Regulation of glycogen metabolism in cultured human muscles by the glycogen phosphorylase inhibitor CP-91149

Carlos LERÍN*[1], Eulàlia MONTELL*, Teresa NOLASCO*, Mar GARCÍA-ROCHA†, Joan J. GUINOVART*† and Anna M. GÓMEZ-FOIX*

*Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, E-08028 Barcelona, Spain, and †Institut de Recerca Biomèdica de Barcelona–Parc Científic de Barcelona, Universitat de Barcelona, E-08028 Barcelona, Spain

Pharmacological inhibition of liver GP (glycogen phosphorylase), which is currently being studied as a treatment for Type II (non-insulin-dependent) diabetes, may affect muscle glycogen metabolism. In the present study, we analysed the effects of the GP inhibitor CP-91149 on non-engineered or GP-overexpressing cultured human muscle cells. We found that CP-91149 treatment decreased muscle GP activity by (1) converting the phosphorylated AMP-independent *a* form into the dephosphorylated AMP-dependent *b* form and (2) inhibiting GP *a* activity and AMP-mediated GP *b* activation. Dephosphorylation of GP was exerted, irrespective of incubation of the cells with glucose, whereas inhibition of its activity was synergic with glucose. As expected, CP-91149 impaired the glycogenolysis induced by glucose deprivation. CP-91149 also promoted the dephosphorylation and activation of GS (glycogen synthase) in non-engineered or GP-overexpressing cultured human muscle cells, but exclusively in glucose-deprived cells. However, this inhibitor did not activate GS in glucose-deprived but glycogen-replete cells overexpressing PTG (protein targeting to glycogen), thus suggesting that glycogen inhibits the CP-91149-mediated activation of GS. Consistently, CP-91149 promoted glycogen resynthesis, but not its overaccumulation. Hence, treatment with CP-91149 impairs muscle glycogen breakdown, but enhances its recovery, which may be useful for the treatment of Type II (insulin-dependent) diabetes.

Key words: glycogenolysis, glycogen phosphorylase, glycogen resynthesis, protein phosphatase 1, skeletal muscle.

INTRODUCTION

The inhibition of liver GP (glycogen phosphorylase) decreases hepatic glucose output [1] and is therefore envisaged as a therapeutic strategy for Type II (non-insulin-dependent) diabetes. Nevertheless, pharmacological inhibitors of GP are not selective to the hepatic isoform, so they may also affect muscle glycogen metabolism. MGP (muscle GP) and liver GP, although encoded by separate genes, are highly similar. Both enzymes are interconverted between an active *a* and an inactive *b* form by phosphorylation–dephosphorylation reactions respectively. Dephosphorylation, which is mainly catalysed by PP1 (protein phosphatase 1), activates GS (glycogen synthase), while it inactivates GP. Both liver GP and MGP are additionally modulated by allosteric effectors. These isoforms are inhibited by purine analogues, such as caffeine, by binding at the purine inhibitory site, whose physiological ligand is unknown, and glucose, which binds at the active site and competes with glucose-1-phosphate. Glucose and purine inhibitions are synergistic [2]. Distinctively, MGP is activated allosterically by AMP, which acts at a specific site in the N-terminal region [2,3].

CP-91149 is a pharmacological inhibitor of liver GP and MGP *in vitro*, with an IC_{50} of 0.13 and 0.20 μM respectively [4]. It is structurally related to caffeine and its inhibitory activity *in vitro* is synergistic to that of glucose. When added to hepatocytes, CP-91149 inhibits glucagon-stimulated glycogenolysis, whereas *in vivo* administration to diabetic mice decreases liver glycogen breakdown [5]. CP-91149 also inhibits MGP functionality when expressed in liver cells [6] and blocks glycogenolysis in human myoblasts [7]. Muscle glycogen is the energetic reservoir for working fibres and it is also one of the main factors that modulate whole-body glucose tolerance. Thus the study of the impact of pharmacological inhibition of GP on human muscle glycogen metabolism is a crucial issue. In the present study, we report the effects of CP-91149 on GP and GS activity in primary cultured human muscle myotubes, either non-engineered or overexpressing MGP, over a range of metabolic conditions.

EXPERIMENTAL

Human muscle primary cultures and treatments

Human muscle primary cultures were initiated from satellite cells from muscle biopsies of three patients tested negative for muscle disease after diagnostic studies (biopsies were obtained with the informed consent and approval of the Human Research Ethics Committee of the Hospital Clínic, Barcelona, Spain). Aneural muscle cultures were established in a monolayer as described elsewhere [8]. Cultures were grown in Dulbecco's modified Eagle's medium/M-199 medium (3:1, v/v), supplemented with 10 % (v/v) foetal bovine serum, 10 μg/ml insulin (Sigma), 2 mM glutamine (Sigma), 25 ng/ml fibroblast growth factor and 10 ng/ml epidermal growth factor (BD Biosciences, Erembodegem, Belgium). Immediately after myoblast fusion, cells were rinsed in Hanks balanced salt solution, and the medium was replaced with a medium devoid of fibroblast growth factor, epidermal growth factor and glutamine before the experiments were performed. All cultures were kept at 37 °C in a humidified 5 % CO_2 atmosphere.

Abbreviations used: GP, glycogen phosphorylase; GS, glycogen synthase; MGP, muscle GP; PP1, protein phosphatase 1; PTG, protein targeting to glycogen.

[1] To whom all correspondence should be addressed. Present address: Department of Cell Biology, School of Medicine, Johns Hopkins University, 725 N Wolfe St., 104 Physiology, Baltimore, MD 21205, U.S.A. (e-mail clerin1@jhmi.edu).

All experiments were performed with at least two biopsies. CP-91149 was kindly provided by Pfizer (Groton, CT, U.S.A.). The synthesis and pharmacological properties of CP-91149 are described in [5].

Transduction with recombinant adenovirus

Adenovirus containing the β-galactosidase cDNA was used as a control. Adenovirus bearing the rabbit MGP cDNA (AdCMV-MGP) has been described elsewhere [9]. Adenovirus containing the mouse PTG (protein targeting to glycogen) cDNA has been described previously [10]. The recombinant viruses were amplified in HEK-293 cells (human embryonic kidney 293 cells), and viral stocks of 1×10^{10} pfu (plaque-forming unit)/ml were prepared in 10 % foetal bovine serum/Dulbecco's modified Eagle's medium. Gene delivery to muscle cultures was achieved by exposing 10-day-old fibres to the virus for 2 h at an moi (multiplicity of infection) of 10. The studies were performed at the time indicated after infection.

Assays of enzymic activity

To measure GS and GP activities, frozen plates were scraped with 100 μl of homogenization buffer consisting of 10 mM Tris/HCl (pH 7.0), 150 mM KF, 15 mM EDTA, 600 mM sucrose, 15 mM 2-mercaptoethanol, 10 μg/ml leupeptin, 1 mM benzamidine and 1 mM PMSF, and the collected cells were subsequently sonicated. The resulting homogenates were used for the determination of enzymic activities. Protein concentration was measured by the Bio-Rad protein assay. GP activity was determined by measuring the incorporation of [U-^{14}C]glucose 1-phosphate into glycogen in the absence or presence of AMP (5 mM) [11]. The GS activity ratio was determined by measuring the incorporation of [U-^{14}C]UDP-glucose into glycogen in the absence or presence of 10 mM glucose 6-phosphate, as described in [12].

Metabolite determinations

To measure the glycogen content, cell monolayers were scraped into 100 μl of 30 % (w/v) KOH and boiled for 15 min. An aliquot of the homogenates was used for the measurement of protein concentration as described above. Homogenates were spotted on to Whatman 3T paper, and glycogen was precipitated by immersing the papers in ice-cold 66 % (v/v) ethanol. The papers were washed twice with 66 % ethanol and dried papers containing precipitated glycogen were incubated in 0.4 M acetate buffer (pH 4.8) containing 25 units/ml α-amyloglucosidase (Sigma) for 90 min at 37 °C. Glucose released from glycogen was measured enzymically in a Cobas Fara II autoanalyser using a GlucoQuant kit (Roche, Barcelona, Spain).

Non-denaturing gel analysis

Cell monolayers were scraped into 100 μl of homogenization buffer as described above, and the homogenates were sonicated and centrifuged at 10 500 ***g*** for 15 min. Pellets were discarded, and an aliquot of supernatants was used to measure the protein concentration as described above. Protein (50 μg) was loaded on to a 5 % (w/v) acrylamide gel polymerized in the presence of 100 mM Tris/HCl (pH 8.8) and 0.5 % glycogen. The electrophoresis buffer consisted of 25 mM Tris/HCl and 200 mM glycine at pH 8.8. A 30 mA current was applied for 60 min at 4 °C, and gels were then incubated for 20 h in a solution containing 50 mM glucose 1-phosphate, 1 % glycogen, 5 mM dithiothreitol, 5 mM EDTA and 100 mM Tris/HCl at pH 6.8 in the presence or absence of 5 mM AMP. Gels were washed three times with distilled water to remove dithiothreitol and incubated with 0.4 % KI and 0.2 % I_2 to reveal glycogen. Purified GP *a* and GP *b* were obtained from Sigma.

Western-blot analysis

Cell monolayers were scraped into 100 μl of homogenization buffer as described above and the homogenates were sonicated. Protein concentration was measured as described above. Protein (25 μg) was loaded on to an 8 % acrylamide gel. Immunoblot analysis was performed using the rabbit polyclonal antibody MGS3 to the muscle isoform of GS. MGS3 was generated against a synthetic peptide containing the nine C-terminal amino acids of the protein (^{729}TSSLGEERN^{737}) and was effective in immunoprecipitating GS activity in homogenates from rat muscles. In Western blots, MGS3 recognized only a band of protein that corresponded to the molecular mass of the enzyme [13]. The primary antibody was detected using the ECL® Plus kit (Roche).

Statistical analysis

Results were analysed for statistical significance by the Student's *t* test.

RESULTS AND DISCUSSION

Inhibition and dephosphorylation of GP by CP-91149

To facilitate ulterior analysis of the enzymic activity and the activation state, we examined the effects of CP-91149 on GP in non-engineered human muscle cells or cells overexpressing MGP after adenoviral transduction. In all the experiments, we used a concentration of 10 μM CP-91149; this concentration inhibits the muscle isoform of GP [6,7]. In the presence of glucose, GP AMP-independent activity (*a* form) was decreased by 58 and 90 % in control and GP-overexpressing cells respectively 2 min after the addition of the inhibitor, and remained constant for at least 3 h. Since this inhibitor is more potent in the presence of glucose when assayed *in vitro* [5], the effect of CP-91149 was determined in cells incubated with or without glucose. In Figure 1(A), we show the inhibition of GP *a* in extracts from control and MGP-overexpressing cells treated with 10 μM CP-91149 for 3 h. In both these cell types, inhibition was higher in glucose-incubated cells compared with glucose-deprived cells (i.e. 55 and 44 % inhibition respectively in control cells). This observation appeared to confirm the synergy of inhibition [5]. However, we could not rule out that part of this smaller inhibition in glucose-deprived cells was due to prior inactivation of GP.

To determine whether CP-91149 inhibited GP *a* allosterically or via its covalent modification, namely conversion into GP *b*, we analysed GP activity in native gels, which allow discrimination between the *a* and *b* forms (Figure 1B, lanes 5 and 6). Endogenous GP activity was not detected in these gels, possibly owing to the low sensitivity of this technique. In contrast, a faint band of the *a* form (AMP-independent activity) was detected in MGP-overexpressing cells incubated with or without glucose (Figure 1B, lanes 1 and 3). A very intense band was detected after development in the presence of AMP. Since GP *a* is not activated by AMP, as shown with purified GP *a* protein (lane 5), these results show that, in MGP-overexpressing cells, most of the enzyme was in the *b* form. After treatment with the inhibitor, the band activity corresponding to the *a* form was blunted in both glucose-deprived and glucose-incubated cells (lanes 2 and 4). This observation indicates that CP-91149 promoted the conversion of GP *a* into GP *b*, according to a model proposed in hepatocytes [6].

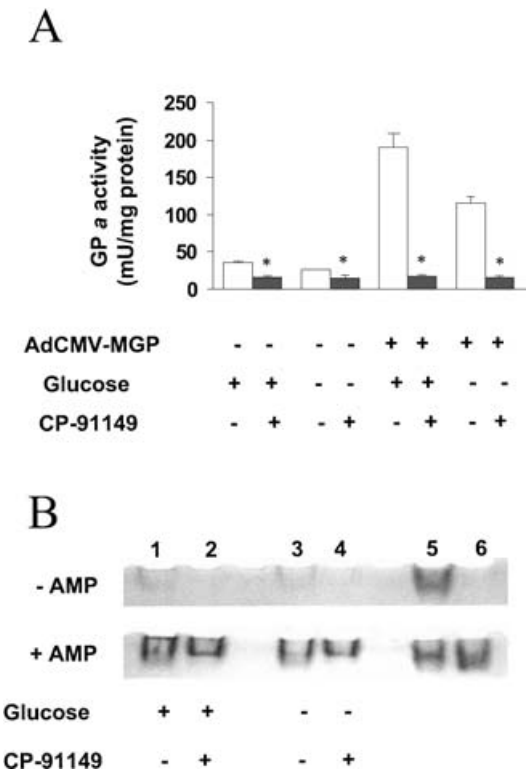

Figure 1 Effect of CP-91149 on muscle-cell AMP-independent GP activity

Cells were transduced with adenoviruses and incubated in the presence of 25 mM glucose for 2 days. They were then either transferred to a glucose-depleted medium (glucose −) or maintained in the same medium (glucose +) for 18 h. Finally, cells were incubated with (black bar) or without (open bar) 10 μM CP-91149 for 3 h. (**A**) GP *a* activity was measured in the absence of AMP by a radioisotopic technique. Differences between cells incubated with CP-91149 and untreated cells are significant ($^*P < 0.00001$). Results are the means ± S.E.M. for at least three independent experiments performed in duplicate. (**B**) Samples from AdCMV-MGP-treated cells were electrophoresed on non-denaturing gels, which were then incubated with the reaction mixture with or without the addition of 5 mM AMP to reveal the GP *a* or total GP activity. Lanes 5 and 6, purified GP *a* and *b* proteins without any treatment. A representative gel from five independent experiments is shown.

We also assessed whether CP-91149 impairs the allosteric activation of GP *b* by AMP, which cannot be ascertained in native gels. For this purpose, AMP-dependent GP activity was examined in muscle-cell extracts (Figure 2), which mostly contained the *b* form (Figure 1B). Total GP activity was decreased by approx. 30% in control cells or by 80% in MGP-overexpressing cells incubated with CP-91149, irrespective of incubation with glucose. These results indicate that the agent blocks AMP activation of GP *b*. Total GP activity was only approx. 20% higher compared with the *a* form in control and GP-overexpressing cells. This observation indicates a higher *a*-form to *b*-form ratio compared with that deduced from native gels. Since the basis of the activity assay is equivalent in both systems, this divergence may be explained by the presence of soluble factors in cell extracts, removed by electrophoresis, which positively influence GP *b* activity, thereby contributing to AMP-independent activity.

Impairment of glycogenolysis by CP-91149

Since CP-91149 promoted the inactivation and inhibition of GP, we next examined its effect on glycogenolysis. Cells were deprived of glucose to increase intracellular AMP and to activate GP allosterically [2,3,14], since, in the presence of glucose, GP

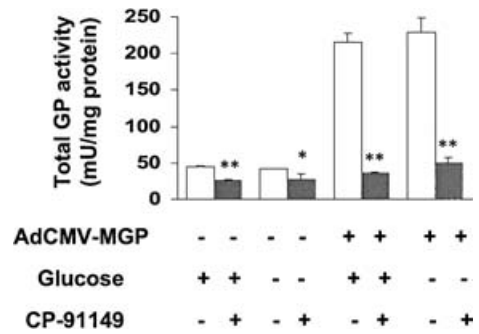

Figure 2 Effect of CP-91149 on muscle-cell total GP activity

Cells were exposed to AdCtrl (adenovirus containing the β-galactosidase cDNA) or AdCMV-MGP and incubated in the presence of 25 mM glucose for 2 days. They were then either transferred to a glucose-depleted medium or maintained in the same medium for 18 h. Finally, cells were incubated in the absence (open bar) or presence (black bar) of 10 μM CP-91149 for 3 h and GP total activity was measured by the radioisotopic technique. Differences between cells incubated with CP-91149 and untreated cells are significant ($^*P < 0.005$ and $^{**}P < 0.00005$). Results are the means ± S.E.M. for at least three independent experiments performed in duplicate.

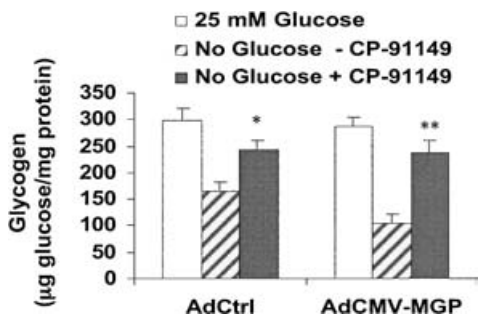

Figure 3 Reduction by CP-91149 of the glycogenolytic response to glucose depletion

Cells transduced with adenoviruses were maintained in 25 mM glucose for 3 days. Glycogen content (open bar) was determined. Cells were then incubated in the absence of glucose for 4 h with (black bar) or without (hatched bar) 10 μM CP-91149. Differences between cells incubated with CP-91149 and untreated cells are significant ($^*P < 0.001$ and $^{**}P < 0.00005$). Results are the means ± S.E.M. for three independent experiments performed in triplicate.

activity is allosterically inhibited and glycogen levels are constant [16]. Consistently, glycogen content decreased to 55% of the initial value in control cells and to 36% in MGP-overexpressing cells (Figure 3). Treatment with the GP inhibitor prevented glycogen mobilization in both cell types, although inhibition was higher in MGP-overexpressing cells (74%) compared with control cells (60%).

Stimulation of GS activation and glycogen resynthesis by CP-91149

We next checked whether CP-91149 affected GS activation by assessing the activity ratio in the absence or presence of the allosteric activator glucose-6-phosphate. This was examined in cells incubated with glucose or cells deprived of glucose to exhaust glycogen, which exerts a feedback inhibition on synthase phosphatase activity but not on phosphorylase phosphatase [15,16]. In cells incubated with glucose, which have high glycogen levels, CP-91149 did not affect the GS activity ratio in control or MGP-overexpressing cells, which showed very similar activity levels (Figure 4A). Moreover, an immunoblot analysis showed that the electrophoretic mobility of GS, which depends on the phosphorylation state of the enzyme, was not altered (Figure 4B).

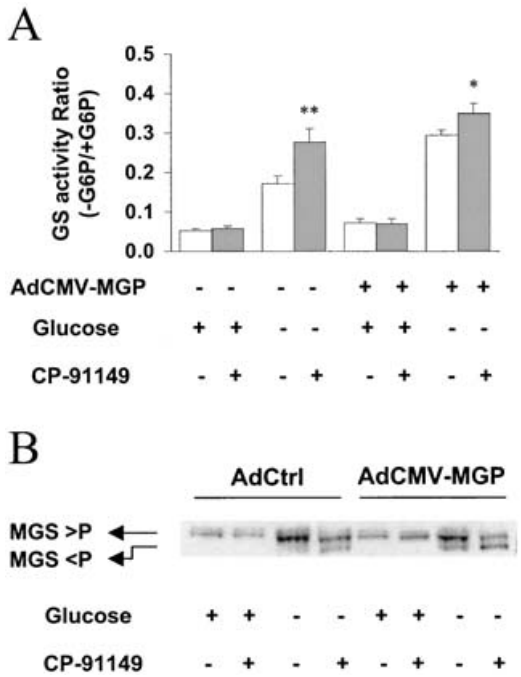

Figure 4 Regulation of GS activity by CP-91149

Cells were exposed to adenoviruses and incubated in the presence of 25 mM glucose for 2 days. They were then either switched to a glucose-depleted medium (glucose −) or maintained in the same medium (glucose +) for 18 h. Finally, cells were incubated in the absence (open bar) or presence (black bar) of 10 μM CP-91149 for 3 h. (**A**) The GS activity ratio was determined. (**B**) MGS was detected by Western-blot analysis. (**A**) Differences between cells incubated with CP-91149 and untreated cells are significant ($^*P < 0.05$ and $^{**}P < 0.00005$). Results are the means ± S.E.M. for six independent experiments performed in duplicate. G6P, glucose 6-phosphate. (**B**) A representative autoradiogram from five independent experiments is shown. GS < P, GS with greater mobility, indicative of a lower phosphorylation state; GS > P, GS with lower mobility, indicative of a higher phosphorylation state.

Table 1 Role of glycogen levels in CP-91149-mediated GS activation

Cells with high glycogen levels were prepared by incubating cells with 25 mM glucose for 2 days after AdCMV-PTG transduction, followed by an 18 h incubation in a glucose-depleted medium. Cells with low glycogen levels were obtained by transduction with AdCMV-PTG and concomitant incubation in a glucose-depleted medium for 18 h. Glycogen levels were determined. Finally, cells were incubated in the absence or presence of 10 μM CP-91149 for 3 h and the GS activity ratio was determined. Results are the means ± S.E.M. for at least three independent experiments performed in triplicate (glycogen levels) or duplicate (GS activity); G6P, glucose 6-phosphate.

	Glycogen (μg of glucose/mg of protein)	GS activity ratio (− G6P/+ G6P)	
		− CP-91149	+ CP-91149
High glycogen level	1864 ± 108	0.29 ± 0.02	0.29 ± 0.03
Low glycogen level	104 ± 12	0.32 ± 0.01	0.40 ± 0.05*

$^*P < 0.05$, differences between cells incubated with CP-91149 and untreated cells are significant.

In cells incubated without glucose, which have depleted glycogen stores, GS was more active, and the activation state was further enhanced by MGP overexpression in correlation with lower glycogen content. This observation is consistent with the above notion that glycogen inhibits GS activation [16]. In glucose-deprived cells, CP-91149 caused the additional activation of GS in both cell types, although activation was higher in control cells compared with MGP-overexpressing cells (Figure 4A). Activation of GS in all these situations was associated with a downward shift in the electrophoretic mobility of GS on SDS/polyacrylamide gels, indicating dephosphorylation of the enzyme (Figure 4B).

Overall, these results demonstrate that treatment of muscle cells with CP-91149 activated GS only in glucose-deprived cells, which have low glycogen levels, and not in glucose-incubated cells, which have high glycogen levels. To determine whether the inhibition of the CP-91149 effect was caused by the presence of glucose or the high glycogen content, cells were genetically modified to modulate glycogen stores. For this purpose, cells were transduced with adenovirus bearing the PTG cDNA. Consistent with results of our previous study [17], overexpression of PTG in muscle cells activated GS and increased glycogen levels up to 2429 ± 189 μg of glucose/mg of protein, 2 days after adenoviral infection. Treatment of glucose-incubated PTG-overexpressing cells with CP-91149 did not modify the GS activity ratio (results not shown). Cells were then incubated in the absence of glucose for 18 h, and glycogen levels decreased to 1864 ± 108 μg of glucose/mg of protein. Treatment with CP-91149 under these conditions (high glycogen levels) did not modify the GS activity ratio (Table 1), which differs from the behaviour in both control and MGP-overexpressing cells incubated in the absence of glucose (see Figure 4A). Moreover, cells were transduced with AdCMV-PTG and concomitantly switched to a glucose-depleted medium to deplete glycogen levels. Under these conditions (low glycogen levels), treatment with the inhibitor activated GS (Table 1), indicating that PTG overexpression by itself did not affect the CP-91149-mediated GS activation. These results demonstrate that glycogen blocks the GS activation induced by CP-91149 treatment. Since glycogen is a potent inhibitor of synthase phosphatase activity in muscle cells, we hypothesize that CP-91149 activates a GS phosphatase activity.

In conclusion, our results suggest that CP-91149 may activate a phosphatase activity that can act on both glycogen-metabolizing enzymes. Remarkably, the activity of this synthase phosphatase would be inhibited by glycogen, whereas the activity of phosphorylase phosphatase would not. Dephosphorylation of GS and GP is primarily catalysed by PP1 activity, which is modulated by regulatory subunits. In muscle cells, at least three PP1 regulatory subunits are expressed, namely G_M, PTG and PPP1R6, whereas, specifically in the human muscles, expression of G_L was also reported previously [18]. A direct effect of GP on synthase phosphatase activity is well characterized in liver [19], which expresses high levels of G_L [20], and it can also be triggered by the muscle isoform of GP when expressed in hepatocytes [6]. The active phosphorylated form of GP, GP *a*, binds with high affinity to a site in the C-terminal domain of G_L, and inhibits PP1 activity on GS [21]. Thus, as we and others have shown previously [6,22], inactivation of GP in the liver releases the inhibitory action of GP *a* on PP1-G_L, thereby activating GS. This mechanism may also operate in muscles and thus underlie the activation of GS by CP-91149 treatment. Against this argument is the observation that the activation of synthase phosphatase by the GP inhibitor in muscle cells is glycogen-dependent, whereas the effects of G_L [23] or CP-91149 [6] in hepatocytes were not affected by glycogen concentration. Furthermore, MGP overexpression and consequently GP *a* increment do not inhibit GS in muscle cells as it does in hepatocytes [6]. Thus the involvement of muscle-specific isoforms, G_M or PPP1R6, primarily expressed in this tissue, is more plausible.

To test whether CP-91149 could enhance glycogen resynthesis, after glycogen depletion (113 ± 8 μg of glucose/mg of protein), non-engineered cells were re-incubated with glucose in the

absence or presence of CP-91149 for 3 h. Treatment with CP-91149 enhanced glycogen accumulation (167 ± 7 versus 145 ± 2 μg of glucose/mg of protein in CP-91149-treated and untreated cells respectively) according to the GS activation observed, although we cannot rule out that inhibition of GP may have contributed to the increase in glycogen deposition.

In summary, inhibition and inactivation of GP by CP-91149 in muscle cells lead to a decrease in the glycogenolytic response to glucose deprivation, which mimics the demand for energy in exercising muscles. Nevertheless, CP-91149 activates GS in glycogen-deprived cells, indicating that this compound may favour glycogen recovery after exercise. These observations are of importance in the context of treatment of Type II (non-insulin-dependent) diabetes, since conversion of glucose into muscle glycogen is a main contributor to whole-body glucose disposal.

We thank Maribel Coca for her technical assistance, and Tanya Yates for correcting the English in the manuscript. A. M. G.-F. was supported by grant no. SAF2000-0193 from the Dirección General de Investigación, Ministerio de Ciencia y Tecnología, Spain, and J. J. G. by grant no. PB98-0992 from the Dirección General de Enseñanza Superior, Ministerio de Educación y Cultura, Spain. This work was partially supported by a grant from the Fondo de Investigación Sanitaria of the Instituto de Salud Carlos III, Red de Centros en Metabolismo y Nutrición (C03/08), Madrid, Spain. A. M. G.-F. and C. L. were recipients of a fellowship from the Comissió Interdepartamental de Recerca i Innovació Tecnológica, Generalitat de Catalunya, Spain.

REFERENCES

1 Treadway, J. L., Mendys, P. and Hoover, D. J. (2001) Glycogen phosphorylase inhibitors for treatment of type 2 diabetes mellitus. Expert Opin. Investig. Drugs **10**, 439–454
2 Sprang, S. R., Withers, S. G., Goldsmith, E. J., Fletterick, R. J. and Madsen, N. B. (1991) Structural basis for the activation of glycogen phosphorylase *b* by adenosine monophosphate. Science **254**, 1367–1371
3 Newgard, C. B., Hwang, P. K. and Fletterick, R. J. (1989) The family of glycogen phosphorylases: structure and function. Crit. Rev. Biochem. Mol. Biol. **24**, 69–99
4 Hoover, D., Lefkowitz-Snow, S., Burgess-Henry, J. L., Martin, W. H., Armento, S. J., Stock, I. A., McPherson, R. K., Genereux, P. E., Gibbs, E. M. and Treadway, J. L. (1998) Indole-2-carboxamide inhibitors of human liver glycogen phosphorylase. J. Med. Chem. **41**, 2934–2938
5 Martin, W. H., Hoover, D. J., Armento, S. J., Stock, I. A., McPherson, R. K., Danley, D. E., Stevenson, R. W., Barrett, E. J. and Treadway, J. L. (1998) Discovery of a human liver glycogen phosphorylase inhibitor that lowers blood glucose *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **95**, 1776–1781
6 Aiston, S., Hampson, L., Gomez-Foix, A. M., Guinovart, J. J. and Agius, L. (2001) Hepatic glycogen synthesis is highly sensitive to phosphorylase activity: evidence from metabolic control analysis. J. Biol. Chem. **276**, 23858–23866
7 Halse, R., Fryer, L. G., McCormack, J. G., Carling, D. and Yeaman, S. J. (2003) Regulation of glycogen synthase by glucose and glycogen: a possible role for AMP-activated protein kinase. Diabetes **52**, 9–15
8 Askanas, V. and Engel, W. K. (1992) Cultured normal and genetically abnormal human muscle. In Handbook of Clinical Neurology: Myopathies (Rowland, L. P. and DiMauro, S., eds.), pp. 85–115, North-Holland, Amsterdam
9 Gomez-Foix, A. M., Coats, W. S., Baque, S., Alam, T., Gerard, R. D. and Newgard, C. B. (1992) Adenovirus-mediated transfer of the muscle glycogen phosphorylase gene into hepatocytes confers altered regulation of glycogen metabolism. J. Biol. Chem. **267**, 25129–25134
10 Berman, H. K., O'Doherty, R. M., Anderson, P. and Newgard, C. B. (1998) Overexpression of protein targeted to glycogen (PTG) in rat hepatocytes causes profound activation of glycogen synthesis independent of normal hormone- and substrate-mediated regulatory mechanisms. J. Biol. Chem. **273**, 26421–26425
11 Gilboe, D. P., Larson, K. L. and Nuttall, F. Q. (1972) Radioactive method for the assay of glycogen phosphorylases. Anal. Biochem. **47**, 20–27
12 Thomas, J. A., Schlender, K. K. and Larner, J. (1968) A rapid filter paper assay for UDP-glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. Anal. Biochem. **25**, 486–499
13 Gomis, R. R., Cid, E., Garcia-Rocha, M., Ferrer, J. C. and Guinovart, J. J. (2002) Liver glycogen synthase but not the muscle isoform differentiates between glucose 6-phosphate produced by glucokinase or hexokinase. J. Biol. Chem. **277**, 23246–23252
14 Buchbinder, J. L. and Fletterick, J. (1996) Role of the active site gate of glycogen phosphorylase in allosteric inhibition and substrate binding. J. Biol. Chem. **271**, 22305–22309
15 Bloch, G., Chase, J. R., Meyer, D. B., Avison, M. J., Shulman, G. I. and Shulman, R. G. (1994) *In vivo* regulation of rat muscle glycogen resynthesis after intense exercise. Am. J. Physiol. **266**, E85–E91
16 Montell, E., Arias, A. and Gomez-Foix, A. M. (1999) Glycogen depletion rather than glucose 6-P increments controls early glycogen recovery in human cultured muscle. Am. J. Physiol. **45**, R1489–R1495
17 Lerin, C., Montell, E., Berman, H. K., Newgard, C. B. and Gomez-Foix, A. M. (2000) Overexpression of protein targeting to glycogen in cultured human muscle cells stimulates glycogen synthesis independent of glycogen and glucose 6-phosphate levels. J. Biol. Chem. **275**, 39991–39995
18 Munro, S., Cuthbertson, D. J. R., Cunningham, J., Sales, M. and Cohen, P. T. W. (2002) Human skeletal muscle expresses a glycogen-targeting subunit of PP1 that is identical to the insulin-sensitive glycogen-targeting subunit G(L) of liver. Diabetes **51**, 591–598
19 Mvumbi, L., Dopere, F. and Stalmans, W. (1983) The inhibitory effect of phosphorylase *a* on the activation of glycogen synthase depends on the type of synthase phosphatase. Biochem. J. **212**, 407–416
20 Armstrong, C. G., Doherty, M. J. and Cohen, P. T. W. (1998) Identification of the separate domains in the hepatic glycogen-targeting subunit of protein phosphatase 1 that interact with phosphorylase a, glycogen and protein phosphatase 1. Biochem. J. **336**, 699–704
21 Alemany, S. and Cohen, P. (1986) Phosphorylase *a* is an allosteric inhibitor of the glycogen and microsomal forms of rat hepatic protein phosphatase-1. FEBS Lett. **198**, 194–202
22 Stalmans, W., De Wulf, H., Hue, L. and Hers, H. G. (1974) The sequential inactivation of glycogen phosphorylase and activation of glycogen synthetase in liver after the administration of glucose to mice and rats. The mechanism of the hepatic threshold to glucose. Eur. J. Biochem. **41**, 117–134
23 Gasa, R., Jensen, P. B., Berman, H. K., Brady, M. J., DePaoli-Roach, A. A. and Newgard, C. B. (2000) Distinctive regulatory and metabolic properties of glycogen-targeting subunits of protein phosphatase-1 (PTG, GL, GM/RGI) expressed in hepatocytes. J. Biol. Chem. **275**, 26396–26403

Received 30 June 2003/13 November 2003; accepted 3 December 2003
Published as BJ Immediate Publication 3 December 2003, DOI 10.1042/BJ20030971