflow during the second and third stimulations was diminished to the same extent in the presence as in the absence of circulating norepinephrine (not shown). Presumably norepinephrine could not be taken up under the conditions used or norepinephrine depletion was not the cause for diminished overflow during repetitive nerve stimulations.

The apparent lack of correlation between the neurotransmitter overflow and the metabolic and hemodynamic nerve effects was further corroborated in experiments using a sympathetic agonist, an α- and β-antagonist and reuptake inhibitor (table 1). In the presence of all these agents the kinetics of the overflow were very similar to those of the control experiment, yet different peak values were reached (fig. 2). The agonist norepinephrine increased glucose output slightly, yet reduced neurotransmitter overflow. The α-antagonist phentolamine abolished the metabolic and hemo-

### Table 1

Substrate exchange, portal flow and norepinephrine overflow following electrical nerve stimulation in the presence of adrenergic agonists, antagonists and reuptake inhibitors in the rat liver perfused in situ

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Increase in output of Glucose (µmol (C₆)·min⁻¹·g liver⁻¹)</th>
<th>Decrease in portal flow (ml·min⁻¹·g liver⁻¹)</th>
<th>Norepinephrine overflow (µmol·min⁻¹·g liver⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.07</td>
<td>0.56</td>
<td>50.35</td>
</tr>
<tr>
<td>1st stimulation</td>
<td>(+ 0.07)</td>
<td>(+ 0.07)</td>
<td>(+ 2.89)</td>
</tr>
<tr>
<td>Control</td>
<td>0.88</td>
<td>0.48</td>
<td>11.29b</td>
</tr>
<tr>
<td>3rd stimulation</td>
<td>(+ 0.12)</td>
<td>(+ 0.05)</td>
<td>(+ 3.65)</td>
</tr>
<tr>
<td>Agonist</td>
<td>1.36</td>
<td>0.60</td>
<td>34.91a</td>
</tr>
<tr>
<td>norepinephrine</td>
<td>(+ 0.27)</td>
<td>(+ 0.07)</td>
<td>(+ 7.31)</td>
</tr>
<tr>
<td>α-Antagonist phentolamine</td>
<td>0.15b</td>
<td>0.09b</td>
<td>105.75a</td>
</tr>
<tr>
<td>β-Antagonist propranolol</td>
<td>0.05</td>
<td>0.04</td>
<td>32.67b</td>
</tr>
<tr>
<td>Reuptake inhibitor</td>
<td>0.89</td>
<td>0.48</td>
<td>30.93b</td>
</tr>
<tr>
<td>Calcium-poor</td>
<td>2.11b</td>
<td>0.49</td>
<td>77.43a</td>
</tr>
</tbody>
</table>

Electrical stimulation of perivascular nerve bundles was performed for 5 min starting 40 min after the onset of perfusion. The effectors were added and omitted (calcium) at the start of the experiment (0 min), respectively. Final concentrations were 5 nM norepinephrine, 50 µM phentolamine, 10 µM propranolol and 1 µM desipramine. The metabolic, hemodynamic and overflow values represent the difference between the basic and the peak values (see fig. 1). Values are means ± SEM given in parentheses of 3 expt each except for the control and norepinephrine which were 4 each.

Student's *t*-test: *P < 0.05, †P < 0.01

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Fig. 2. Kinetics of norepinephrine overflow after nerve stimulation in the presence of adrenergic antagonists and a reuptake inhibitor in the rat liver perfused in situ. The hatched column represents the period of stimulation. Values are means of 3 expt each; the SEM is not shown to improve legibility of the figure, it is given for the peak values in table 1. For further experimental information see also table 1.

Abbreviations: αBA, α-blocking agent (phentolamine); β-BA, β-blocking agent (propranolol); DMI, desipramine (reuptake inhibitor)

dynamic nerve effects but increased the overflow. The β-antagonist propranolol had no effects on the metabolic and hemodynamic parameters, yet the neurotransmitter overflow was reduced. Finally, the reuptake inhibitor desipramine clearly increased the nerve-dependent glucose output, left the lactate exchange and the flow reduction unaltered and enhanced the overflow. With a calcium-poor medium (overall level ≤ 300 μmol/l) nerve stimulation did neither elicit a change of the substrate balance and of the portal flow nor any norepinephrine overflow.

4. DISCUSSION

Here we show that repetitive stimulation of the hepatic nerves resulted in essentially the same increase of glucose and lactate output and decrease of portal flow (fig. 1). Apparently, even during 120 min perfusion including 15 min neurostimulation, the functional neurotransmitter content in the hepatic nerve endings was kept over the level required for normal signal propagation, although with the amino acid and neurotransmitter free medium used, replenishment by synthesis or by uptake was unlikely or even impossible. Both the metabolic and hemodynamic effects showed an ‘escape’ phenomenon [22], in that the maximal changes reached could not be maintained in spite of a continued neurostimulation. After a 20 min interval the liver was no longer refractory to another stimulation period. The mechanism of the development and disappearance of this refractoriness is not understood.

Stimulation of hepatic nerves was accompanied by an overflow of norepinephrine, which was clearly diminished during the second and third stimulation period, although the metabolic and hemodynamic effects remained the same (fig. 1). If the neurotransmitter overflow is proportional to release [13,18], the finding indicates that at least during the first stimulation period but possibly also during the second and third period more neurotransmitter was released than required for signal transmission.

During all consecutive stimulation periods (fig. 1) and during the stimulation in the presence of the sympathetic agonist, the α- and β-antagonist and the reuptake inhibitor, overflow of norepinephrine was progressively reduced after an initial peak with the same kinetics (fig. 2). This phenomenon, termed ‘depletion response’, has been observed also during sustained sympathetic stimulation of the heart in anaesthetized dogs [24,25]. It might be due to negative feedback inhibition of release via presynaptic α2-receptors [26]. However, the unaltered depletion kinetics during blockade of α-receptors with phentolamine are at variance with this possibility. The phenomenon might also be caused by a gradual increase of neurotransmitter reuptake. Yet, the unchanged overflow kinetics in the presence of the reuptake inhibitor desipramine do not support this mechanism. Furthermore, it has been postulated that most of the norepinephrine in the nerve endings is contained in a storage pool and that only a minor part is in a secretory pool, which is depleted rapidly upon nerve stimulation, but replenished rather slowly from the storage pool
The present findings are not in contradiction to the 'two pools' proposal, but they do not prove it.

Finally, it was found that under a variety of conditions the metabolic and hemodynamic nerve effects were not correlated to norepinephrine overflow (table 1). Neurotransmitter overflow alone was in accord with expectations [18, 23]: It was decreased by the α-agonist norepinephrine and increased by the α-antagonist phenolamine; both can be explained by the negative feedback of release via pre-synaptic α-receptors. It was decreased by the β-antagonist propranolol, which indicates a blockade of the positive feedback of release via pre-synaptic β-receptors. It was increased by desipramine, which shows that reuptake is involved in the complex events leading to overflow. It was completely suppressed at low calcium concentrations, which is in line with the calcium requirement of the release process in [27, 28]. It thus remained open how the metabolic and hemodynamic nerve effects are quantitatively linked to the synaptic neurotransmission. Maybe the cotransmitter to norepinephrine which is not known in this system [19] plays a key role.

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REFERENCES