Effects of Prolactin \textit{in Vitro} on Fatty Acid Synthesis in Rat Adipose Tissue*

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In preliminary experiments we have shown that ovine prolactin \textit{in vitro} stimulates the oxidation of glucose carbon to CO\textsubscript{2} by adipose tissue from normal fed rats \textit{ad libitum} (1). This increased glucose utilization is accompanied by an increased incorporation of glucose carbon into long chain fatty acid (1). These effects \textit{in vitro} of prolactin are qualitatively similar to those of insulin in the same tissue (2). Further experiments which form the basis of this report indicate that the effects \textit{in vitro} of prolactin on fatty acid synthesis in adipose tissue are dependent upon its primary effects on glucose metabolism.

EXPERIMENTAL

Male albino rats of the Wistar strain weighing between 125 and 150 g and fed Purina rat pellets (Ralston Purina Company) \textit{ad libitum} were used throughout these studies. Alloxan diabetic rats were prepared by the rapid intravenous injection of 45 mg per kilogram of alloxan monohydrate after a 24-hour fast; the animals were not used until at least 2 weeks after the administration of alloxan, and unless random blood glucose values exceeded 300 mg per 100 ml. Uniformly labeled glucose-\textsubscript{C\textsuperscript{14}}, glucose-1-\textsubscript{C\textsuperscript{14}}, glucose-6-\textsubscript{C\textsuperscript{14}}, acetate-1-\textsubscript{C\textsuperscript{14}}, and pyruvate-2-\textsubscript{C\textsuperscript{14}} were obtained from the Volk Radio-Chemical Company. These materials were chromatographically pure. Glucagon-free insulin was provided through the courtesy of Dr. W. R. Kirtley of the Lilly Research Laboratories. Ovine prolactin prepared by the Armour Laboratories was a gift of the Endocrinology Study Section of the National Institutes of Health.

The animals were killed by decapitation and the epididymal fat pads removed as previously described (2) with the exception that the pads were placed directly into the incubation vessels without prior weighing. At no time were the pads exposed to chilled buffer. In each instance one of each pair of epididymal fat pads was used as a control in order to minimize effects due to the variability in baseline metabolic activity of adipose tissue from one animal to another. Incubation was carried out in Stanley-Tracewell vessels as previously described (1). The vessels containing the appropriate substrate in 3.0 ml of Krebs bicarbonate or Krebs phosphate buffer were placed in a Dubnoff metabolic shaker set at 37°, 80 cycles per minute, and exposed to the proper gas phase before the animals were killed. After the adipose tissue had been placed in the vessels, exposure to the proper gas phase (5% CO\textsubscript{2} in 95% O\textsubscript{2} for Krebs bicarbonate buffer and 100% O\textsubscript{2} for Krebs phosphate buffer) was continued for an additional 5 minutes. The vessels were then stopped with rubber stoppers and kept closed for the remainder of the incubation period. Substrates were present in the medium at the following concentrations: glucose 20 mmoles per liter; pyruvate 40 mmoles per liter, and acetate 60 mmoles per liter. When glucose was added in the presence of acetate or pyruvate its concentration was 10 mmoles per liter.

Glucose present in the medium was determined by the use of glucose oxidase (3). Tissue nitrogen was determined by a micro-Kjeldahl procedure. The methods for the determination and calculation of substrate specific activity, carbon dioxide production from labeled substrate, and the incorporation of substrate carbon into long chain fatty acid were as previously described (1). All results have been expressed in terms of micromoles of substrate carbon oxidized to CO\textsubscript{2} or incorporated into fatty acid per mg of tissue nitrogen.

RESULTS

As shown in Table I, ovine prolactin \textit{in vitro} in a final concentration of 0.5 to 1.0 mg per ml increases the production of CO\textsubscript{2} from glucose by adipose tissue from normal fed rats. This increased glucose utilization is accompanied by an increased incorporation of glucose carbon into long chain fatty acid. Although a consistent and significant effect of prolactin \textit{in vitro} could be demonstrated on glucose oxidation to CO\textsubscript{2} and on the incorporation of glucose carbon into long chain fatty acid, the magnitude of this effect varied markedly from animal to animal, particularly at the higher hormone concentration. This marked variation in the magnitude of the response to prolactin \textit{in vitro} resembles the marked variability in the magnitude of the response to the addition of insulin \textit{in vitro} to this same tissue (2).

When insulin (0.1 unit per ml) was added to one of a pair of epididymal fat pads from a fed rat, both pads being incubated with prolactin (1.0 mg per ml), an additional increase in CO\textsubscript{2} production from uniformly labeled glucose-C\textsubscript{14} resulted (Table II). Moreover, fatty acid synthesis from glucose in the pad incubated in the presence of both insulin and prolactin was much greater than in the control pad incubated in the presence of prolactin alone.

Prolactin \textit{in vitro} increased CO\textsubscript{2} production from glucose by adipose tissue from alloxan diabetic rats as shown in Table III.
However, prolactin did not correct the defect in fatty acid synthesis from glucose in this tissue. In contrast, insulin in vitro corrects the defect in fatty acid synthesis from glucose in the adipose tissue of alloxan diabetic rats (2).

When adipose tissue from normal fed rats was incubated with either acetate-1-C\textsuperscript{14} or pyruvate-2-C\textsuperscript{14} prolactin in vitro had no consistent effect on the oxidation of acetate or pyruvate to CO\textsubscript{2}, effect.

\begin{table}[h]
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\begin{tabular}{lcccc}
\hline
Animal No. & Oxidation of glucose carbon to CO\textsubscript{2} & Incorporation of glucose carbon into fatty acids & \\
          & Control & Prolactin & Prolactin effect & Control & Prolactin & Prolactin effect \\
\hline
\textbf{Prolactin (1.0 mg per ml)\textsuperscript{+}} & & & & & & \\
\textbf{Mean 1-6 s.e.} & 1.41 & 5.98 & +1.57 & 0.40 & 2.57 & +2.17 \\
\textbf{Prolactin (0.5 mg per ml)} & & & & & & \\
1 & 1.91 & 3.49 & +1.58 & 0.19 & 0.53 & +0.34 \\
2 & 2.05 & 3.38 & +1.33 & 0.47 & 0.99 & +0.52 \\
3 & 0.60 & 2.52 & +1.15 & 0.11 & 0.24 & +0.13 \\
4 & 1.24 & 2.82 & +1.38 & 0.33 & 0.53 & +0.40 \\
5 & 2.08 & 3.39 & +1.31 & 0.52 & 0.57 & +0.05 \\
\textbf{Mean s.e.} & 1.64 & 3.12 & +1.48 & 0.28 & 0.57 & +0.29 \\
\end{tabular}
\caption{Effects of ovine prolactin added in vitro on metabolism of uniformly labeled glucose-C\textsuperscript{14} by rat adipose tissue*}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{lcccc}
\hline
Animal No. & Oxidation of glucose carbon to CO\textsubscript{2} & Incorporation of glucose carbon into fatty acids & \\
          & Control & Prolactin & Prolactin effect & Control & Prolactin & Prolactin effect \\
\hline
1 & 0.30 & 0.43 & +0.13 & 0.00 & 0.00 & +0.00 \\
2 & 0.00 & 0.89 & +0.89 & 0.00 & 0.00 & +0.00 \\
3 & 0.56 & 1.60 & +1.04 & 0.00 & 0.33 & +0.33 \\
4 & 0.20 & 0.79 & +0.59 & 0.00 & 0.00 & +0.00 \\
5 & 0.23 & 1.37 & +1.04 & 0.00 & 0.00 & +0.00 \\
\textbf{Mean s.e.} & 0.24 & 1.56 & +1.32 & 0.00 & 0.05 & +0.05 \\
\end{tabular}
\caption{Effects of ovine prolactin added in vitro on metabolism of uniformly labeled glucose-C\textsuperscript{14} by adipose tissue from alloxan diabetic rats*}
\end{table}

* All values expressed as amoles of glucose carbon per mg of tissue nitrogen. Incubation carried out for 3 hours in Krebs bicarbonate buffer containing 20 mmoles of glucose per liter. Prolactin concentrations, when present, 1.0 mg per ml. All experiments are paired. Statistical analysis of the differences listed under \textit{Effect} indicates the significance of the hormonal effect.
or on long chain fatty acid synthesis from these substrates (Table IV).

When unlabeled glucose (10 mmoles per liter) was present in the medium in addition to acetate-1-C\textsubscript{14} or pyruvate-2-C\textsubscript{14} prolactin in \textit{vitro} stimulated fatty acid synthesis from these two precursors of acetyl coenzyme A in adipose tissue from normal fed rats (Table V). It was concluded, therefore, that the effects of ovine prolactin \textit{vitro} on fatty acid synthesis from acetate or pyruvate are secondary to its effects on glucose metabolism in this tissue.

In Table VI are summarized experiments in which one member of a pair of epididymal fat pads was incubated with glucose-1-C\textsubscript{14}, and the other with glucose-6-C\textsubscript{14} under conditions previously described (1) which permit the hourly collection of the \textit{C}\textsubscript{14}O\textsubscript{2} evolved. Pads were incubated without added hormone for 1 hour to obtain control values for the relative rates of appearance of carbon atoms 1 and 6 of glucose in \textit{C}\textsubscript{14}O\textsubscript{2}. Prolactin (1.0 mg per ml) was then added. The data show that in the first hour under control conditions, 5 to 10 times as much carbon 1 of glucose was isolated in \textit{C}\textsubscript{14}O\textsubscript{2} as was carbon 6. After the addition of prolactin \textit{vitro} the amounts of both carbon 1 and carbon 6 appearing in \textit{C}\textsubscript{14}O\textsubscript{2} per hour were increased. However, in every experimental period after the addition of prolactin more carbon 1 appeared in \textit{C}\textsubscript{14}O\textsubscript{2} than did carbon 6. These data indicate the participation of the phosphoglucone oxidative pathway in the increased glucose utilization stimulated by prolactin \textit{vitro}.

\textbf{DISCUSSION}

The paired epididymal fat pads of the rat have proven to be a suitable system \textit{vitro} for the study of adipose tissue metabolism when undue handling or chilling is avoided (2). It has been shown that insulin \textit{vitro} markedly increases glucose uptake, the oxidation of glucose to \textit{C}\textsubscript{14}O\textsubscript{2}, and the incorporation of glucose carbon into long chain fatty acid in the epididymal fat of fed normal or alloxan diabetic rats (2). Insulin \textit{vitro} has no effect on fatty acid synthesis from acetate-1-C\textsubscript{14} or pyruvate-2-C\textsubscript{14} when these substrates are present alone in the medium. However, when unlabeled glucose is present in addition to either acetate or pyruvate a marked stimulation of fatty acid synthesis from these two precursors of acetyl coenzyme A is observed (2). It was concluded that the effects of insulin on fatty acid synthesis in rat adipose tissue are secondary to its effects on glucose metabolism.

The Embden-Meyerhof and phosphoglucone oxidative pathways are known to be operative in adipose tissue (4, 5), and there is also evidence suggestive of the presence of the uronic acid pathway as well (1). When paired epididymal fat pads are incubated with glucose-1-C\textsubscript{14} and glucose-6-C\textsubscript{14} and the appearance of carbon atoms 1 and 6 of glucose in \textit{C}\textsubscript{14}O\textsubscript{2} followed hourly over a 4-hour period the amount of carbon 1 appearing in \textit{C}\textsubscript{14}O\textsubscript{2} markedly exceeds that of carbon 6 (1). The addition of insulin \textit{vitro} after a 1-hour control period in such experiments produces a striking increase in the appearance of carbon 1 in
have been interpreted as indicating the participation of the phosphogluconate oxidative pathway in the increased glucose oxidation stimulated by insulin in vitro.

Bovine growth hormone in concentrations of 0.2 to 1.0 mg per ml has also been found to increase glucose oxidation to CO₂ in adipose tissue from fed normal or alloxan diabetic rats (1). However, this increased glucose utilization is not accompanied by an increase in fatty acid synthesis from glucose (1). Moreover, growth hormone in vitro stimulates glucose utilization in a manner which results in the more rapid appearance of carbon 6 of glucose in CO₂ than of carbon 1 (1). From these studies it appeared that only certain specific pathways of glucose metabolism lead to an increase in long chain fatty acid synthesis in adipose tissue.

Bovine serum albumin and commercial adrenocorticotropic hormone (Armour Laboratories) had no consistent effect in vitro on glucose oxidation or fatty acid synthesis from glucose in adipose tissue from normal fed rats (1). Although it is unlikely that adipose tissue is a major site of prolactin action, the use of this hormone in vitro provides a tool for the further exploration of the relationship between glucose metabolism and fatty acid synthesis in adipose tissue. The data herein reported show that prolactin in vitro has no direct effect on fatty acid synthesis from acetate or pyruvate unless glucose is also present in the medium. Prolactin stimulation of fatty acid synthesis from these two precursors of acetyl coenzyme A thus is dependent upon concomitant glucose utilization.

The effects of prolactin in vitro on CO₂ production by adipose tissue from glucose-1-C¹⁴ and glucose-6-C¹⁴ indicate the participation of the phosphogluconate oxidative pathway in the increased glucose utilization stimulated by this hormone. These prolactin effects resemble those of insulin in this tissue (1, 4), but differ from those observed with bovine growth hormone (1). Bovine growth hormone stimulates glucose utilization in adipose tissue in a manner which results in the more rapid appearance of carbon 6 in CO₂ than of carbon 1, suggesting that the phosphogluconate oxidative pathway does not participate to a major extent in the increased CO₂ formation from glucose. Both insulin and prolactin in vitro stimulate the synthesis of long chain fatty acid from glucose, whereas growth hormone does not. The data suggest that the differences in the effects of these hormones on fatty acid synthesis are related in part to the differences in their effects on carbohydrate metabolism in adipose tissue. The defect in fatty acid synthesis from glucose in adipose tissue from alloxan diabetic rats is not corrected by prolactin in vitro. This may mean its action is dependent upon the presence of insulin, or that its effect is not of sufficient magnitude to permit detection under the conditions employed. It may be argued that these effects in vitro of prolactin represent a delaying effect on the degradation of insulin bound to the tissue at the time of removal from the animal (6). Another possibility is that these effects reflect the action of prolactin in peripheral tissues but are modified by the relative insensitivity of adipose tissue to this hormone, and also perhaps by the factor of the species specificity of certain pituitary hormones (7). These same factors of relative insensitivity of the target tissue, and species specificity of pituitary hormones may account for the high concentrations of prolactin required to demonstrate these effects. The effects of prolactin in vitro cannot be distinguished from those of insulin on the glucose metabolism of adipose tissue from normal fed rats and may introduce uncertainty in the use of this tissue as a bioassay for insulin (8).

Whether these effects in vitro prove to be of physiological significance or not with respect to the action of prolactin on adipose and other tissues, the data raise the interesting possibility that any agent, physiological or pharmacological, which can stimulate the metabolism of glucose in adipose tissue in a manner similar to insulin will also stimulate long chain fatty acid synthesis from glucose, acetate, or pyruvate.

**SUMMARY**

The effects of ovine prolactin in vitro on the metabolism of glucose, acetate, and pyruvate by rat adipose tissue has been investigated with the use of paired epididymal fat pads. Ovine prolactin increased the oxidation of glucose carbon to CO₂ and enhanced the incorporation of glucose carbon into long chain fatty acid by adipose tissue from normal fed rats. Insulin (0.1 unit per ml) added in vitro to one of a pair of epididymal fat pads from normal fed rats, both pads being incubated in the presence of prolactin, stimulates a further increase in glucose oxidation to CO₂ and in fatty acid synthesis from glucose. Ovine prolactin in vitro increased CO₂ production from glucose by adipose tissue from alloxan diabetic rats but did not correct the defect in long chain fatty acid synthesis from glucose in this condition.

Prolactin in vitro had no effect on the synthesis of long chain fatty acid from acetate-1-C¹⁴ or pyruvate-2-C¹⁴ when these substrates were present alone in the medium. In the presence of added unlabeled glucose prolactin in vitro stimulates fatty acid synthesis from acetate-1-C¹⁴ and pyruvate-2-C¹⁴ by adipose tissue from normal fed rats. It was concluded that the effects of prolactin in vitro on long chain fatty acid synthesis in rat adipose tissue are dependent upon the effects of this hormone on glucose metabolism.

Paired epididymal fat pads from normal fed rats were incubated with glucose-1-C¹⁴ and glucose-6-C¹⁴ in Stanley-Tracewell vessels to permit hourly collections of CO₂ production. During an initial 1-hour control period the production of CO₂ from carbon 1 was 5 to 10 times greater than that from carbon 6. At the end of the control period prolactin was added in vitro; CO₂ production from both carbons 1 and 6 was increased, but the amount of carbon 1 appearing in CO₂ always markedly exceeded that of carbon 6. This observation indicates the participation of the phosphogluconate oxidative pathway in the increased glucose utilization stimulated by prolactin in vitro.

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