Regulation by glucagon (cAMP) and insulin of the promoter of the human phosphoenolpyruvate carboxykinase gene (cytosolic) in cultured rat hepatocytes and in human hepatoblastoma cells

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A promoter fragment (−457 to +65) of the human cytosolic phosphoenolpyruvate carboxykinase gene, which by analogy to the rat promoter contains regulatory regions conferring glucagon (cAMP) and insulin responsiveness to the phosphoenolpyruvate carboxykinase gene, was cloned into a luciferase expression vector and transfected into cultured rat hepatocytes and human hepatoblastoma cells (HepG2) to study the regulation of the transgene by glucagon (cAMP) and insulin. A reporter gene that contained the rat promoter sequence from −493 to +33 was used for comparison. In cultured rat hepatocytes glucagon and its second messenger cAMP increased luciferase expression 4–6-fold over basal levels. Insulin reduced this effect by 40–70%.

Luciferase expression was also stimulated by the combination of dexamethasone and cAMP in HepG2 cells and this effect was inhibited by insulin. The phosphoinositide 3-kinase (PI 3-kinase) inhibitor, wortmannin, abolished this action of insulin in cultured rat hepatocytes. The results show that the promoter of the human phosphoenolpyruvate carboxykinase gene mediates the stimulatory action of glucagon and its second messenger cAMP. The inhibitory action of insulin was exerted through the PI 3-kinase pathway in cultured rat hepatocytes.

Key words: gluconeogenesis, HepG2 cells, liver, phosphoinositide 3-kinase.

INTRODUCTION

Phosphoenolpyruvate carboxykinase (GTP) (PCK; EC 4.1.1.32) converts oxaloacetate into phosphoenolpyruvate, which is a key regulatory step in the control of hepatic and kidney gluconeogenesis. In different species the gene is expressed in both the cytosol (PCK1) and the mitochondria (PCK2) to different extents. In human liver the cytosolic and the mitochondrial enzymes are expressed to almost the same amount, in rat liver the cytosolic and the mitochondrial enzymes are 

required for its stimulation by cAMP [18], are highly conserved in the human promoter. An insulin-responsive region, which has been mapped between positions −416 and −407 in the rat promoter [19,20], is completely conserved in the human promoter corresponding to positions −395 to −386 [13]. However, cotransfection experiments with an expression plasmid encoding the catalytic subunit of the cAMP-dependent protein kinase, and a chloramphenicol acetyltransferase (CAT) reporter gene under the control of the human PCK gene promoter, revealed no clear stimulation of CAT expression in H4IIIE rat hepatoma cells. No results from experiments using insulin as an antagonist are available [13].

The aim of the present study was to investigate the function of the human cytosolic PCK gene promoter by glucagon (cAMP) and insulin. The human PCK gene promoter responds to glucagon or cAMP in cultured rat hepatocytes, and to cAMP and/or dexamethasone in HepG2 cells. The stimulation was antagonized in either cell type by insulin.

EXPERIMENTAL PROCEDURES

Animals and chemicals

Male Wistar rats, supplied by Harlan-Winkelmann (Borchen, Germany), with a body weight of 200–250 g and fed with a standard diet (Altromin), were used for preparation of hepatocytes. Chemicals of ‘pro analysis’ quality were purchased from

Abbreviations used: PCK, phosphoenolpyruvate carboxykinase (GTP); CPT-cAMP, chlorophenylthio-cAMP; PI 3-kinase, phosphoinositide 3-kinase; CRE, cAMP-responsive element; CAT, chloramphenicol acetyltransferase; MEM, minimum essential medium; IRS, insulin-responsive sequence.

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local suppliers. Collagenase A, chlorophenylthio-cAMP (CPT-
cAMP), newborn calf serum and fetal calf serum were supplied by
Boehringer Mannheim (Mannheim, Germany) and hormones
and wortmannin by Sigma (Heidelberg, Germany). Culture
medium M199, minimum essential medium (MEM) with Earle’s
salts, non-essential amino acids and trypsin were supplied by Life
Technologies (Eggenstein, Germany) and Percoll by Pharmacia
Biotech (Freiburg, Germany). HepG2 cells were provided kindly
by Dr Thomas Kietzmann (Institut für Biochemie und
Molekulare Zellbiologie, Göttingen, Germany).

Preparation and culture of primary rat hepatocytes

Hepatocytes were prepared by the collagenase perfusion tech-
nique and separated from non-parenchymal liver cells and debris
by a Percoll density gradient. They were cultured for 48 h at
a density of 4.5 × 10⁴ cells/cm² in 1.5 ml of medium M199 on 60-
mm-diameter culture dishes under a gas atmosphere of 16 %
O₂/5 % CO₂/79 % N₂. The medium was supplemented with
100 nM dexamethasone, 0.5 nM insulin and, for the first 5 h,
with 4 %, newborn calf serum to support cell attachment. Cells
were cultured further in 2.5 ml of serum-free M199 containing
dexamethasone and insulin. The medium was changed after 24 h.
After a total of 48 h of culture the cells were washed twice with
insulin-free M199 with dexamethasone. Experiments were then
started by applying fresh medium containing hormones at the
concentrations given in the Figures.

Growth and culture of HepG2 cells

Human HepG2 hepatoblastoma cells were grown to confluence
in tissue-culture bottles (175 cm²) in 25 ml of MEM containing
10 % fetal calf serum. For subcultivation, trypsin-treated cells
were diluted in MEM containing 10 % fetal calf serum and
plated again on 60-mm-diameter culture dishes at a density of
1.4 × 10⁴ cells/cm². On the next day fresh serum-containing
medium was applied and cells were transfected after another 4 h.
Then, 24 h after transfection the cells were washed twice with
serum-free medium and experiments were started by applying
fresh medium containing hormones at the concentrations indi-
cated in the Figures.

Plasmids

Plasmid pGL-rPCK−493/+33, which contains the rat promoter
segment from −493 to +33 relative to the transcriptional start
site of the rat PCK gene, was constructed by cloning a PCR-
generated DNA fragment into the SmaI site of pUC19. The
fragment was cut out again with BamHI and KpnI and inserted
into the BglII- and KpnI-digested luciferase expression vector
pGL3 (Promega, Madison, WI, U.S.A.). The plasmid was kindly
provided by Dr Jutta Bratke (Institut für Biochemie und
Molekulare Zellbiologie, Göttingen, Germany). Plasmid pGL-
hPCK−457/+65 containing a human promoter fragment from
−457 to +65 relative to the transcriptional start site of the
human PCK gene was generated by digestion of HPP-CAT
(where HPP is human PCK promoter) [13] with BglII and
subcloning of the resulting DNA fragment into the BglII site of
pGL3. HPP-CAT was provided kindly by Dr R. O’Brien
(Department of Molecular Physiology and Biophysics,
Vanderbilt University Medical School, Nashville, TN, U.S.A.).

Mutation of a putative ‘insulin-responsive element’ in the human
PCK gene promoter

The plasmid pGL-hPCK−457/+65mut was generated by a
PCR approach using two different primer pairs carrying
the mutations in the putative insulin-responsive sequence
(IRS). pGL-hPCK−457/+65 was used as the template. One
primer pair comprised sense primer RVprimer3 in the PGL3
vector (Promega) and the mutated antisense primer 5’-
CAAAAGGGACCCAGCTATAAGATGTCACCCC-3’ (bases
−415 to −386 in the human promoter, with the mutation
underlined). The second primer pair comprised mutated sense
primer 5’-GTCCTTCTTTGCAACCAGCAGCTCTTGGTA-3’
(bases −395 to −366, with the mutation underlined) and the
antisense primer GLprimer2 in the PGL3 vector. The resulting
two PCR fragments were linked together by the use of the primer
pair RVprimer3 and GLprimer2. The resulting DNA fragment
was digested with HindIII and MluI and subcloned into the
HindIII/MluI-digested pGL3 vector. The TGGTG→GTCCC
mutation from position −395 to −391 was confirmed by
sequencing of the final plasmid.

Transfection protocol

The plasmids pGL-hPCK−457/+65, pGL-hPCK−457/
+65mut and pGL-rPCK−493/+33 were transfected into rat
hepatocytes and human HepG2 hepatoblastoma cells by the
calcium phosphate DNA precipitation method [21]. Plasmid
DNA (2 μg) was precipitated in 150 μl of 2× concentrated
Hepes (1× concentrated is made of 25 mM Hepes, pH 7.05,
140 mM NaCl and 0.75 mM NaHPO₄; buffer A) with 125 mM
CaCl₂ and added to 1.5 ml of a freshly isolated hepatocyte cell
suspension on a 60-cm-diameter culture dish. For the transfection
of HepG2 cells 2.5 μg of plasmid DNA in 150 μl of buffer A were
used and the precipitate was added to the cells on a 60-mm-
diameter culture dish. In both protocols the cells and the plasmid
DNA precipitate were mixed thoroughly. After transfection,
medium was changed and incubation continued as described
above. The protocol for the transfection of primary hepatocytes
has been used widely in different applications and with a variety
of plasmids. A promoter-less control luciferase transgene is not
expressed. The non-stimulated PCK gene promoter/luciferase
transgene is expressed at basal levels. Its expression can be
stimulated by glucagon or CPT-cAMP maximally between 24
and 48 h of culture. In the present study a 48 h culture period
was employed, because after that time the hepatocytes had completely
recovered from the isolation procedure. After 48 h of culture the
expression of the transgene was stimulated with glucagon or
CPT-cAMP for 8 h. During this stimulation period the expression
of the luciferase gene increased linearly with time [22–24].

Other assays and data analysis

The Promega luciferase bioluminescence assay was performed as
described by the supplier. Luminescence was measured with a
LB953 luminometer from Berthold (Wildbad, Germany).

The relative light units were normalized to protein content of the
cell extracts. Protein concentration was determined by the Bradford
method using a commercially available dye reagent (Bio-Rad,
München, Germany).

The results were calculated from a number of different cell
cultures, each run in triplicate, as indicated in the Figure legends.
They are expressed as means ± S.E.M. Significance of differences
between the means with glucagon alone and in the presence of
insulin was calculated by the use of Student’s t test applied to paired values. Significant inhibition by insulin was defined by the P values as indicated in the Figure legends.

RESULTS AND DISCUSSION

Stimulation by glucagon (cAMP) of the human and rat PCK gene promoters in cultured rat hepatocytes: inhibition of the stimulation by insulin

In cultured rat hepatocytes the expression of the luciferase transgene under the control of the human PCK gene promoter was stimulated 4-fold over the basal non-stimulated expression by 5 nM glucagon. Half-maximal stimulatory glucagon concentrations were reached at 0.11 nM. In the presence of 100 nM insulin the maximal stimulation by 5 nM glucagon was only 2.7-fold over non-stimulated values, which corresponds to a 43 % inhibition of the maximal stimulation observed in the absence of insulin. Again, half-maximal stimulation was reached at 0.11 nM glucagon. Insulin alone did not affect basal, non-stimulated luciferase expression. Thus insulin inhibited the maximal stimulation but did not shift the half-maximal stimulatory concentrations of glucagon (Figure 1A). When the cAMP analogue CPT-cAMP was used instead of glucagon, the expression of the luciferase transgene was stimulated by 10 μM CPT-cAMP 4.7-fold over the basal non-stimulated expression. In the presence of 100 nM insulin this stimulation was only 2.5-fold, which corresponds to a 58 % inhibition. Half-maximal stimulation was reached at 1.2 μM CPT-cAMP in the absence of insulin and at 1 μM in the presence of 100 nM insulin. Therefore the maximal stimulation was inhibited by insulin. The half-maximal stimulatory concentration of cAMP was unaffected. Again insulin did not affect PCK gene promoter-driven basal, non-stimulated luciferase expression (Figure 1B).

For comparison, a reporter gene consisting of the promoter of the rat PCK gene directing the expression of the luciferase reporter gene was transfected into cultured rat hepatocytes. Glucagon, at 5 nM, resulted in a 6.2-fold increase in luciferase expression over the basal, non-stimulated expression. Half-maximal stimulation was reached at 0.26 nM glucagon. In the presence of insulin the maximal stimulation was only 3.5-fold, which corresponds to a 51 % inhibition. Half-maximal stimulation was achieved at 0.3 nM glucagon. Basal, non-stimulated expression of the luciferase gene was not affected by insulin (Figure 1C). CPT-cAMP, at 10 μM, stimulated luciferase expression 5-fold over the basal, non-stimulated expression. Half-maximal stimulation was achieved at 0.29 μM CPT-cAMP. In the presence of 100 nM insulin the maximal stimulation by 10 μM CPT-cAMP was only 3.7-fold, which corresponds to a 32 % inhibition. The half-maximal stimulation was reached at 0.44 μM CPT-cAMP. Basal expression was not affected by insulin (Figure 1D).

Thus the maximal activation of the human and rat PCK gene promoters by glucagon or CPT-cAMP was inhibited by insulin. Insulin did not change the half-maximal stimulatory concentrations of glucagon or cAMP, which were in the same range for both promoters.

In the rat cytosolic PCK gene the CRE1, CRE2, P3 and P4 promoter elements mediate cAMP responsiveness [11]. Computer analysis revealed that these regions are highly conserved in the human promoter, thus it should also be regulated by glucagon or its second messenger, cAMP. However, functional analysis has only been carried out by overexpressing the catalytic subunit of protein kinase A along with a human PCK promoter-driven CAT reporter gene [13]. The results presented in this paper demonstrate that the human promoter is stimulated by glucagon and cAMP in cultured rat hepatocytes. Therefore, the stimulation

Figure 1 Concentration dependency of the glucagon- or cAMP-stimulated increase in human and rat PCK gene promoter activity in cultured rat hepatocytes: inhibition by insulin

Cells were transfected either with plasmid pGL-hPCK — 457/+65 (A, B) or with plasmid pGL-rPCK — 493/+33 (C, D), in which the luciferase gene is under the control of either a human or a rat PCK gene promoter fragment. After 48 h of culture the expression of the transgene was stimulated for 8 h with glucagon (A, C) or CPT-cAMP (B, D) at the concentrations indicated. Insulin at 100 nM was added where indicated. Then cells were harvested and luciferase activity was measured in cell lysates by the use of a luminometer. Relative luciferase activity was calculated as the increase over non-stimulated activity in the absence of glucagon or CPT-cAMP, set to 1. Values are the means ± S.E.M. from three to ten separate cell-culture experiments, each run in triplicate. Statistics: Student’s t test for paired values; values in the presence of insulin are different from values in the absence of insulin with * P ≤ 0.05 and ** P ≤ 0.01.
in response to glucagon is very likely to be mediated by the glucagon-stimulated increase in intracellular cAMP concentrations.

Insulin, the physiological antagonist of glucagon action, decreases the glucagon-stimulated increase in cAMP by the activation of phosphodiesterase, thereby attenuating the stimulation by glucagon of PCK gene expression [14,15,22]. In the present study insulin inhibited the stimulation by glucagon or cAMP of luciferase expression from the human as well as from the rat cytosolic PCK gene promoter, indicating that insulin was antagonistic through both promoters. However, because insulin is also inhibitory when the non-hydrolysable cAMP analogue CPT-cAMP is used, the effect must occur downstream from cAMP hydrolysis, as has been suggested previously [22,25].

Hence, from the present results it may be argued that the human promoter, like the rat promoter, responds to cAMP, the signal that represents increased blood glucagon concentrations. The result is an increased rate of PCK gene expression and hepatic gluconeogenesis during fasting periods. The increase in insulin concentrations after a carbohydrate-rich meal attenuates hepatic PCK gene expression and gluconeogenesis [26]. Thus the human PCK gene promoter may contribute to the glucostat function of the liver in humans. Because no antagonism by insulin was observed in human and rat hepatoma cells after stimulation of human PCK promoter/CAT reporter gene expression with the catalytic subunit of cAMP-dependent protein kinase [13], primary cultured hepatocytes may serve as a more adequate model system to investigate the regulation of the human PCK gene promoter by glucagon and insulin.

Stimulation by CPT-cAMP and dexamethasone of the human and rat PCK gene promoters in HepG2 cells: inhibition of the stimulation by insulin

In HepG2 cells luciferase expression under the control of the human PCK gene promoter was not significantly stimulated by CPT-cAMP alone (1.4-fold) but was increased 2.0-fold over basal non-stimulated expression by dexamethasone alone. The combination of cAMP and dexamethasone stimulated luciferase expression 2.8-fold over basal non-stimulated values. Insulin inhibited the dexamethasone-stimulated luciferase expression by 59%, and the CAMP/dexamethasone-stimulated expression by 76% (Figure 2A). Luciferase expression under the control of the rat PCK gene promoter was hardly stimulated by dexamethasone alone (1.4-fold) and was stimulated 2.6-fold over basal by CPT-cAMP alone. Dexamethasone and CAMP in combination stimulated luciferase expression by 4.3-fold. This stimulation was inhibited by 48% in the presence of insulin (Figure 2B). Basal non-stimulated luciferase expression from both promoters was not affected by insulin.

Stimulation by glucagon (cAMP) of the human PCK gene promoter containing a mutation in a putative IRS in cultured rat hepatocytes and HepG2 cells: inhibition of the stimulation by insulin

The rat PCK gene promoter contains two different cis-acting elements that mediate the inhibitory action of insulin on PCK gene transcription. One element is located between –437 and –402 and the other between –271 and +69 [12]. Attempts to more precisely define the IRS by using PCK gene promoter/CAT fusion genes with progressively shortened promoter fragments yielded inconsistent results in transient-transfection experiments. By the use of H4IIE cells stably transfected with PCK gene promoter/CAT transgenes an IRS was located between –416 and –407 in the rat PCK gene promoter. Mutational analysis in the context of a heterologous thymidine kinase promoter was used to confirm that this element mediates the insulin inhibition of the induction of a PCK promoter/CAT transgene by dexamethasone and cAMP. However, mutation of this element in the context of the otherwise wild-type rat PCK gene promoter did not abrogate insulin responsiveness [19,20]. The rat IRS is perfectly conserved in the human PCK gene promoter between –395 and –386 [13]; thus it was of interest to see whether this element behaved like the respective sequence in the rat promoter, i.e. mutation of the IRS in the context of the otherwise wild-type human promoter should not abrogate insulin responsiveness. By the use of a PCR approach the sequence TGGTG between –395 and –391 in the human promoter was converted into GTCCC. A reporter gene consisting of the mutated PCK promoter directing luciferase transgene in HepG2 cells by 2.6-fold, which corresponds to a 47% inhibition. In the absence and presence of insulin, half-maximal expression was reached at 0.09 and 0.12 nM glucagon, respectively (Figure 3A). Thus, as with the wild-type human PCK gene promoter, the mutation of the putative insulin-responsive region did not affect the inhibition by insulin of glucagon stimulation. When CPT-cAMP was used instead of glucagon the expression of the mutated promoter was stimulated maximally 3.2-fold over basal expression. In the presence of insulin this stimulation was only 2.2-fold, which corresponds to a 45% inhibition of the maximal response. Half-maximal stimulation was reached at 0.7 µM CPT-cAMP in the absence of insulin and at 1.25 µM CPT-cAMP in the presence of insulin (Figure 3B). Therefore, as with the wild-type human PCK gene promoter, a mutation of the putative insulin-responsive region did not affect the inhibition by insulin of stimulation by cAMP.

The combination of 100 µM CPT-cAMP and 500 nM dexamethasone stimulated the expression from the mutated human PCK gene promoter/luciferase transgene in HepG2 cells by 2.6-
Cells were transfected with plasmid pGL-hPCK—457/+65mut, which has a TGGTG → GTCCC conversion in the putative insulin-responsive region located between −395 and −391 in the human PCK gene promoter. Cells were processed as described in Figure 1. Expression of the luciferase transgene was stimulated with either glucagon (A) or CPT-cAMP (B) at the indicated concentrations in the absence or presence of 100 nM insulin. Relative luciferase activity was calculated as the increase over non-stimulated activity in the absence of glucagon or CPT-cAMP, which was set to 1. Values are the means±S.E.M. from three or four separate cell-culture experiments, each run in triplicate. Statistics: Student’s t test for paired values; values in the presence of insulin are different from values in the absence of insulin with *P < 0.05.

Figure 3  Concentration dependency of the glucagon- or cAMP-stimulated increase in activity of the mutated human PCK gene promoter in cultured rat hepatocytes: inhibition by insulin

Figure 4  Stimulation of the mutated human PCK gene promoter by CPT-cAMP and dexamethasone in human HepG2 cells: inhibition by insulin

HepG2 cells were transfected with the plasmid pGL-hPCK—457/+65mut, described in Figure 3. Cells were incubated for 24 h with 100 μM CPT-cAMP and 500 nM dexamethasone (Dex) in the absence (white bars) or presence (black bars) of 100 nM insulin. The expression of luciferase was measured in cell lysates using a luminometer. Relative luciferase activity was calculated as the increase over basal non-stimulated activity in the absence of CPT-cAMP and dexamethasone, set to 1. Values are the means±S.E.M. from three separate cell-culture experiments, each run in triplicate. Statistics: Student’s t test for paired values; values in the presence of insulin are different from values in the absence of insulin with *P < 0.05.

fold over the non-stimulated value. This increase of expression was prevented by 100 nM insulin (Figure 4). Hence, as in the rat promoter, the IRS in the human PCK gene promoter is not sufficient on its own to confer full insulin responsiveness to the promoter either in cultured rat hepatocytes or in HepG2 human hepatoblastoma cells. These results validate findings from previous studies with the rat PCK gene promoter, which showed that the deletion of the distal IRS between −437 and −402 alone did not abrogate insulin responsiveness. Insulin repression of the PCK gene promoter also requires the proximal IRS located between −271 and +69 [19,20].

Experiments to identify insulin responsiveness of the rat PCK gene promoter have been carried out in the rat hepatoma cell line H4IIE [19,20]. Therefore, these cells were also used to define the regulation by insulin of the human promoter. However, very low expression of a transfected luciferase transgene under the control of the human wild-type or mutated promoter was observed (results not shown), which corroborated the previous findings that only retinoic acid weakly stimulated the activity of the human promoter in H4IIE rat hepatoma cells; dexamethasone and the co-transfected catalytic subunit of protein kinase A as well as insulin were without effect [13]. Hence, obviously the functionality of the human PCK gene promoter depends on the proper cellular background. Therefore, constructs used in the present study could not be tested in H4IIE cells. Up to now no comprehensive results on the cell-specific regulation of PCK gene expression in rat hepatocytes and hepatoma cells are available. Because the rat PCK gene promoter carrying mutations in the insulin-responsive regions has never been investigated in cultured rat hepatocytes it will be a goal of future studies to define the cell-specific determinants of the regulation of PCK gene expression by comparing the stimulation by glucagon (cAMP) as well as the inhibition by insulin of the wild-type and mutant rat and human promoters in primary rat hepatocytes and in hepatoma cell lines.

A sequence that shares high homology with a prospective IRS was identified in the human PCK gene promoter between −295 and −283 by computer analysis [13]. This element has not been functionally investigated and is not conserved in the rat promoter. The role of this element, on its own or in co-operation with the upstream sequence, was not explored. It also was not within the scope of the present study to identify functionally and structurally the insulin-responsive regions in the human PCK gene promoter, which will be investigated in a separate study. However, the preliminary results shown here suggest that the human and rat promoters are very similar.

Involvement of phosphoinositide 3-kinase (PI 3-kinase) in insulin inhibition of the human and rat PCK gene promoters in cultured rat hepatocytes

Studies in cultured rat hepatocytes and in rat hepatoma cells showed that stimulation of the expression of the rat PCK gene by glucagon or cAMP was inhibited by insulin through the PI 3-
Farndehyde pathway [27–30]. The luciferase expression vector controlled by the human promoter was transfected into rat hepatocytes to investigate whether PI 3-kinase was also required for the insulin inhibition of the human PCK gene promoter. Inhibition of the glucagon (cAMP)-dependent activation by insulin was studied in the absence and presence of wortmannin, a PI 3-kinase inhibitor. The rat promoter was investigated for comparison. CPT-cAMP or glucagon stimulated luciferase expression from the reporter construct by 2.7- and 3.8-fold over basal non-stimulated expression. Insulin inhibited this stimulation by 71 and 51 %, respectively. Wortmannin alone had no significant effect on the stimulation of luciferase expression by CPT-cAMP or glucagon but it abrogated the inhibition by insulin (Figure 5A). When the luciferase gene was driven by the rat PCK gene promoter, CPT-cAMP or glucagon stimulated luciferase expression 4.5- or 5.1-fold over basal non-stimulated expression. Insulin inhibited this stimulation by 46 and 47 %, respectively, and this effect was abolished by wortmannin. The stimulation of luciferase expression by CPT-cAMP was increased further by 1.7-fold in the presence of wortmannin alone (Figure 5B). The data indicate that in cultured rat hepatocytes the glucagon (cAMP)-stimulated activation of the human and rat PCK gene promoters is inhibited by insulin through activation of the PI 3-kinase pathway.

Transcription of the rat PCK gene is inhibited by insulin in both primary cultured rat hepatocytes and rat hepatoma cells [12,15], and this appears to require an intact PI 3-kinase pathway [27–30]. The data presented here show that the human PCK gene promoter is also inhibited by insulin through activation of the PI 3-kinase pathway in primary cultured rat hepatocytes. The downstream PI 3-kinase target that ultimately mediates the transcriptional inactivation of the PCK gene promoter has not been identified. However, it must be at a site downstream of cAMP, because insulin inhibition of the CPT-cAMP stimulation of the rat and human promoters was abrogated by wortmannin.

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