INHIBITION OF MUSCLE PYRUVATE DEHYDROGENASE BY A POLYPEPTIDE FROM GROWTH HORMONE

J.H. Aylward, J. Bornstein, M.K. Gould and Solveiga Hall

Department of Biochemistry
Monash University
Clayton, Victoria 3168, Australia

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SUMMARY. InG*, a polypeptide derived from growth hormone, inhibited the oxidation of labelled pyruvate by isolated rat soleus muscle. The oxidation of β-hydroxybutyrate was not inhibited, suggesting that InG affected the pyruvate dehydrogenase reaction. InG did not directly inhibit pyruvate dehydrogenase but inhibited the net conversion of pyruvate dehydrogenase from the inactive to the active form.

Bornstein and his co-workers (1) have shown that many of the insulin antagonistic effects of growth hormone could be reproduced by InG, a polypeptide prepared from purified growth hormone by hydrolysis at pH 2.3. Thus, InG inhibited glucose uptake by muscle (2), fatty acid synthesis in liver and adipose tissue, and stimulated lipolysis in adipose tissue (3).

The experiments presented in this paper have shown that InG inhibits the oxidation of pyruvate but not β-hydroxybutyrate by rat soleus muscle *in vitro*. Further, using the pyruvate dehydrogenase assay system of Wieland *et al*. (4), it has been shown that the effect of InG is not directly on the enzyme but on its activation.

METHODS

Rat soleus muscles weighing approximately 30 mg were incubated in Krebs bicarbonate medium, pH 7.4, gas phase 95% O_2 - 5% CO_2, at 37°. Glucose

* InG refers to a polypeptide derived from growth hormone which depresses glucose uptake by muscle and adipose tissue, fatty acid synthesis in liver and adipose tissue; potentiates lipolysis; and apparently specifically inhibits glyceraldehyde 3-phosphate dehydrogenase, pyruvic dehydrogenase and acetyl CoA carboxylase.

AcG refers to a similarly derived polypeptide which accelerates glucose uptake and fatty acid synthesis, inhibits lipolysis, and reverses or prevents the InG effect on the above enzymes.

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TABLE 1 EFFECT OF InG ON GLUCOSE UPTAKE AND LACTATE PRODUCTION

Soleus muscles were incubated for 60 min at 37° in Krebs-bicarbonate medium containing 1×10^{-2} M glucose $\pm$ 1 μg/ml of InG. Values are mean of 4 determinations $\pm$ S.D.

	Glucose Uptake (μmoles/g/h)	Lactate Produced (μmoles/g/h)
Control	15.4 $\pm$ 2.4	24 $\pm$ 2
InG	8.3 $\pm$ 3.8	37 $\pm$ 2

uptake and medium lactate were determined as described previously (2). For the measurement of ^{14}C-labelled CO_2 production from pyruvate, muscles were preincubated for 30 min at 37°, gassed with 95% O_2 - 5% CO_2, and sealed with rubber caps. The medium contained 5×10^{-2} M pyruvate or β-hydroxybutyrate and, where appropriate, InG $\pm$ AcG (1). Following preincubation, radioactive substrate (1 μCi) was added in a volume of 10 μl. The flasks were re-gassed with O_2 - CO_2, sealed, and incubated for 60 min at 37°. CO_2 was recovered using hyamine as a trapping agent, using standard manometric techniques, and counted in a Nuclear Chicago Unilux scintillation counter. In every experiment control flasks, containing all components except the tissue, were included in order to measure the amount of labelled CO_2 released from the substrate.

Pyruvate dehydrogenase assay

Rat muscle mitochondria prepared according to Bullock et al. (5) were suspended in potassium phosphate buffer, 5×10^{-3} M, pH 7.0, and disrupted by repeated freezing and thawing. Activation of the enzyme was effected by incubating the disrupted mitochondria (3.0 mg protein/ml) for 30 min at 25° in the presence of 1×10^{-2} M $CaCl_2$, 1×10^{-2} M $MgCl_2$, and 0.167 mg/ml of pig heart phosphatase (4). The activity of pyruvate dehydrogenase was assayed by the method of Wieland et al. (4), modified in that the concentration of p-nitroaniline was 1×10^{-4} M and that of mercaptoethanol was 3×10^{-3} M.

TABLE 2 EFFECT OF InG AND AcG ON OXIDATION OF PYRUVATE AND β-HYDROXYBUTYRATE

Evolution of ^{14}C-labelled CO_2 from [U-^{14}C] pyruvate or [4-^{14}C] β-hydroxybutyrate was determined as described in METHODS. Where indicated, InG ± AcG was also present at a concentration of 1 μg/ml. Values are mean of 4 determinations ± S.D.

	μmoles substrate ⟶ [^{14}C] CO_2/g muscle		
Substrate	Control	InG	InG + AcG
Pyruvate	1.2 ± 0.1	0.5 ± 0.1	1.5 ± 0.3
β-hydroxybutyrate	2.2 ± 0.2	3.7 ± 0.5	-

InG and AcG were prepared from ovine growth hormone (prepared by the method of Wilhelmi (6)) as previously described (2). Radioactive substrates were obtained from the Radiochemical Centre, Amersham, England.

RESULTS

In contrast to earlier experiments which showed that InG inhibited lactate production in cell-free muscle extracts (2), the polypeptide stimulated lactate production by soleus muscle whilst inhibiting glucose uptake (Table 1). This suggested that InG may have inhibited glucose metabolism at a point beyond pyruvate. As shown in Table 2, InG inhibited the oxidation of pyruvate, and this was reversed by AcG. The oxidation of β-hydroxybutyrate was, however, stimulated by InG, presumably by inhibiting the conversion of glucose intermediates arising from residual glycogen stores to acetyl CoA.

Thus it was concluded that InG inhibits the conversion of pyruvate to acetyl CoA.

The effect of InG on the pyruvate dehydrogenase activity of muscle mitochondria was investigated and, as seen in Table 3, the peptide did not affect

TABLE 3 EFFECT OF InG ON THE ACTIVATION OF PYRUVATE DEHYDROGENASE

Pyruvate dehydrogenase activity was assayed in skeletal muscle mitochondria under the following conditions :
Mitochondrial protein 0.90 mg ± Phosphatase 50 μg ± InG 0.6 μg.
Volume during activation 0.3 ml. Volume during assay 3 ml.

	[InG]			
	During Activation Period (μg/ml)	During Enzyme Assay (μg/ml)	nmoles Acetyl CoA/min/ mg mitochondrial protein ± S.D.	
Before Activation	-	0	0.58 ± 0.05	(5)
	-	0.2	0.58 ± 0.05	(3)
After Activation (30 min at 25°)	0	0	1.06 ± 0	(3)
	0	0.2	1.06 ± 0.05	(3)
	2	0.2	0.74 ± 0	(3)

the basal activity of pyruvate dehydrogenase, but strongly inhibited activation by phosphatase, and had no action on the fully activated enzyme.

DISCUSSION

Reports from a number of sources have indicated that growth hormone inhibited the oxidation of pyruvate *in vivo* (7,8) and *in vitro* (9). Similarly, it has also been reported that the ability to oxidise pyruvate may be diminished in both clinical (1) and experimental (11) diabetes; and, more specifically, Haft (12) has shown that formation of acetyl CoA from pyruvate was depressed in the liver of alloxan diabetic rats. The present observation that InG derived from growth hormone inhibited pyruvate oxidation by the rat soleus is consistent with the above reports. Recent studies have shown that pyruvate dehydrogenase exists in an active (dephosphorylated) and an inactive (phosphorylated) form

(4), and that the proportion of the enzyme in the active form is depressed in starvation (4) and diabetes (13). Weil et al. (7) and Wieland et al. (4) have suggested that these effects may be due to mobilisation of fatty acids, but Goodman (9) has shown that the inhibition of pyruvate metabolism by growth hormone was independent of adipose tissue lipolysis. Although elevation of fatty acids might be expected to contribute to the inhibition of pyruvate dehydrogenase, the experiments presented in this communication indicate that there may also be a direct effect of growth hormone on pyruvate dehydrogenase acting through the InG part sequence.

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