Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 241-248

BROWN ADIPOSE TISSUE IS A MAJOR SITE OF GLUCOSE UTILISATION IN C57B1/6 ob/ob MICE TREATED WITH A THERMOGENIC β-ADRENOCEPTOR AGONIST

P. Young, M.A. Cawthorne and S.A. Smith

Beecham Pharmaceuticals Research Division, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, England

Received April 22, 1985

Chronic treatment of C57B1/6 ob/ob mice with the thermogenic β-adrenoceptor agonist BRL 26830 increased the maximal activities of hexokinase and phosphofructokinase 2- and 3-fold respectively in interscapular brown adipose tissue. Combining measurements of whole body glucose turnover with measurements of the uptake of 2-deoxyglucose by tissues in vivo has allowed tissue rates of glucose utilisation to be calculated. Under basal conditions, brown adipose tissue accounts for only 1.6% of the whole body rate of glucose utilisation. This is increased to 3.7% in mice given a single dose of BRL 26830. In ob/ob mice treated chronically with BRL 26830, which causes brown adipose tissue hypertrophy, glucose utilisation by this tissue was increased to 4.1% of the whole body rate under the basal condition. In these mice, an additional acute treatment with BRL 26830 increased brown adipose tissue glucose utilisation rate to 12.5% of the whole body rate.

Brown adipose tissue is well recognised as the tissue site of both cold- and diet-induced thermogenesis in rodents (1,2). Recently, it has been suggested that brown adipose tissue might also play an important role in the control of blood glucose in rodents (3,4,5).

Genetically obese (ob/ob) mice have a high metabolic efficiency (6) and show extreme sensitivity to cold (7,8). These disorders are due to functional atrophy of brown adipose tissue (9). Obese mice are also markedly glucose intolerant. To investigate whether decreased glucose utilisation by brown adipose tissue could make an important contribution to the development of the impaired glucose tolerance of obese (ob/ob) mice, the effects of the thermogenic β-adrenoceptor agonist, BRL 26830 (10), which causes hypertrophy of brown adipose tissue when dosed chronically (11), on whole body glucose turnover in obese mice has been determined. In addition, the effects of this agent on the maximal activity of glycolytic enzymes in various tissues and on

0006-291X/85 $1.50

the uptake of a tracer dose of 2-deoxy-[^{14}C]-glucose in vivo has been measured. Such data have allowed us to calculate rates of glucose uptake for individual tissues and demonstrate that brown adipose tissue can become a quantitatively important site of glucose utilisation in these mice.

MATERIALS AND METHODS

Male C57Bl/6 ob/ob mice were obtained from OLAC 1976 Ltd., Bicester, Oxon. The mice were purchased at 4-5 weeks of age and were maintained at 23+1°C under a 12h light - 12h dark cycle and were 12 weeks old at the start of the studies. They were fed ad-lib throughout on Oxoid rat and mouse breeders diet (H.C. Styles, Bewdley, Worcs.).

D-[6-^{3}H]-glucose (500 mCi/mmol), D-[U-^{14}C]-glucose (291 mCi/mmol) and 2-deoxy-D-[1-^{14}C]-glucose (59 mCi/mmol) were obtained from Amersham International, Amersham, Bucks. The radiochemicals were administered to mice at their original specific activities.

In experiments to study the effects of chronic treatment with a thermogenic β-agonist, BRL 26830, (R*,R*)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino] propyl] benzoate, (E)-2-butenedioate (2:1) salt, (1 mg/kg or 10 mg/kg) was given once daily for 3 weeks by oral gavage. The mice were killed 24h after the last dose of BRL 26830. The maximal activities of hexokinase, 6-phosphofructokinase and 2-oxoglutarate dehydrogenase were measured spectrophotometrically (12,13,14). Since liver possesses both hexokinase and glucokinase activities, the total activity of the two enzymes in liver was determined in the presence of 100 mM glucose. This is not necessary for brown adipose tissue since glucokinase is absent (4).

To determine glucose turnover rates, mice that had been treated for 3 weeks with either BRL 26830 (1 mg/kg/day) or water were fasted for 5h from 08.00h and then groups of mice from each treatment were subdivided and given either an additional dose of BRL 26830 or water. After a further 60 mins, each mouse received by tail vein injection D-[6-^{3}H]/[U-^{14}C]-glucose (1 ml/100 g; 100 μCi/ml and 10 μCi/ml respectively) in 0.9% sterile saline. Blood glucose concentration (15), blood glucose specific radioactivity and glucose turnover rates were determined as previously described (16).

To determine 2-deoxyglucose uptake into various tissues, mice that had followed the same dosage protocol as outlined in the glucose turnover experiments were given a tracer dose of 2-deoxy-D-[1-^{14}C]-glucose (6 μCi/kg) by tail vein injection. After 45 mins, the mice were killed and tissues rapidly dissected and samples taken for combustion in a tissue oxidiser (5).

RESULTS AND DISCUSSION

It has been demonstrated previously that BRL 26830 stimulates thermogenesis in C57Bl/6 ob/ob mice (10,17). In addition, chronic treatment of C57Bl/6 ob/ob mice results in a restoration of normal glucose tolerance (18). In 5h-fasted ob/ob mice (Table 1), acute treatment with BRL 26830 had no effect on the plasma insulin concentration but elevated blood glucose

TABLE 1

EFFECT OF BRL 26830 ON BLOOD GLUCOSE AND GLUCOSE TURNOVER IN C57Bl/6 ob/ob MICE

	Control	BRL 26830 acute	BRL 26830 chronic	BRL 26830 chronic & acute
Body wt. (g)	45.5±1.0	42.6±1.3	43.6±1.1	44.3±1.4
Blood glucose concentration (mM) at intervals following acute treatment:				
20 mins	15.9±1.7	20.7±3.3	12.9±1.8	10.4±1.3*
40 mins	15.4±1.6	21.2±4.0	12.0±1.5	9.1±1.1**
60 mins	12.8±1.5	19.6±4.0	11.0±1.9	8.5±1.3
80 mins	12.7±1.7	17.8±2.5	10.8±1.8	9.3±0.9
Rate constant for [6-^{3}H]-glucose decay (min^{-1})	0.031±0.003	0.030±0.004	0.039±0.004	0.046±0.002**
Rate constant for [U-^{14}C]-glucose (min^{-1})	0.024±0.003	0.020±0.003	0.034±0.003	0.039±0.002**
Rd (µmol/min/100 g body wt.)	20.9±3.2	30.0±3.8	15.5±2.0	17.6±2.8

Male C57Bl/6 ob/ob mice were given BRL 26830 (1 mg/kg) as indicated in Methods. Results are as mean ± S.E.M. of 6 mice. Statistical significance (Student's t-test) relative to control ob/ob mice is indicated by *P<0.05, **P<0.01.

slightly, although the latter effect was not significant statistically. Chronically-treated mice had a significantly lower plasma insulin concentration (59±5 ng/ml v controls 130±25 ng/ml; P<0.05) and a slightly, but not significantly, lower blood glucose concentration. The decrease in blood glucose concentration was significant in those mice that also received a further acute dose of BRL 26830.

In agreement with previous studies (16) that have used [6-^{3}H] and [U-^{14}C]-glucose to measure glucose turnover in rats, the rate constant (k min^{-1}) for the decay of [6-^{3}H]-glucose was greater than for [U-^{14}C]-glucose indicating significant recycling of glucose through the Cori cycle. Although there was some variability in the level of recycling, none of the treatments significantly affected the rate of recycling (data not shown). The rate constant

for glucose decay was increased significantly in the chronically treated mice that were given a further acute dose of BRL 26830 (Table 1). However, although the whole-body fractional turnover rate of glucose was increased, by virtue of the fact that blood glucose concentration was also reduced, the net mass of glucose utilised (Rd) per unit time was the same as that of the control mice.

Chronic treatment of C57Bl/6 ob/ob mice with BRL 26830 increased the maximum activity in brown adipose tissue of hexokinase and 6-phosphofructokinase, but not that of oxoglutarate dehydrogenase (Table 2). The former provide respectively a quantitative index of the maximum capacity of glycolysis from glucose and glycolysis from glycogen. In contrast to brown adipose tissue, the activity of 6-phosphofructokinase in quadriceps muscle and heart was not affected.

It is not possible to equate directly increases in the maximum capacity of the glycolytic pathway (as measured by enzyme activity) with the actual flux through that pathway. To provide a qualitative index of the relative flux of glucose into various tissues, the net uptake of a tracer dose of 2-

TABLE 2

EFFECT OF BRL 26830 ON THE MAXIMUM CATALYTIC ACTIVITIES (μmoles/min per tissue) OF HEXOKINASE, PHOSPHOFRUCTOKINASE AND OXOGLUTARATE DEHYDROGENASE IN VARIOUS TISSUES OF OBESE MICE

	Treatment	Oxoglutarate Dehydrogenase	Phospho-fructokinase	Hexokinase & Glucokinase
Interscapular Brown Adipose Tissue	Control	0.79±0.08	0.88±0.09	0.34±0.02
	BRL 26830	1.01±0.11	2.89±0.27***	0.65±0.04***
Liver	Control	5.38±0.22	9.50±0.75	4.12±0.75
	BRL 26830	7.23±0.60*	14.28±1.28**	6.41±0.69*
Heart	Control	1.64±0.07	1.49±0.08	0.79±0.06
	BRL 26830	1.57±0.14	1.32±0.08	0.67±0.04
Quadriceps Muscle	Control	not detected	1.98±0.16	not detected
	BRL 26830		2.01±0.17	

Mice were treated with BRL 26830 (1 mg/kg) for 21 days and killed 24h after the last dose. Enzyme activities are presented as the mean ± S.E.M. for 6 mice and statistical significance (Student's t-test) is indicated by *P<0.05, **P<0.01, ***P<0.001.

TABLE 3

EFFECT OF ACUTE AND CHRONIC TREATMENT OF OBESE MICE WITH BRL 26830 ON TISSUE UPTAKE OF 2-DEOXYGLUCOSE

	Tissue 2-Deoxyglucose Content (% Injected)			
	Control	BRL 26830 (acute)	BRL 26830 (1 mg/kg/day chronic)	BRL 26830 (1 mg/kg/day chronic) + BRL 26830 (1 mg/kg acute)
Interscapular brown adipose tissue	0.31±0.06	0.74±0.06*	0.93±0.04***	2.73±0.47***
Epididymal white adipose tissue (per g)	0.39±0.04	0.70±0.08**	0.44±0.02	0.63±0.07*
Heart	1.68±0.29	1.30±0.14	1.97±0.24	1.56±0.16
Quadriceps muscle (per g)	0.41±0.06	0.25±0.02	0.4±0.05	0.36±0.03
Liver	6.08±0.2	6.54±0.32*	3.92±0.12***	4.49±0.05***
Brain	not determined	not determined	0.66±0.04	0.74±0.14

Obese mice were treated as outlined in the text. Each value is the mean ± S.E.M. of 5 mice. Statistical significance (Student's t-test) relative to control ob/ob mice is indicated by *P<0.05, **P<0.01, ***P<0.001.

deoxyglucose in vivo was determined (5) in mice that had received identical treatments to those adopted in the glucose turnover experiments (Table 3). Expressing the data as 2-deoxyglucose trapped in each tissue as a function of 2-deoxyglucose injected, a single dose of BRL 26830 increased 2-deoxyglucose uptake from 0.31% to 0.74% in interscapular brown adipose tissue. In mice given BRL 26830 (1 mg/kg) chronically, interscapular brown adipose tissue consumed 0.93% of the available 2-deoxyglucose label. When a further acute dose of BRL 26830 was given to these chronically-treated mice, the uptake of 2-deoxyglucose into interscapular brown adipose tissue was 2.73%. No other tissue showed any increase in 2-deoxyglucose uptake with the exception of epididymal white adipose tissue, which showed up to a 2-fold increase.

It is generally accepted that interscapular brown adipose tissue comprises 25% of the total brown fat in the mouse (19). Using this value, it is

TABLE 4

CALCULATED TISSUES RATES OF GLUCOSE UTILISATION
IN C57Bl/6 ob/ob MICE GIVEN BRL 26830

	Control	BRL 26830 (acute) 1 mg/kg	BRL 26830 (chronic) 1 mg/kg	BRL 26830 (chronic & acute) 1 mg/kg
Whole body glucose utilisation Rd (μmoles/min/mouse)	8.1±1.2	12.3±1.6	6.3±0.8	6.9±1.1
Tissue glucose uptake (% of whole body utilisation)				
†Brown adipose tissue	1.6	3.7	4.1	12.5
Epididymal white adipose tissue (per g)	0.5	0.9	0.5	0.6
Heart	2.4	1.6	2.3	1.8
Quadriceps muscle (per g)	1.7	1.8	2.1	2.5
‡Liver	7.4	8.2	4.6	4.6
Brain	not determined	not determined	0.8	0.9

Tissue uptake of glucose was calculated from the equation ^{14}C-2-deoxyglucose in tissue/total ^{14}C-2-deoxyglucose uptake x whole body Rd, where total 2-deoxyglucose uptake was equal to 2-deoxyglucose injected minus 2-deoxyglucose remaining in whole body glucose space.

†Interscapular brown adipose tissue is assumed to be 25% of the total brown adipose tissue in the ob/ob mouse.

‡This is an underestimate since liver can lose 2-deoxyglucose-6-phosphate via the action of glucose-6-phosphatase.

possible from the glucose turnover and 2-deoxyglucose uptake data, which were obtained under identical conditions, to derive individual tissue contributions to the total rate of glucose utilisation (see legend to Table 4). Under basal conditions, brown adipose tissue only accounted for 1.6% of the whole body glucose utilisation rate but this increased to 3.7% in animals given a single acute treatment with BRL 26830. In mice that received BRL 26830 (1 mg/kg) chronically, the basal rate of glucose uptake of brown adipose tissue was 4.1% of the whole animal rate and this was increased to 12.5% in mice given a further acute dose of BRL 26830.

The present data therefore provide evidence that brown adipose tissue can be a quantitatively important site of glucose utilisation in mice and supports the recent qualitative studies (20) that have demonstrated increased net uptake of 2-deoxyglucose into brown adipose tissue in cold-exposed lean and 26-day old ob/ob mice.

The mechanism by which glucose uptake by brown adipose tissue is preferentially increased relative to other tissues is not completely understood. Chronic treatment of ob/ob mice with BRL 26830 induces hypertrophy of brown adipose tissue (11) and this may well account for the increased glucose uptake in the chronically-treated mice relative to the untreated controls. However, it cannot account for the increase in glucose uptake that was produced by the acute treatments. Previously (21), it has been demonstrated that there is heterogeneity in the insulin sensitivity of various tissues. We suggest that such differential insulin sensitivity, which might be modulated by local hormones such as adenosine (22) or prostaglandins (23) could regulate the flux of glucose into brown adipose tissue. Thus, during acute thermogenic stimulation a selective increase in insulin sensitivity of brown adipose tissue would allow increased glucose uptake and utilisation by this tissue. In contrast, a relative decrease in insulin sensitivity in brown adipose tissue would reduce glucose uptake and this might be associated with a reduced level of dietary induced thermogenesis and the development of obesity.

ACKNOWLEDGEMENTS

We wish to thank Miss L. King and Miss H. Chapman for their excellent technical assistance and Mrs. J.S. Hodder for her help in the preparation of this manuscript. We are grateful to Drs. P. Trayhurn and S.W. Mercer of the Dunn Nutrition Unit, Cambridge, for making an unpublished manuscript available to us.

REFERENCES

1. Smith,R.E. (1961) Physiologist 4, 113.
2. Rothwell, N.J. and Stock, M.J. (1979) Nature 281, 31-35.
3. McCormack, J.G. (1982) Prog. Lipid Res. 21, 195-222.
4. Cooney, J.G. and Newsholme, E.A. (1982) FEBS Letts 148, 198-200.
5. Young, P., Cawthorne, M.A., Levy, A.L. and Wilson, K. (1984) FEBS Letts 176, 16-20.

6. Thurlby, P. and Trayhurn, P. (1979) Br. J. Nutr. 42, 377-385.
7. Davis, T.R.A. and Mayer, J. (1954) Am. J. Physiol. 177, 222-226.
8. Trayhurn, P., Thurlby, P.L. and James, W.P.T. (1977) Nature (Lond.) 266, 60-62.
9. Trayhurn, P. and James, W.P.T. (1978) Pflügers Arch. 373, 189-193.
10. Arch, J.R.S. and Ainsworth, A.T. (1983) Am. J. Clin. Nutr. 38, 549-558.
11. Young, P., Wilson, S. and Arch, J.R.S. (1984) Life Science 34, 1111-1117.
12. Crabtree, B. and Newsholme, E.A. (1972) Biochem. J. 126, 49-58.
13. Opie, L.H. and Newsholme, E.A. (1967) Biochem. J. 103, 391-399.
14. McCormack, J.G. and Denton, R.M. (1979) Biochem. J. 189, 533-544.
15. Schmidt, F.H. (1973) in 3rd International Donau Symposium uber Diabetes Mellitus. Verlag W. Mandrich, Wien-Munchen-Bern.
16. Okajima, F. and Ui, M. (1979) Biochem. J. 182, 565-575.
17. Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Sennitt, M.V., Thody, V.E., Wilson, C. and Wilson, S. (1984) Nature (Lond.) 309, 163-165.
18. Sennitt, M.V., Arch, J.R.S., Levy, A.L., Simson, D.L., Smith, S.A. and Cawthorne, M.A. (1984) Biochem. Pharmac. (in press).
19. Thurlby, P.L. and Trayhurn, P. (1980) Pflügers Arch 385, 193-201.
20. Mercer, S.W. and Trayhurn, P. (1984) Biosc. Rep. 4, 933-940.
21. Hom, F.G. and Goodner, C.J. (1984) Diabetes 33, 153-159.
22. Budohoski, L., Challiss, R.A.J., Cooney, G.J., McManus, B. and Newsholme, E.A. (1984) Biochem. J. 224, 327-330.
23. Challiss, R.A.J., Lozeman, F.G., Budohoski, L. and Newsholme, E.A. (1985) Biochem. Soc. Trans. 13, 268-269.