THE EFFECTS OF STARVATION AND INSULIN ON THE RELEASE OF GLUCONEOGENIC SUBSTRATES FROM THE EXTRA-SPLANCHNIC TISSUES IN VIVO

Perry J. BLACKSHEAR
Nuffield Department of Clinical Medicine, The Radcliffe Infirmary, Oxford, England

and

Paul A. H. HOLLOWAY and K. G. M. M. ALBERTI*
Chemical Pathology, Southampton General Hospital, Tremona Road, Southampton S09 4XY, England

Received 26 September 1974

1. Introduction

The predominant gluconeogenic substrates in the rat and man are lactate, pyruvate, glycerol, alanine and other amino acids [1]. One of the factors regulating gluconeogenesis in both starvation and diabetes in the availability of these substances. However, the control of gluconeogenic substrate flux from the peripheral tissues is still unclear. We have recently shown that sodium dichloroacetate decreases the net extra-splanchnic release of lactate, pyruvate and alanine in both starved and diabetic rats [2,3]. These data are consistent with the activation of pyruvate dehydrogenase (PDH) by dichloroacetate, as described in the perfused rat heart by Whitehouse and Randle [4]. Wieland et al. [5] have shown that starvation and diabetes decrease the proportion of active PDH in rat heart and kidney, and that refeeding and insulin treatment, respectively, restore this activity to normal levels. These observations suggested a mechanism by which starvation, through inactivation of PDH in some tissues, could increase lactate and pyruvate flux from the peripheral tissues to the liver; it has generally been accepted, however, that lactate release from the extra hepatic tissues is *decreased* in starvation [6]. The results presented in this paper show the opposite; the flux of gluconeogenic substrates from the peripheral tissues to the liver was *doubled* after 24 hr of starvation.

* To whom corresponce should be addressed.

2. Methods and materials

The animals, methods of cannulation, anaesthesia, blood sampling and metabolite determinations used in these studies were as described in detail previously [2]. The experimental design involved excluding the liver and gut from the circulation and measuring the rates of accumulation of metabolites in blood for 30 min. This functional hepatectomy preparation involves the simultaneous ligation of the coeliac axis and the superior mesenteric arteries, and the hepatic portal vein. In the first experiment described, arterial blood metabolite concentrations were measured after functional hepatectomy in fed rats or rats starved for 24 hr. In the second experiment, control values from starved rats were compared with those starved animals which received 2 units of soluble insulin (Burroughs Wellcome and Co. Ltd., London, UK) as a single intravenous bolus, immediately after functional hepatectomy. In other experiments, starved rats received either 2 mg of 2,3-dimethylpyrazole (Fluka AG, Buchs, Switserland) or 0.5 ml of 0.1 M *n*-butyrate (BDH Chemicals Ltd., Poole, Dorset, UK) as a single intravenous injection immediately following functional hepatectomy.

3. Results and discussion

Fig. 1 shows the slight increase in glucose disappearance and the marked suppression of glucose precursor

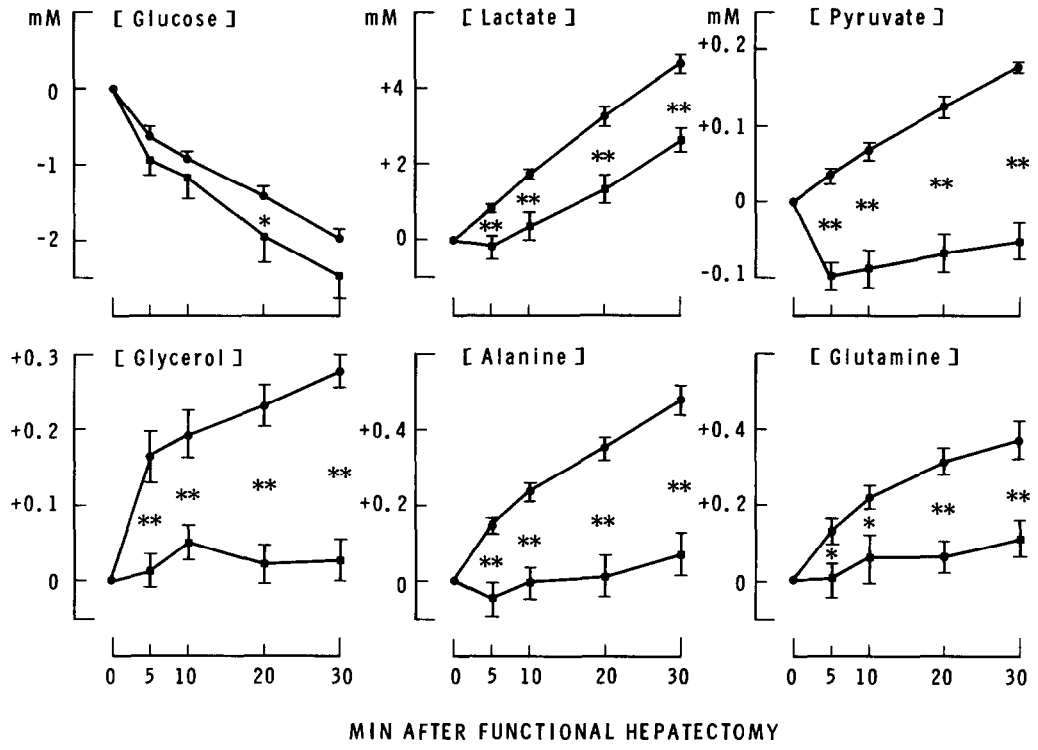

Fig. 1. Arterial blood metabolite concentration changes after functional hepatectomy in fed (■—■—■) and starved (●—●—●) rats. The vertical axes respresent the *change* in mM from time 0 min. Each point represents the mean of determinations from 5–11 animals ± S.E.M. *, < p 0.05; **, < p 0.01 when comparing values from fed and starved rats at a given time point using Student's t-test. Original (0 min) concentrations were (mM): glucose: fed, 7.73 ± 0.30; starved, 4.08 ± 0.22 ($p < 0.01$); lactate: fed, 1.65 ± 0.33; starved, 0.57 ± 0.03 ($p < 0.01$); pyruvate: fed, 0.20 ± 0.03; starved, 0.06 ± 0.00 ($p < 0.01$); glycerol: fed, 0.17 ± 0.01; starved, 0.21 ± 0.01 ($p < 0.05$); alanine: fed, 0.59 ± 0.08; starved, 0.17 ± 0.02 ($p < 0.01$); glutamine: fed, 0.59 ± 0.04; starved, 0.51 ± 0.04 ($p < 0.05$). For other details, see the text.

accumulation after functional hepatectomy in fed rats when compared with animals starved for 24 hr. In starvation, lactate accumulation was increased by 80%, while both alanine and pyruvate accumulated in significant amounts compared with negligible changes in fed animals.

Thus the net release of gluconeogenic substrates (lactate, pyruvate, alanine and glycerol) from the extra-splanchnic tissues was increased 100% in the starved compared with the fed state. With the exception of glycerol, these substances are directly related to pyruvate metabolism in muscle and other tissues, and a decrease in pyruvate availability should decrease their net release. Two possibilities could explain this pattern of decreased glucose uptake and increased lactate, pyruvate and alanine output in starvation: decreased muscle glycogen formation, or decreased pyruvate oxidation. Pyruvate oxidation is known to be inhibited in starvation and diabetes [7,8]; further, Wieland et al. [5] have shown that the proportion of PDH in the active form in heart muscle is decreased by about 80% in starvation and 85% in untreated diabetes. We previously found that dichloroacetate caused marked decreases in lactate, pyruvate and alanine accumulation after functional hepatectomy in both starved and diabetic rats [2,3]. This suggests by analogy that PDH inactivation could be responsible for the changes seen in the starved animals.

It is known that increased ratios of acetyl-CoA/CoA and NADH/NAD inhibit the PDH complex in rat liver [9,10]. In this way, oxidation of fatty acids and

ketone bodies can inhibit PDH in muscle. Insulin, which has been shown to activate PDH in adipose tissue [12], could activate this enzyme in muscle either directly or via its antilipolytic effects.

Insulin mimicked the effects of dichloroacetate in that it also decreased the net peripheral release of lactate (60%), pyruvate (30%), and alanine (50%) in starved rats (fig. 2). That insulin was exerting an antilipolytic effect in this situation can be seen by the 70% decrease in glycerol accumulation (fig. 2). Thus, insulin decreased the net release of gluconeogenic substrates from the peripheral tissues by 60%. Possible mechanisms again include increased muscle glycogen formation or the activation of PDH, either directly or by decreasing tissue lipolysis. The latter possibility is supported by the observation that insulin treatment of diabetic rats or refeeding of starved rats restored the proportion of active PDH to normal levels in heart and kidney [5]. Houghton [13] also noted that perfusion of the hindquarter from starved rats with 5 mM glucose in the presence of insulin increased glucose uptake 10-fold, increased O_2 consumption, but *decreased* lactate output by 20% when compared with glucose perfusions alone.

An attempt was made to mimick this effect of insulin using an antilipolytic agent, 3,5-dimethylpyrazole [14]. This compound decreased glycerol accumulation after functional hepatectomy by 66%. It also inhibited the net peripheral release of lactate (45%) and alanine (22%), although not of pyruvate. Thus, even short-term inhibition of lipolysis can mimick some of the effects of insulin, dichloroacetate, and feeding in decreasing the net peripheral release of gluconeogenic substrates. Wieland et al. [5] found that treatment of starved rats with nicotinic acid also increased the amount of active PDH in heart and kidney to the normal, fed range.

The injection of butyrate, which would be expected to increase peripheral tissue acetyl-CoA/CoA and NADH/NAD ratios, slightly inhibited glucose uptake.

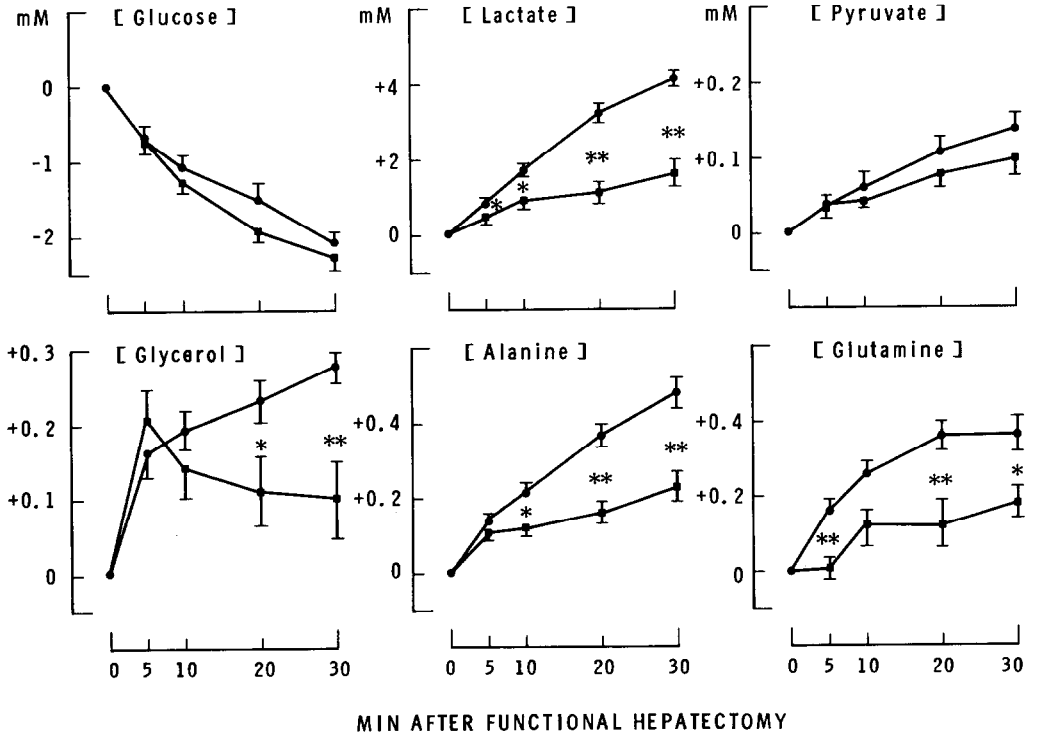

Fig. 2. Arterial blood metabolite concentration changes after functional hepatectomy in starved rats treated with NaCl (●—●—●) or insulin (■—■—■). Original concentrations were similar to those listed for starved animals in the legend for fig. 1; other details are as in fig. 1.

It did not affect lactate or pyruvate accumulation, however. This observation, together with the fact that the rates of lactate, pyruvate and alanine release were similar in severely diabetic and starved animals [2,3], suggests that PDH may be 'maximally' inactive in both conditions.

Glutamine has been shown to be an effective gluconeogenic precursor in the perfused liver [15] and in vivo after feeding with a high casein diet [16]. However, recent studies suggest that most of the glutamine released from muscle is taken up by the gut rather than by the liver (17–19). In the present experiments, glutamine release was suppressed by the same treatments which inhibited the release of alanine, e.g. feeding (70%), insulin injection (50%), and 3,5-dimethylpyrazole injection (25%). The mechanisms of these effects on glutamine release are as yet unknown.

The above results indicate that in the fed state, the net extra-splanchnic release of the glucose precursors lactate, pyruvate, alanine and glycerol is suppressed. The data further suggest that insulin may contribute to this effect, through increased glycogen formation and/or increased pyruvate oxidation. Thus, insulin may control gluconeogenesis in vivo in two distinct ways: by inhibiting hepatic gluconeogenesis directly [20]; and by decreasing the flux of glucose precursors to the liver. In this way gluconeogenic substrate availability would be decreased in the fed state, and increased in gluconeogenic situations such as starvation and diabetes.

These results do not support the conclusion of Kreisberg et al. [6] that lactate, quantitatively the most important gluconeogenic substrate, is released in decreased amounts from the extra-hepatic tissues in starvation. Further work is required to elucidate the mechanism of this effect, as well as the tissue of origin of the large amount of lactate produced in the starved state.

Acknowledgements

We would like to thank Dr D. H. Williamson, Dr B. D. Ross and Professor H. A. Krebs for critical comments and advice, and Professor P. B. Beeson for laboratory facilities. We are grateful to the Rhodes Trust, the Wellcome Trust and the Medical Research Council for generous financial aid.

References

[1] Exton, J. H. (1972) Metabolism 21, 945–990.
[2] Blackshear, P. J., Holloway, P. A. H. and Alberti, K. G. M. M. (1974) Biochem. J. 142, 279–286.
[3] Blackshear, P. J., Holloway, P. A. H. and Alberti, K. G. M. M. (1974) submitted for publication.
[4] Whitehouse, S. and Randle, P. J. (1973) Biochem. J. 134, 651–653.
[5] Wieland, O., Siess, E., Schulze-Wethmar, F. H., von Funcke, H. G. and Winton, B. (1971) Arch. Biochem. Biophys. 143, 593–601.
[6] Kreisberg, R. A., Pennington, L. F. and Boshell, B. R. (1970) Diabetes 19, 53–63.
[7] Garland, P. B. and Randle, P. J. (1963) Nature (Lond.) 199, 381–383.
[8] Garland, P. B., Newsholme, E. A. and Randle, P. J. (1964) Biochem. J. 93, 665–678.
[9] Garland, P. B. and Randle, P. J. (1964) Biochem. J. 91, 6c–7c.
[10] Nicholls, D. G. and Garland, P. B. (1966) Biochem. J. 100, 77p–78p.
[11] Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., Denton, R. M. and Pogson, C. I. (1966) Rec. Prog. Horm. Res. 22, 1–48.
[12] Weiss, L., Löffler, G., Schirmann, A. and Wieland, O. (1971) FEBS Lett. 15, 229–231.
[13] Houghton, C. R. S. (1971) D. Phil. Thesis, Oxford University.
[14] Bizzi, A., Tacconi, M. T. and Garattini, S. (1966) Experentia 22, 664.
[15] Ross, B. D., Hems, R. and Krebs, H. A. (1967) Biochem. J. 102, 942–951.
[16] Aikawa, T., Matsutaka, H., Yamamoto, H., Okuda, T., Ishikawa, E., Kawano, T. and Matsumura, E. (1973) J. Biochem. (Tokyo) 74, 1003–1017.
[17] Matsutaka, H., Aikawa, T., Yamamoto, H. and Ishikawa, E. (1973) J. Biochem. (Tokyo) 74, 1019–1029.
[18] Hills, A. G., Reid, E. L. and Kerr, W. D. (1972) Am. J. Physiol. 223, 1470–1476.
[19] Felig, P., Wahren, J. and Räf, L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1775–1779.
[20] Exton, J. H., Harper, S. C., Tucker, A. L. and Ho, R.-J. (1973) Biochim. Biophys. Acta 329, 23–40.