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Differential effect of glucagon on gluconeogenesis in periportal and pericentral regions of the liver lobule

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The effect of glucagon on gluconeogenesis was measured in periportal and pericentral regions of the liver lobule by monitoring changes in rates of O_2 uptake on the surface of the perfused liver with miniature O_2 electrodes after infusion of lactate. When lactate (2 mM) was infused into livers from starved rats perfused in the anterograde direction, O_2 uptake was increased 2.5-fold more in periportal than in pericentral regions, reflecting increased energy demands for glucose synthesis. Under these conditions, glucagon infusion in the presence of lactate increased O_2 uptake exclusively in periportal regions of the liver lobule. Thus, when perfusion is in the physiological anterograde direction, the metabolic actions of glucagon predominate in periportal regions of the liver lobule under gluconeogenic conditions in the starved state. When livers were perfused in the retrograde direction, however, glucagon stimulated O_2 uptake exclusively in pericentral regions. Thus glucagon only stimulates gluconeogenesis in 'upstream' regions of the liver lobule irrespective of the direction of flow.

INTRODUCTION

It is well known that glucagon interacts with receptors on the liver surface, which leads to an activation of adenylate cyclase and an increase in intracellular cyclic AMP (Exton *et al.*, 1971). The increases in glycogenolysis, gluconeogenesis, urea synthesis and oxidative phosphorylation, and decreases in glycolysis, result, at least in part, from increases in this second messenger. The mechanism of the stimulation of glycogen breakdown by cyclic AMP is well understood (Exton *et al.*, 1971); however, the actions of glucagon and cyclic AMP on many other metabolic systems remain unclear. One possible explanation for the inhibition of glycolysis by glucagon involves inactivation of phosphofructokinase 2 via a cyclic AMP-dependent protein kinase (Hers & Hue, 1983). This enzyme is responsible for the synthesis of fructose 2,6-bisphosphate, an activator of phosphofructokinase 1. The actions of glucagon on hepatic glucose synthesis are also unclear; however, increases in pyruvate carboxylation have been reported (Yamazaki & Haynes, 1975). Furthermore, it is known that mitochondria isolated from glucagon-treated liver have higher rates of O_2 uptake than in controls (Adam & Haynes, 1969). The phosphorylation of a mitochondrial component in the cytochrome c_1 region by glucagon treatment has been reported (Halestrap, 1982).

Over the past decade, data have accumulated from histochemical studies suggesting that cells in periportal and pericentral regions of the hepatic lobule contain different activities of key metabolic enzymes (Rappaport, 1976; Jungermann & Katz, 1982). Using micro-dissection techniques, Guder & Schmidt (1976) demonstrated clear quantitative differences in many enzyme activities in tissues from periportal and pericentral zones of the liver lobule. Major enzymes involved in gluconeogenesis are localized preferentially in periportal regions of the lobule (Babcock & Candell, 1976; Sasse *et al.*, 1975), whereas several major glycolytic enzymes predominate in pericentral areas (Sasse *et al.*, 1975; Hilderbrand, 1980). On the basis of these differences, Jungermann *et al.* (1977) suggested that gluconeogenesis is localized in periportal regions and that glycolysis predominates in pericentral areas of the liver lobule. However, the rate of carbon flux in the gluconeogenic pathway in intact cells cannot be deduced solely from differences in maximal activity of a few enzymes. Substrate concentrations, rates of cofactor supply and local concentrations of intracellular modulators are also important (Thurman & Kauffman, 1985).

To determine metabolic flux rates in different regions of the liver lobule, miniature O_2 electrodes have been used to measure changes in rates of O_2 uptake in periportal and pericentral regions when glucose or lactate is infused into livers, perfused in the anterograde directions, from starved rats (Matsumura & Thurman, 1983, 1984; Matsumura *et al.*, 1984). Such studies demonstrated that the overall processes of glycolysis and gluconeogenesis indeed predominate in pericentral and periportal regions of the liver lobule respectively. When livers were perfused in the retrograde direction, however, maximal glycolytic and gluconeogenic activites were switched rapidly to cells in the opposite region of the lobule (Matsumura & Thurman, 1984; Matsumura *et al.*, 1984). Thus it appears that factors other than maximal enzyme activity are important in the short-term regulation of hepatic carbohydrate metabolism.

Hormones also have differential effects on cells in different zones of the hepatic lobule. Adrenaline stimulates gluconeogenesis approx. 3-fold more in pericentral than in periportal regions of the liver lobule in perfusions in the anterograde direction (Matsumura *et al.*, 1984). Since glucagon has profound effects on O_2 and carbohydrate metabolism, the purpose of the experiments described in the present study was to quantify the effects of glucagon on O_2 uptake and gluconeogenesis in periportal and pericentral regions of

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the liver lobule. Preliminary accounts of this work have appeared in abstract form (Kinugasa & Thurman, 1985).

METHODS

Animals and liver perfusion

Female Sprague–Dawley rats (200–250 *g*) were employed in these studies. Rats were starved for 24 h before perfusion. Livers were perfused with Krebs–Henseleit bicarbonate buffer (pH 7.4, 37 °C) saturated with O_2/CO_2 (19:1) in a non-recirculating system as described previously (Scholz *et al.*, 1973). Perfusate was pumped into the liver via a cannula inserted in the portal vein (anterograde) and effluent perfusate was collected via a cannula inserted in the inferior vena cava. This order was reversed for perfusions in the retrograde direction. Sodium lactate (Sigma, St. Louis, MO, U.S.A.) was diluted with buffer, adjusted to pH 7.4, and infused into the liver at concentrations described in the text and Figure legends.

Glucagon (Sigma) was dissolved in 0.1 M-HCl containing bovine serum albumin (1%, w/v), diluted with buffer containing albumin (1%) and infused into the liver at a final concentration of 10 nM, a concentration that has maximal effects on carbohydrate metabolism (Kimmig *et al.*, 1983*a*,*b*).

Rates of oxygen uptake in periportal and pericentral regions of the liver lobule

Miniature O_2 electrodes were constructed from 50 μm-diameter platinum wire pulled under heat in a glass capillary (Corning no. 770). They were coated with an O_2-permeable acrylic ester polymer (Rhoplex) to form a membrane as described elsewhere (Matsumura & Thurman, 1983). The finished electrodes had tip diameters of 50–60 μm, much smaller than the average diameter of the liver lobule. The electrodes were positioned on periportal or pericentral regions of the liver lobule by utilizing the contrast in pigmentation for placement. After steady-state O_2 concentration was established, the inflow and outflow were stopped briefly (2–3 s) and decreases in O_2 concentration in periportal and pericentral regions were monitored. Rates of O_2 uptake in periportal and pericentral regions of the liver lobule were calculated from rates of change in O_2 concentration (e.g., see Fig. 2) and the fluid content of the liver as described elsewhere in detail (Matsumura & Thurman, 1983).

Analytical

Samples of effluent perfusate were collected and analysed for glucose, lactate and pyruvate by standard enzymic procedures (Bergmeyer, 1979). The O_2 concentration in the effluent perfusate was monitored continuously with a Teflon-shielded Clark-type platinum electrode. Rates of uptake of O_2 or production of glucose, lactate or pyruvate by the liver were calculated from influent–effluent concentration differences and the constant flow rate and were normalized to the wet weight of liver.

Values are expressed as means ± S.D. Statistical comparisons were performed with Student's matched-paired *t* test.

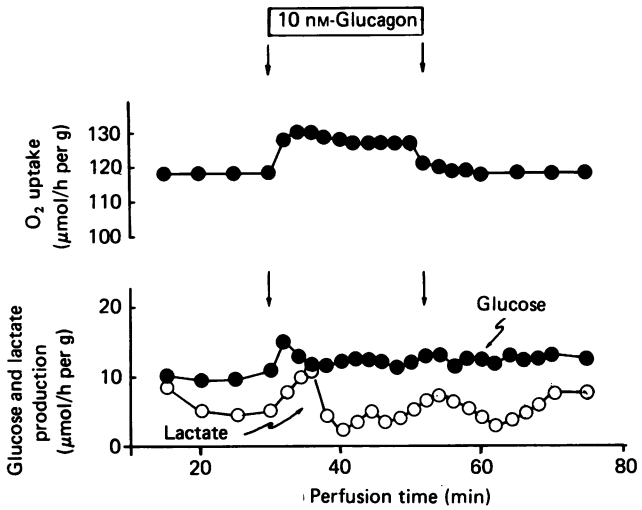

Fig. 1. Effect of glucagon on oxygen uptake and the production of glucose and lactate in livers from starved rats

Rats were deprived of food for 24 h before experiments. Experimental conditions were as described in Table 1, with perfusion in the anterograde direction. The upper panel shows O_2 uptake. Values are expressed as means for 11 experiments. The lower panel shows production of glucose (●) and lactate (○). Values are expressed as means for five experiments.

RESULTS

Effect of glucagon in the starved state

In contrast with livers from fed rats, carbohydrate output was minimal in livers from starved rats perfused in the anterograde direction (Fig. 1, Table 1). Under these conditions, infusion of glucagon had little effect on glucose output or rates of glycolysis as reflected by lactate output (Fig. 1, Table 1); however, O_2 uptake by the whole organ was increased about 12 μmol/h per g. This increase appeared to predominate in pericentral regions of the liver lobule (Table 1). When glucagon infusion was terminated, the increase in O_2 uptake in pericentral areas returned to the baseline rapidly (Table 1).

Effect of glucagon on local rates of gluconeogenesis

When lactate (2 mM) was infused into livers from normal starved rats perfused in the anterograde direction, rates of O_2 uptake by the organ increased rapidly from 110 to 148 μmol/h per g (Fig. 2, Table 2). A further increment of about 30 μmol/h per g was observed when glucagon was infused in the presence of lactate (Fig. 2, Table 2). Infusion of lactate increased glucose output from 9 to 55 μmol/h per g and pyruvate production by about an additional 30 μmol/h per g. Glucagon increased glucose production from 55 to 72 μmol/h per g, but had no effect on rates of pyruvate production (Table 2, Fig. 2). When lactate and glucagon infusions were terminated, all parameters returned to their respective baselines (Fig. 2).

Lactate increased rates of O_2 uptake predominantly in periportal regions of the liver lobule in perfusions in the anterograde direction (Table 2), confirming earlier work by Matsumura *et al.* (1984). In this study, the increase was 52 μmol/h per g in periportal areas and 21 μmol/h per g in pericentral regions. Glucagon infusion under these conditions increased O_2 uptake by an additional

Table 1. Effect of glucagon on glucose production and oxygen uptake in periportal and pericentral regions of livers from starved rats

Normal rats were starved for 24 h before experiments. Experimental design is described in the legend of Fig. 1. Perfusion was in the anterograde direction. O_2 uptake in distinct areas was measured by the stopped-flow method as described in the Methods section. O_2 uptake by the whole organ was calculated from influent−effluent O_2 concentration differences, the flow rate and the liver wet weight. Glucose was measured enzymically in the effluent perfusate. Local rates of O_2 uptake were measured before and 5 and 20 min after infusion of 10 nM-glucagon as well as 20 min after termination of glucagon infusion. Values are expressed as means±S.D. for 11 livers for O_2 uptake and for five livers for glucose production. $*P < 0.05$ for comparison with value appearing direct above in the same column.

Addition	O_2 uptake (μmol/h per g)			Glucose production (μmol/h per g)
	Periportal	Pericentral	Whole organ	
None	118±17	61±13	118±15	10±2
5 min after glucagon	125±20	77±16*	130±18*	15±3
20 min after glucagon	112±19	74±17	127±17	12±2
Glucagon off for 20 min	105±14	64±14*	118±17	13±13

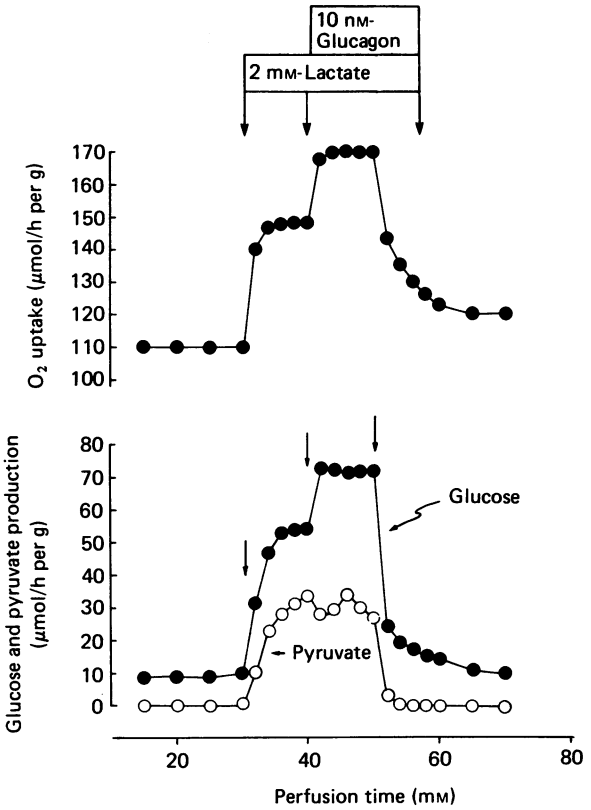

Fig. 2. Effect of lactate and glucagon on oxygen uptake and the production of glucose and pyruvate

Normal rats were starved for 24 h before experiments. Livers were perfused in the anterograde direction. Lactate and glucagon were perfused in the concentrations indicated, with precision infusion pumps. The upper panel shows O_2 uptake. The lower panel shows production of glucose (●) and pyruvate (○). The data are mean values for five experiments.

31 μmol/h per g exclusively in periportal areas (Table 2). When glucagon and lactate infusions were terminated, O_2 uptake declined to near-basal values in periportal areas, yet remained elevated in pericentral regions.

Effect of glucagon on gluconeogenesis from lactate in perfusions in the retrograde direction

When lactate and glucagon were infused into livers from starved rats perfused in the retrograde direction, O_2 uptake and glucose and pyruvate production were increased by about the same amounts as when perfusions were in the anterograde direction (Fig. 3). However, under these conditions, the stimulation of O_2 uptake by lactate occurred predominantly in pericentral regions of the liver lobule (Table 3; Matsumura *et al.*, 1984). Further, in sharp contrast with data obtained from perfusions in the anterograde direction, glucagon increased O_2 uptake by an additional 48 μmol/h per g exclusively in 'upstream' pericentral regions of the liver lobule (Table 3). When the infusions were terminated, O_2 uptake declined to near the original baseline in both regions.

DISCUSSION

Effect of glucagon in the starved state

In livers from starved rats perfused in the anterograde direction, glucagon increased O_2 uptake by about 10 μmol/h per g in the whole organ (Fig. 1), mainly in pericentral regions of the liver lobule (Table 1). Enhanced futile cycling of phosphorylated glycolytic intermediates seems unlikely, owing to the extremely low rate of glycolytic flux in the starved state (Fig. 1; Van Schaftingen *et al.*, 1980). It is possible that this increase is due to a direct effect of glucagon on mitochondria (Adam & Haynes, 1969; Halestrap, 1982; Yamazaki, 1975).

The stoichiometry between extra O_2 consumed and glucose synthesis is well established: 6 mol of ATP are needed to convert 2 mol of lactate into 1 mol of glucose. With a P/O ratio of 2.7, this becomes 0.9 mol of O_2 required for each mol of glucose synthesized. In our previous work (Matsumura *et al.*, 1984; see Table 1), this stoichiometry between energy requirement and glucose synthesis is evident. Furthermore, we know from densitometric scans of colour photographs of the liver surface that periportal and pericentral regions each occupy about 50% of the liver lobule (Lemasters *et al.*, 1986). Thus it is possible to test the hypothesis that the stoichiometry between extra O_2 consumed and glucose

Table 2. Effect of lactate and glucagon on glucose production and oxygen uptake in periportal and pericentral regions of livers from starved rats

Livers were perfused in the anterograde direction. Experimental conditions are described in the legend of Fig. 2. Values are expressed as means ± S.D. for ten livers for O_2 uptake and five livers for glucose production. Statistical comparisons were as in Table 1 with the value appearing directly above in the same column: $^{**}P < 0.01$; $^{***}P < 0.001$.

Addition	O_2 uptake (μmol/h per g)			Glucose production (μmol/h per g)
	Periportal	Pericentral	Whole organ	
None	111 ± 13	59 ± 14	110 ± 7	9 ± 2
2 mM-lactate	163 ± 20***	80 ± 16***	148 ± 13***	55 ± 14***
Lactate + 10 nM-glucagon	194 ± 31***	80 ± 19	178 ± 18***	72 ± 18**
Lactate + glucagon off for 20 min	123 ± 26***	78 ± 23	120 ± 14***	10 ± 3***

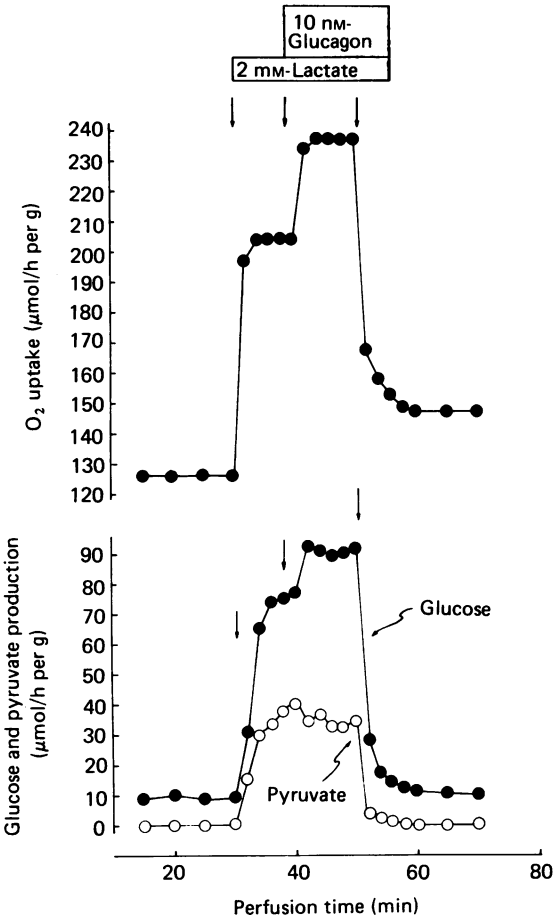

Fig. 3. Effect of lactate and glucagon on oxygen uptake and the production of glucose and pyruvate during perfusion in the retrograde direction

Livers from starved rats were perfused in the retrograde direction. Other experimental conditions were as in Fig. 2. The upper panel shows O_2 uptake. The lower panel shows production of glucose (●) and pyruvate (○). The data are mean values for five experiments.

produced holds in any given region of the liver lobule under the influence of glucagon, with a simple calculation. For example, in the absence of gluconeogenic precursors, glucagon increases whole-organ O_2 uptake by 12 μmol/h per g in perfusions in the anterograde direction (Table 1). In periportal regions the increase was 7 μmol/h per g and in pericentral areas it was 16 μmol/h per g. Therefore, $7+16 = 23$ and $23/2 = 11.5\mu$mol/h per g. Thus local changes measured with miniature O_2 electrodes nearly equal changes measured with the Clark-type electrode for the whole organ (12 μmol/h per g). Subsequent changes after the addition of a gluconeogenic precursor should therefore reflect the nearly 1:1 stoichiometry between O_2 consumption and glucose production if corrected for the small effect of glucagon on O_2 uptake in the absence of lactate. For example, the increase in O_2 uptake by the whole organ by glucagon in the presence of lactate in retrograde experiments was 33 μmol/h per g (Table 3). After correction for the direct effect of glucagon, 21 $(33-12)$ μmol of extra O_2/h per g should have been taken up for the synthesis of about 19 μmol of glucose/h per g. Indeed, the increase in glucose synthesis observed was 16 μmol/h per g. Thus the energy demands for glucose synthesis are reflected closely by extra O_2 consumption by the whole organ. Furthermore, such calculations hold for local regions. In periportal regions, an extra 7 μmol of O_2/h per g was taken up when glucagon was infused (Table 3). When corrected for the effect of glucagon alone, this effect is negligible. In pericentral regions, however, 48 μmol of extra O_2/h per g was consumed in the presence of glucagon. When corrected for the 16 μmol/h per g caused by glucagon alone, this gives 32 μmol/h per g; of $32/2 = 16$ μmol/h per g, which is very close to the value of 14 μmol of extra glucose/h per g produced when hormone was infused (Table 3). Similar calculations can be made for perfusions in the anterograde direction. Thus we conclude that extra O_2 consumption is a good index of local rates of gluconeogenesis.

In this work, we confirmed (Table 2) a previous study which demonstrated that gluconeogenesis measured from changes in O_2 uptake predominated in periportal regions of the liver lobule when perfusion was in the anterograde direction (Matsumura *et al.*, 1984). Further, because gluconeogenesis could be shifted rapidly to pericentral areas when perfusion was in the retrograde direction, it was concluded that the differential distribution of gluconeogenic enzymes in various regions of the liver lobule is not an important determinant of the overall rate of gluconeogenic flux (Table 3; Matsumura *et al.*, 1984). This conclusion contrasts with calculations of gluconeogenic flux made from the distribution of some key enzymes of gluconeogenesis and appropriate kinetic

Table 3. Effect of lactate and glucagon on glucose production and oxygen uptake in periportal and pericentral regions of livers from starved rats during perfusion in the retrograde direction

Livers were perfused in the retrograde direction. Experimental conditions are described in the legend of Fig. 3. Values are expressed as means ± S.D. from ten livers for O_2 uptake and five livers for glucose production. Statistical comparisons are as in Table 2 with the value appearing directly above in the same column.

Addition	Oxygen uptake (μmol/h per g)			Glucose production (μmol/h per g)
	Periportal	Pericentral	Whole organ	
None	45±5	122±13	126±15	9±2
2 mM-lactate	74±5***	190±38***	204±19***	75±15***
Lactate + 10 nM-glucagon	81±12	238±35**	237±26***	91±20**
Lactate + glucagon off for 20 min	50±9***	142±24***	147±17***	10±4***

constants (Jungermann & Katz, 1982). Further, since lactate was supplied in excess to hepatocytes in both regions of the liver lobule, substrate supply was ruled out as the responsible factor.

In the present study, in perfusions in both the retrograde and the anterograde direction, we observed that glucagon only stimulated gluconeogenesis in 'upstream' regions of the liver louble [e.g. the region responding exclusively to glucagon could be switched rapidly by changing the direction of the flow (Figs. 1 and 3; Tables 2 and 3)]. Why is the effect of this hormone localized to hepatocytes in 'upstream' regions of the liver lobule? At our present level of knowledge, we do not know; however, from the experiments in this study, several factors can be ruled out. One possibility is that 'downstream' regions of the liver lobule do not respond to glucagon because they are hypoxic. This alternative appears unlikely, however, since $[O_2]$ was over 200 μM in 'downstream' areas, values far above the K_m of cytochrome oxidase for oxygen (Jones & Mason, 1978). Moreover, adrenaline stimulated gluconeogenesis predominantly in 'downstream' regions (Matsumura *et al.*, 1984), which could not occur if hepatocytes in 'downstream' regions were hypoxic. Moreover, since glucagon increased O_2 uptake in 'downstream' regions in livers from fed rats (results not shown), it must reach all cells in the liver lobule. Several additional factors can also be ruled out on the basis of the observation that the region stimulated by glucagon could be switched rapidly in less than 30 s by reversing the direction of flow. We can rule out the distribution of glucagon receptors, adenylate cyclase and protein kinases across the liver lobule in the specific effect of glucagon on gluconeogenesis in 'upstream' regions of the liver lobule. In fact, glucagon-stimulated adenylate cyclase activity is slightly higher in 'downstream' pericentral regions of the liver lobule (Zierz & Jungermann, unpublished work). The stimulation of glucose synthesis in 'upstream' regions by glucagon may involve the cyclic-AMP-dependent phosphorylation of several enzymes in carbohydrate metabolism, such as pyruvate kinase and phosphofructokinase 1 and 2, leading to more efficient glucose synthesis. This possbility also seems unlikely, however, since local enzyme activity is most probably not a major factor regulating regional glucose synthesis (Matsumura *et al.*, 1984).

It is now apparent that hormones do not have similar effects on hepatocytes in different regions of the liver louble. Adrenaline stimuates gluconeogenesis to a greater extent in 'downstream' than in 'upstream' regions of the liver lobule (Matsumura *et al.*, 1984). In contrast, glucagon stimulates glucose synthesis exclusively in 'upstream' regions (Tables 2 and 3). Glucagon acts via cyclic AMP, whereas adrenaline works predominantly via α_1-adrenergic receptors to produce phosphoinositol-dependent increases in cytosolic Ca^{2+} (Joseph *et al.*, 1984). Because glucagon stimulates glucose synthesis exclusively in periportal areas (Table 2), whereas adrenaline acts predominantly in pericentral regions (Matsumura *et al.*, 1984), we speculate that cyclic-AMP-dependent effects reside predominantly in 'upstream' areas, whereas Ca^{2+}-mediated actions occur mainly in 'downstream' regions of the liver lobule. It is possible that hormone actions in the liver are determined by the local $[O_2]$; however, more work will be needed to substantiate this speculation.

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