Effects of prolonged elevation of plasma adrenaline concentration in vivo on insulin-sensitivity in soleus muscle of the rat*

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1. Prolonged elevation of the plasma adrenaline concentration was produced in rats by implantation of adrenaline-releasing retard-tablets. With this technique, a hyperadrenalaemic state is maintained for at least 5 days. 2. At 6 h after implantation of the retard-tablet it was found that plasma glucose and fatty acid concentrations increased and insulin concentration decreased compared with values obtained from placebo-tablet-implanted rats. Administration of a subcutaneous glucose load demonstrated an impaired glucose tolerance in vivo, and incubation of soleus muscle strips from 6 h-hyperadrenalaenic rats in vitro demonstrated a decreased sensitivity of the rates of glycolysis and glucose transport to insulin. 3. The sensitivities of the rates of glycolysis, glucose transport and glycogen synthesis to insulin were determined for the incubated soleus muscle preparation isolated from animals after 48 h, 72 h and 120 h duration of hyperadrenalaemia. At 48 h after retard-tablet implantation, the sensitivity of the processes of glucose transport and glycolysis was decreased; at 72 h, the insulin-sensitivities of the rates of glycolysis and glucose transport in skeletal muscle were similar to those determined for control animals; at 120 h, however, the sensitivities of the processes of glucose transport and glycolysis were both statistically significantly increased. In contrast, no changes in the sensitivity of the process of glycogen synthesis were observed at any of the time intervals studied. 4. The possible biochemical basis for the observed changes in skeletal-muscle insulin-sensitivity with prolonged hyperadrenalaemia is discussed.

INTRODUCTION

The acute administration of β-adrenoceptor agonists to animals has profound effects on glucose metabolism. Adrenaline infusion induces hyperglycaemia in both man and experimental animals, due, at least in part, to stimulation of hepatic glucose output (Sherline et al., 1972; Kneer et al., 1974) and inhibition of peripheral glucose utilization (Deibert & DeFronzo, 1980; Sacca et al., 1982). In addition, acute hyperadrenalaemia inhibits insulin secretion (Coore & Randle, 1964; Porte, 1967; Altszuler et al., 1967).

Acute exposure of isolated muscle to adrenaline or the β-adrenoceptor agonist isoprenaline causes an activation of glycogenolysis, an elevation of hexose monophosphate concentrations and a consequent inhibition of the rate of glucose phosphorylation (Sloan et al., 1978; Chiasson et al., 1981; Challiss et al., 1986). This may be one mechanism by which the rate of peripheral glucose utilization is decreased in vivo.

Little information is available on the effects of prolonged administration of catecholamines on the effects of insulin on glucose metabolism. Chronic administration of the β2-adrenoceptor agonist terbutaline sulphate to humans increased insulin-stimulated glucose utilization (Scheidegger et al., 1984). In lean and obese rats of the Zucker strain, chronic treatment with a novel β-adrenoceptor agonist (BRL 26830A) increased glucose tolerance and insulin-sensitivity in vivo (Cawthorne et al., 1984; Smith et al., 1985). These effects are considered to be due to an adaptive increase in peripheral glucose utilization (Scheidegger et al., 1984; Smith et al., 1985). Furthermore, in skeletal muscle isolated from animals treated chronically with the β-adrenoceptor agonist BRL 26830A and incubated in vitro, there was a profound increase in the sensitivity of the rate of glucose utilization to insulin (Challiss et al., 1985). For this reason the effect of chronically elevated blood adrenaline concentration in rats on the sensitivity of glucose utilization, glucose transport and glycogen synthesis to insulin in the incubated soleus muscle from these animals has been studied. The hyperadrenalaemia was achieved by implanting adrenaline-releasing retard-tablets subcutaneously (Porta et al., 1979; Korsatko et al., 1982).

METHODS

Animal manipulations

Male Wistar rats (160–200 g) were used for the study. Under light diethyl ether anaesthesia, a small dorsolateral incision was made and a retard-tablet was implanted subcutaneously at least 3 cm below the interscapular brown adipose tissue; the wound was sutured, and animals recovered within 15 min. Control animals received a placebo tablet. The preparation of the adrenaline-releasing retard-tablets and the time course and rate of adrenaline release have been reported previously (Korsatko et al., 1982; Porta et al., 1984). In the present study retard-tablets that release adrenaline at
obtained from the tail plasma glucose concentrations.

Subsequent administration, animals with tolerance tests periods 72 or 120 tail the artery, for metabolic and hormone determinations. A subcutaneous dose of glucose (1 g/kg body wt.) was given, as a 50% (w/v) glucose solution, and plasma glucose concentrations were determined for the subsequent 2 h period by using 20 μl blood samples obtained from the tail artery.

Skeletal-muscle incubations

Rats were starved for 12 h before the experiment. Experiments were performed in rats 6, 48, 72 or 120 h after adrenaline-tablet implantation. Stripped soleus muscles were prepared and incubated as described by Crettaz et al. (1980), with the modifications given by Challiss et al. (1983). Additions of insulin (1-10000 μunits/ml) were made at the start of the incubation period. Rates of lactate production, [14C]lactate production and 14C incorporation into glycogen were measured as described previously (Espinola et al., 1983a; Challiss et al., 1984). The rate of hexose oxidation to CO2 was measured as described by Leighton et al. (1985), and was never greater than 15% of the rate of lactate formation under any of the incubation conditions investigated (results not shown).

For the investigation of glycogen transport into soleus muscle strips, using 3-O-methyl[U-14C]glucose, it was considered important to carry out experiments under conditions identical with those used previously. Thus the radiolabelled compounds were added to 5.5 mM-glucose in the incubation medium, so that the actual concentrations of 3-O-methylglucose were negligible. [3H]Inulin was also added to enable the intercellular space in muscle to be measured (for details, see Challiss et al., 1986). Preliminary experiments established that the rates of lactate formation, glycogen synthesis and 3-O-methyl[U-14C]glucose transport increased linearly over the 60 min time course of the experiment (results not shown).

Metabolite and hormone determinations

Insulin concentrations were determined in plasma samples (Hales & Randle, 1963). For other assays, blood samples were deproteinized with 4% (w/v) HClO4. Glucose concentration was determined by the method of Bergmeyer et al. (1973); fatty acid concentration was measured by the method of Shimizu et al. (1979). The concentrations of adrenaline and noradrenaline were determined after extraction on alumina of 250 μl plasma samples, by the h.p.l.c. method of Boulloum et al. (1985). Glycogen content in soleus muscle was determined by the method of Keppler & Deer (1973). Intramuscular triacylglycerol was measured by chloroform/methanol extraction (Folch et al., 1957) of soleus muscle after freeze-drying and removal of residual extracellular adipose tissue. After an overnight extraction, the organic phase was dried by nitrogen evaporation, and the

**Table 1. Effects of adrenaline retard-tablet implantation on plasma concentrations of metabolites and hormones, and glycogen and triacylglycerol concentrations in soleus muscle**

<table>
<thead>
<tr>
<th>Time after implantation (h)</th>
<th>Glucose (mmol/l)</th>
<th>Fatty acid (μmol/ml)</th>
<th>Adrenaline (pg/ml)</th>
<th>Noradrenaline (pg/ml)</th>
<th>Insulin (μunits/ml)</th>
<th>Triacylglycerol (μmol/g dry wt.)</th>
<th>Glycogen (μmol/g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.7 ± 0.2 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>7.20 ± 0.15 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>15 ± 6 (15)</td>
</tr>
<tr>
<td>6</td>
<td>3.9 ± 0.2 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>7.20 ± 0.15 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>15 ± 6 (15)</td>
</tr>
<tr>
<td>24</td>
<td>4.1 ± 0.2 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>7.20 ± 0.15 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>15 ± 6 (15)</td>
</tr>
<tr>
<td>96</td>
<td>3.1 ± 0.2 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>7.20 ± 0.15 (15)</td>
<td>0.51 ± 0.02 (15)</td>
<td>37.3 ± 3 (9)</td>
<td>15 ± 6 (15)</td>
</tr>
</tbody>
</table>

Results are presented as means ± s.e.m., with numbers of observations given in parentheses. Statistical significance was determined by Student's t test for unpaired observations for comparison of each time point versus values for placebo implantation; *P < 0.05; **P < 0.01; ***P < 0.001; n.d., not determined.
was integral glucose hyperglycaemic 48, area are decreased taken from contrast, group. for 6-120 plasma noradrenaline concentration data therefore the parameters the adrenaline plasma hydrolysis (Eggstein 1973).

RESULTS

It has previously been demonstrated that plasma adrenaline concentrations are elevated for at least 48 h after implantation of adrenaline-releasing retard-tablets into rats (Korsatko et al., 1982). The concentration of plasma adrenaline was also elevated 72 h and 120 h after retard-tablet implantation, but there was no change in plasma noradrenaline concentration (Table 1).

Preliminary experiments showed that implantation of the placebo tablet into animals had no effect on any of the parameters at any of the time points investigated, and therefore data from animals receiving the placebo pellet for 6–120 h were 'pooled' to provide a single control group.

Retard-tablet implantation for 6 h increased the plasma concentrations of glucose and fatty acids and decreased that of insulin. For longer periods of time after implantation, the concentrations of plasma glucose and insulin were normal, but that of plasma non-esterified fatty acids was increased throughout the time course of investigation (Table 1).

The muscle glycogen contents of soleus muscle were decreased at 6 h, 24 h and 48 h after retard-tablet implantation, but at 72 h and 120 h were not significantly different from the glycogen content of soleus muscles taken from animals receiving placebo tablets (Table 1). In contrast, triacylglycerol content of soleus muscle was decreased only 120 h after implantation of the adrenaline retard-tablet.

The results of glucose tolerance tests performed 0, 6, 24, 48, 72 and 120 h after adrenaline-pellet implantation are shown in Table 2. The simplest indicator of the hyperglycaemic response to a subcutaneous glucose load is the area under the response curve, taking the plasma glucose concentration at zero time as the baseline. The integral values are given in Table 2: glucose tolerance was impaired 6 h after adrenaline-pellet implantation; the area under the glucose tolerance curve was 39% greater after hyperadrenalinaemia for 6 h than that of animals receiving the placebo implant. However, with longer duration of hyperadrenalinaemia, glucose tolerance was normal; indeed after 72 and 120 h there was an improvement in tolerance, although it did not reach statistical significance (Table 2).

We have previously used the isolated incubated stripped soleus muscle as a skeletal-muscle preparation in vitro to investigate physiological changes in the sensitivity of the rates of glycolysis and glycogen synthesis to insulin (Budohoski et al., 1984a; Challiss et al., 1984). For control muscle incubations insulin increased the rate of lactate formation about 2-fold, and the concentration of insulin that produced a half-maximal stimulation of the rate of lactate formation was about 75 μunits/ml; the rate of glycogen synthesis was increased about 3-fold by a maximal concentration of insulin, with a half-maximal effect achieved at about 125 μunits of insulin/ml (Table 3). These results are similar to those obtained in previous studies (Budohoski et al., 1984a,b; Challiss et al., 1984, 1986).

Adrenaline retard-tablet implantation for 6 h produced a dramatic effect on the sensitivity of the rate of lactate formation to insulin; the concentration of insulin required to produce half-maximal stimulation was increased to about 750 μunits/ml. In contrast, neither the basal nor the maximal rates of lactate formation were altered, so that it was the sensitivity and not the range of response to insulin that was affected by acute hyperadrenalinaemia. There was no effect on the sensitivity of glycogen synthesis to insulin (Table 3), but there was an increase in the rates of glycogen synthesis at 100, 1000 and 10000 μunits of insulin/ml. This may be a consequence of the low content of glycogen in the soleus muscles of these animals (Table 1), since glycogen content can be an important determinant of its own rate of synthesis (Danforth, 1965; Fell et al., 1982). The decrease in sensitivity of the rate of lactate formation to insulin was present in muscles from animals 48 h after adrenaline-tablet implantation (Table 3), approx. 600 μunits of insulin/ml being required to elicit a half-maximal stimulation.

<table>
<thead>
<tr>
<th>Time after glucose administration (min)</th>
<th>Time after retard-tablet implantation (h) . . .</th>
<th>Glucose (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 . . . (15)</td>
<td>6 . . . (15)</td>
</tr>
<tr>
<td></td>
<td>2.92±0.23</td>
<td>7.16±0.43</td>
</tr>
<tr>
<td>15</td>
<td>5.16±0.45</td>
<td>9.02±0.47</td>
</tr>
<tr>
<td>30</td>
<td>4.89±0.21</td>
<td>9.39±0.61</td>
</tr>
<tr>
<td>45</td>
<td>3.91±0.16</td>
<td>9.18±0.61</td>
</tr>
<tr>
<td>60</td>
<td>3.50±0.17</td>
<td>8.73±0.72</td>
</tr>
<tr>
<td>90</td>
<td>3.15±0.17</td>
<td>7.67±0.58</td>
</tr>
<tr>
<td>120</td>
<td>3.05±0.16</td>
<td>6.94±0.58</td>
</tr>
<tr>
<td>Area under curve J6^20</td>
<td>100±7</td>
<td>139±12**</td>
</tr>
</tbody>
</table>
Table 3. Effects of insulin on rates of lactate and glycogen formation by the stripped-soleus-muscle preparation isolated from rats which had received adrenaline retard-tablets implanted 0–120 h before study

Results are presented as means ± s.e.m., with the minimum number of separate muscle incubations for each condition in parentheses. Statistical significance was established by Student's t test (for unpaired observations); comparisons are for each hyperadrenalinaemic period with control values at each given insulin concentrations: *P < 0.05, **P < 0.001.

<table>
<thead>
<tr>
<th>Insulin (units/ml)</th>
<th>Time after retard-tablet implantation (h)</th>
<th>Rate (μmol/h per g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
<td>6</td>
</tr>
<tr>
<td>Lactate formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.91 ± 0.63 (19)</td>
<td>6.58 ± 0.73 (7)</td>
</tr>
<tr>
<td>10</td>
<td>8.62 ± 0.35</td>
<td>6.58 ± 1.03</td>
</tr>
<tr>
<td>100</td>
<td>12.10 ± 0.43</td>
<td>5.66 ± 0.82**</td>
</tr>
<tr>
<td>1000</td>
<td>15.39 ± 0.52</td>
<td>11.91 ± 0.49**</td>
</tr>
<tr>
<td>10000</td>
<td>15.24 ± 1.03</td>
<td>13.33 ± 0.33</td>
</tr>
<tr>
<td>Glycogen synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.65 ± 0.11 (14)</td>
<td>1.72 ± 0.20 (7)</td>
</tr>
<tr>
<td>10</td>
<td>1.62 ± 0.10</td>
<td>1.96 ± 0.27</td>
</tr>
<tr>
<td>100</td>
<td>2.82 ± 0.08</td>
<td>3.87 ± 0.40**</td>
</tr>
<tr>
<td>1000</td>
<td>4.07 ± 0.35</td>
<td>6.57 ± 0.90**</td>
</tr>
<tr>
<td>10000</td>
<td>4.51 ± 0.14</td>
<td>6.10 ± 0.30**</td>
</tr>
</tbody>
</table>

Hyperadrenalinaemia for 72 h resulted in less marked effects on the rates of lactate formation than on glycogen synthesis. Prolonged hyperadrenalinaemia (120 h) had profound effects on the rate of lactate formation, but the sensitivity of the process was still greater than that observed for muscles from control animals. The rate of lactate formation (mg/l) was higher in insulin-treated muscles than in control muscles (Chaloupka et al., 1986). However, because of the high rate of glycogen synthesis, the sensitivity of the process was less than that observed for muscles from control animals. The rate of glycogen formation (mg/g) was lower in insulin-treated muscles than in control muscles. However, because of the high rate of glycogen synthesis, the sensitivity of the process was less than that observed for muscles from control animals. The rate of glycogen formation (mg/g) was lower in insulin-treated muscles than in control muscles.

Fig. 1. Effects of insulin on rates of 3-O-methyl-D-14Cglucose transport from rats receiving adrenaline-retard-tablets for 0–120 h. Each data point represents the mean of at least six separate muscle-stripe incubations for control (○), 6 h (△), 48 h (□), 72 h (●), and 120 h (▴) after retard-tablet implantation. Error bars are SEMs. Error bars have been omitted for clarity. Student's t test for statistical significance of differences between experimental and control values determined by Student's t test for unpaired observations: *P < 0.05; **P < 0.001.
The range of response was unaffected by hyperadrenalinaemia for any of the times investigated (Fig. 1). The concentration of insulin that stimulated the rate of 3-O-methylglucose transport half-maximally varied dramatically: 6 and 48 h after implantation of adrenaline retard-tablet, the sensitivity of glucose transport was decreased; after 72 h the sensitivity was similar to that observed for muscle strips from control rats, and after 120 h sensitivity to insulin was dramatically increased (Fig. 1).

**DISCUSSION**

Plasma catecholamine concentrations may be elevated under various physiological conditions, e.g. exercise-training (Galbo et al., 1975; Maron et al., 1977; Winder et al., 1978) and cold-exposure (Leduc, 1961; Depocas &Behrens, 1978; Picotti et al., 1981); several studies have addressed the question of whether increased catecholamine concentrations are responsible for some of the changes seen in these conditions. These studies have involved decreasing the 'effectiveness' of catecholamines by surgical (Galbo et al., 1978; Richter, 1984; Henriksen et al., 1985), chemical (Galbo et al., 1978; Richter et al., 1980) or pharmacological (Irving et al., 1974; Galbo et al., 1975; Ji et al., 1986) methods, or by increasing the 'effectiveness' of catecholamines by administration of adrenoceptor agonists (Harri & Valtola, 1975; Thibault et al., 1979; Fell et al., 1981; Racotta et al., 1986). The role of catecholamines in causing changes in glucose metabolism and tissue sensitivity to insulin has received little attention, which is surprising, since exercise-training (Richter et al., 1982; Espinal et al. 1983b) and cold-exposure (Vallerand et al., 1983; Budohoski et al., 1984b) are characterized by an increased peripheral sensitivity to insulin.

In the present study, we have used adrenaline-releasing retard-tablets (Korsatko et al., 1982), implanted subcutaneously, to bring about a chronic state of hyperadrenalinaemia, which is maintained for at least 5 days after implantation. In common with many other studies (Shikama & Ui, 1975; Deibert & DeFronzo, 1980; Sacca et al., 1982), we found that acute elevation of plasma adrenaline concentrations (6 h) caused an elevation of plasma glucose, a decrease in plasma insulin concentrations, decreased glucose tolerance and a decrease in the sensitivity to insulin of glucose utilization in skeletal muscle. A decrease in insulin-sensitivity of glucose utilization of skeletal muscle exposed to β-adrenoceptor agonists in vitro has been shown to be due to an increased rate of glycogenolysis causing an increase in tissue hexose monophosphate concentrations and a consequent inhibition of the rate of glucose phosphorylation (Chiasson et al., 1981; Challiss et al., 1986), but the rate of glucose transport into the muscle is itself not affected by the presence of β-adrenoceptor agonists (Challiss et al., 1986). However, short-term elevation of the adrenaline concentration in vivo (6 h and 48 h) resulted in a decrease in the sensitivity not only of glycogenesis but also of glucose transport in the isolated soleus muscle preparation. Furthermore, the indirect mechanism by which adrenaline decreases the sensitivity of the glycogenolytic rate in vitro does not provide an explanation of the data presented here: comparison of the rate of lactate formation measured spectrophotometrically and radiochemically suggests that the glycogenolytic rate observed in incubated muscles from 6 h-hyperadrenalinaemic rats is similar to that observed in muscles from control rats (L. Budohoski, unpublished work).

The quintessential finding in the present study is that, with increasing duration of hyperadrenalinaemia, the initially decreased sensitivity of glucose transport, and hence glycogenolysis, to insulin is reversed, to produce a dramatic increase in the sensitivity of these processes to insulin. Thus 72 h after retard-tablet implantation the sensitivity of glucose transport in soleus muscle is similar to that observed in muscles from control rats, and after 120 h it is increased. It is important to note that the changes in sensitivity to insulin are observed for the processes of lactate formation and glucose transport, but not for the process of glycogen synthesis, which suggests a post-receptor interaction between the insulin and adrenaline transduction mechanisms. This view is supported by the finding that the number and affinity of insulin receptors in soleus muscle are unaffected by 120 h hyperadrenalinaemia (L. Budohoski, unpublished work).

To our knowledge, this is the first report that prolonged (120 h) elevation of the plasma concentration of adrenaline increases peripheral tissue sensitivity to insulin. Several studies have shown that β-adrenoceptor agonists, when administered chronically, improve insulin-stimulated glucose disposal in man (Scheidegger et al., 1984) and improve glucose tolerance and insulin-sensitivity both in vivo in genetically obese rats (Cawthorne et al., 1984; Smith et al., 1985) and in isolated muscles from lean and obese animals in vitro (Challis et al., 1985). Our results therefore suggest that the effects of terbutaline sulphate and BRL 26830A are mediated by the β-adrenoceptor-agonist activity of these agents and that this effect may be a common feature of β-adrenoceptor agonists when administered chronically.

Evidence has been obtained that a decrease in the local concentration of adenosine in isolated soleus muscle increases the sensitivity of the rates of glucose transport, but not glycogen synthesis, to insulin (Espinal et al., 1983b; Budohoski et al., 1984a). Since chronic elevation of plasma adrenaline concentration for 120 h has a similar effect, it is tempting to speculate that adrenaline exerts its effects on the sensitivity of glucose transport to insulin by decreasing the concentration of adenosine in skeletal muscle or changes in the properties of skeletal-muscle adenosine receptors, and this postulate requires investigation.

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**REFERENCES**

Coore, H. G. & Randle, P. J. (1964) Biochem. J. 93, 66–78
Hales, C. N. & Randle, P. J. (1963) Biochem. J. 88, 137–146

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