Vanadate Induction of L-Type Pyruvate Kinase mRNA in Adult Rat Hepatocytes in Primary Culture

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In primary culture of adult rat hepatocytes, vanadate in the presence of glucose stimulates the expression of the liver (L-type) pyruvate kinase gene. Glucose by itself was inactive, and vanadate, like insulin, was also inefficient in the absence of glucose. Similar results were obtained on glucokinase gene expression. An analogue of cAMP, 8-(4-chlorophenylthio)-cAMP, inhibited the production of L-type pyruvate kinase and glucokinase mRNAs in the presence of glucose plus vanadate. *Diabetes* 40:462–64, 1991

Vanadate has been reported to be a potent insulin mimetic agent in many cells (1–7). Oral administration of this compound to diabetic animals normalizes blood glucose concentration (8) and restores liver glycogen and fructose-2,6-bisphosphate levels and glucokinase and 6-phosphofructo-2-kinase activities (9). As a result of vanadate action, a switch from gluconeogenesis to glycolysis is produced in the diabetic rat liver.

The activity of liver (L-type) pyruvate kinase and its mRNA concentration fluctuate markedly according to the nutritional and hormonal status of the animal. Diabetes (10–12) and fasting (13,14) cause a strong decrease in activity and mRNA levels. Insulin administration to diabetic rats (10–12) or refeeding fasted rats with a high-carbohydrate diet (13,14) produces an increase in both parameters. Insulin, glucocorticoids, and thyroid hormones appear to play a "permissive" role in the induction of L-type pyruvate kinase mRNA synthesis in response to carbohydrate feeding (14,15). It has been shown that glucagon and its second messenger, cAMP, block transcription of the gene for L-type pyruvate kinase (12,14,15).

Decaux et al. (16) developed a model of adult rat hepatocytes in primary culture to study the action of different effectors on the expression of the L-type pyruvate kinase gene. In these cells, this gene is expressed only when glucose and insulin are present together, each of them being inactive by itself (16). Using the same model, we investigated the effect of vanadate on L-type pyruvate kinase gene expression.

RESEARCH DESIGN AND METHODS

All reagents were of the best grade available commercially. Sodium orthovanadate (Na_3VO_4) was from Fisher (Silver Spring, MD).

Hepatocytes were isolated from 72-h-starved male Wistar rats (wt 200–250 g) as described previously (16). After removing nonparenchymal cells and debris, hepatocytes were suspended in medium 199 containing 5.5 mM glucose, 1 nM insulin, 1 μM thyroid hormones, and 10% fetal calf serum, and 9×10^6 cells were plated in 5 ml of medium 199. Cultures were maintained at 37°C under an atmosphere of air/CO_2 (19:1). After 4 h, the medium was removed and replaced by 10 ml of fresh medium 199 containing 1 μM thyroid hormones, 1 μM dexamethasone, 10 nM insulin, 10 mM glucose, and no fetal calf serum, for 5 days. Then, the cells were cultured for 24 h in the presence of 10 mM lactate alone, without glucose or insulin. Culture was extended after removing the medium containing lactate and by addition of vanadate with or without glucose, insulin with glucose, or glucose alone for 24 h. The effect of an analogue of cAMP, 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), was tested in the presence of vanadate plus glucose or insulin plus glucose also for 24 h. The medium was changed every 24 h. All experiments were performed in the presence of glucocorticoids and thyroid hormones as permissive hormones (14,15). After these different treatments, cells were washed in isotonic saline solution, scraped out, and frozen at −80°C.

The extraction procedure of total RNA and Northern-blot analysis were performed (16). Filters were hybridized with

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L-type pyruvate kinase and glucokinase cDNA probes (16). The L-type pyruvate kinase cDNA probe corresponded to a 388–base pair (bp) *Taq* I–*Pst* I restriction fragment of the 11C6 clone, which was subcloned into the single-stranded phage M13 (17). The recombinant phage was used as a template for the synthesis of the complementary strand in the presence of the four deoxynucleotides, including a limited amount of [α-^{32}P]dCTP. The glucokinase cDNA probe, provided by Iynedjian (18), was 1800 bp long and was labeled by random priming.

RESULTS

To study the effects of vanadate on the expression of the L-type pyruvate kinase gene, we used the same experimental procedure as described by Decaux et al. (16). After 5 days of culture in the presence of 10 mM glucose and 10 nM insulin, the cells were cultured for 24 h in a medium containing 10 mM lactate but not glucose or insulin. Hepatocytes were then cultured for 24 h in the presence of vanadate with or without glucose or with glucose plus insulin.

When hepatocytes were cultured for 24 h with vanadate plus lactate in the absence of glucose, after deprivation of glucose and insulin, no accumulation of L-type pyruvate kinase mRNA was observed (Fig. 1). Glucose alone was inefficient in stimulating the expression of the L-type pyruvate kinase gene, as previously demonstrated (16). L-type pyruvate kinase gene was only expressed when glucose and vanadate were present together, as occurred with insulin plus glucose (16; Fig. 1). However, the increase of L-type pyruvate kinase mRNA levels in the presence of vanadate plus glucose was much less than that observed with insulin plus glucose. The accumulation of L-type pyruvate kinase mRNA in the presence of insulin plus glucose represented in vitro <10% of that reached in vivo (16; Fig. 1); this figure decreased to 2% when insulin was replaced by vanadate.

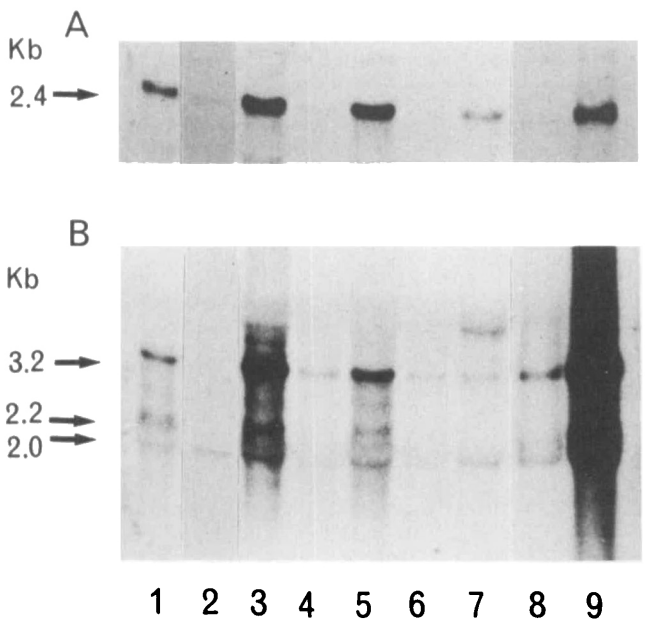

FIG. 1. Northern-blot analysis of total cellular RNAs after different culture conditions. Total cellular RNAs were isolated from pooled hepatocytes of 6–10 dishes for each culture condition. RNAs were denatured with methylmercury hydroxide and electrophoresed in 1.5% agarose gel (load of total cellular RNA was 30 μg/lane). RNAs were then transferred to nylon filter and hybridized with ^{32}P-labeled liver (L-type) pyruvate kinase (*B*) or with [^{32}P]glucokinase (*A*) cDNA probes. Similar results were obtained in 2 other experiments. *Lane 1*, 24 h of culture in presence of 10 mM lactate alone, without glucose and insulin; *lane 2*, same as *lane 1*, then 24 h of culture in presence of 10 mM glucose; *lane 3*, same as *lane 1*, then 24 h of culture in presence of 10 mM glucose and 10 nM insulin; *lane 4*, same as *lane 1*, then 24 h of culture in presence of 10 mM glucose, 10 nM insulin, and 50 μM cAMP analogue 8-(4-chlorophenylthio)-cAMP (CPT-cAMP); *lane 5*, same as *lane 1*, then 24 h of culture in presence of 10 mM glucose and 25 μM vanadate; *lane 6*, same as *lane 1*, then 24 h of culture in presence of 10 mM glucose, 25 μM vanadate, and 50 μM CPT-cAMP; *lane 7*, same as *lane 1*, then 24 h of culture in presence of 10 mM lactate and 25 μM vanadate; *lane 8*, liver from fasted rat; and *lane 9*, liver from carbohydrate-refed rat. Kb, kilobase.

The reaccumulation of L-type pyruvate kinase mRNA in the presence of glucose plus vanadate or glucose plus insulin was totally blocked by the addition of 50 μM CPT-cAMP (Fig. 1). This transcriptional inhibitory effect of cAMP was not counteracted by glucose plus vanadate or glucose plus insulin (16; Fig. 1), as has been shown in refed animals (14).

To confirm these results, the Northern blots were also hybridized with a cDNA probe specific to hepatic glucokinase mRNA (18), because it has been previously demonstrated that vanadate treatment of diabetic rats, like insulin administration (19–21), restored the decreased hepatic glucokinase activity, probably by inducing the synthesis of the enzyme (9). This hypothesis is supported by the fact that there is a good correlation between mRNA levels, rate of synthesis, and activity of glucokinase enzyme in the experimental conditions in vivo (19–21) and in vitro (22–24). Hepatocytes cultured for 24 h in the presence of insulin plus glucose or vanadate plus glucose showed an increase in glucokinase mRNA levels compared with those after 24 h of culture in a "lactate" medium (Fig. 1). Addition of CPT-cAMP inhibited the production of the specific mRNA in both experimental conditions. These results are in line with those of Iynedjian et al. (24).

DISCUSSION

Our results show that vanadate, in addition to the previously reported insulin mimetic effects (1–9), can also reproduce the hormone action on the expression of L-type pyruvate kinase gene. Like insulin, in the absence of glucose, vanadate is inefficient in stimulating the expression of the L-type pyruvate kinase gene. This result indicates that neither vanadate nor insulin has a direct role on transcription of the L-type pyruvate kinase gene by itself, because glucose is required for its mechanism of action. The observation that the insulin receptor has tyrosine-specific protein kinase activity after insulin binding (25) and the fact that vanadate is a potent and selective inhibitor of phosphotyrosyl–protein phosphatases (26) suggest that both agents could alter the phosphorylation state of relevant substrates involved in insulin action. Studies in vivo clearly show that insulin action on the transcription of the L-type pyruvate kinase gene may not be direct, because protein inhibitors impair the induction of the gene caused by this hormone (12). In addition, fructose could stimulate transcription of the L-type pyruvate kinase gene in vivo in the absence of insulin probably by stimulating accumulation of a glycolytic intermediate (10,12,27). As Decaux et al. (16) have hypothesized for the insulin-glucose–dependent stimulation of the gene, these results allow us to postulate that the vanadate-glucose–dependent increase of L-type pyruvate kinase levels also could

depend on the presence of a transcriptional activator derived from carbohydrate metabolism and accumulated in the presence of vanadate plus glucose.

In conclusion, our results show that vanadate acts similarly to insulin on gene expression. In addition, the use of hepatocyte cultures allows us to exclude an indirect mechanism of vanadate in vivo, mediated by an increase in insulin secretion.

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