Regulation of oxygen consumption and microcirculation by α-sympathetic nerves in isolated perfused rat liver

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In isolated rat liver perfused at constant flow with erythrocyte-free Krebs-Henseleit bicarbonate buffer containing 5 mM glucose and 2 mM lactate, perivascular stimulation of the hepatic nerves caused a rapid decrease of oxygen uptake, a decrease of the periportal and, after a transient rise, of the perivenous tissue p_{O_2} of surface acini, an increase of portal pressure, and an enhancement of glucose output. Furthermore, nerve stimulation changed the intrahepatic distribution of the perfusate drastically. Infusion of trypan blue 20 s after nerve stimulation resulted in a heterogeneous staining of the liver both at the surface and in cross-sections, while it led to a homogeneous distribution in non-stimulated controls. It is concluded that the major component in the mechanism of the nerve-dependent decrease of oxygen uptake is the microcirculatory change rather than a metabolic effect.

Oxygen consumption *Rat liver* *Sympathetic nerve* *Microcirculation* *Zonal tissue* p_{O_2}

1. INTRODUCTION

In intact animals postganglionic stimulation of the hepatic nerves was found to increase hepatic glucose output, to decrease conductance in both the hepatic artery and the portal vein and to transiently lower hepatic oxygen uptake (review [1]). In perfused liver preparations, stimulation of the hepatic nerves around the portal vein and the hepatic artery was also reported to enhance glucose release [2–5], to lower portal flow [3–5] and, in a preliminary report, to decrease oxygen uptake [6]. The nerve action was predominantly α-sympathetic, since it could be inhibited by the α-blocker phentolamine [3,6].

In the perfused liver [6–9] and in isolated hepatocyte suspensions [10–12], phenylephrine or noradrenaline were found to increase oxygen uptake. The discrepancy between the decrease of oxygen consumption by the stimulation of α-sympathetic nerves and the increase of oxygen consumption by a circulating α-agonist may be due to a complex mechanism with opposing components: metabolic effects may lead to an increase, and hemodynamic, microcirculatory effects to a decrease in oxygen uptake.

Therefore the purpose of the present investigation was to study in the perfused rat liver the effect of sympathetic nerves on oxygen consumption, on microcirculation and on tissue oxygen tension in periportal and perivenous areas of surface acini, which has become possible with the recent development of miniature oxygen electrodes [13].

It was found that the nerve-dependent decrease of oxygen uptake was accompanied by an increase of the portal pressure and by a drastic change of the intrahepatic distribution of the perfusate. This microcirculatory change rather than a metabolic effect appears to be primarily responsible for the decrease of oxygen consumption.

2. MATERIALS AND METHODS

All chemicals were reagent grade purchased

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from commercial sources. Noradrenaline was from Fluka (CH-9470 Buchs), and phentolamine was a gift from Ciba Geigy (D-7867 Wehr). Enzymes were from Boehringer (D-6800 Mannheim).

Male Wistar rats (150–230 g) were kept on a 12 h day-night rhythm with free access to food, standard diet 1320 of Altromin (D-4937 Lage).

2.1. *Liver perfusion*

Livers were isolated under pentobarbital anaesthesia (60 mg/kg) and perfused via the portal vein with Krebs-Henseleit bicarbonate buffer (5 mM glucose; 2 mM lactate; 95% O_2/5% CO_2; 37°C; constant flow without recirculation, 5 $ml \cdot g^{-1} \cdot min^{-1}$). The liver perfusion technique employed was virtually identical with the one in [14], except that the position of the liver was inverted (the intestinal aspect up). The venous oxygen tension was continuously monitored with a Clark-type electrode and the portal pressure with a pressure transducer. Effluent samples were taken every minute to analyze glucose concentration by an enzymatic method.

2.2. *Electrical stimulation*

The hepatic nerves were stimulated with a bipolar platinum wire electrode placed perivascularly around both the portal vein and the hepatic artery which was not perfused but was still joined to the portal vein (20 V, 2 ms, 20 Hz).

2.3. *Periportal and perivenous oxygen tension*

A miniature oxygen electrode was prepared with a 50 μm-diameter platinum wire sealed in a glass capillary. The tip was formed by pulling under heat using a pipette puller (David Kopf, CA) and was covered with an oxygen permeable membrane (Rhoplex® , Rohm and Hass). The tip diameter was 30–50 μm. This electrode was used in conjunction with an Ag/AgCl reference electrode.

The periportal and perivenous regions of the liver acini were identified by the natural pigmentation of the liver surface: light areas = 'periportal', dark brown spots = 'perivenous' [13]. One miniature electrode each was placed on a periportal and a perivenous region of the liver surface and the respective oxygen tensions were measured continuously before, during and after electrical stimulation. To avoid oxygen exchange between the liver surface and surrounding air, the liver surface was covered with mineral oil. At the end of the experiments, the electrodes were calibrated on the surface of the liver fixed with 1% formaldehyde by perfusing the fixed liver with medium equilibrated with 95% N_2/5% CO_2 or 95% O_2/5% CO_2. The sensitivity of the O_2 electrode was reduced 2- to 3-fold when placed on the liver surface as compared to the sensitivity determined in a saline solution [13,15].

2.4. *Visualization of microcirculation by trypan blue infusion*

To examine microcirculatory changes during electrical stimulation, trypan blue was infused into the portal vein at a final concentration of 0.2%. Photographs were taken of the liver surface at 20 s, 5 min and 10 min after the initiation of the electrical stimulation. Infusion of the dye was initiated at zero time, at 4 min 40 s or 9 min 40 s (the lag time for arrival of the dye at the liver was 10 s). Livers were then cut into sections about 5 mm thick and immersed in a 10% formaldehyde solution. Subsequently photographs of cross-sections of the liver were taken.

3. RESULTS

3.1. *Oxygen consumption*

Electrical stimulation for 5 min resulted in a decrease in the rate of hepatic oxygen consumption reaching a minimum (−26%) after 1.1 min; the rate then began to return to the pre-stimulation level indicating an 'escape' phenomenon [16] (fig.1A, table 1). The periportal oxygen tension decreased from about 540 to 110 torr in 1 min. In contrast, the perivenous oxygen tension first increased sharply from about 235–340 torr within 10–15 s and then fell to 10–20 torr in about 1 min (fig.1B, table 1). In about half the measurements the decreased tension began to rise slightly during the latter part of the electrical stimulation.

The portal pressure was enhanced reaching a maximum (+124%) in 1.2 min followed by a gradual decrease which was probably related to the 'vascular escape from neurogenic vasoconstriction' [16] (fig.1C, table 1). The rate of glucose output first decreased slightly (−10%) and subsequently increased to a maximum (+250%) at about 4 min (fig.1D, table 1). All the nerve effects

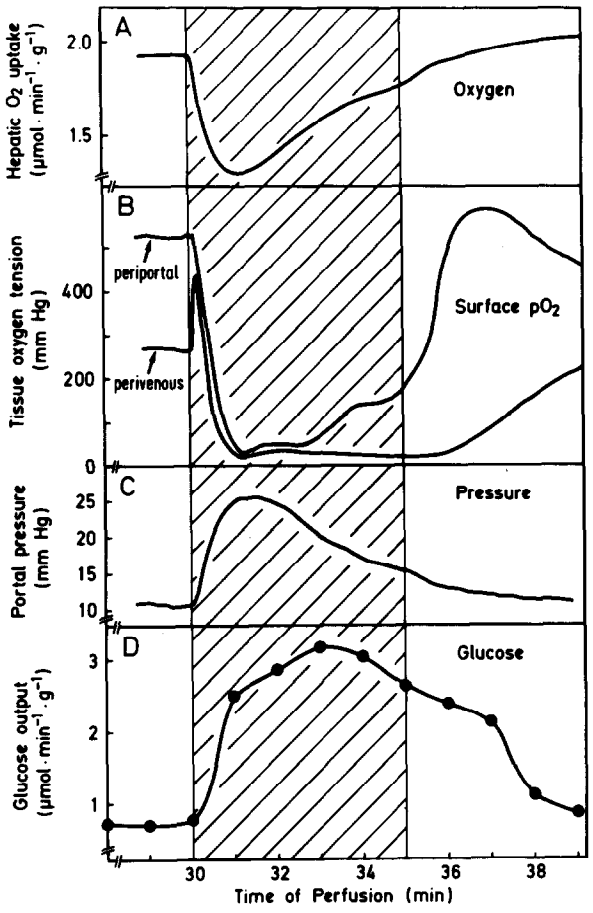

Fig.1. Kinetics of oxygen uptake, zonal tissue oxygen tension, portal pressure and glucose output in the perfused rat liver during electrical nerve stimulation. The hatched column represents the period of stimulation. A typical experiment is shown. The mean values of 4 experiments are given in table 1.

were completely abolished by the α-blocker phentolamine at 50 μM (not shown).

3.2. *Microcirculation*

Infusion of trypan blue into the liver led within 20 s to a homogeneous staining of the parenchyma both at the lobar surface and in cross-sections (fig.2, middle). However, infusion of the dye after 20 s of electrical stimulation of the hepatic nerves resulted in a heterogeneous staining of the liver at the surface and in cross-sections (fig.2, bottom). Apparently, nerve stimulation caused an intrahepatic redistribution of flow at constant

Table 1

Oxygen uptake, glucose output, zonal tissue oxygen tension and portal pressure of the perfused rat liver following electrical nerve stimulation

Parameters	*n*	Changes	
		Absolute (μmol·min^{-1}·g^{-1})	Relative (% of basal)
Hepatic O_2 uptake	5	−0.6± 0.2	−26.0± 6.8
Glucose output	5	+2.1± 0.3	+250 ±53
Tissue O_2 tension		mm Hg	
Periportal	4	−432 ±34	−79.7± 9.3
Perivenous			
Transient	3	−108 ±20	+52 ±14
Steady state	3	−231 ±53	−99 ± 1
Portal pressure	5	+12 ± 0.6	+124 ±24

The differences between the basal and the peak values (see fig.1) are given; they are means ± SE of the number of experiments indicated (*n*)

overall flow; many tissue areas were no longer adequately perfused. These inaccessible areas constituted – roughly estimated – 30% of the total tissue.

Infusion of the dye after 5 min of nerve stimulation still resulted in a heterogeneous staining, which was less pronounced than after 20 s. A homogeneous staining was observed again 10 min after the onset of nerve stimulation, which lasted for 5 min (not shown). Thus, the nerve-dependent microcirculatory change was reversible.

4. DISCUSSION

It was shown in the present investigation that stimulation of the hepatic nerves led to a decrease of oxygen consumption of the liver at constant oxygen delivery (fig.1A), to an enhancement of glucose output (fig.1D) and to an increase of portal pressure (fig.1C) combined with a redistribution of flow within the liver lobes (fig.2).

4.1. *Nervous regulation of intrahepatic microcirculation*

Stimulation of the hepatic nerves apparently 'shut down' some tissue areas, so that they could no longer be adequately perfused, while it left other areas 'open', so that they could be perfused.

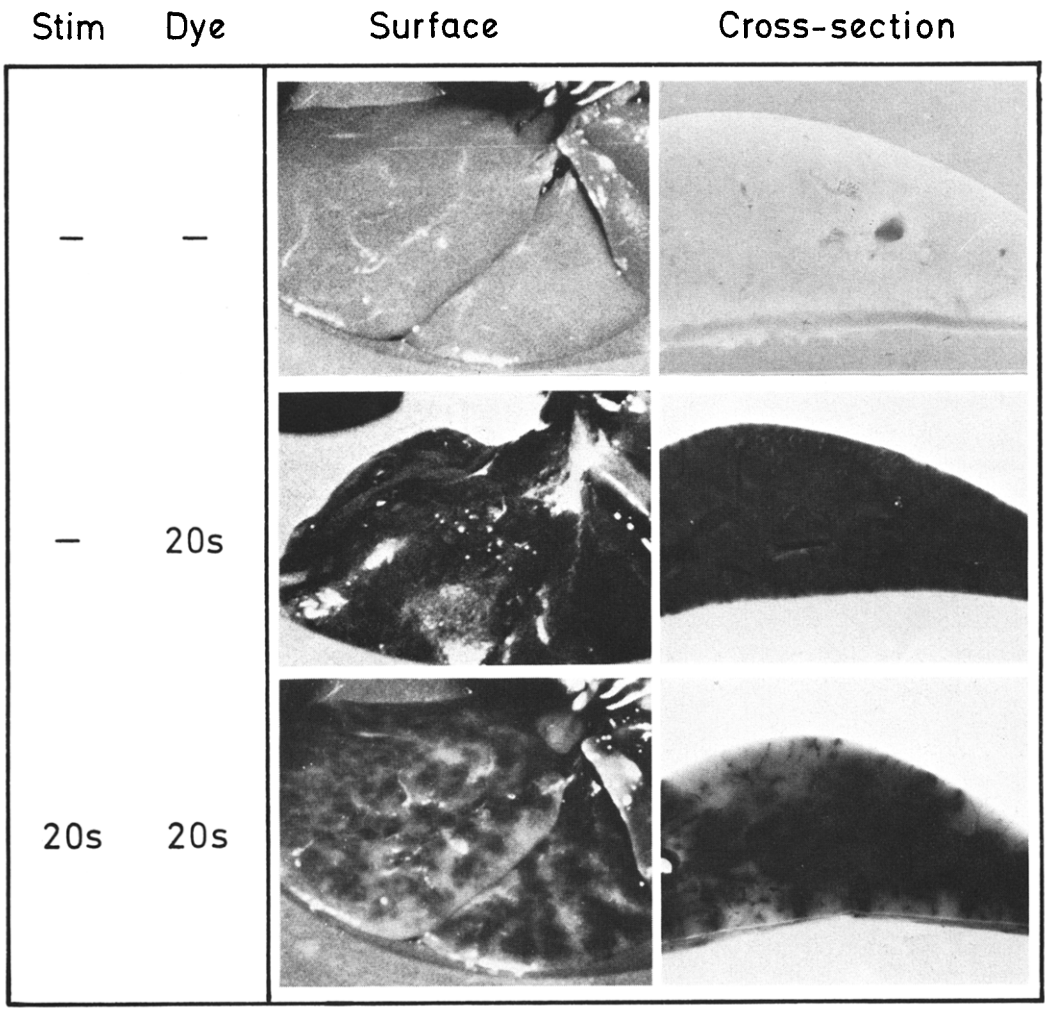

Fig.2. Intrahepatic distribution of trypan blue in perfused rat liver during electrical stimulation. The dye was infused for 20 s. The photographs of the liver surface and of cross-sections were taken at zero time without (top) and with (middle) dye infusion and at 20 s after the onset of nerve stimulation with dye infusion initiated at zero time (bottom).

Since the overall perfusion flow was kept constant, the decreased flow through the shut down parts of the tissue was balanced by an increased flow through the open parts.

A similar change of the intrahepatic circulation was observed in livers perfused at constant flow after infusion of noradrenaline (preliminary results). Such a microcirculatory alteration at unchanged overall flow has not been considered in previous work on the action of α-agonists on liver metabolism [7–10].

The redistribution of flow in the perfused rat liver after nerve stimulation (fig.2) most probably corresponds to two previous observations:

(i) Using X-ray angiography a 'restricted' circulation of portal venous blood was seen in anaesthetized animals including rats after electrical stimulation of the nerve plexus around the hepatic artery or after injection of adrenaline [17];

(ii) Using thermocouples to measure tissue heat conduction a stronger reduction of flow after nerve stimulation was observed in peripheral than in central regions of the right medial liver lobe of anaesthetized dogs [18]. However, no redistribution of arterial and portal flows after

nerve stimulation was detected in dogs and cats using radiolabelled microspheres [19].

Apparently, there were two types of acini, the nerve-insensitive, open and the nerve-sensitive, shut-down type. The open acini formed aggregates proximal to major vessels (fig.2, bottom) suggesting that they were supplied from vessels of lower order, perhaps 4th–6th branching [20]. The shut down acini appeared to be located distal from major vessels. These areas may be supplied from vessels of higher order; i.e., 7th–10th branching. There is some indication that the acini lying within 200–300 μm from the lobar surface belong almost exclusively to the shut down type. This would provide a logical explanation for the observation that the surface tissue oxygen tension in periportal and perivenous zones measured with the miniature oxygen electrode invariably decreased during electrical stimulation to steady state values close to zero (fig.1B).

The observation that the perivenous but not the periportal tissue p_{O_2} increased transiently upon nerve stimulation (fig.1B) cannot readily be explained. It could be due to a transient increase of the oxygen supply (via shunting?), to a transient inhibition of cellular respiration in the perivenous zone or to yet another mechanism.

4.2. *Nervous regulation of metabolism*

Hepatic O_2-uptake could be decreased by stimulation of α-sympathetic liver nerves (fig.1A, see also [1]); in contrast it appeared to be increased by α-agonists [6–9]. With the decrease of O_2-uptake elicited by nerve stimulation the major component was the microcirculatory change (fig.2). A much smaller nerve-dependent increase of O_2-consumption – similar to the noradrenaline stimulated O_2-uptake in isolated hepatocytes [10–12] – might have occurred simultaneously in the open acini. This possibility remains to be studied. Conversely, it must be assumed that with the increase of O_2-uptake elicited by circulating α-agonists [6–9] the hemodynamic change was the minor component.

Hepatic glycogenolysis can be enhanced by stimulation of α-sympathetic liver nerves (fig.1D, see also [2–5]) and by circulating catecholamines (review [21]). Two major modes of action were envisaged for the catecholamines [22]. Adrenaline or noradrenaline may act directly at the parenchymal cells or indirectly at the vasculature leading to vasoconstriction and thus to hypoxia, which causes glycogenolysis by an as yet unknown mechanism [23]. Similarly, 3 major modes of action were proposed for the nerve effects ([3], see also [24]). The nerves may act directly at the parenchymal cells or indirectly via norepinephrine overflow from the vasculature or via hemodynamic changes causing hypoxia. Apparently, no attempts have been made to evaluate the contribution of hemodynamic changes to the activation of glycogenolysis by catecholamines in the perfused liver, maybe because the effect was observed also in isolated hepatocytes [12,25], in which hemodynamic changes are excluded. In the activation of glycogenolysis by nerve stimulation the observed alterations of the intrahepatic circulation (fig.2) should play a major role. However, nerve stimulation led to an increase of glycogenolysis also, when a decrease of flow was prevented by sodium nitroprusside [3]; this finding would not support an indirect metabolic nerve effect via vasoconstriction. Yet, as long as a microcirculatory change at an overall constant flow – due to sodium nitroprusside – cannot be excluded, the problem remains open.

In conclusion, our findings would suggest that the mechanism of action of sympathetic nerves and of circulating catecholamines, both on glycogenolysis and O_2-uptake, is very complex involving direct and indirect, microcirculatory components, which become effective maybe at different populations of acini. Future research may reveal important biochemical differences between these populations.

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REFERENCES

[1] Lautt, W.W. (1980) Can. J. Physiol. Pharmacol. 58, 105–123.

[2] Seydoux, J., Brunsmann, M.J.A., Jeanrenaud, B. and Girardier, L. (1979) Am. J. Physiol. 236, E 323–327.

[3] Hartmann, H., Beckh, K. and Jungermann, K. (1983) Eur. J. Biochem. 123, 521–526.

[4] Beckh, K., Hartmann, H. and Jungermann, K. (1982) FEBS Lett. 146, 69–72.

[5] Beckh, K., Balks, H.-J. and Jungermann, K. (1982) FEBS Lett. 149, 261–265.

[6] Beckh, K., Hartmann, H., Scholz, R. and Jungermann, K. (1983) Hoppe Seyler's Z. Physiol. Chem. 346, 1096.

[7] Scholz, R. and Schwabe, U. (1980) in: Alcohol and Aldehyde Metabolizing Systems – IV (Thurman, R.G. ed) pp.601–618, Plenum, New York.

[8] Reinhart, P.H., Taylor, W.M. and Bygrave, F.L. (1982) J. Biol. Chem. 257, 1906–1912.

[9] Taylor, W.M., Reinhart, P.H. and Bygrave, F.L. (1983) Biochem. J. 212, 555–565.

[10] Dehaye, J.P., Hughes, B.P., Blackmore, P.F. and Exton, J.H. (1981) Biochem. J. 194, 949–956.

[11] Binet, A. and Claret, M. (1983) Biochem. J. 210, 867–873.

[12] Blackmore, P.F., Hughes, B.P., Charest, R., Shuman, E.A. and Exton, J.H. (1983) J. Biol. Chem. 258, 10488–10494.

[13] Ji, S., Lemasters, J.J., Christensin, V. and Thurman, R.G. (1982) Proc. Natl. Acad. Sci. USA 79, 5415–5419.

[14] Scholz, R., Hansen, W. and Thurman, R.G. (1983) Eur. J. Biochem. 38, 64–72.

[15] Baumgärtl, H. and Lübbers, D.W. (1983) in: Polarographic Oxygen Sensors (Gnaiger, X. and Forstner, Y. eds) pp.37–65, Springer Verlag, Berlin-Heidelberg.

[16] Lautt, W.W. (1977) Am. J. Physiol. 232, H 652–656.

[17] Daniel, P.M. and Prichard, M.M.L. (1951) J. Physiol. 114, 538–548.

[18] Ungvary, G. and Varga, B. (1971) Acta Physiol. Acad. Scient. Hungar. 40, 347–357.

[19] Greenway, C.V. and Oshira, G. (1972) J. Physiol. 227, 487–501.

[20] Ungvary, G. (1977) Functional Morphology of the Hepatic Vascular System, p.146, 184, Akademiai Kiado, Budapest.

[21] Whitton, P.D. (1981) in: Short-term Regulation of Liver Metabolism (Hue, L. and Van de Werve, G. eds) pp.45–62, Elsevier, Amsterdam.

[22] Sherline, P., Lynch, A. and Glinsmann, W. (1972) Endocrinology 91, 680–690.

[23] Schmelck, P.H. and Hanoune, J. (1980) Mol. Cell. Biochem. 33, 35–48.

[24] Bollen, M., DeRuysscher, D. and Stalmans, W. (1983) Abstr.15th FEBS Meeting, Bruxelles, SO4-TH-034.

[25] Hue, L., Blackmore, P.F. and Exton, J.H. (1981) J. Biol. Chem. 256, 8900–8903.