BIOCHEMICAL LETTERS

Insulin action on the lactating-rat mammary gland: intracellular glucose and its phosphorylation

In their excellent study of the metabolic targets of insulin in the mammary gland of hyperinsulinaemic, euglycaemic, lactating rats, Burnol *et al.* (1988) identify the activation of phosphofructokinase-1 as important for enhancing glucose uptake. They propose that the observed fall in [glucose 6-phosphate] relieves an inhibition of hexokinase. The observed fall in tissue [glucose] is then ascribed to the increased rate of its phosphorylation.

Such de-inhibition is supported by experiments carried out in our laboratory several years ago (N. J. Pyne & N. J. Kuhn, unpublished work). Fig. 1 shows that the hexokinase activity of extracts of lactating-rat mammary tissue, assayed by a direct radiochemical method (News-holme *et al.*, 1967), was markedly inhibited by physiological concentrations of glucose 6-phosphate, and enhanced by the inclusion of glucose-6-phosphate dehydrogenase + NAD⁺. Intracellular [glucose 6-phosphate] in freeze-clamped mammary glands from rats given intravenous [¹⁴C]sucrose as extracellular marker was 0.15 mM and 0.13 mM in two fed rats, and 0.40 mM and 0.47 mM in two 16 h-starved rats.

On the other hand the 75% fall in tissue glucose reported by Burnol *et al.* (1988) cannot possibly be, as they claim, a decreased intracellular [glucose], the pool of which contributes at most 20% of the total (Page & Kuhn, 1987). The determination of intracellular [glucose] in lactating mammary gland is clearly of importance in learning how its enormous glucose consumption is controlled. In particular, it can throw light on the regulatory contribution made by transport across the plasma membrane. However, the technical difficulty in assessing this small intracellular pool by correction for extracellular glucose is exacerbated by uncertainty over the concentration of the latter in a tissue that extracts 30–60% of arterial glucose. A previous use of whole arterial blood [glucose] clearly gave incorrect values for intracellular [glucose] (Jones *et al.*, 1984). Arterial plasma [glucose] is too high since it gives nonsensical results. However venous plasma [glucose] gives a sensible range of calculated intracellular [glucose] values (0–0.4 mM) that agree with values arrived at by different approaches (Page & Kuhn, 1987). Therefore the decreased whole tissue glucose reported by Burnol *et al.* (1988) in euglycaemic rats must reflect a decreased venous plasma [glucose], consequent on the enhanced rate of uptake by the tissue. Alone, it gives no information on the intracellular

Fig. 1. Inhibition of hexokinase by added glucose 6-phosphate (a) and relief by added glucose-6-phosphate dehydrogenase and NAD⁺ of inhibition by endogenously generated glucose 6-phosphate (b)

Reaction mixtures containing 25 mM-Tris/HCl buffer, pH 7.4, 10 mM-mercaptoethanol, 5 mM-MgSO₄, 0.5 mM-EDTA, 4 mM-ATP, 0.2 mM (a) or 2.0 mM (b) [U-¹⁴C]glucose (7000 d.p.m./nmol) and 2.5 (or as shown) μl of mammary high speed supernatant in a final volume of 0.5 ml were incubated at 25 °C for 40 min. NAD⁺-specific glucose-6-phosphate dehydrogenase (G-6-PDH; 60 μunits) from *Leuconostoc mesenteroides* was added where indicated in panel (b).

[glucose] or on the rate-limiting status of glucose transport.

Nicholas J. KUHN

School of Biochemistry,
University of Birmingham,
P.O. Box 363,
Birmingham B15 2TT, U.K.

- Burnol, A. F., Ebner, S., Ferre, P. & Girard, J. (1988) *Biochem. J.* **254**, 11–14
 Jones, R. G., Illic, V. & Williamson, D. H. (1984) *Biochem. J.* **223**, 345–351
 Newsholme, E. A., Robinson, I. & Taylor, K. (1967) *Biochim. Biophys. Acta* **132**, 338–346
 Page, T. & Kuhn, N. J. (1987) *Biochem. Soc. Trans.* **15**, 1095–1096

Received 21 September 1988

Insulin action in the lactating mammary gland: a reply

In his letter, showing an interest in our work that we do appreciate, Kuhn (1989) expresses concern for our interpretation of the decreased mammary gland [glucose] during the euglycaemic hyperinsulinaemic clamp. He raises the question of which process (transport or metabolism) possesses the highest control strength on the pathway of glucose utilization in the mammary gland. In addition, Dr. Kuhn presents data on the inhibition of hexokinase by glucose 6-phosphate in the mammary gland, supporting the idea suggested in our paper that the decreased intracellular [glucose 6-phosphate] during the clamp could induce a de-inhibition of hexokinase. Indeed, intracellular [glucose 6-phosphate] (0.06 mM), calculated from whole tissue glucose-6-phosphate content (Burnol *et al.*, 1988) and intracellular water content (Page & Kuhn, 1987), is in the range of concentration having a small inhibitory effect on hexokinase activity (Kuhn, 1989).

It is clear that [glucose] measured in the whole mammary gland does not represent the intracellular [glucose], due to the contamination by the large extracellular glucose pool. Kuhn (1989) suggests that venous rather than arterial plasma [glucose] allows calculation of valid intracellular glucose concentrations (Page & Kuhn, 1987). The observed decrease in whole tissue [glucose] during the clamp (Burnol *et al.*, 1988) might thus reflect diminished venous [glucose] rather than a real decrease in intracellular [glucose] in the mammary gland. During the euglycaemic hyperinsulinaemic clamp, the blood [glucose] measured in the pudic-epigastric vein is decreased by only 38% under insulin stimulation (our unpublished results), whereas whole tissue [glucose] was decreased by 75% (Burnol *et al.*, 1988). This suggests that intracellular [glucose] was also decreased. The decrease in venous blood [glucose] during the clamp is in the range of values previously found during the re-activation of the mammary gland in the starvation–

refeeding transition (Jones & Williamson, 1984). The relatively small decrease in venous blood [glucose] is not in contradiction to the several-fold increase in glucose extraction, since glucose extraction is reflected by the arteriovenous difference and not by the absolute venous blood [glucose]. Moreover, an increase in the mammary blood flow when the mammary gland is active (Hanwell & Linzell, 1973; Jones & Williamson, 1984; Mercer & Williamson, 1986) probably minimizes the changes in the venous blood glucose concentration. We thus think that although we have not determined the intracellular [glucose] in the mammary gland, the decreased [glucose] in the whole gland reflects a real decrease in intracellular [glucose].

In our paper we stated that it was not possible, taking into account a potential decrease in the intracellular [glucose], to assess the relative importance of a stimulation of glucose transport by insulin or by the increased glucose gradient across the acinar membrane. However, we have not questioned a stimulatory effect of insulin on glucose transport (Threadgold & Kuhn, 1984). In any case, the decrease in [glucose 6-phosphate] in the presence of an increased glucose flux is sufficient in itself to conclude that the 'pull' mechanism has a greater importance in the insulin stimulation of mammary gland glucose metabolism than a 'push' mechanism attributable to glucose transport stimulation (a similar conclusion was reached by Jones *et al.*, 1984).

Finally, it could be argued that the euglycaemic hyperinsulinaemic glucose clamp is an unphysiological situation and that in a situation where the mammary gland is activated (fed state), an increase in blood [glucose] favours the 'push' mechanism. However, it has been shown that blood [glucose] excursions throughout the day are minimal (Carrick & Kuhn, 1978) and that in the starved–refed transition the arterial blood [glucose] is only marginally increased (Jones & Williamson, 1984).

Anne-Françoise BURNOL, Sylvie EBNER,
Pascal FERRÉ and Jean GIRARD

Centre de Recherche sur la Nutrition du CNRS,
9 rue Jules Hetzel, 92190 Meudon-Bellevue, France

- Burnol, A. F., Ebner, S., Ferré, P. & Girard, J. (1988) *Biochem. J.* **254**, 11–14
 Carrick, D. T. & Kuhn, N. J. (1978) *Biochem. J.* **174**, 319–325
 Hanwell, A. & Linzell, J. L. (1973) *J. Physiol. (London)* **233**, 111–125
 Jones R. G. & Williamson, D. H. (1984) *Biosci. Rep.* **4**, 421–426
 Jones R. G., Illic, V. & Williamson, D. H. (1984) *Biochem. J.* **223**, 345–351
 Kuhn, N. J. (1989) *Biochem. J.* **257**, 933–934
 Mercer, S. W. & Williamson, D. H. (1986) *Biochem. J.* **239**, 489–492
 Page, T. & Kuhn, N. J. (1987) *Biochem. Soc. Trans.* **15**, 1095–1096
 Threadgold, L. C. & Kuhn, N. J. (1984) *Biochem. J.* **218**, 213–219

Received 1 November 1988