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Zonation of the action of glucagon on gluconeogenesis studied in the bivascularly perfused rat liver

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Abstract We have measured the action of glucagon, infused into the hepatic artery, on gluconeogenesis from lactate in the rat liver, bivascularly perfused in both the anterograde and retrograde modes. Concerning glucose production and oxygen uptake per unit cell space, the response of the periportal cells reached via the hepatic artery in retrograde perfusion to glucagon is superior to the response of the cells reached via the same vessel in anterograde perfusion. This phenomenon, however, most probably reflects zonation of gluconeogenesis rather than zonation of the hormonal action. The latter conclusion is based on the observation that the fractional change caused by the hormone is the same for all liver cells.

Key words: Bivascular liver perfusion; Metabolic zonation; Glucagon; Gluconeogenesis; Oxygen uptake; Lactate

1. Introduction

Zonation of the action of glucagon in the liver is controversial. According to Kinugasa and Thurman [1], under gluconeogenic conditions, glucagon increases oxygen uptake solely in regions of high oxygen tension. Under normal, physiological conditions, high oxygen tension occurs in periportal cells. The action of glucagon should, thus, predominate in periportal hepatocytes [1]. The distribution of the activities of glucagon-sensitive adenylate cyclase and phosphodiesterase, however, reveals little differences along the hepatic acinus [2,3]. For this reason, Jungermann and collaborators believe that most cells respond equally to the hormone. Zonation of the action of glucagon would be predominantly a consequence of a concentration gradient along the sinusoids [2–4]. Consistent with this view are the experiments of Keppens and De Wulf, showing that periportal and perivenous isolated hepatocytes respond equally to glycogenolytic agonists, including glucagon [5]. The controversy still persists, however, because factors lost upon isolation of hepatocytes could be responsible for zonation in the intact organ.

The present paper describes experiments in which zonation of the action of glucagon on gluconeogenesis was investigated in the bivascularly perfused rat liver. With this technique, a fraction of periportal hepatocytes can be reached via the hepatic artery in the retrograde mode of perfusion. It was shown with this experimental technique that gluconeogenesis from lactate predominates in periportal hepatocytes and that this prevalence is determined primarily by a heterogeneous distribution of key enzymes along the hepatic acinus and not by oxygen concentration gradients [8].

When investigating zonation of the action of hormones it is important to distinguish between zonation of the metabolic route and zonation of the hormonal effects. In the case of glucagon and gluconeogenesis, it is necessary to verify the fractional increased caused by the hormone in periportal and perivenous cells. If the fractional changes are the same for both regions, zonation of the action of glucagon is improbable, even though the absolute changes caused by the hormone, because of the unequal distribution of gluconeogenesis, may be different in periportal and perivenous regions.

2. Experimental

Male albino rats (Wistar), weighing 150–200 g, were fed ad libitum with a standard laboratory diet (Purina). For the surgical procedure, the rats were fasted for 24 h and anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg).

Hemoglobin-free, non-recirculating bivascular liver perfusion was performed either in the anterograde mode or in the retrograde mode as described elsewhere [7]. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment (37°C). The flow through the portal vein (anterograde) or the hepatic vein (retrograde) was between 28 and 32 ml/min. The flow through the hepatic artery was between 2 and 3 ml/min.

Glucagon was infused into the hepatic artery at a rate of 0.035 $nmol \cdot min^{-1} \cdot$ (g liver wet weight)$^{-1}$ (final concentration 10 nM). Lactate was infused into the hepatic artery at a rate of 10 $\mu mol \cdot min^{-1} \cdot g^{-1}$ (final concentration 2.5 mM). [^{3}H]Water was infused simultaneously with lactate and glucagon in order to monitor recovery of the perfusion fluid pumped into the hepatic artery [6]. Preparations with less than 85% recovery were discarded.

Samples of the effluent perfusion fluid were collected according to the experimental protocol and analyzed for glucose and pyruvate by standard enzymatic procedures [9,10]. The oxygen concentration in the outflowing perfusate was monitored continuously, employing a teflon-shielded platinum electrode. Metabolic rates were calculated from the concentrations in the effluent perfusate and the total flow rates and were referred to the wet weight of the liver or to the intracellular space accessible in each perfusion mode [7,8].

The radioactivity of [^{3}H]water was measured by liquid scintillation spectroscopy. The following scintillation solution was used: toluene/ethanol (2:1) containing 5 g/l PPO (2,5-diphenyloxazole) and 0.15 g/l POPOP (2,2′-*p*-phenylene-bis(5-phenyloxazole)).

The statistical significance of the differences between parameters was evaluated by means of Student's *t*-test. Analysis was performed employing the Primer program (version 1.0; McGraw-Hill, 1988) and the results are mentioned in the text as the *P* values. $P < 0.05$ was adopted as a criterion of significance.

3. Results and discussion

In the hemoglobin-free bivascularly perfused rat liver from fasted rats, as determined by the multiple-indicator dilution technique, 0.59 ml aqueous cell space per g liver wet weight is

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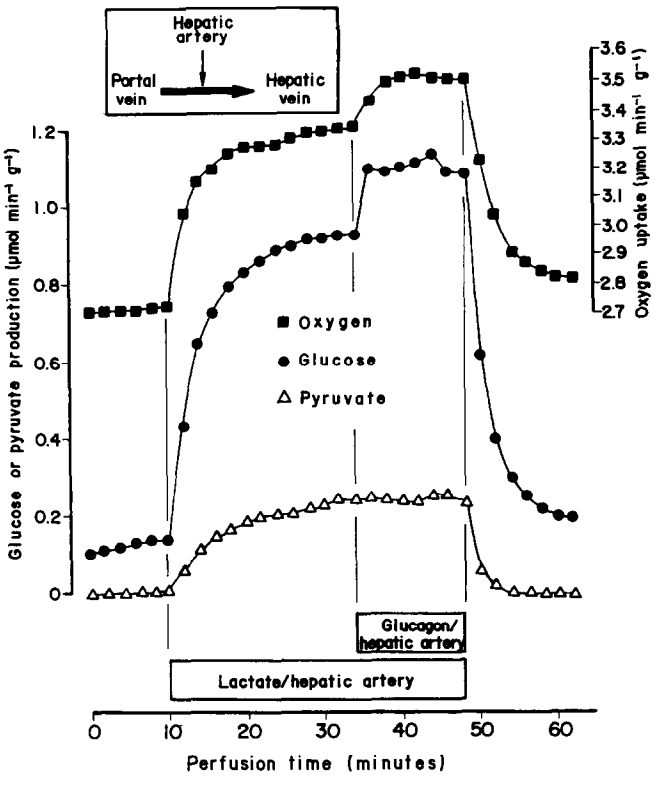

Fig. 1. Effects of glucagon infused into the hepatic artery on gluconeogenesis from lactate infused into the hepatic artery in anterograde perfusion. A liver from a 24-h fasted rat was perfused in the anterograde mode as described in section 2. Lactate (10 μmol·min^{-1}·g^{-1}) and glucagon (0.035 nmol·min^{-1}·g^{-1}) were infused at the times indicated by the horizontal bars. [^{3}H]Water was infused simultaneously for monitoring the recovery of the fluid pumped into the hepatic artery (not shown). Samples were taken for the measurement of glucose and pyruvate concentration. Oxygen concentration was measured polagraphically.

accessible via the hepatic artery in anterograde (normograde) perfusion; in retrograde perfusion, only 0.25 ml/g can be reached [7]. The metabolic responses of the rat liver when lactate and glucagon are given to these cell spaces is illustrated in Figs. 1 and 2. Fig. 1 shows the results of a typical experiment in which lactate and glucagon were infused into the hepatic artery in anterograde perfusion; and Fig. 2 the results obtained in a similar experiment in retrograde perfusion. Lactate was infused for 38 min (10–48 min), and glucagon for 14 min (34–48 min), as indicated by the horizontal bars. In both cases, the rates of lactate and glucagon infusion were 10 μmol·min^{-1}·g^{-1} and 0.035 nmol·min^{-1}·g^{-1}, respectively. These infusion rates result in mean sinusoidal concentrations of 2.5 mM and 10 nM, respectively. These saturating concentrations were chosen in order to minimize concentration gradients along the hepatic acinus.

In qualitative terms, the responses to lactate infused into the hepatic artery are those expected, i.e. glucose production, pyruvate production and oxygen consumption are increased [7]. Glucagon, when introduced into the hepatic artery, further increases glucose production and oxygen consumption in both perfusion modes. Pyruvate production, however, is not affected in anterograde perfusion and inhibited in the retrograde mode.

Quantitatively, the responses per unit liver weight tend to be smaller in the retrograde, partly due to the smaller cell spaces that are accessible via the hepatic artery in this perfusion mode. Confirming previous work, however, proportionality is lacking [8]. When the increases in glucose production and oxygen consumption produced by lactate infusion are expressed in terms of the accessible cellular spaces (μmol·min^{-1}·ml^{-1}), both parameters predominate in the cells that can be reached in retrograde perfusion (periportal cells [8]).

Table 1 shows a quantitative analysis of the effects of glucagon on glucose production, oxygen consumption and pyruvate production in both perfusion modes. The changes produced by glucagon can be expressed in three different ways: (i) as the absolute change referred to the liver wet weight (μmol·min^{-1}·g^{-1}), (ii) as the absolute change referred to the accessible cell spaces (μmol·min^{-1}·ml^{-1}), and (iii) as the relative change expressed as a percentage of the control. In case (ii), the reates expressed as μmol·min^{-1}·g^{-1} were divided by the aqueous cell spaces (ml/g) determined in previous work [7]. Glucagon up to 10 nM does not affect these spaces [11]. In case (iii), the rates of gluconeogenesis from lactate measured in the presence of glucagon were divided by the rates measured before hormone infusion. Corrections were made for the basal rates, i.e. the metabolic activity in the absence of lactate.

As revealed in Table 1, the increases in glucose production and oxygen consumption caused by glucagon were very similar

Table 1
Changes in glucose production, oxygen consumption and pyruvate production from lactate under gluconeogenic conditions caused by clucagon in the bivascularly perfused liver from fasted rats

Metabolic parameter	Units	Anterograde mode (n = 9)	Retrograde mode (n = 17)	Student's t-test (P)
Change in glucose production	μmol·min^{-1}·g^{-1}	0.191 ± 0.026	0.145 ± 0.021	0.19
	μmol·min^{-1}·ml^{-1}	0.320 ± 0.044	0.570 ± 0.082	0.045
	% activation	24.9 ± 2.3	27.5 ± 5.1	0.73
Change in oxygen consumption	μmol·min^{-1}·g^{-1}	0.164 ± 0.023	0.149 ± 0.019	0.83
	μmol·min^{-1}·ml^{-1}	0.274 ± 0.039	0.596 ± 0.077	0.008
	% activation	27.0 ± 4.0	36.0 ± 5.0	0.24
Change in pyruvate production	μmol·min^{-1}·g^{-1}	0.012 ± 0.011	−0.027 ± 0.006	0.003
	μmol·min^{-1}·ml^{-1}	0.019 + 0.017	−0.107 ± 0.022	< 0.001
	% change	5.0 ± 5.0	−55.0 ± 11.0	< 0.001

Glucagon and lactate were infused at 0.035 nmol·min^{-1}·g^{-1} and 10 nmol·min^{-1}·g^{-1}, respectively, into the hepatic artery in anterograde or retrograde perfusion according to the protocol illustrated by Figs. 1 and 2. The changes produced by glucagon on glucose production, oxygen consumption and pyruvate production under steady-state conditions were evaluated and expressed as μmol·min^{-1}· (g liver wet weight)$^{-1}$ and μmol·min^{-1}· (ml accessible aqueous cell space)$^{-1}$. The accessible cell spaces in the anterograde and retrograde modes, 0.59 and 0.25 ml/g, respectively, were determined elsewhere [5]. The percent changes produced by glucagon were calculated after subtracting the basal rates (e.g. glucose release before lactate infusion).

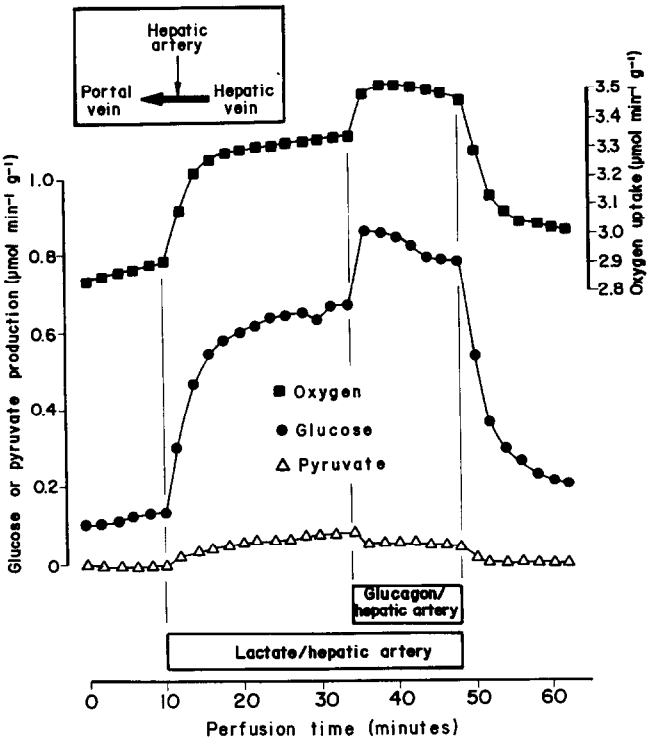

Fig. 2. Effects of glucagon infused into the hepatic artery on gluconeogenesis from lactate infused into the hepatic artery in retrograde perfusion. A liver from a 24-h fasted rat was perfused in the retrograde mode as described in section 2. Lactate (10 μmol·min^{-1}·g^{-1}) and glucagon (0.035 nmol·min^{-1}·g^{-1}) were infused at the times indicated by the horizontal bars. [^{3}H]Water was infused simultaneously for monitoring the recovery of the fluid pumped into the hepatic artery (not shown). Samples were taken for the measurement of glucose and pyruvate concentration. Oxygen concentration was measured polagraphically.

in both perfusion modes when expressed as μmol·min^{-1}·g^{-1}. In the anterograde mode these changes tend to be greater, but statistical significance is lacking. When referred to the accessible cell space, however, the changes caused by glucagon are clearly more pronounced in retrograde perfusion, meaning also that they are more pronounced in periportal hepatocytes. The percent activations caused by glucagon in both perfusion modes, however, are not different. This is quite clear for gluconeogenesis. In the case of oxygen uptake the percent changes tend to be somewhat greater in retrograde perfusion, but statistical significance is lacking, in spite of the relatively large number of perfusion experiments. Thus, irrespective of the cell type, the action of glucagon on gluconeogenesis is almost proportional to the flux through this metabolic pathway.

A different picture emerges in the case of pyruvate release. In this case, as revealed in Table 1, the action of glucagon is different: not effect in anterograde perfusion (changes and standard errors are equal) and inhibition in retrograde perfusion. The percent changes are clearly different; no effect in the case of anterograde perfusion and 55% inhibition in retrograde perfusion.

The data in Table 1 seem to indicate that the action of glucagon on gluconeogenesis reflects zonation of this metabolic pathway rather than zonation of the receptor-elicited hormonal action. In principle, at least, the effects produced by glucagon in periportal and perivenous cells can be produced by similar receptor densities, adenylate cyclase activities and phosphodiesterase activities [2–4]. The different effects of glucagon on pyruvate release in periportal and perivenous cells do not contradict this conclusion. Pyruvate is an intermediate, not a final product, and its rate of release under steady-state conditions reflects its cytosolic concentration. The latter, in turn, results from a complex interplay of factors, mainly unidirectional fluxes and redox potentials, which are probably different in periportal and perivenous cells [4].

The proposition of Kinugasa and Thurman [1] that glucagon increases oxygen uptake under gluconeogenic conditions solely in regions with high oxygen tensions was not confirmed by our results. The periportal cells reached via the hepatic artery in retrograde perfusion were under low oxygen tensions, but the oxygen uptake increase per unit cell space was higher than that found in anterograde perfusion. In anterograde perfusion, many cells under high oxygen tensions are reached. If the increases in oxygen uptake caused by glucagon were strictly dependent on oxygen concentration, the changes per unit cell space measured in retrograde perfusion should be smaller than those found in anterograde perfusion. It seems, thus, that zonation of the action of glucagon in the liver depends on the zonation of the metabolic pathways. Zonation as a consequence of concentration gradients is equally possible [2–4]. In the present work, glucagon was infused at high rates in order to minimize concentration gradients. In vivo, however, portal–venous concentration gradients may be important, especially if combined with gradients of other hormones, such as insulin for example [12].

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References

[1] Kinusaga, A. and Thurman, R.,G. (1986) Biochem. J. 246, 425–430.
[2] Zierz, S. and Jungermann, K. (1984) Eur. J. Biochem. 145, 499–504.
[3] Runge, D. and Jungermann, K. (1991) Histochemistry 96, 87–92.
[4] Jungermann, K. and Thurman, R.G. (1992) Enzyme 46, 33–58.
[5] Keppens, S. and De Wulf, H. (1988) FEBS Lett. 233, 47–50.
[6] Pang, S., Cherry, W.F., Accaputo, J., Schwab, A.J. and Goresky, C.A. (1988) J. Pharmacol. Exper. Ther. 247, 690–700.
[7] Suzuki-Kemmelmeier, F., Ishii-Iwamoto, E.L. and Bracht, A. (1992) Biochim. Biophys. Acta 1116, 275–282.
[8] Bracht, A., Constantin, J., Ishii-Iwamoto, E.L. and Suzuki-Kemmelmeier, F. (1994) Biochim. Biophys. Acta 1199, 298–304.
[9] Bergmeyer, H.U. and Bernt, E. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) pp. 1205–1215, Verlag Chemie-Academic Press, New York.
[10] Czok, R. and Lamprecht, W. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 1446–1451, Verlag Chemie-Academic Press, New York.
[11] Bracht, A. (1994) The action of glucagon and inhibitors of oxidative phosphorylation on some metabolic parameters in the bivascularly perfused liver. Full-Professor Thesis, Maringá.
[12] Jungermann, K. and Katz, N. (1986) in: Regulation of Hepatic Metabolism: Intra- and Intercellular Compartmentation (Thurman, R.G., Kauffman, F.C. and Jungermann, K., eds.) pp. 211–235, Plenum Press, New York.