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Modulation by somatostatin of nerve-mediated activation of glycogenolysis in the perfused rat liver

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Perivascular nerve stimulation of rat livers perfused in situ with erythrocyte-free Krebs-Henseleit buffer at constant pressure in a non-recirculating system resulted in an increase of glucose and lactate production and in a decrease of portal flow. Infusion of somatostatin in different concentrations (2×10^{-7}, 10^{-8}, 10^{-9} mol·l^{-1}) reduced the nerve-mediated activation of glucose release maximally to 66%. There was only a slight effect on the lactate output, the nerve-mediated reduction of portal flow was unaltered. In controls, somatostatin alone had no effect on the metabolic and hemodynamic parameters. In order to differentiate between a presynaptic and postsynaptic mechanism, the noradrenaline overflow was calculated. The unaltered release of the neurotransmitter in the presence or absence of somatostatin excluded a presynaptic mechanism. To mimic the nerve effects on the carbohydrate metabolism and on the hemodynamics, noradrenaline (2×10^{-7} mol·l^{-1}) was infused instead of the nerve stimulation over a period of 5 min. Somatostatin did not change the endocrine effects of the catecholamine under these conditions. The nerve-dependent effect of somatostatin suggests that other neurotransmitters (e.g. VIP) or mediators (e.g. prostanoids) may be influenced by somatostatin.

Perfused liver; Noradrenaline; Somatostatin; Carbohydrate metabolism; (Rat, Hepatic nerve)

1. INTRODUCTION

The liver parenchyma is innervated by sympathetic and parasympathetic fibers of the autonomic nervous system [1]. Various metabolic pathways were reported to be regulated by the hepatic nerves [2–4]. In the in situ perfused rat liver electrical stimulation of the hepatic nerves around the hepatic artery and portal vein was shown to increase glucose and lactate output [5] as well as urate and allantoin formation [6] and to reduce ketogenesis [7], urea release and ammonia uptake [8] as well as oxygen consumption [9,10]. The nerve action could be blocked by the α-blocking agent phentolamine and therefore, it was predominantly mediated by α-receptors [5]. The effects on the carbohydrate metabolism were reported to be modulated by insulin and glucagon [11]. Insulin inhibited the nerve-mediated activation of glucose release in a dose-dependent manner, had no effects on the lactate output and the portal flow. Dependent on the hormone concentration, the effects of glucagon and nerve stimulation on the glucose output were additive, on the lactate balance antagonistic.

In vivo, the autonomic nerves interact with circulating hormones and mediators of hormone effects. The investigations dealing with the interfering actions of hormones and nerves are supposed to show aspects of the in vivo situation. The interactions should not only be focused on the effects of the neurotransmitter, e.g. noradrenaline

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Abbreviations: HPLC, high-pressure liquid chromatography; VIP, vasoactive intestinal polypeptide

Enzymes: Glucose dehydrogenase (EC 1.1.1.47); glutamate-pyruvic transaminase (EC 2.6.1.2); lactate dehydrogenase (EC 1.1.1.27)

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for the sympathetic nervous system, and the circulating hormones, but also on modulating effects of co-transmitters histochemically described in the liver parenchyma as VIP (vasoactive intestinal peptide) and neurotensin [12,13].

The present study shows that somatostatin modulates the nerve effects. Possible mechanisms of the hormone action were characterized.

2. MATERIALS AND METHODS

2.1. *Materials*

All chemicals were reagent grade and from commercial sources. Enzymes and bovine serum albumin were purchased from Boehringer, D-6800 Mannheim. Synthetic cyclic somatostatin were from Serono, D-7800 Freiburg; L-noradrenaline from Sigma, D-8020 Taufkirchen.

2.2. *Animals*

Male Wistar rats (180–240 g body wt) were obtained from Versuchstieranstalt, D-3000 Hannover. They were kept on a 12 h day-night rhythm with free access to food (standard diet 1320 of Altromin, D-4937 Lage) and to water.

2.3. *Liver perfusion*

All experiments were started between 9 and 11 a.m. when the livers are still full of glycogen [14]. The rats were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body wt). Livers were perfused in situ with a medium containing a Krebs-Henseleit-bicarbonate buffer equilibrated with 95% (v/v) oxygen and 5% (v/v) carbon dioxide. The perfusion pressure was constant at about 10 cm H_2O with a flow rate of 4 $ml \cdot min^{-1} \cdot g^{-1}$ liver.

2.4. *Nerve stimulation and infusion of noradrenaline*

The perivascular hepatic nerves were stimulated by a bipolar platinum electrode placed around the hepatic artery and portal vein (20 V, 20 Hz, 2 ms) for over 5 min. In order to mimic the nerve effects noradrenaline was infused in a concentration of 2×10^{-7} $mol \cdot l^{-1}$ over the same period. In different concentrations (10^{-9}, 10^{-8}, 2×10^{-7} $mol \cdot l^{-1}$), the infusion of somatostatin was started 5 min before the onset of nerve stimulation or before the start of infusion of noradrenaline over a period of 10 min.

2.5. *Determination of metabolites and noradrenaline*

Glucose and lactate were determined by standard enzymatic techniques using glucose dehydrogenase and lactate dehydrogenase, glutamate-pyruvic transaminase, respectively. Noradrenaline was quantitated by electrochemical detection after separation with high-pressure liquid chromatography [15].

3. RESULTS

Perivascular stimulation of the hepatic nerves over a period of 5 min resulted in an increase of glucose and lactate output reaching the maximum value after about 5 min. The portal flow was decreased to 70% after 2 min. During the nerve stimulation, the portal flow began to return to the prestimulation level (fig.1A). In the presence of somatostatin (2×10^{-7} $mol \cdot l^{-1}$), nerve stimulation led to the activation of glucose release, however, to a minor extent (66%, area under the curve calculated). Somatostatin also reduced the increase of lactate output. Almost the same results were obtained for the glucose and lactate output in the presence of 10^{-8} $mol \cdot l^{-1}$ somatostatin (fig.2). Infusing a lower concentration of somatostatin (10^{-9} $mol \cdot l^{-1}$) the effects were less pronounced (fig.2).

The diminution of the portal flow after nerve stimulation was not influenced by somatostatin excluding a hemodynamic action of the hormone in this model. The data show that somatostatin modulates the nerve-mediated effects on the carbohydrate metabolism in a dose-dependent manner.

Somatostatin acts either on the presynaptic or on the postsynaptic site of the nerve action. The presynaptic interaction of the nerve release and somatostatin was determined by measuring the noradrenaline overflow. The noradrenaline overflow was reported to be a good parameter for the quantification of noradrenaline released into the synaptic cleft [16]. The data show that the hor-

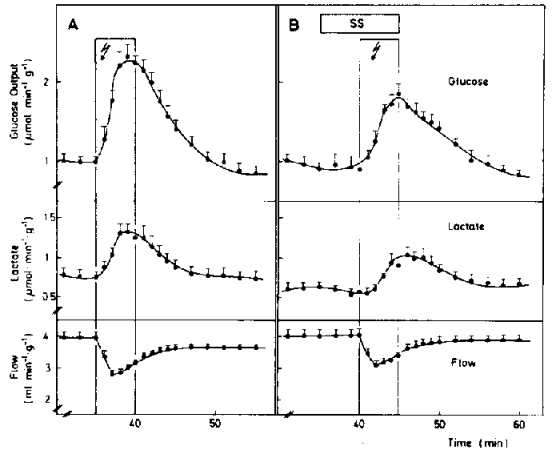

Fig.1. Increase of glucose and lactate output and decrease of portal flow after nerve stimulation (20 Hz, 20 V, 2 ms) over a period of 5 min in the perfused rat liver in the absence (A) and presence (B) of 2×10^{-7} $mol \cdot l^{-1}$ somatostatin. The values given are means ± SE of 7 and 6 experiments, respectively.

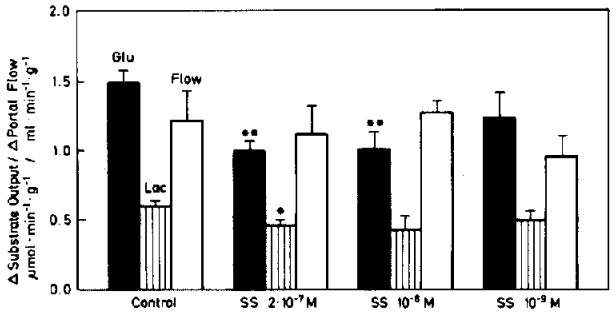

Fig.2. Increase of glucose (Glu) and lactate (Lac) output (area calculated under the curve) and decrease of portal flow (Flow) after nerve stimulation over a period of 5 min in the perfused rat liver in the absence and presence of somatostatin (SS) at different concentrations (2×10^{-7}, 10^{-8}, 10^{-9} mol·l^{-1}). Values are means ± SE of 7, 6, 5 and 3, respectively. Asterisks denote significant differences (*$p < 0.05$, ** $p < 0.005$) as compared to the control group.

mone did not change the noradrenaline overflow (table 1) and therefore exclude a presynaptic effect of somatostatin.

Previous studies [5] demonstrated that the nerve effects on the carbohydrate metabolism and the hemodynamics could be mimicked by the infusion of noradrenaline into the portal vein. The equipotent concentration was about 2×10^{-7} mol·l^{-1} [5]. In the absence or presence of the high concentration of somatostatin (2×10^{-7} mol·l^{-1}), the metabolic changes on glucose and lactate output by infused noradrenaline did not significantly differ (table 2). These results exclude an antagonism of the neurotransmitter noradrenaline and somatostatin on the receptor/postreceptor level.

Table 1

The maximum noradrenaline overflow in the outflow after nerve stimulation in the perfused rat liver in the absence and presence of somatostatin (SS)

Conditions	Number of experiments	Noradrenaline (pmol·min^{-1}·g^{-1})
Control	5	60.46 ± 15.40
+ SS (2×10^{-7} mol·l^{-1})	4	56.00 ± 4.76
+ SS (10^{-8} mol·l^{-1})	4	72.80 ± 15.96

Values are given as means ± SE

Table 2

Increase of glucose and lactate output and decrease of portal flow after infusion of noradrenaline (2×10^{-7} mol·l^{-1}) in the perfused rat liver in the absence and presence of somatostatin (SS)

Infusion of	Glucose (μmol·min^{-1}·g^{-1})	Lactate (μmol·min^{-1}·g^{-1})
Noradrenaline alone	1.21 ± 0.16	0.48 ± 0.03
Noradrenaline + SS (2×10^{-7} M)	1.22 ± 0.11	0.44 ± 0.04

Values are given as means ± SE of 4 experiments, each

4. DISCUSSION

In the present investigation it was shown that somatostatin modulated the nerve-mediated activation of glucose release in the perfused rat liver.

Somatostatin reduced the enhancement of hepatic glucose production without inhibiting the alteration of portal flow. The basal glucose release of the liver was not changed by the peptide. These effects are comparable to the action of insulin. Insulin was reported to inhibit the enhancement of glycogenolysis triggered by the hepatic nerves [11]. The effects of somatostatin on the basal and hormone-stimulated glucose production of the liver are discussed controversially. There are studies reporting no effect of somatostatin on the carbohydrate metabolism in the perfused rat liver [17], rat liver slices [18] and isolated rat hepatocytes [19]. In contrast to these findings an inhibition of glucagon-stimulated glycogenolysis was reported in the perfused liver [20] and isolated rat hepatocytes [21,22].

The physiological role of circulating somatostatin is difficult to determine. Many effects of the hormone were described using pharmacological doses in a range of 10^{-7} mol·l^{-1} [20–22]. In the portal vein plasma levels of maximal 500 pg/ml were reported [23]. This corresponds to about 2×10^{-10} mol·l^{-1} taking into account that somatostatin-14 and somatostatin-28 are the two principal biologically active forms of the hormone. However, the values given in the literature have to be critically interpreted because somatostatin in plasma is rapidly degraded. In the present study an effect of somatostatin was observed in the range of 10^{-8} mol·l^{-1}. This suggests that somatostatin is not active in a physiological range. However, it is well known that isolated systems as

the perfused rat liver is less sensitive to hormone actions than the in vivo organ.

In the present study there was no antagonism between the glycogenolytic hormone noradrenaline and somatostatin. The mechanism of nerve action is more complex than an interaction of two hormones. Probably, the nerve effects are mediated by interactions of the nerve endings of the neurons, the non-parenchymal cells and parenchymal cells [13,24]. The presynaptic site of action was excluded by the determination of the unaltered overflow of noradrenaline. However, a second transmitter which is involved in the nervous regulation of hepatic carbohydrate metabolism could be influenced by somatostatin. One candidate is VIP (vasoactive intestinal peptide) functioning as a neurotransmitter especially in the gastrointestinal tract. Anatomical studies demonstrated VIP immunohistochemically in the liver [12]. VIP was shown to activate the glycogenolysis in isolated hepatocytes. The enhanced glucose production could be inhibited by somatostatin [25]. Therefore, one may speculate that somatostatin blocks the release of VIP acting as a co-transmitter which reduces the nerve-mediated activation of glucose output. In the present study VIP was not detected in the outflow of the perfused liver (data not shown). This could be due to a release of VIP after nerve stimulation too little to be detectable and/or to a rapid degradation in the synaptic cleft.

Recently, it was shown that inhibitors of the synthesis of prostanoids reduced the nerve-mediated effects [13]. Prostaglandins were reported to be released after nerve stimulation [25]. These studies demonstrated the role of prostanoids synthesized by non-parenchymal cells in mediating the effects of hepatic nerves. In the present study one may suggest that somatostatin interferes with the synthesis or release of these mediators.

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