The temporal relationship between glycogen phosphorylase and activation of the pyruvate dehydrogenase complex during adrenaline infusion in resting canine skeletal muscle

Paul A. Roberts, Susan J. G. Loxham*, Simon M. Poucher*, Dumitru Constantin-Teodosiu and Paul L. Greenhaff

School of Biomedical Sciences, Queen’s Medical Centre, University of Nottingham, Nottingham, NG7 2UH and *Cardiovascular and Gastrointestinal Global Discovery Research Department, AstraZeneca Pharmaceuticals, Alderley Park, SK10 4TG, UK

The present study examined the effect of adrenaline infusion on the activation status of glycogen phosphorylase and the pyruvate dehydrogenase complex (PDC) and on the accumulation of glucose-6-phosphate (G-6-P) and acetylcarnitine in resting canine skeletal muscle. The study was performed in an effort to gain some insight into the temporal relationship between glycogen phosphorylase and PDC activation in vivo in skeletal muscle, which is currently unresolved. Multiple muscle samples were obtained from canine brachial muscle (n = 10) before and during (1, 3, 7 and 15 min) adrenaline infusion (0.14 μg (kg body mass)⁻¹ min⁻¹, i.v.). Adrenaline infusion increased glycogen phosphorylase ‘a’ by > 2-fold above basal levels after 3 min (pre-infusion = 9.2 ± 1.1 vs. 3 min = 22.3 ± 4.0 mmol glucosyl units (kg dry muscle)⁻¹ min⁻¹, P < 0.05). The concentration of G-6-P increased transiently from its basal concentration at 1 min (pre-infusion = 1.5 ± 0.2 vs. 1 min = 4.4 ± 0.9 mmol kg dry muscle)⁻¹, P < 0.01), declined to its pre-infusion concentration at 3 min (P < 0.05), and then increased again after 7 min of infusion (P < 0.05). The PDC was activated following 7 min of adrenaline infusion (pre-infusion = 0.22 ± 0.04 vs. 7 min = 1.04 ± 0.15 mmol acetyl-CoA (kg wet muscle)⁻¹ min⁻¹, P < 0.01), and this degree of activation was maintained for the duration of infusion. During the first 3 min of infusion, the concentration of acetylcarnitine declined (pre-infusion = 3.8 ± 0.3 vs. 3 min = 1.6 ± 0.2 mmol (kg dry muscle)⁻¹, P < 0.05), before transiently increasing at 7 min above the 3 min concentration (3 min = 1.6 ± 0.2 vs. 7 min = 5.1 ± 1.0 mmol (kg dry muscle)⁻¹, P < 0.01). This is the first study to demonstrate that adrenaline can indirectly activate the PDC in skeletal muscle in vivo at rest. The results demonstrate that adrenaline increased glycogen phosphorylase activation and glycolytic flux within 3 min of infusion, but took several more minutes to activate the PDC. This temporal relationship, combined with a probable adrenaline-induced increase in metabolic rate (and thereby resting ATP demand), resulted in the biphasic changes in G-6-P and acetylcarnitine with infusion time.

(Resubmitted 22 March 2002; accepted after revision 29 August 2002; first published online 27 September 2002)

Corresponding author P. A. Roberts: E Floor, School of Biomedical Sciences, Queen’s Medical Centre, University Of Nottingham, Nottingham, NG7 2UH, UK. Email: p.roberts@nottingham.ac.uk

Glycogen phosphorylase and the pyruvate dehydrogenase complex (PDC) occupy pivotal positions within carbohydrate metabolism. Glycogen phosphorylase catalyses the rate limiting step in glycogenolysis, whereby muscle glycogen is broken down leading to the formation of glucose-6-phosphate (G-6-P), and therefore sets the potential upper limit for glycolytic flux and increases in pyruvate availability (Chasiotis et al. 1982; Chasiotis, 1988). The PDC catalyses the physiologically irreversible reaction that commits the glycolytic product pyruvate to its oxidative fate within mitochondria, through its conversion into mitochondrial acetyl-CoA (Wieland, 1983). The resulting acetyl groups can subsequently be utilised by the tricarboxylic acid (TCA) cycle to form reduced intermediates or (when acetyl-CoA availability exceeds its rate of TCA cycle utilisation) can be transferred towards carnitine forming acetylcarnitine (Childress et al. 1966).

The interplay between glycogen phosphorylase and PDC is central in dictating glycolytic flux, lactate accumulation and mitochondrial acetyl-CoA availability in skeletal muscle, and it is not surprising therefore that abnormalities of each can be directly linked to a variety of disease states, including McArdle’s disease and congenital lactic acidosis (Sahlin et al. 1995; Stacpoole et al. 1997). The temporal relationship between the transformation of glycogen phosphorylase to its more active ‘a’ form (Phos) and the activation of the PDC in vivo is currently unclear in skeletal muscle at rest and during contraction. Whilst a
considerable body of knowledge is available regarding glycogen phosphorylase and PDC activation in isolation of one another (Chasiotis et al. 1982; Wieland, 1983), to date the interplay between the two enzymes has only been investigated during skeletal muscle contraction (Howlett et al. 1998; Parolin et al. 1999, 2000; Watt et al. 2001). It is true to say that the interaction between glycogen phosphorylase and PDC is extremely complex during contraction, due to the independent activation of both enzymes through increases in cellular calcium availability and other modulators, e.g. cell energy state (Denton et al. 1972; Chasiotis et al. 1982; Hansford, 1994). Indeed, with this point in mind, the recent conclusion of Watt and coworkers (Watt et al. 2001), that adrenaline can activate the PDC during moderate exercise in human skeletal muscle, can be called into question given that no differences in PDC activation existed at any time point during contraction between the adrenaline- and saline (control)-treated groups (Watt et al. 2001).

In a recent study by our group, adrenaline was infused into healthy human volunteers at rest in an attempt to activate the PDC, largely independently of calcium involvement, via an increase in intramuscular pyruvate availability (Constantin-Teodosiu et al. 1999). Despite a significant increase in anaplerotic flux (a term coined by Kornberg (1966) referring to the replenishment of TCA cycle intermediates), no significant increase in PDC activation or accumulation of acetylcarnitine was observed from the basal state (Constantin-Teodosiu et al. 1999). Noting that a muscle biopsy sample was not obtained until after 30 min of adrenaline infusion, the authors reasoned that the lack of an effect of adrenaline on PDC activation and acetyl group accumulation could have been due to temporal changes in the activation status of the enzyme complex, such that the effect of adrenaline on PDC activation was missed (Constantin-Teodosiu et al. 1999).

The aim of the present study therefore was to examine concomitantly the time course of skeletal muscle glycogen phosphorylase and PDC activation during a 15 min infusion of adrenaline at rest. By examining the interplay between activation and flux through the two enzymes, reflected by changes in G-6-P and acetylcarnitine concentrations with infusion time, the present study sheds new light on the factors that regulate intermediary metabolism in vivo.

METHODS

Animals

All in vivo procedures were performed in full accordance with UK legislation and Home Office approval. Following an overnight fast, ten female beagle dogs (Animal Breeding Unit, AstraZeneca Pharmaceuticals, Alderley Park, Cheshire, UK; body mass 9.2 ± 0.3 kg) were pre-medicated with morphine sulphate (10 mg, i.m.), 30 min prior to the induction and maintenance of anaesthesia with sodium pentobarbital (pentobarbitone, Sagatal, Rhône Merieux, Harlow, UK). Anaesthesia was induced by an i.v. bolus of pentobarbitone (45.9 ± 0.4 mg (kg body mass)⁻¹) followed by a continuous infusion throughout each experiment (0.11 ± 0.01 mg kg⁻¹ min⁻¹, i.v.). Once adequate anaesthesia was established, the trachea was intubated and the animals were artificially ventilated with room air (24 cycles min⁻¹, tidal volume 13–15 ml kg⁻¹, Model 16/24, Palmer Bioscience, London, UK).

Surgical procedures

The right carotid artery was cannulated and mean arterial blood pressure was recorded using a pressure transducer (PDCR 75, Druck, Barendecht, The Netherlands) and an eight-channel chart recorder (Graphyte Linearacorder, mk8 WR3500, Nantwich, UK). The right brachial artery and antecubital vein were cannulated for the collection of arterial blood samples; for the monitoring of blood pH, $P_{\text{CO}_2}$ and $P_{\text{O}_2}$ (280 Blood Gas Systems, Ciba-Corning, Medfield, MA, USA), and for the venous infusion of adrenaline. The left brachial muscle was exposed by the removal of overlying skin and superficial fascia. The systemic arterial and venous blood supply of the brachial muscle bed was left intact. This marked the end point of surgery.

Experimental protocol

Each animal was infused with 2.1 μg (kg body mass)⁻¹ of adrenaline, i.v. (n = 10), made up in physiologically buffered saline with the addition of ascorbic acid to prevent oxidation of the treatment. Adrenaline was infused over a 15 min time period at a rate of 0.14 μg (kg body mass)⁻¹ min⁻¹, i.v., with mean arterial blood pressure and heart rate recorded throughout the infusion. Following the completion of the experiment, each animal was killed humanely under anaesthesia with an i.v. infusion of pentobarbitone and saturated potassium chloride.

Blood sampling and analyses

Arterial blood samples were obtained from the contralateral (right) brachial muscle immediately prior to adrenaline treatment and after 1, 3, 7 and 15 min of the infusion. Three 1 ml blood samples were obtained at each time point. The first was mixed with residual heparin, the second with 0.1 ml EGTA (200 mM, pH 7.5) and the third with 0.1 ml of glutathione (0.1 M). Heparinised blood was immediately used for pH, $P_{\text{O}_2}$ and $P_{\text{CO}_2}$ determinations (280 Blood Gas System, Ciba-Corning, Medfield, MA, USA), and haemoglobin concentrations (Co-oximiter, Instrumental Laboratory, Lexington, USA). Samples mixed with EGTA were transferred to flip-top Eppendorf tubes and centrifuged (15 g, 3 min). The resultant plasma was stored, at −80°C and used at a later date to determine glucose, lactate (YSI 2300 STATPlus analyser, YSI, Yellow Springs, OH, USA) and non-esterified free fatty acid (NEFA) concentrations (NEFA C kit, Wako Chemicals, Wako, Germany). Samples mixed with glutathione were transferred to flip-top Eppendorf tubes and centrifuged (15 g, 3 min), the resultant plasma was stored at 80°C and used for the determination of adrenaline concentration at a later date using HPLC (Forster & Macdonald, 1998).

Muscle sampling and analyses

Immediately prior to the onset of adrenaline infusion, a muscle biopsy was excised from the distal end of the exposed left brachial muscle using a scalpel blade and forceps. Subsequent biopsy samples were taken following 1, 3, 7 and 15 min of adrenaline treatment and after 1, 3, 7 and 15 min of the infusion. Three 1 ml blood samples were obtained at each time point. The first was mixed with residual heparin, the second with 0.1 ml EGTA (200 mM, pH 7.5) and the third with 0.1 ml of glutathione (0.1 M). Heparinised blood was immediately used for pH, $P_{\text{O}_2}$ and $P_{\text{CO}_2}$ determinations (280 Blood Gas System, Ciba-Corning, Medfield, MA, USA), and haemoglobin concentrations (Co-oximiter, Instrumental Laboratory, Lexington, USA). Samples mixed with EGTA were transferred to flip-top Eppendorf tubes and centrifuged (15 g, 3 min). The resultant plasma was stored, at −80°C and used at a later date to determine glucose, lactate (YSI 2300 STATPlus analyser, YSI, Yellow Springs, OH, USA) and non-esterified free fatty acid (NEFA) concentrations (NEFA C kit, Wako Chemicals, Wako, Germany). Samples mixed with glutathione were transferred to flip-top Eppendorf tubes and centrifuged (15 g, 3 min), the resultant plasma was stored at 80°C and used for the determination of adrenaline concentration at a later date using HPLC (Forster & Macdonald, 1998).
All biopsy samples were divided into two equal portions under liquid nitrogen. Subsequently, one portion was freeze-dried, dissected free from visible blood and connective tissue and powdered. Following extraction in 0.5 M perchloric acid containing 1 mM EDTA, the supernatant was neutralised with 2.2 M KHCO₃ and used for the spectrophotometric determination of ATP, phosphocreatine (PCr), creatine, G-6-P and lactate concentrations (Harris et al. 1974). The same extract was also used for the determination of muscle free carnitine and acetylcarnitine concentrations using radioisotopic substrates as described previously (Cederblad et al. 1990). Freeze-dried muscle powder was also used for the spectrophotometric determination of muscle glycogen concentration, following alkaline extraction (Harris et al. 1974). Glycogen phosphorylase ‘a’ activity and total activity were determined spectrophotometrically using freeze-dried muscle powder (Holmes & Mansour, 1968). The remaining portion of frozen, wet muscle was used to assess the activation status of the PDC using the previously described radioisotopic method of Constantin-Teodosiu et al. (1991).

With the exception of lactate, the content of all muscle metabolites was adjusted to the mean total creatine concentration within each individual animal. By doing so it was possible to compensate for any admixture of connective tissue and other non-muscular constituents within each muscle biopsy sample (Gilbert et al. 1971; Harris, 1981).

Calculations and statistics
All data are reported as means ± s.e.m. Comparisons between infusion time points, for both absolute concentrations and changes from rest, were carried out using a one-way analysis of variance (ANOVA). When a significant F value was obtained (P < 0.05) an LSD post hoc test was used to locate differences (SPSS base v8 for Microsoft Windows 95). Significance was accepted at the 5% level, unless otherwise stated in the text.

RESULTS
Cardiovascular data
Heart rate increased after 1 min of adrenaline infusion (pre-infusion = 152 ± 5 vs. 1 min = 170 ± 5 beats min⁻¹, P < 0.05), and this tachycardia was maintained for the remainder of the infusion (P < 0.05, Table 1). Similarly, mean arterial blood pressure increased from basal levels following 1 min of adrenaline treatment (pre-infusion = 111.1 ± 2.8 vs. 1 min = 129.1 ± 2.7 mmHg, P < 0.05), and remained elevated for the duration of the infusion (P < 0.05, Table 1).

Plasma adrenaline and metabolite concentrations
Plasma adrenaline was increased from basal levels following 1 min of infusion (pre-infusion = 3.92 ± 0.88 vs. 1 min = 13.33 ± 3.35 nmol l⁻¹, P < 0.01, Table 1) and remained elevated for the remainder of the infusion (P < 0.01, Table 1).

Plasma glucose was elevated above basal levels following 7 min of infusion (pre-infusion = 5.00 ± 0.20 vs. 7 min = 5.86 ± 0.22 mmol l⁻¹, P < 0.01), with this increase being maintained for the remainder of the infusion (Table 1).
Plasma lactate and NEFA concentrations were unchanged throughout the infusion period (Table 1).

**Muscle glycogen phosphorylase transformation and glucose-6-phosphate accumulation**

Glycogen phosphorylase was transformed to its more active 'a' form following 3 min of the infusion ($P < 0.05$, Fig. 2A). The amount of glycogen phosphorylase existing in its 'a' moiety increased further from rest at 7 min ($P < 0.01$) and was ~3-fold higher than the pre-infusion value following 15 min of treatment ($P < 0.01$, Fig. 2A).

No change in the 'total' activity of glycogen phosphorylase ($\text{Phos}_{\text{total}}$) was observed over the time course of infusion (Table 2).

Glycogen phosphorylase's transformation increased G-6-P concentration transiently during the first minute of the infusion ($P < 0.01$), after which it declined towards its pre-infusion concentration ($P < 0.05$, Fig. 2B). Following 3 min of infusion, G-6-P accumulated in an almost linear manner (Fig. 2B), increasing above its pre-infusion concentration following 7 min of the infusion ($P < 0.05$).

### Table 2. Muscle total glycogen phosphorylase activity ($\text{Phos}_{\text{total}}$) and metabolite concentrations during 15 min adrenaline infusion

<table>
<thead>
<tr>
<th></th>
<th>Pre-Infusion</th>
<th>1 min</th>
<th>3 min</th>
<th>7 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Phos}_{\text{total}}$</td>
<td>101.1 ± 5.1</td>
<td>104.1 ± 7.0</td>
<td>104.2 ± 6.3</td>
<td>102.5 ± 8.1</td>
<td>106.6 ± 4.8</td>
</tr>
<tr>
<td>Glycogen</td>
<td>241 ± 11</td>
<td>235 ± 10</td>
<td>237 ± 11</td>
<td>250 ± 10</td>
<td>228 ± 12</td>
</tr>
<tr>
<td>ATP</td>
<td>25.5 ± 0.5</td>
<td>23.8 ± 0.6</td>
<td>24.4 ± 0.9</td>
<td>24.7 ± 1.1</td>
<td>26.3 ± 1.1</td>
</tr>
<tr>
<td>PCr</td>
<td>61.2 ± 1.0</td>
<td>61.7 ± 1.4</td>
<td>64.2 ± 2.3</td>
<td>60.4 ± 1.5</td>
<td>62.8 ± 1.5</td>
</tr>
<tr>
<td>Creatine</td>
<td>64.2 ± 4.6</td>
<td>58.8 ± 1.7</td>
<td>56.3 ± 1.3</td>
<td>62.0 ± 1.4</td>
<td>57.7 ± 2.5</td>
</tr>
<tr>
<td>Lactate</td>
<td>7.9 ± 0.7</td>
<td>6.8 ± 0.7</td>
<td>6.0 ± 1.2</td>
<td>8.3 ± 0.9</td>
<td>9.8 ± 1.3†</td>
</tr>
<tr>
<td>Carnitine</td>
<td>15.4 ± 0.6</td>
<td>16.8 ± 0.7</td>
<td>17.6 ± 0.6*</td>
<td>14.0 ± 1.1†</td>
<td>16.8 ± 0.5‡</td>
</tr>
<tr>
<td>Total carnitine</td>
<td>19.1 ± 1.5</td>
<td>20.1 ± 1.4</td>
<td>20.2 ± 1.3</td>
<td>19.3 ± 1.5</td>
<td>20.1 ± 1.5</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E.M. ATP, PCr, creatine, carnitine, total carnitine (acetyl-carnitine + carnitine), glycogen and lactate concentrations expressed as mmol (kg dry muscle)$^{-1}$ and $\text{Phos}_{\text{total}}$ as mmol glucosyl units (kg dry muscle)$^{-1}$ min$^{-1}$; *Significantly different from pre-infusion time point ($P < 0.05$); † significantly different from 1 min time point ($P < 0.01$); ‡ different from 7 min time point ($P < 0.05$).
After 15 min of treatment G-6-P concentration was >3-fold higher than its pre-infusion concentration ($P < 0.01$, Fig. 2B).

**Muscle PDC activation and acetylcarnitine accumulation**

PDC activity was unchanged from basal levels over the initial 3 min of infusion, after which it increased >4-fold between 3 and 7 min ($P < 0.01$, Fig. 3A). PDC activity was maintained relatively constant between 7 and 15 min of infusion (Fig. 3A).

Acetylcarnitine declined from its pre-infusion concentration during the first 3 min of the infusion ($P < 0.05$), after which it increased between 3 and 7 min ($P < 0.01$, Fig. 3B). During the final 8 min of infusion, acetylcarnitine declined back towards its pre-infusion concentration ($P < 0.05$, Fig. 3B). Changes in the concentration of acetylcarnitine were mirrored by fluctuations in the concentration of free-carnitine (Table 2). No change in the concentration of the total carnitine pool (acetylcarnitine + carnitine) was observed over the time course of infusion (Table 2).

**Muscle metabolites**

Muscle glycogen concentration was maintained throughout infusion with no significant fall from the pre-infusion concentration being observed at any time point during treatment (Table 2). Muscle ATP, PCr and free-creatine concentrations were maintained over the course of infusion (Table 2). Muscle lactate concentration was maintained throughout infusion (Table 2) with only a trend for its concentration to increase from the pre-infusion time point following 15 min of treatment (pre-infusion = 7.9 ± 0.7 vs. 15 min = 9.8 ± 1.3 mmol (kg dry muscle)$^{-1}$, $P = 0.18$). However, following 15 min of infusion, the concentration of lactate had increased above the 3 min time point (3 min = 6.0 ± 1.2 vs. 15 min = 9.8 ± 1.3 mmol (kg dry muscle)$^{-1}$, $P < 0.01$, Table 2).

**DISCUSSION**

The present study examined the effect of adrenaline infusion on glycogen phosphorylase and PDC activation status and flux, reflected by changes in G-6-P and acetylcarnitine concentration, in resting canine skeletal muscle. The principal finding of the study was that glycogen phosphorylase activation preceded PDC’s conversion to its active ‘a’ form (PDC$_a$) by several minutes in vivo. This temporal relationship, coupled to the probable adrenaline-induced increase in metabolic rate, was also reflected in the biphasic changes in G-6-P and acetylcarnitine concentrations that occurred during the infusion period. This is the first study to show that adrenaline can indirectly activate the PDC in skeletal muscle at rest, and concomitantly examine the temporal relationship between glycogen phosphorylase and PDC activation in vivo. These observations were made largely independently of any change in cellular energy charge and calcium availability, as experienced during muscular contraction.

**Figure 3. Muscle pyruvate dehydrogenase complex ‘a’ (PDC$_a$) activity (A) and acetylcarnitine concentration (B) during 15 min of adrenaline infusion**

Results are expressed as means ± S.E.M, with units of mmol acetyl-CoA (kg wet muscle)$^{-1}$ min$^{-1}$ at 37°C for the pyruvate dehydrogenase complex and mmol (kg dry muscle)$^{-1}$ for acetylcarnitine. *Significantly different from the pre-infusion timepoint ($P < 0.05$); † significantly different from 3 min time point ($P < 0.05$); ‡ significantly different from 7 min time point ($P < 0.05$).
Adrenaline is a well known and potent activator of glycogen phosphorylase in vivo (Chasiotis et al. 1983), that acts by inducing the transformation of glycogen phosphorylase from its less active ‘b’ form to its more active ‘a’ moiety (Posner et al. 1962; Chasiotis et al. 1983). In the present study, glycogen phosphorylase was converted to Phos, at the onset of the infusion (i.e. Phos, was significantly higher than its basal value following 3 min of adrenaline infusion, $P < 0.05$, Fig. 2A) and the molar fraction of Phos, increased further during the remainder of the infusion ($P < 0.05$, Fig. 2A). These observations are consistent with the work of Chasiotis and colleagues (Chasiotis et al. 1983), who reported a similar resting Phos, mole fraction and rate of transformation during 2 min of adrenaline infusion in human skeletal muscle, using a dose similar to the present study (0.15 $\mu$g (kg body mass)$^{-1}$ min$^{-1}$). The transformation of glycogen phosphorylase during the first 3 min of adrenaline infusion was reflected by a significant increase in G-6-P concentration after 1 min ($A 3 \text{ mmol (kg dry muscle)}^{-1}$, $P < 0.01$, Fig. 2B). This observation indicates that glycogen phosphorylase did not have to be extensively converted to its ‘a’ form to have a significant impact upon flux at the onset of adrenaline infusion. The increase in G-6-P concentration at 1 min was, however, only transient and it fell back towards its pre-infusion concentration after 3 min of treatment ($A 2.3 \text{ mmol (kg dry muscle)}^{-1}$, $P < 0.05$, Fig. 2B); despite there being a trend for Phos, to increase further from 1 to 3 min ($1 \text{ min} = 13.36 \pm 1.99 \text{ vs. } 3 \text{ min} = 22.31 \pm 3.95 \text{ mmol glycosyl units (kg dry muscle)}^{-1} \text{ min}^{-2}$, $P = 0.09$, Fig. 2A). This observation could indicate reduced flux through glycogen phosphorylase at this time, thereby reducing G-6-P production, or alternatively, increased flux through phosphofructokinase and/or PDC, thereby reducing the concentration of upstream glycolytic intermediates, i.e. lowering G-6-P concentration. Although the activation status of the PDC at 3 min of infusion had doubled compared with that measured at 1 min ($P = 0.22$, Fig. 3A), acetylcarnitine concentration declined in an almost linear manner from basal values to 3 min of infusion ($P < 0.05$, Fig. 3B). This decline indicates that flux through the PDC could not match the increased demands of the TCA cycle at the onset of adrenaline infusion, such that acetyl groups were sequestered from acetylcarnitine to sustain TCA cycle flux. As the decline in acetylcarnitine during the first 3 min was nearly linear ($r^2 = 0.89$), we can assume that the rate of acetyl group sequestration from acetylcarnitine towards the TCA cycle remained constant throughout this period. Moreover, if flux through the PDC had increased from 1 to 3 min of infusion, in line with the doubling of its activation status, then we could have expected acetylcarnitine concentration to increase as acetyl-CoA accumulated above the demands of the TCA cycle. It would appear therefore that the fall in G-6-P concentration between 1 and 3 min of the infusion was due to either a reduction in glycogen phosphorylase flux, in the face of an increased Phos, moiety, or reflected increased flux through phosphofructokinase during this time. The former is the most likely, due to post-transformational regulation of glycogen phosphorylase flux by the rise in G-6-P concentration at 1 min and/or to the concomitant reduction in the availability of inorganic phosphate – a substrate for the glycogen phosphorylase reaction (Chasiotis et al. 1983; Constantin-Teodosiu et al. 1999). Indeed, in a similar study by Chasiotis and colleagues, it was shown that resting skeletal muscle inorganic phosphate concentration fell from its basal value following 2 min of adrenaline infusion (Chasiotis et al. 1983) and to a concentration range that could have markedly affected Phos, flux. Alternatively, the fall in G-6-P concentration at 3 min could be due to a G-6-P-mediated increase in the content of fructose-6-phosphate, a known positive modulator of phosphofructokinase flux, at 1 min.

The fall in acetylcarnitine concentration during the first 3 min of infusion was unexpected, particularly as the 2.2 mmol (kg dry muscle)$^{-1}$ decline equates to the formation of ~30 mmol ATP equivalents (kg dry muscle)$^{-1}$ (Fig. 3B). However, it is known that adrenaline can cause a dose-dependent increase in resting energy expenditure (Staten et al. 1987; Matthews et al. 1990), that this increase can be sustained for several hours following infusion (Ratheiser et al. 1998) and is fuelled by an increase in carbohydrate oxidation, as determined from the respiratory exchange ratio (Ratheiser et al. 1998). In the present study it appears, therefore, that adrenaline increased basal metabolic rate at the onset of infusion, which was met by an increase in acetyl group utilisation, resulting in the observed decline in acetylcarnitine concentration during the first 3 min of infusion (Fig. 3B). Indirect evidence that an increase in basal metabolic rate occurred in the present study is provided by the observed increase in heart rate after 1 min of adrenaline infusion ($P < 0.01$), which remained elevated for the duration of infusion (Table 1).

Following 3 min of infusion the PDC became activated above basal levels ($P < 0.01$, Fig. 3A), resulting in acetyl-CoA accumulation and hence the observed 3.5 mmol (kg dry muscle)$^{-1}$ increase in acetylcarnitine concentration from 3 to 7 min of infusion ($P < 0.01$, Fig. 3B). The increased activation status of the PDC was probably due to an increase in muscle pyruvate availability, which serves as both a reaction substrate and a positive allosteric modulator of PDC flux in vivo (Linn et al. 1969; Wieland, 1983). Indeed, the concentration of pyruvate would only have to increase marginally to result in PDC activation, as 0.5 mM pyruvate has been shown to inhibit pyruvate dehydrogenase kinase activity by ~50% and to activate the PDC in vitro (Linn et al. 1969). In a recent study by our group, the infusion of an identical dose of adrenaline into resting human skeletal muscle, resulted in a marked...
expansion in the concentration of the muscle TCA cycle intermediates from basal levels (~45%, \( P < 0.05 \); Constantin-Teodosiu et al. 1999). As this expansion occurred principally via the alanine amino transferase reaction, which is pyruvate-requiring, it stands to reason that adrenaline must increase glycolytic flux and thereby, pyruvate availability towards the PDC (Constantin-Teodosiu et al. 1999). Following PDC activation, the concentration of G-6-P started to accumulate almost linearly during infusion (\( r^2 = 0.94 \)); being significantly higher than at the pre-infusion (\( P < 0.01 \)) and 3 min (\( P < 0.01 \)) time points after 15 min (Fig. 2B). This increase in G-6-P concentration supports the notion that there was a better matching between glycogen phosphorylase and PDC flux after 3 min of infusion. This point is supported by the observed increase in muscle lactate (3 min = 6.03 ± 1.18 vs. 15 min = 9.82 ± 1.35 mmol (kg dry muscle)\(^{-1}\), \( P < 0.01 \), Table 2) and acetylcarnitine (3 min = 1.58 ± 0.22 vs. 7 min = 5.10 ± 1.05 mmol (kg dry muscle)\(^{-1}\), \( P < 0.01 \), Fig. 3B) concentrations between 3 and 7 min of infusion. This indicates that flux through both glycogen phosphorylase and PDC increased, and to an extent where neither was rate limiting towards the metabolic demands of the muscle.

The concentration of acetylcarnitine failed to increase further following 7 min of infusion, and actually fell towards its basal concentration after 15 min of treatment (7 min = 5.10 ± 1.05 vs. 15 min = 2.33 ± 0.48 mmol (kg dry muscle)\(^{-1}\), \( P < 0.01 \), Fig. 3B). In the face of the increase in G-6-P concentration from 7 to 15 min, this implies that flux through the PDC must have fallen from 7 min onwards and was unable to meet the increased demands of the TCA cycle as a result of adrenaline infusion, despite the activation status of the PDC remaining constant from 7 min onwards (Fig. 3A).

In conclusion, the present study has detailed the temporal relationship between glycogen phosphorylase and PDC activation during adrenaline infusion in resting canine skeletal muscle. This is the first study to show that adrenaline can indirectly activate the PDC in vivo at rest. The most significant finding of the present study was that glycogen phosphorylase activation preceded PDC activation by several minutes. This temporal relationship, coupled to the probable adrenaline-induced increase in resting energy expenditure, produced the biphasic changes in G-6-P and acetylcarnitine concentrations during infusion.

REFERENCES


**Acknowledgements**

This work was supported by the Medical Research Council.