Inhibition of hepatic glycogen phosphorylase is a promising treatment strategy for attenuating hyperglycemia in type 2 diabetes. Crystallographic studies indicate, however, that selectivity between glycogen phosphorylase in skeletal muscle and liver is unlikely to be achieved. Furthermore, glycogen phosphorylase activity is critical for normal skeletal muscle function, and thus fatigue may represent a major development hurdle for this therapeutic strategy. We have carried out the first systematic evaluation of this important issue. The rat gastrocnemius-plantaris-soleus (GPS) muscle was isolated and perfused with a red cell suspension, containing 3 μmol/l glycogen phosphorylase inhibitor (GPi) or vehicle (control). After 60 min, the GPS muscle was snap-frozen (rest, n = 11 per group) or underwent 20 s of maximal contraction (n = 8, control; n = 9, GPi) or 10 min of submaximal contraction (n = 10 per group). GPi pretreatment reduced the activation of the glycogen phosphorylase a form by 16% at rest, 25% after 20 s, and 44% after 10 min of contraction compared with the corresponding control. AMP-mediated glycogen phosphorylase activation was impaired only at 10 min (by 21%). GPi transiently reduced muscle lactate production during contraction, but other than this, muscle energy metabolism and function remained unaffected at both contraction intensities. These data indicate that glycogen phosphorylase inhibition aimed at attenuating hyperglycaemia is unlikely to negatively impact muscle metabolic and functional capacity. Diabetes 54: 2453–2459, 2005

Glycogen phosphorylase catalyzes the breakdown of glycogen to glucose-1-phosphate in liver and tissues with high and fluctuating energy demands. Glycogen phosphorylase exists in two interconvertible forms (a and b); the proportion that exists in each form is regulated by phosphorylation. The less active b form is transformed by phosphorylation to the more active a form. In skeletal muscle, the transformation from b to a is regulated by changes in intracellular concentrations of AMP, inosine monophosphate (IMP), and inorganic phosphate and by glycogen phosphorylase kinase activation, all of which increase during contraction. Glycogen phosphorylase kinase also exists in two forms, glycogen phosphorylase kinase a and glycogen phosphorylase kinase b. Glycogen phosphorylase kinase a activity is increased in the presence of elevated intracellular calcium (Ca$^{2+}$) and cAMP, mediated by contraction (1–4) and adrenaline (5–7), respectively, which then phosphorylates and activates glycogen phosphorylase kinase a. Phosphorylated glycogen phosphorylase kinase a then phosphorylates and transforms glycogen phosphorylase b to its more active a form. On cessation of contraction, calcium is sequestered back into the sarcoplasmic reticulum, and the elevated concentrations of ADP, AMP, and inorganic phosphate are used to regenerate muscle ATP and phosphocreatine (PCr) stores. Glycogen phosphorylase a form is subsequently dephosphorylated by the action of protein phosphatases, whereby glycogen phosphorylase is returned to its less active b form.

Pharmacological inhibitors of glycogen phosphorylase have been developed and studied as a potential therapy for attenuating hyperglycemia associated with type 2 diabetes (8,9). The current study has used the compound CP-316819, an analogue of CP-91149 (10), which binds at an “regulatory” pocket some 33 Å from the catalytic site (where glucose binds). These compounds principally bind to this regulatory inhibitor site on the less-active b form of glycogen phosphorylase, so preventing its transformation to the more active a form of the enzyme. In a murine model of diabetes, administration of glycogen phosphorylase inhibitors (GPs) has been shown to reduce liver glycogen.
phosphorylase activity and to dramatically attenuate hyperglycemia without producing hypoglycemia (11,12). These inhibitors have also been shown to be more potent at reducing hepatic glucose output in the presence of high glucose concentrations, thus protecting against rebound hypoglycemia (13). There is also evidence that this class of G Pi s may be cardioprotective (14–16), and thus the profile of these compounds renders them extremely attractive for the treatment of type 2 diabetic patients who typically die from adverse cardiovascular events.

One potential major limit of the current GPi s is that the available compounds do not demonstrate specificity toward hepatic tissue. This reflects the observation that there is significant homology between hepatic and skeletal muscle glycogen phosphorylase isoenzymes and that the novel binding site for this class of inhibitor is conserved across isoenzymes (10). It is therefore a concern that administration of GPi s, in addition to inhibiting hepatic glucose output, will also limit muscle glycogen degradation during contraction. This in turn could be detrimental to patient quality of life if it impaired muscle function and hence patient mobility. The impact of reduced carbohydrate flux on muscle function is illustrated by those studies that have described the negative effects of lowering preexercise muscle glycogen concentration (17,18) and β-adrenoceptor blockade (19) on exercise tolerance. The latter has been shown to blunt cAMP activation of glycogen phosphorylase in skeletal muscle (20), in addition to having direct cardiac effects. The marked impairment of exercise capacity in McArdle’s disease (21–23), due to heterogeneous nonsense mutations in the PYGM gene (24), also supports the contention that impaired carbohydrate flux can limit skeletal muscle function.

All of these scenarios are associated with a significant increase in muscle adenine nucleotide loss during exercise (21–23,25,26) and consequently the development of muscle fatigue (17,18,25–28). Based on the emerging likelihood that GPi s will be used clinically in the treatment of type 2 diabetes, we felt it important to address the effects of glycogen phosphorylase administration on skeletal muscle energy metabolism and function. This is especially relevant as patients with type 2 diabetes are advised by their medical advisor to increase their level of physical activity. It was therefore the aim of the present study to investigate the effect of GPi, CP-316819, on muscle glycogen phosphorylase activation status, metabolism, and function in skeletal muscle during maximal and submaximal contraction using an in situ rat hindlimb model. We felt that these two contraction protocols would best demonstrate the potential impact of a reduced glycogenolytic flux because during both conditions glycogen would be the obligate ligature. This resulted in the G Pi s muscle group remaining fixed to the limb on the dorsal side of the knee joint. The femoral artery and vein were then cannulated, and heparinized saline (10 units/ml) was slowly flushed through the vasculature of the G Pi s muscle group. An arterial cannula was then attached to a primed perfusion system that was contained in an enclosed chamber that maintained an ambient temperature of 37°C, and the muscle group was perfused with previously prepared perfusate medium (see below for details). The animal was then killed humanely (according to U.K. Home Office Guidelines) and placed with the ventral surface down to enable the tibia to be secured using a clamp fixed to a stereotaxical frame, after which the thread from the Achilles tendon was attached to an isotonic force transducer (Grass Instruments, Warwick, RI). Clamping the tibia facilitated the measurement of muscle force production during contraction by minimizing inertia generated by movement of the animal.

The perfusion media contained isolated porcine erythrocytes suspended in a modified Krebs buffer containing 5% BSA, insulin (100 μU/ml), and 0.15 mmol/l pyruvate (adjusted to pH 7.4, hematocrit, 47%; and 6 mmol/l glucose). In one group of animals (G Pi s, n = 30), CP-316819 (Pfizer Global R&D; IC50 against glycogen phosphorylase, 100 mmol/l; IC50 on cell glycogenolysis, 1.5 μmol/l, dissolved in DMSO) was added to the perfusion medium to give an unbound free drug concentration of 3 μmol/l and DMSO concentration of 0.01%, whereas the control group (n = 29) received vehicle only. The perfusion media were perfused with their respective perfusate medium at the rate of 8 ml/min (15 ml · min−1 · 100 g−1 wet muscle) before undergoing, in a randomized order, one of the following: 1) immediate excision of the G Pi s muscle group, which was then immediately snap-frozen in liquid nitrogen (n = 11 per group, rested muscle); 2) 20 s of maximal intensity isometric contraction (30 Hz, 200 ms, 10 V; control, n = 8; G Pi s, n = 9); or 3) 10 min of submaximal intensity isometric contraction (1 Hz, 0.3 ms, 2 V; n = 10 per group). Muscle contraction was achieved via direct electrical stimulation of the sciatic nerve using a hook electrode (In Vivo Instruments, North Reading, MA). Muscle activity was recorded throughout (Mac Lab 400; AD Instruments, Castle Hill, NSW, Australia). Muscle blood flow was increased twofold from the resting rate at the start of the 10 min of submaximal contraction but was left unchanged from the resting rate for muscles undergoing maximal intensity contraction because it was felt that increasing flow under these conditions would have minimal impact on metabolism and function. Arterial and venous blood samples were collected at rest and every 2 min during the 10 min of submaximal intensity contraction and were used to determine perfuse lactate concentration (YSI Bioanalytical Products, Yellow Springs, OH). Arterial and venous lactate concentrations were not determined during maximal intensity contraction because of the short duration of the contraction protocol. Immediately after contraction, the G Pi s muscle group was rapidly excised and snap-frozen in liquid nitrogen. The muscle group was weighed and then stored in liquid nitrogen for subsequently analysis. Lactate efflux was calculated based on the flow rate and the difference between initial buffer lactate concentration and “venous” sampled buffer lactate concentration.

Muscle metabolite analysis. The G Pi s muscle group was crushed under liquid nitrogen and thoroughly mixed to create a homogenous mix of fiber types. A aliquot of this pool of muscle was then freeze-dried overnight and then stored at −80°C until powdered and extracted (30) for determination of muscle ATP, ADP, AMP, IMP, inosine (INO), hypoxanthine (HYP), xanthine (XAN), and uric acid concentrations as previously described by high-performance liquid chromatography (31) and muscle PhoX, free creatine, hexose monophosphate (HMP), lactate, and glycogen concentrations by spectrophotometry, as previously described (30,32).

Glycogen phosphorylase activation status. Freeze-dried powdered aliquots of muscle tissue were extracted at −35°C (60% glycerol, 30 mmol/l Na–β-glycerophosphate, 50 mmol/l NaF, 5 mmol/l EDTA, and 0.06% BSA, pH 7.0, containing 30 mmol/l dithiothreitol [DTT]) and used for determination of both fractional and maximal (by incubation with AMP) glycogen phosphorylase activity. This was achieved by measuring the generation of HMPs from both fractional and maximal (by incubation with AMP) glycogen phosphorylase activity. The rate of glycogen breakdown was determined from aliquots of incubation medium taken over time and measured for HMP accumulation; from this, glycogen phosphorylase activity was calculated. In brief, 40 μl muscle homogenate was added to a prewarmed incubation buffer (800 μl assay buffer [30 mmol/l Na–β-glycerophosphate+3 H2O, 15 mmol/l NaF, 2 mmol/l EDTA, 11 mmol/l KH2PO4], 10 mmol/l DTT, and 60 μl distilled H2O) for measurement of a form status and to an incubation buffer (800 μl assay buffer [as above], 10 mmol/l DTT, 2 mmol/l AMP, and 10 μl distilled H2O) for measurement of total (a + b) status, before the addition of glycogen and subsequent aliquot removal for HMP analysis by spectrophotometry (32).

Statistics. A comparison of differences between means was performed using Student’s unpaired t test or two-way ANOVA when appropriate. When the
Black circles = Control
White circles = GPI

FIG. 1. Muscle isometric force production expressed as area under the curve (AUC) during 20 s of maximal intensity contraction after pretreatment with control or CP-316819. ○, control; □, GPI. Data are means ± SE.

ANOVA resulted in a significant F statistic, a least-significant-difference post hoc test was used to locate differences. Significance was set at P < 0.05.

RESULTS

Muscle function. Isometric force production over the 20 s of maximal contraction is presented as the area under the tension-time curve for every 2-s period of contraction throughout the 20-s contraction protocol (Fig. 1). Peak tension was 46.7 ± 4.7 and 40.8 ± 4.5 kg/100 g wet muscle mass in control and GPi-treated groups, respectively. Muscle tension development declined in a linear-like fashion throughout the 20 s of contraction in both the control and GPi-treated groups. After 10 s of contraction, force output was significantly lower than that recorded after 2 s and remained lower thereafter (P < 0.05). There was no difference in tension development between control and GPi treatments at any time point during contraction.

Figure 2 shows the initial peak tension and isometric tension development every minute during the 10 min of submaximal contraction after control or GPi treatment. The control group produced a mean peak tension of 12.00 ± 0.96 kg/100 g wet muscle mass, which declined as contraction duration increased. Tension development was significantly reduced from the initial peak tension after 4 min of contraction (P < 0.05) and continued to decline thereafter until, at 10 min, it was 64% lower than at the onset of contraction (P < 0.001). After GPi treatment, the initial mean peak tension was 11.96 ± 0.87 kg/100 g wet muscle mass, which was no different from control. Tension then declined in an identical manner to that observed in the control group.

Glycogen phosphorylase activation status. Figure 3 shows glycogen phosphorylase a form activation status in rat GPS muscle at rest, after 20 s of maximal contraction, and after 10 min of submaximal contraction in control and GPi-treated muscles. Glycogen phosphorylase a form in the control group at rest was 57.6 ± 3.5 mmol·kg⁻¹·min⁻¹ and increased to 79.2 ± 5.1 and 44.4 ± 3.0 mmol·kg⁻¹·min⁻¹ after 20 s (P < 0.05) and 10 min (P < 0.05) of contraction, respectively. In the GPi-treated group, glycogen phosphorylase a form increased from 48.3 ± 2.4 mmol·kg⁻¹·min⁻¹ at rest to 60.0 ± 4.0 mmol·kg⁻¹·min⁻¹ after 20 s of contraction (P < 0.05) and 25.0 ± 2.1 mmol·kg⁻¹·min⁻¹ after 10 min of contraction (P < 0.001). Glycogen phosphorylase a form status in the GPi-treated muscle was significantly lower at rest (16%, P < 0.05) and after 20 s (25%, P < 0.05) and 10 min (44%, P < 0.05) of contraction when compared with respective controls.

Figure 3 also shows glycogen phosphorylase activity measured after incubation of muscle samples with AMP. This reflects the ability of CP-316819 to inhibit glycogen phosphorylase in the presence of a stimulus that under normal circumstances maximally activates the enzyme and also reflects the carryover of drug from muscle to the assay buffer, either tightly bound to glycogen phosphorylase or as free drug. AMP-mediated activation of glycogen phosphorylase did not differ between treatments in resting muscle (111.5 ± 2.0 [control] vs. 108.1 ± 2.1 [GPi] mmol·kg⁻¹·min⁻¹) or in muscle obtained after 20 s of maximal intensity contraction (118.4 ± 2.1 [control] vs. 115.7 ± 3.1 [GPi] mmol·kg⁻¹·min⁻¹). However, GPi treatment reduced AMP-mediated activation of glycogen phosphorylase in muscle obtained after 10 min of submaximal intensity contraction compared with control (112.5 ± 2.9 [control] vs. 88.0 ± 3.6 [GPi] mmol·kg⁻¹·min⁻¹, P < 0.001).

Muscle metabolism. Table 1 contains the concentrations of muscle adenine nucleotides and products of adenine nucleotide pool degradation at rest, after 20 s of maximal intensity contraction, and after 10 min of submaximal intensity contraction after control or GPi treatment. Contraction, irrespective of intensity, induced ATP degradation in both treatment groups when compared with corresponding resting values. This was reflected by the increase in concentration of adenine nucleotide degradation products after contraction. However, no difference in the concentrations of adenine nucleotides or their breakdown products (individual or pooled) was observed between treatments at rest or after 20 s or 10 min of

FIG. 2. Muscle force production expressed as tension per kilogram per 100 g wet muscle during 10 min of submaximal intensity contraction after pretreatment with control or CP-316819. ○, control; □, GPI. Data are means ± SE.
contraction. Similarly, PCr and glycogen hydrolysis were no different between treatments after maximal or submaximal intensity contraction, and total creatine was the same within and between treatments (Table 2). Contraction increased HMP accumulation from rest in both treatment groups. This increase was greatest after maximal intensity contraction but was no different between control and GPi-treated muscle during maximal or submaximal intensity contraction (Table 2). Muscle lactate concentration was not different between treatments at rest. However, muscle lactate was lower in the GPi group after 20 s of maximal contraction compared with control (Table 2; P < 0.05). Similarly, after 10 min of submaximal intensity contraction, there was a trend for muscle lactate to be lower in the GPi-treated group compared with control (P = 0.09). Muscle lactate efflux (measured every 2 min during submaximal intensity contraction) was lower in the GPi-treated group at 2 min compared with control. However, from 4 min onward, there was no difference between treatments (Fig. 4).

**DISCUSSION**

Failure of insulin to suppress hepatic glucose output is a critical component of type 2 diabetes. Great interest has been generated regarding the potential benefits of GPis for the treatment of type 2 diabetes because their mode of action may avoid hyperglycemic episodes (8,9,11,12,33). Studies have demonstrated that in animal models of type 2 diabetes, GPis can attenuate liver glycogenolysis, thereby reducing hyperglycemia. One desirable observation has been that the most commonly studied compounds are more potent in the presence of high blood glucose concentration (13). This appears to afford a self-regulating mechanism, whereby a high blood glucose concentration enhances liver phosphorylase inhibition. When normoglycemia is approached, inhibition of liver glycogen phosphorylase is attenuated, thus avoiding the potential for rebound hypoglycemia.

Currently, drug development efforts have failed, however, to discover GPis that specifically bind to the hepatic glycogen phosphorylase isoenzyme. Although protection from “reflex” hypoglycemia provides an attractive safety profile, glycogen phosphorylase also plays an important role in skeletal muscle energy metabolism. Substantial inhibition of glycogen phosphorylase in skeletal muscle may induce glycogen depletion/β-adrenoceptor blockade/ McArdle’s-like syndromes (20–23,25,26), producing impaired exercise tolerance, lethargy, and the risk of renal damage. Furthermore, impaired muscle function would impair quality of life and patient physical activity levels, further compounding their insulin resistance. We therefore decided to characterize the impact of the GPi CP-316819 on skeletal muscle metabolism and function during contraction. Our study is the first to investigate the integrative effects of selective and direct pharmacological inhibition of glycogen phosphorylase on skeletal muscle function.

**TABLE 1**

Muscle adenine nucleotides and their breakdown products at rest, after 20 s of maximal intensity contraction, and after 10 min of submaximal contraction after pretreatment with control or CP-316819.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GPi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>20 s</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>ATP</td>
<td>27.82 ± 0.41</td>
<td>24.15 ± 0.65</td>
</tr>
<tr>
<td>ADP</td>
<td>3.25 ± 0.19</td>
<td>3.55 ± 0.19</td>
</tr>
<tr>
<td>AMP</td>
<td>0.27 ± 0.07</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>IMP</td>
<td>0.47 ± 0.05</td>
<td>3.30 ± 0.68</td>
</tr>
<tr>
<td>INO</td>
<td>3.37 ± 0.12</td>
<td>3.24 ± 0.08</td>
</tr>
<tr>
<td>HYP</td>
<td>0.07 ± 0.02</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>XAN</td>
<td>0.23 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>UA</td>
<td>1.36 ± 0.07</td>
<td>0.90 ± 0.06</td>
</tr>
</tbody>
</table>

Data are means ± SE mmol/kg dry muscle. UA, uric acid.
phosphorylase activation status, energy metabolism, and function in situ.

We have presented evidence that CP-316819 was able to reduce muscle phosphorylase a form activation status in situ at rest (16%) and during maximal (24%) and submaximal (44%) intensity isometric contraction (Fig. 3). In addition, we were able to demonstrate a modest reduction in lactate production. However, despite this blunting of phosphorylase activation, there was no effect of GPi on muscle IMP accumulation, adenine nucleotide homeostasis (Tables 1 and 2), or function during maximal (Fig. 1) and submaximal intensity (Fig. 2) contraction. Thus, the magnitude of glycogen phosphorylase inhibition attained during contraction was not sufficient to precipitate a clinically meaningful reduction in muscle function (20–22,26). This in turn explains why we did not observe a significant effect on muscle metabolism and function in the present study.

Interestingly, when AMP (an allosteric activator of glycogen phosphorylase) was used to maximally activate phosphorylase, using muscle tissue from each group, no difference in phosphorylase a activity was seen between control and GPi-treated tissues at rest or after 20 s of maximal intensity contraction, despite the observed inhibition of the a form in situ. These data indicate that the rate of glycogenolysis required to sustain ATP turnover during maximal intensity contraction was achieved, probably because of sufficient calcium and AMP-mediated activation of glycogen phosphorylase during contraction to override the inhibitory effect of CP-316819. However, the ability of AMP to activate glycogen phosphorylase was impaired by 22% in the samples obtained after 10 min of submaximal intensity contraction (Fig. 3). Our explanation for this observation is that the lower demand on glycogenolysis to maintain ATP turnover at this lower contraction intensity, compared with maximal intensity, would have resulted in comparatively less glycogen phosphorylase activation by intracellular calcium and AMP transients, which may therefore have enabled CP-316819 to be more effective at dampening glycogen phosphorylase activation in situ and in the presence of AMP. Irrespective of this interpretation, the clear lack of any effect of CP-316819 on muscle HMP accumulation, adenine nucleotide homeostasis, and function demonstrates that the transient effect of GPi observed in situ was insufficient to have any functionally relevant or metabolic consequences.

There was a trend for muscle lactate concentration to be lower (P = 0.09) after submaximal contraction in the GPi-treated group compared with control, whereas muscle lactate efflux was significantly reduced early during contraction (Fig. 4, P < 0.05). This indicates that muscle glycolysis was lower during the first 2 min of contraction at this intensity. These data are also consistent with the modest reduction in muscle lactate concentration observed after 20 s of maximal stimulation. However, neither of these responses were of a sufficient magnitude to cause a measurable reduction in muscle HMP accumulation or a sparing of muscle glycogen stores (7 mmol/kg dry muscle lactate, equals only 3.5 mmol/kg dry muscle glycogen) (Table 2). This indicates that CP-316819 was capable of producing inhibition of glycogen phosphorylase, even during intense contraction. However, this effect appears to have been short-lived, supporting our contention that even when pharmacological inhibition of muscle glycogen phosphorylase is achieved (an important aspect of our study), this is insufficient to impinge significantly on the physiological activation of glycogen phosphorylase. This conclusion is supported when one considers the metabolic interactions of AMP and its breakdown products with glycogen phosphorylase. AMP and IMP both activate muscle phosphorylase b through the AMP binding site, whereas metabolites of IMP (INO, HYP, and XAN) are inhibitors of muscle glycogen phosphorylase through the purine binding site (the site where caffeine binds). Our data show that a marked increase in the muscle IMP concentration occurred in the face of minor changes in

### TABLE 2

| Muscle PCr, total creatine, HMPs, glycogen, and lactate concentrations at rest, after 20 s of maximal intensity contraction, and after 10 min of submaximal contraction after pretreatment with control or CP-316819 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Control         | GPi             |                 |                 |
|                 | Rest            | 20 s            | 10 min          | Rest            | 20 s            | 10 min          |
| n               | 11              | 8               | 10              | 11              | 9               | 10              |
| PCr             | 58.97 ± 4.05    | 29.07 ± 2.69    | 30.82 ± 3.39    | 57.9 ± 3.6      | 32.8 ± 1.7      | 39.0 ± 4.6      |
| Total creatine  | 91.2 ± 1.5      | 91.4 ± 1.6      | 90.4 ± 1.4      | 89.0 ± 1.0      | 87.8 ± 1.2      | 90.6 ± 1.1      |
| HMP             | 4.4 ± 0.3       | 14.8 ± 2.0      | 9.2 ± 0.7       | 5.2 ± 0.6       | 14.8 ± 1.2      | 8.0 ± 0.5       |
| Glycogen        | 148.3 ± 6.3     | 115.9 ± 6.7     | 110.5 ± 7.4     | 158.3 ± 3.3     | 113.5 ± 8.2     | 116.1 ± 5.9     |
| Lactate         | 14.2 ± 2.3      | 44.3 ± 2.9      | 58.8 ± 3.9      | 16.0 ± 2.4      | 37.1 ± 1.8*     | 47.6 ± 5.1      |

Data are means ± SE mmol/kg dry muscle. *Significantly different from corresponding control value, P < 0.05.

![FIG. 4. Muscle lactate efflux during 10 min of submaximal contraction after pretreatment with control or CP-316819. Black bars = Control, White bars = GPi.](image)
metabolites downstream of INO. Thus the net “metabolite activation” of phosphorylase by IMP is consistent with the lack of metabolic perturbation in the presence of the GPi. Similarly, no difference was observed between treatment groups in muscle PCr and adenine nucleotide concentrations during contraction, thus confirming that muscle ATP homeostasis was unaffected by CP-316819.

We have shown that muscle function and energy metabolism during maximal and submaximal intensity contraction are unaffected by the presence of GPi when studied in situ in a pump-perfused rat hindlimb model. It is plausible that GPi may compromise glycogen metabolism at low contraction intensities, i.e., when contraction-induced muscle glycogen phosphorylase activation is lower. Furthermore, the potency of CP-316819 may be moderately enhanced if intracellular muscle glucose concentration increases, such as during more prolonged low intensity contraction. This interpretation may also help explain the greater level of glycogen phosphorylase inhibition seen after submaximal contraction in the present study compared with maximal intensity contraction. However, it is also plausible that this trend reflected the longer duration of muscle tissue exposure to CP-316819 (the contraction duration was 30-fold longer). Given that in both experiments, muscle was perfused with 3 μmol/l unbound CP-316819 for 60 min before the onset of contraction, it is plausible that there was at least some sustained concentration-dependent drug uptake into muscle during the submaximal contraction period.

In conclusion, pharmacological inhibition of hepatic glycogen phosphorylase has the potential to be an effective therapeutic strategy for the treatment of type 2 diabetes, as evidenced by studies showing glucose-lowering effects of these compounds (8,9,11,12). We have shown that CP-316819 administration reduced glycogen phosphorylase activation in situ in rat skeletal muscle at rest and during maximal and submaximal contraction and consequently produced a modest reduction in muscle lactate production. In contrast, CP-316819 had no effect on muscle HMP accumulation, adenine nucleotide homeostasis, or function at either contraction intensity. Collectively, these observations are in keeping with the view that the extent of glycogen phosphorylase inhibition that occurred during contraction was not of a magnitude sufficient to measurably influence glycogen metabolism, possibly because of the contraction-mediated increase in positive modulators of glycogen phosphorylase. Based on the these findings, it would appear that concerns relating to potential negative effects of glycogen phosphorylase inhibition on quality of life due to impaired muscle function are currently unwarranted. Further studies aimed at establishing the effects of glycogen phosphorylase inhibition after chronic oral dosing and under alternative exercise protocols are required to fully clarify the impact these compounds have on skeletal muscle metabolism and function.

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