Impaired sensitivity to insulin of rat livers perfused with blood of diminished haematocrit

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1. In livers from fed rats perfused with homologous whole blood of a haematocrit value of 37%, insulin decreased the perfusate concentrations of glucose and amino acids, production of ketone bodies (3-hydroxybutyrate + acetoacetate) and increased bile flow.
2. Perfusion with blood diluted with buffer to a haematocrit value of 17% decreased hepatic O₂ consumption by 40–50%. Perfusate concentrations of glucose and lactate, the rate of ketogenesis and the ratios [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] were all increased.
3. In livers perfused with blood of diminished haematocrit, effects of insulin on perfusate glucose and amino acids, ketogenesis and bile flow were abolished.

In livers from fed rats perfused with homologous whole blood, rapid effects of insulin have been obtained on a variety of metabolic processes including lipogenesis (Topping & Mayes, 1972, 1976a), fatty-acid esterification (Bates *et al.*, 1977; Topping & Mayes, 1972) and triacylglycerol secretion (Topping & Mayes, 1972, 1976a). In this system the hormone also inhibits ketogenesis and decreases perfusate glucose and amino acids and increases bile flow (D. L. Topping, D. G. Clark, R. J. Illman, M. Brinkman, R. P. Trimble & S. D. Neville, unpublished work).

Most perfused liver preparations employ media containing either homologous or heterologous erythrocytes at haematocrit values at least one-third lower than whole blood and commonly at haematocrit values of 18–20% (for a review see Ross, 1972). Studies by some groups with these media have shown effects of insulin. These include hypoglycaemia (e.g. Mortimore, 1963; Haft, 1967, 1968) and inhibition of amino-acid release (e.g. Mortimore & Mondon, 1970) but usually with perfusates of haematocrit values considerably in excess of 20%. Other workers have reported either inconsistent effects of the hormone (Hems *et al.*, 1972; Woodside & Heimberg, 1976) or non-responsiveness of the liver to insulin (Patzelt *et al.*, 1973; Assimacopoulos-Jeannet *et al.*, 1977). Based largely on the findings of these latter studies, reviewers have generally concluded that effects of insulin were either extrahepatic (Levine & Fritz, 1956; Nikkilä, 1969) or mediated via other (unidentified) factors (Levine & Fritz, 1956) or due to a diminution in the effects of catabolic hormones (Hems, 1979).

We considered it possible that reported discrepancies in insulin sensitivity might reflect those in perfusate erythrocyte content. Accordingly, we have examined acute metabolic effects of the hormone in livers perfused with whole rat blood at normal (37%) and diminished (17%) haematocrit values.

Materials and methods

Liver perfusion

Livers from fed rats (295–305 g body wt.) of the Hooded Wistar strain were perfused *in situ* for an experimental period of 40 min. The perfusate was 80 ml of defibrinated whole blood from the rat, dialysed for 24 h at 0–4°C as described by Mayes & Felts (1966). The dialysis buffer consisted of two changes (each of 2 litres) of a modified Krebs & Henseleit (1932) bicarbonate buffer containing glucose (10 mM), mixed amino acids (500 mg/litre) and 50% of the specified concentration of Ca²⁺. This perfusate has a haematocrit value of approx. 37%, roughly equivalent to 13.5 g of haemoglobin/100 ml (Topping, 1975). In perfusions with blood of diminished haematocrit, sufficient buffer was added before dialysis to give a haematocrit value of approx. 17%. In all experiments the rate of perfusion was 0.9–1.0 ml/min per g of liver and the pO₂ was kept at 90–100 mm Hg (12–13 kPa).

After an initial equilibration period of 10 min, crystalline bovine insulin (Sigma Chemical Co., St. Louis, MO, U.S.A.) was infused to maintain a concentration of 100 ng/ml of cell-free perfusate. The start of the infusion was designated zero time

and control livers were infused with 0.15M-NaCl only.

Bile was collected at 20min intervals as described previously (Topping *et al.*, 1978).

Analytical techniques

Samples of perfusate were taken at zero time and at 20 and 40 min. Each sample was rapidly chilled in ice until further processing immediately after the experiment. At the end of the experimental period the liver was excised, blotted dry and weighed for calculation of rates of metabolism per unit weight of tissue. In deriving net rates of metabolite production and utilization, corrections were applied for additions to and removals from the perfusate (Topping & Mayes, 1972, 1976b).

Blood glucose was measured by a commercial glucose oxidase method (Boehringer Mannheim Pty. Ltd., Mt. Waverley, Victoria 3149, Australia). Enzymic methods were used for the determination of L-(+)-lactate (Hohorst, 1963), pyruvate (Czok & Lamprecht, 1974) and ketone bodies (Williamson *et al.*, 1962) in whole perfusate. Plasma amino acids were concentrated by ion-exchange chromatography (Topping *et al.*, 1979) and measured by g.l.c. (Mackenzie & Tenaschuk, 1974).

Haematocrit values were determined by using a DAMON/IEC MB micro-haematocrit centrifuge. At the end of the experiment perfusate samples were taken with gas-tight syringes from the cannulae entering and leaving the liver and their O₂ content was determined polarographically (Topping, 1975).

Statistical methods

The statistical significance of differences between experimental groups was determined by the analysis of variance (Brownlee, 1949). Results are means $\pm$ S.E.M. and the numbers of observations are given in parentheses.

Results and discussion

In livers perfused with whole blood the mean haematocrit value was $37 \pm 1\%$ (9) for control and insulin-infused livers combined. Hepatic O₂ consumption was unaffected by insulin with mean values of 4.1 ± 0.1 (5) and 4.3 ± 0.2 (5) $\mu\text{mol/min}$ per g of liver for control and insulin groups respectively. These rates of utilization are in close agreement with previous observations under similar conditions (Topping, 1975; Mayes & Felts, 1976). The haematocrit value was $17 \pm 1\%$ (9) in livers perfused with diluted blood and O₂ consumption was correspondingly decreased ($P < 0.001$) with mean values of 2.2 ± 0.1 (5) and 2.6 ± 0.3 (4) $\mu\text{mol/min}$ per g of liver in livers perfused with and without insulin respectively. The small increase due to insulin in these livers was not statistically significant.

Fig. 1. Concentrations of perfusate glucose in perfused rat liver

Livers from fed rats were perfused with either whole rat blood or blood diluted with buffer and in the presence and absence of insulin infused as described in the text. Values of perfusate glucose are shown as means $\pm$ S.E.M. for the numbers of observations shown in parentheses. The groups were: A, whole blood (O); B, whole blood + insulin (●); C, diluted blood (Δ); D, diluted blood + insulin (▲). P values (for concentrations at 40 min), <0.01 (A versus B) <0.001 (A versus C,D) <0.001 (B versus C,D).

Concentrations of perfusate glucose in livers perfused with whole blood declined during the experiment, finally reaching $7.5 \mu\text{mol/ml}$ (Fig. 1). As observed recently (D. L. Topping, D. G. Clark, R. J. Illman, M. Brinkman, R. P. Trimble & S. D. Neville, unpublished work), insulin infusion significantly decreased final glucose concentrations by 17%. Perfusion with diluted blood resulted in significantly higher concentrations of perfusate glucose. This finding agrees with those of Brauer *et al.* (1963). These authors observed that the perfused liver could not stabilize glucose concentrations at physiological concentrations unless the perfusate was fully oxygenated whole blood. Indeed, they concluded that the equilibrium glucose concentrations of liver preparations *in vitro* could be expressed solely as a function of O₂ consumption. In addition to increasing perfusate glucose, the perfusions with diluted blood considerably diminished the hypoglycaemic effects of insulin (Fig. 1).

Mean concentrations of perfusate lactate or pyruvate were unaffected by insulin in livers perfused with whole or diluted blood (Table 1). In

Table 1. Concentrations of perfusate lactate, pyruvate and amino acids, the rate of ketogenesis and the redox state in perfused rat liver

Livers from fed rats were perfused with rat blood (whole or diluted with buffer) in the presence and absence of insulin infused as described in the text. Concentrations of perfusate lactate, pyruvate and amino acids and the ratio [lactate]/[pyruvate] were determined at 40 min, and the rate of ketogenesis and the ratio [3-hydroxybutyrate]/[acetoacetate] were averaged for the whole experiment. Values are means $\pm$ S.E.M. for the numbers of observations shown in parentheses.

Group	Blood [lactate] ($\mu\text{mol/ml}$)	Blood [pyruvate] ($\mu\text{mol/ml}$)	[Lactate]/[pyruvate]	Ketogenesis ($\mu\text{mol/h per g}$)	[3-Hydroxybutyrate]/[acetoacetate]	Plasma amino acids ($\mu\text{mol/ml}$)
(A) Whole blood	1.33 ± 0.14 (5)	0.17 ± 0.04 (5)	7.3 ± 0.6 (5)	3.0 ± 0.3 (5)	0.44 ± 0.08 (5)	3.46 ± 0.41 (5)
(B) Whole blood + insulin	1.09 ± 0.08 (5)	0.17 ± 0.03 (5)	8.1 ± 1.0 (5)	1.9 ± 0.2 (5)	0.36 ± 0.07 (5)	2.50 ± 0.27 (5)
(C) Diluted blood	3.10 ± 0.10 (4)	0.21 ± 0.02 (4)	13.9 ± 0.6 (4)	4.2 ± 0.3 (5)	0.82 ± 0.13 (5)	3.63 ± 0.43 (5)
(D) Diluted blood + insulin	3.11 ± 0.23 (5)	0.21 ± 0.02 (5)	14.4 ± 0.9 (5)	3.8 ± 0.4 (5)	0.97 ± 0.06 (5)	2.85 ± 0.42 (4)
P values	<0.001 (A versus C, A versus D, B versus C, B versus D)		<0.001 (A versus C, A versus D, B versus C, B versus D)		<0.01 (A versus C) <0.01 (B versus C) <0.001 (A versus D, B versus D)	

livers perfused with whole blood, final concentrations of these metabolites were close to those observed *in vivo*. Perfusion with diluted blood significantly increased both perfusate [lactate] and the ratio [lactate]/[pyruvate]. It is notable that the increase in lactate concentration and less oxidized redox state occurred in spite of the diminished supply of lactate from erythrocyte glycolysis.

In livers perfused with whole blood, infusion of insulin decreased ketogenesis by 38% (Table 1). Perfusion with diluted blood significantly increased the rate of ketone-body formation and in these livers the anti-ketogenic effects of insulin were virtually abolished. In neither group was the ratio [3-hydroxybutyrate]/[acetoacetate] affected by infusion of the hormone, but the mean ratio was increased from 0.40 to 0.89 by perfusion with diluted blood.

It is thought that ketone bodies are formed when the supply of acetyl-CoA to the liver exceeds the capacity of the citric acid cycle (Mayes & Felts, 1967). The direct anti-ketogenic effect of the hormone, which occurs *in vivo* (Elkeles *et al.*, 1978), may be due to a net decrease in hepatic fatty-acid oxidation due to either diminished lipolysis (Topping & Mayes, 1972) or enhanced esterification (Bates *et al.*, 1977). Alternatively the decrease may reflect a stimulation, by insulin, of lipogenesis with a consequent increase in [malonyl-CoA]. This would inhibit the entry of fatty acids into the mitochondrion for oxidation (McGarry & Foster, 1979). Clearly the increased ketogenesis in livers perfused with blood of diminished haematocrit need

not reflect an increase in total fatty-acid oxidation, merely a diminished supply of O_2 (also mirrored by the more reduced redox state).

The concentrations of plasma amino acids at the end of perfusion are also shown in Table 1. In livers perfused with whole blood, infusion of insulin significantly decreased concentrations of total amino acids. This decrease is believed to reflect a direct inhibition, by insulin, of hepatic proteolysis (Mortimore & Mondon, 1970). In livers perfused with blood of diminished haematocrit, considerable variation was observed in perfusate amino acids. Although insulin infusion significantly decreased concentrations of imino, aromatic and basic amino acids (results not shown), total amino acids were not significantly altered. It is, however, apparent that in the latter case concentrations were still lower than in the corresponding controls. Clearly these metabolic effects remain more sensitive to insulin on diminution of perfusate haematocrit than the other parameters studied in the present investigation.

The data we have obtained in these studies confirm the hypothesis that the sensitivity to insulin of the perfused rat liver was diminished on decreasing perfusate haematocrit. This impairment of liver function is similar to that observed when hepatic O_2 consumption is decreased by other means such as the formation of carboxyhaemoglobin (Topping, 1975; Gardner *et al.*, 1978) and decreasing perfusate $p\text{O}_2$ (Mayes & Felts, 1976). An explanation for the diminution in insulin sensitivity

may be sought in the observations of Brauer *et al.* (1963) that the isolated liver could not equilibrate $[K^+]$ unless the perfusate is fully oxygenated whole blood. Given the role of intracellular ions in the expression of acute hormonal effects on the liver (Hems, 1979; Exton, 1979), it is possible that diminution in haematocrit affected the ionic balance. This is supported by the observation that in livers perfused with whole blood, insulin significantly ($P < 0.01$) increased bile flow from 67 ± 2 (4) to 76 ± 3 (5) $\mu\text{l/g}$ of liver per h. The stimulation is believed to reflect increases in ion transport (Larsen & Christensen, 1978) and was abolished in livers perfused with diluted blood, where the corresponding rates of flow were 60 ± 2 (5) and 61 ± 2 (5) $\mu\text{l/g}$ of liver per h for control and insulin-infused livers respectively.

From these experiments it would appear that a possible reason for the insulin insensitivity of some perfused liver preparations is the use of media of considerably diminished haematocrit. These perfusates would seem to be unsuitable for the study of insulin action.

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References

- Assimakopoulos-Jeannet, F., Karakash, C., Le Marchand, Y. & Jeanrenaud, B. (1977) *Biochim. Biophys. Acta* **498**, 91–101
- Bates, E. A., Topping, D. L., Sooranna, S. P., Saggerson, E. D. & Mayes, P. A. (1977) *FEBS Lett.* **54**, 225–228
- Brauer, R. W., Leong, G. F., Holloway, R. J. & Krebs, J. S. (1963) in *Selective Vulnerability of the Brain in Hypoxaemia* (Schadé, J. P. & McMenemey, W. H., eds.), pp. 273–294, Blackwell Scientific Publications, Oxford
- Brownlee, K. A. (1949) *Industrial Experimentation*, 4th edn., p. 74, H.M.S.O., London
- Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 1446–1451, Academic Press, New York and London
- Elkeles, R. S., Chalmers, R. A. & Hambley, J. (1978) *Clin. Sci. Mol. Med.* **55**, 499–504
- Exton, J. H. (1979) *J. Cyclic Nucleotide Res.* **5**, 277–287
- Gardner, R. S., Topping, D. L. & Mayes, P. A. (1978) *Biochem. Biophys. Res. Commun.* **82**, 526–531
- Haft, D. E. (1967) *Am. J. Physiol.* **213**, 219–230
- Haft, D. E. (1968) *Diabetes* **17**, 244–250
- Hems, D. A. (1979) *Clin. Sci. Mol. Med.* **56**, 197–202
- Hems, D. A., Whitton, P. D. & Taylor, E. A. (1972) *Biochem. J.* **129**, 529–538
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 266–272, Academic Press, New York and London
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Larsen, J. A. & Christensen, K. D. (1978) *Acta Physiol. Scand.* **102**, 301–309
- Levine, R. & Fritz, I. B. (1956) *Diabetes* **5**, 209–217
- Mackenzie, S. L. & Tenaschuk, D. (1974) *J. Chromatogr.* **97**, 19–24
- Mayes, P. A. & Felts, J. M. (1966) *Proc. Eur. Soc. Study Drug Toxic.* **7**, 16–29
- Mayes, P. A. & Felts, J. M. (1967) *Nature (London)* **215**, 716–718
- Mayes, P. A. & Felts, J. M. (1976) *Biochem. J.* **156**, 685–689
- McGarry, J. D. & Foster, D. W. (1979) *J. Biol. Chem.* **254**, 8163–8168
- Mortimore, G. E. (1963) *Am. J. Physiol.* **204**, 699–704
- Mortimore, G. E. & Mondon, C. E. (1970) *J. Biol. Chem.* **245**, 2375–2383
- Nikkilä, E. A. (1969) *Adv. Lipid Res.* **7**, 63–134
- Patzelt, C., Löffler, G. & Wieland, O. H. (1973) *Eur. J. Biochem.* **33**, 117–122
- Ross, B. D. (1972) *Perfusion Techniques in Biochemistry*, pp. 135–220, Clarendon Press, Oxford
- Topping, D. L. (1975) *Biochem. J.* **152**, 425–427
- Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311
- Topping, D. L. & Mayes, P. A. (1976a) *Biochem. Soc. Trans.* **4**, 717
- Topping, D. L. & Mayes, P. A. (1976b) *Br. J. Nutr.* **36**, 113–126
- Topping, D. L., Illman, R. J., Dreosti, I. E., Trimble, R. P. & Record, I. R. (1978) *Nutr. Rep. Int.* **18**, 631–637
- Topping, D. L., Clark, D. G., Storer, G. B., Illman, R. J. & Trimble, R. P. (1979) *Biochem. J.* **184**, 97–106
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **108**, 353–361
- Woodside, W. F. & Heimberg, M. (1976) *J. Biol. Chem.* **251**, 13–23