Effects of Treatment of Spontaneously Hypertensive Rats With the Angiotensin-Converting Enzyme Inhibitor Trandolapril and the Calcium Antagonist Verapamil on the Sensitivity of Glucose Metabolism to Insulin in Rat Soleus Muscle In Vitro

Brendan Leighton, Alison L. Sanderson, Martin E. Young, George K. Radda, Ernest A. Boehm, and Joseph F. Clark

We measured the sensitivity of glucose metabolism to insulin in soleus muscle preparations isolated from spontaneously hypertensive (SH) rats and normotensive age-matched Wistar-Kyoto (WKY) rats. SH rats were treated with the angiotensin-converting enzyme (ACE) inhibitor trandolapril (1 mg/kg) and/or a second antihypertensive drug, the calcium antagonist verapamil, alone (100 mg/kg) or as combination therapy (50 mg/kg). Treatment of SH rats with trandolapril or trandolapril in combination with verapamil for 6 weeks normalized the blood pressure. The estimated concentration of insulin required for half-maximal stimulation of glycogen synthesis (i.e., EC_{50} values) was ~500 μU/ml for muscles from both WKY and SH rats. This value is five times higher than the value obtained from soleus muscle preparations isolated from insulin-sensitive Wistar rats. This indicates that glycogen synthesis is insensitive to insulin in SH and WKY rat soleus muscle. Treatment of SH rats with trandolapril with or without verapamil improved the sensitivity of glycogen synthesis to insulin in soleus muscle. Further experiments investigated whether acute exposure (1 h) of insulin-sensitive skeletal muscle with either trandolaprilat (the active metabolite of trandolapril) or bradykinin (levels of which may be raised by ACE inhibition) could affect the insulin-stimulated rate of glucose metabolism. These results show that both trandolaprilat and bradykinin caused a small but significant increase in the rates of glucose metabolism. In conclusion, *1*) SH and WKY rat skeletal muscle was insulin resistant, *2*) chronic treatment of SH rats with trandolapril with or without verapamil normalized blood pressure and improved the response of glycogen metabolism to insulin, and *3*) bradykinin and trandolaprilat acutely caused a small but significant increase in the rate of glycogen synthesis to a submaximal physiological concentration of insulin. *Diabetes* 45 (Suppl. 1):S120–S124, 1996

From the Department of Biochemistry, University of Oxford, Oxford, U.K.

Address correspondence and reprint requests to Dr. Brendan Leighton, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

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ACE, angiotensin-converting enzyme; NIDDM, non-insulin-dependent diabetes mellitus; SH, spontaneously hypertensive.

Insulin rapidly stimulates the uptake and metabolism of glucose in skeletal muscle (1). People with non-insulin-dependent diabetes mellitus (NIDDM) show a markedly decreased sensitivity of glucose metabolism to insulin in skeletal muscle (i.e., they are insulin resistant) (2). Insulin resistance in individuals with NIDDM can be ameliorated by a program of regular exercise (3). A possible mechanism is that kinins (4,5) (e.g., bradykinin) and their second messengers, the prostaglandins (4–9), both of which are active metabolites produced during some forms of exercise, can modulate at least in part the sensitivity of glucose metabolism to insulin. However, some evidence against this mechanism has been reported (10–12). It has also been proposed that the kinin-prostaglandin system might be abnormal in some pathological states (5).

Blood pressure has been correlated with the degree of insulin resistance in skeletal muscle in sedentary people (2). The rate of bradykinin release (which may reflect the rate of formation) from the exercising forearm is decreased in insulin-resistant people with NIDDM (13), but this rate is higher in insulin-sensitive people (14). Consequently, angiotensin-converting enzyme (ACE) inhibitors are useful for slowing down the rate of degradation of kinins and increasing the local concentration of bradykinin in muscle (as perhaps in people with NIDDM or hypertension), which might lead to an increase in the sensitivity of glucose metabolism to insulin. There are some reports that the response of glucose metabolism to insulin is decreased in skeletal muscle from spontaneously hypertensive (SH) rats (15–17); however, these reports have been contested (18–22). Nevertheless, we measured the sensitivity of glucose metabolism to insulin in skeletal muscle from a control group of SH rats and normotensive age-matched Wistar-Kyoto rats (WKY), which acted as an important reference group. We examined the effects of the ACE inhibitor trandolapril on SH rats alone as well as in combination with a second antihypertensive drug, the calcium antagonist verapamil. To control for any effects of verapamil on insulin sensitivity, a group of SH rats was also treated with verapamil. Recent studies have reported that dual administration of an ACE inhibitor with a calcium antagonist has some

TABLE 1
Effects of trandolapril and/or verapamil on lactate release in soleus muscle in vitro

Insulin concentration (μU/ml)	Rates of lactate release (μmol · h^{-1} · g^{-1})				
	WKY	SH	SH-T	SH-V	SH-VT
10	11.39 ± 1.15 (4)	15.07 ± 1.82 (4)	13.16 ± 1.23 (5)	14.32 ± 1.98 (4)	20.89 ± 1.23 (5)†‡
100	14.43 ± 2.19 (4)	15.66 ± 0.57 (5)	17.67 ± 1.98 (5)	17.69 ± 1.53 (5)	18.69 ± 2.32 (5)
200	18.68 ± 1.51 (4)	19.38 ± 0.9 (5)	22.16 ± 1.75 (5)‡	16.35 ± 0.91 (4)	21.54 ± 1.50 (5)‡
1,000	21.47 ± 1.38 (4)*	29.87 ± 0.84 (5)†	23.97 ± 1.11 (5)*	23.51 ± 1.05 (5)*	22.54 ± 1.47 (5)*
10,000	23.26 ± 1.73 (4)	24.58 ± 1.28 (5)	27.19 ± 2.00 (5)	25.56 ± 0.98 (5)	27.58 ± 1.60 (5)

Data are means ± SE (n). *$P < 0.05$ vs. control SH rats; †$P < 0.05$ vs. WKY; ‡$P < 0.05$ vs. SH-V.

therapeutic advantages in terms of lowering blood pressure (23), improving endothelial function (24), and improving insulin sensitivity (25). Therefore the aims of this study were to determine whether chronic treatment of SH rats with the ACE inhibitor trandolapril or combination therapy of trandolapril plus verapamil could alter the response or sensitivity of the rates of lactate release or glycogen synthesis to insulin. Preliminary investigations also examined the acute effects of treating insulin-sensitive skeletal muscle isolated from Wistar rats with trandolaprilat (the active metabolite of trandolapril) or bradykinin to gather information about the potential biochemical mechanism of action of ACE inhibitors in vivo.

RESEARCH DESIGN AND METHODS

Animals. Male SH and normotensive (WKY) rats and Wistar rats (130–140 g; for the in vitro incubation experiments) were purchased at 4 months of age (Harlan-Olac Bicester, U.K.) and kept in the Department of Biochemistry's animal quarters until experimentation. At ~6 months of age, the SH rats were randomized into four groups, with some treated for 6 weeks as follows: age-matched control SH rats not given any hypertensive therapy, SH rats treated with verapamil (SH-V; 100 mg/kg), SH rats treated with the ACE inhibitor trandolapril (SH-T; 1 mg/kg), and SH rats treated with combination therapy of 50 mg/kg verapamil and 1 mg/kg trandolapril (SH-VT). A reference group of nonhypertensive control subjects (WKY) was also included in the study. Rats were fed on a standard diet (supplied by SDS, Whitham, U.K.; digestible carbohydrate 52%, protein 16%, fat 2%, and nondigestible residue 30%, all by weight). The animals were housed in controlled conditions (23 ± 1°C) with a 12-h light-dark cycle and received standard laboratory diet and water ad libitum. Stripped soleus muscles were routinely prepared from nonfasted rats between 0930 and 1030 h. The experiments adhered to the guidelines laid down by the Animals Scientific Procedures Act, U.K., 1986.

Isolation and incubation procedure. Soleus muscle preparations were isolated and prepared as originally described by Crettaz et al. (26). Muscles were tied at resting tension to stainless steel clips and placed in Erlenmeyer flasks containing Krebs-Ringer bicarbonate buffer (pH 7.4) with 5.5 mmol/l D-glucose, 1.35% (wt/vol) defatted bovine serum albumin, and 1.1 mmol/l $CaCl_2$. After a 30-min preincubation, muscle prep-arations were transferred to flasks that contained identical medium plus 0.3 μCi [U-^{14}C]glucose/ml (Amersham PLC, Amersham, U.K.) and bovine insulin (Sigma, Poole, U.K.). The flasks were gassed with O_2-CO_2 (95%-5%) for the whole of the preincubation period and for the first 15 min of the incubation period. After 60 min of incubation, muscles were removed, blotted, and immediately frozen in liquid N_2. In some experiments, stripped soleus muscle preparations were isolated from Wistar rats and incubated with insulin (100 μU/ml) and either bradykinin (in the presence of the protease inhibitor thiophan [2.2 μmol/l] and the ACE inhibitor lisinopril [0.1 mmol/l] used in place of trandolapril, which is a prodrug) or the active metabolite of trandolapril, trandolaprilat (muscles were incubated in the absence of bovine serum albumin in the medium).

Analytic procedures. The concentration of lactate in the incubation medium (spectrophotometric or net) (27) and the rates of incorporation of ^{14}C-labeled glucose into glycogen (28) were measured. It is important to note that the net rate of lactate formation, which is measured by a spectrophotometric assay, yields a measure of the rate of glycolysis from glucose potentially supplied from either muscle glycogen and/or glucose in the incubation medium. However, we have found that the rate of lactate release normally serves as a good indicator of the rate of glucose transport (7,29). All values are presented as means ± SE. Statistically significant differences between groups were determined with Student's t test for unpaired results as appropriate. Tail blood pressure was measured three times per week to monitor the progress of the antihypertensive therapy.

RESULTS

Chronic treatment of SH rats. Generally, except for anomalous values with insulin at 1,000 μU/ml that were significantly lower, there were similar rates of lactate release in isolated stripped soleus muscle preparations isolated from either WKY rats or SH rats treated with trandolapril, verapamil, or verapamil plus trandolapril vs. values from control SH rats (Table 1). However, the rate of lactate release was also significantly increased, compared with values from either SH or WKY rats, with insulin at 10 μU/ml in muscles isolated from SH rats treated with verapamil plus trandolapril. Interestingly, at a submaximal concentration of insulin (200 μU/ml), lactate release is significantly higher in muscles from SH rats treated with trandolapril alone or in combination with verapamil than in muscle preparations isolated from verapamil-treated SH rats.

The rates of glycogen synthesis at all insulin concentrations, except 100 μU/ml, were not significantly different in soleus muscles isolated from control SH rats and WKY rats (Table 2). Indeed, from a plot of the rates of glycogen synthesis vs. the concentrations of insulin (not shown), the estimated concentrations of insulin required for half-maximal inhibition of glycogen synthesis (i.e., EC_{50} values) were ~500 μU/ml for muscles from both WKY and SH rats. Treatment of SH rats with verapamil for 6 weeks resulted in no significant changes in the rates of glycogen synthesis vs. control SH rats, but a significant increase in the rate occurred in response to insulin at 100 μU/ml compared with results from WKY rats. Treatment of SH rats with trandolapril for 6 weeks resulted in a significant increase in the rates of glycogen synthesis in response to insulin at 200 μU/ml (vs. control SH rats) or at 100 or 1,000 μU/ml (vs. WKY rats). The EC_{50} value for glycogen synthesis in incubated soleus muscles isolated from SH-VT rats was estimated to be 300 μU/ml. Treatment of SH rats with trandolapril and verapamil for 6 weeks resulted in a significant increase in the rates of glycogen synthesis in response to insulin at 10, 200, or 1,000 μU/ml (vs. WKY rats) or at 10 and 200 μU/ml (vs. SH rats). The EC_{50} value for glycogen synthesis in incubated soleus muscles isolated from SH-T rats was estimated to be 200 μU/ml.

TABLE 2
Effects of trandolapril and/or verapamil on glycogen synthesis in soleus muscle in vitro

Insulin concentration (μU/ml)	Rates of glycogen synthesis (μmol · h^{-1} · g^{-1})				
	WKY	SH	SH-T	SH-V	SH-VT
10	0.89 ± 0.23 (4)	1.31 ± 0.27 (4)	1.01 ± 0.25 (5)	0.94 ± 0.17 (5)	2.01 ± 0.22 (5)*†
100	1.10 ± 0.09 (4)*	1.94 ± 0.34 (5)†	2.00 ± 0.26 (5)†	1.47 ± 0.16 (5)†	3.30 ± 0.71 (5)†
200	2.03 ± 0.58 (4)	1.93 ± 0.14 (5)	3.66 ± 0.73 (5)*	2.14 ± 0.45 (4)	3.42 ± 0.30 (5)†
1,000	4.25 ± 0.26 (4)	5.44 ± 0.84 (5)	5.45 ± 0.31 (5)†	4.12 ± 0.44 (5)	5.72 ± 0.20 (5)†
10,000	5.57 ± 0.63 (4)	6.47 ± 1.28 (5)	7.23 ± 0.66 (5)	5.29 ± 0.29 (5)	6.45 ± 0.49 (5)

Data are means ± SE (n). *P < 0.05 vs. control SH rats; †P < 0.05 vs. WKY.

Acute treatment of Wistar skeletal muscle. Trandolaprilat, which is the active metabolite of trandolapril, was used for acute in vitro studies. Trandolaprilat (at concentrations of 0.01–1 mmol/l) significantly increased the insulin-stimulated rates of lactate release and glycogen synthesis (Table 3). Furthermore, we considered it important that we incubated muscle preparations with low (0.01 nmol/l) and high (100 nmol/l) concentrations of bradykinin because previous studies have observed significant effects of bradykinin when subnanomolar concentrations are used (30). A high concentration of bradykinin (100 nmol/l) had no effect on the insulin-mediated rate of lactate release or glycogen synthesis (Table 4). A low concentration of bradykinin (0.01 nmol/l) only increased the rate of glycogen synthesis in response to insulin (Table 4).

DISCUSSION

Chronic treatment of SH rats. The present study examined whether the sensitivity of glucose metabolism to insulin is decreased in skeletal muscle isolated from SH rats. A major aim of the study was to determine whether the sensitivity of intracellular glucose metabolism could be improved by chronic treatment of SH rats either with an ACE inhibitor, trandolapril, or with a combination of trandolapril and the calcium antagonist verapamil. To complete the study, we measured the sensitivity of lactate release and glycogen synthesis in skeletal muscle from a reference group, the WKY rats, which are often cited as controls for the hypertensive SH rats, and a group of SH rats treated with verapamil to determine what effects verapamil therapy has on its own.

The SH rats had elevated blood pressure, which remained elevated throughout this study. Treatment of SH rats for 6 weeks with trandolapril or trandolapril in combination with verapamil significantly decreased the blood pressure to within the range of values found for WKY rats (E.A.B, J.F.C., unpublished observations). Verapamil treatment alone did not affect the blood pressure of SH rats. The heart-to-body weight ratio was significantly elevated in the SH rats and SH-V rats, indicating hypertrophy (E.A.B, J.F.C., unpublished observations). This ratio was normalized by treatment of SH rats with either trandolapril or trandolapril plus verapamil. The lack of effect of verapamil both on blood pressure and on the heart-to-body weight ratio may reflect the fact that verapamil has species-specific effects, being without observable effect in the SH rats at these doses. Verapamil is regularly used as an antihypertensive agent.

Our observations on the responsiveness of lactate formation and glycogen synthesis to insulin in skeletal muscle isolated from the control SH rats and the WKY rats are interesting in comparison with published data (7,29,31). In insulin-responsive rats, we have routinely reported EC_{50} values for glycogen synthesis of 100 μU/ml (29,30). Therefore our results suggest that soleus muscle preparations isolated from SH rats are insulin resistant (30) because we estimated the EC_{50} value for insulin to be ~500 μU/ml. A further finding is that the skeletal muscle isolated from WKY rats is also insulin resistant, with a similar EC_{50} value for glycogen synthesis to insulin. The rates of lactate release and glycogen synthesis were generally similar in muscle isolated from SH and WKY rats except for concentrations of insulin of 1,000 μU/ml for lactate release and 10 μU/ml for glycogen synthesis. Whether SH rats are insulin resistant, as measured by the euglycemic clamp technique (15,16,19,20), is not clear. The results from the present study suggest that it is difficult to determine the degree of insulin resistance in skeletal muscle of SH rats when comparing results obtained from WKY rats. WKY rats at 8 months of age exhibit specific differences regarding glucose and glycogen metabolism compared with other rat strains (18). An insulin-mimetic compound, vanadate, lowers blood insulin levels in Wistar and Sprague-Dawley rats but not in WKY rats (18). WKY rats are resistant to the effects of streptozotocin and have marked differences in plasma triglycerides, cardiac function, and heart rate compared with Wistar and Sprague-Dawley rats (18). The insulin resistance of WKY rats at 8 months of age may partially explain the discrepancy in insulin sensitivity in the SH rat of some previous studies (18–22).

Therefore the present study has shown that the rates of glycogen synthesis were markedly insensitive to the effects of insulin in soleus muscle isolated from either WKY or SH rats compared with values obtained in incubated muscles from young Wistar rats (29,30). It is possible that the development of insulin resistance in SH and WKY rats is related to the aging process. We have previously demonstrated that glycogen synthesis becomes insensitive to the effects of insulin in aging Wistar and Sprague-Dawley rats

TABLE 3
Effects of trandolaprilat on lactate release and glycogen synthesis in soleus muscle in vitro

Trandolaprilat concentration (mmol/l)	Rate of lactate release (μmol · h^{-1} · g^{-1})	Rate of glycogen synthesis (μmol · h^{-1} · g^{-1})
0	9.42 ± 0.42 (8)	1.53 ± 0.13 (7)
0.01	11.85 ± 0.99 (5)*	1.83 ± 0.1 (5)*
0.1	11.66 ± 0.69 (4)*	1.63 ± 0.02 (4)
1.0	11.29 ± 0.71 (9)*	1.86 ± 0.07 (8)*

Data are means ± SE (n). Insulin was present in the medium at 100 μU/ml. *P < 0.05 vs. 0 mmol/l.

TABLE 4
Effects of bradykinin on lactate release and glycogen synthesis in soleus muscle in vitro

Bradykinin concentration (nmol/l)	Rate of lactate release ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	Rate of glycogen synthesis ($\mu mol \cdot h^{-1} \cdot g^{-1}$)
0	8.00 ± 0.60 (9)	1.39 ± 0.15 (9)
100	7.72 ± 0.67 (8)	1.46 ± 0.11 (8)
0.01	8.45 ± 0.37 (9)	1.79 ± 0.09 (9)*

Data are means ± SE (n). Insulin was present in the medium at 100 μU/ml. *$P < 0.05$ vs. 0 mmol/l.

(31). However, results from the present study demonstrate that treatment of SH rats with trandolapril plus or minus verapamil caused a normalization of blood pressure, with respect to a reference group of WKY rats, and a significant increase in the response of glycogen synthesis to submaximal and/or maximal concentrations of insulin (Table 2). Thus our results suggest that ACE inhibition not only normalizes blood pressure in hypertensive rats but also may improve the sensitivity of glucose utilization to insulin in skeletal muscle. Further experiments are required to determine whether subantihypertensive doses of ACE inhibitors, including trandolapril, can improve insulin responses in insulin-resistant skeletal muscle. Also, it is important to determine whether ACE inhibitors with or without verapamil can overcome the insulin resistance found in other conditions, such as obesity and physical inactivity. It would also be interesting, therefore, to examine the treatment of WKY rats with trandolapril to observe whether insulin resistance could be reversed in these rats.

The response of the rates of lactate release to insulin were generally not improved by treatment of SH rats with trandolapril alone or in combination with verapamil. However, the rate of lactate release (and glycogen synthesis) was significantly increased with insulin at 10 μU/ml in incubated soleus muscles isolated from SH rats treated with trandolapril and verapamil. Therefore it might be suggested that the contribution of verapamil to the combination therapy is to restore to normal basal glucose metabolism. There is some evidence that insulin resistance in incubated cells is linked to an increase in cytosolic free calcium levels (32), and verapamil added to the incubation medium can reverse this insulin resistance, perhaps by lowering the intracellular calcium levels, which are known to be elevated (32). We do not know whether this mechanism explains the decreased rates of lactate release with insulin at 200 and 1,000 μU/ml in incubated soleus muscle preparations isolated from SH rats treated with verapamil.

Acute treatment of Wistar skeletal muscle. Further studies are necessary to clarify the biochemical mechanism of action of ACE inhibitors in skeletal muscle. Therefore we investigated whether acute treatment (1 h) of skeletal muscle with an ACE inhibitor has any effects on submaximal insulin-mediated responses in soleus muscle preparations isolated from non-insulin-resistant rats. Although the magnitude of the effects of trandolaprilat is small, it might be possible to utilize the isolated incubated soleus muscle preparation obtained from insulin-resistant animals (e.g., SH rats or obese [*fa/fa*] Zucker rats) to delineate the mechanism of action of ACE inhibition. Such a strategy could also be applied to other insulin-resistant animals exposed to chronic ACE inhibition.

Bradykinin has been implicated as an active metabolite that may modulate the sensitivity of glucose metabolism to insulin in skeletal muscle (4,5). Previous studies have reported that bradykinin has no effect on basal or insulin-stimulated 2-deoxyglucose or 3-*O*-methylglucose (nonmetabolizable analogues of glucose used to measure the rate of glucose transport) in isolated incubated skeletal muscle preparations (11,12). However, we decided to incubate rat soleus muscle preparations with bradykinin and two agents that would slow down the rate of degradation of bradykinin (see METHODS). These results suggest that low concentrations of bradykinin might have effects on glycogen metabolism in skeletal muscle. Further experiments are required to clarify whether bradykinin is involved in the improvement of insulin sensitivity of glycogen synthesis in SH rat muscle by ACE inhibition.

In conclusion, we have demonstrated that the response of glycogen synthesis to insulin is markedly decreased in isolated soleus muscle preparations obtained from 8-month-old SH and WKY rats. Treatment of SH rats with trandolapril with or without verapamil normalized blood pressure and the heart-to-body weight ratio and improved the response of glycogen synthesis to insulin. Treatment had no effect, however, on insulin resistance caused by toxemia. Bradykinin (0.01 nmol/l) and the active metabolite of trandolapril, trandolaprilat, caused a small but significant increase in the rate of glycogen synthesis in response to a submaximal concentration of insulin.

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