The effect of insulin and noradrenaline on the uptake of 2-[1-^{14}C]deoxyglucose in vivo by brown adipose tissue and other glucose-utilising tissues of the mouse

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The uptake of 2-[1-^{14}C]deoxyglucose in vivo by brown adipose tissue was greater than that of brain or heart in control mice on a whole tissue basis. When mice were treated with noradrenaline the rate of uptake of 2-[1-^{14}C]deoxyglucose by brown adipose tissue was increased 6-fold with the only other change occurring in heart where a 5-fold increase was observed. After administration of insulin the uptake of 2-[1-^{14}C]deoxyglucose in vivo was increased in heart, brown adipose tissue, white adipose tissue and muscle. The amount of 2-[1-^{14}C]deoxyglucose taken up by brown adipose tissue compared to other tissues and the changes in this uptake after administration of noradrenaline or insulin suggest that brown adipose tissue is capable of playing a quantitatively important role in glucose removal from the blood.

Brown adipose tissue *2-Deoxyglucose* *Insulin* *Noradrenaline* *Glucose uptake*

1. INTRODUCTION

Recent studies have determined that brown adipose tissue (BAT) is responsible for a significant amount of the heat production seen in non-shivering and diet-induced thermogenesis in mammals [1]. The lipogenic capacity of this tissue has also been widely investigated and it has been suggested that in animals fed a high carbohydrate diet, blood glucose is the major percursor for this lipogenesis [2–4]. Although it is generally accepted that the fuel for thermogenesis in BAT is fatty acids, there is no direct evidence that this is always the case. It has been suggested that glucose may be used as a direct fuel for thermogenesis as well as a lipogenic precursor [5], but there are no reports of the extent of glucose uptake in vivo by BAT. The high rates of lipogenesis observed in BAT under certain conditions coupled with the high activities of the glycolytic enzymes hexokinase and 6-phosphofructokinase indicate that despite its small size (1–2% of body weight), BAT could utilise a considerable amount of glucose and therefore be important in removal of glucose from the blood [6,7].

The uptake of the glucose analogue 2-deoxyglucose in vivo has been used as an index of relative glucose uptake in tissues of the rat [8–10]. 2-Deoxyglucose is transported by the glucose transport system and becomes trapped in the tissue after phosphorylation by hexokinase. The accumulation of label thus reflects the overall process of transport and phosphorylation by a tissue [8]. Here, the uptake of 2-[1-^{14}C]deoxyglucose was measured in BAT and in the major glucose utilising tissues of adult mice to give some indication of the relative importance of BAT in whole body glucose utilisation. The effects of noradrenaline and insulin on 2-deoxyglucose uptake were also investigated, since noradrenaline stimulates thermogenic activity in BAT [11] and BAT is known to be an insulin-sensitive tissue [12,13].

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2. MATERIALS AND METHODS

Male QS mice were obtained from the Bosch Animal House, University of Sydney. The mice were maintained at 22°C under a 12 h light/dark cycle and fed on a standard laboratory diet (Rat and Mouse Kubes; Allied Feeds, Rhodes, NSW). For experiments on deoxyglucose uptake mice were lightly anaesthetized with phenobarbitone (50 mg/kg i.p.) and injected intravenously with 2-[1-^{14}C]deoxyglucose (45 μCi/ml Amersham, England) at a dose of 150 μCi/kg. Where indicated noradrenaline (Leverophed, Winthrop Sydney, Australia; 260 μg/kg) and insulin (Actrapid, Novo, Copenhagen, 0.5 units/kg) were injected i.v. with 2-[1-^{14}C]deoxyglucose (total volume 0.2 ml). Samples of blood (5 μl) were taken from the tip of the tail at 1,2,5,10,20 and 30 min after injection of label. These samples were immediately deproteinised by addition of 0.05 ml of 6% $HClO_4$ and centrifuged in an Eppendorf microfuge (12 000 × g, 3 min). The supernatant was used for the determination of blood glucose [14] and blood radioactivity.

After 30 min the mice were killed by cervical dislocation and the heart, liver, brain, interscapular BAT, white adipose tissue (epididymal) and skeletal muscle (quadriceps) removed and quickly frozen in liquid nitrogen. Samples of these tissues (100–400 mg) were homogenised in 1.2 ml of 6% $HC10_4$: 80 mM triethanolamine HCl and centrifuged at 12 000 × g for 5 min in an Eppendorf microfuge. The supernatants were neutralised with KOH and 0.5 ml applied to individual plastic columns of Dowex 2-formate (prepared from Dowex 2-chloride 2 × 8-400 Sigma, St. Louis, MO) for separation of 2-[1-^{14}C]deoxyglucose from 2-[1-^{14}C]deoxyglucose 6-phosphate [15]. Addition of 5 ml H_2O eluted 2-[1-^{14}C]deoxyglucose and subsequent elution with 5 ml of 1 M formic acid/0.3 M ammonium formate yielded 2-[1-^{14}C]deoxyglucose 6-phosphate. Calculation of the recovery of label showed that 95–100% of the radioactivity added to the column was present in the two fractions collected. The uptake of 2-[1-^{14}C]deoxyglucose by tissues is expressed as dpm/total tissue except for white adipose tissue and skeletal muscle where results are expressed as dpm/g wet wt. It was assumed that interscapular BAT accounted for 25% of the total BAT when calculating 2-[1-^{14}C]deoxyglucose uptake in this tissue [16].

The model used to calculate the rate constant (K) of net tissue uptake of 2-[1-^{14}C]deoxyglucose is based upon the assumption that the rate of disappearance of label from the blood is the sum of all individual tissue rates of uptake [10]. For a single injection of tracer where the plasma disappearance of label is treated as a single exponential function:

$$K = \frac{CK_p}{C_{p_o}(1 - e^{-K_p t})}$$

where K is the rate constant for net tissue uptake in a given tissue, C the concentration of label in the tissue (dpm/g), C_{p_o} the initial plasma concentration of radioactivity and K_p the plasma disappearance rate constant. No correction was made for label in the extracellular space of tissues and it was presumed that all 2-[1-^{14}C]deoxyglucose 6-phosphate was intracellular.

3. RESULTS AND DISCUSSION

The blood glucose level of control, noradrenaline-treated and insulin-treated animals over the 30

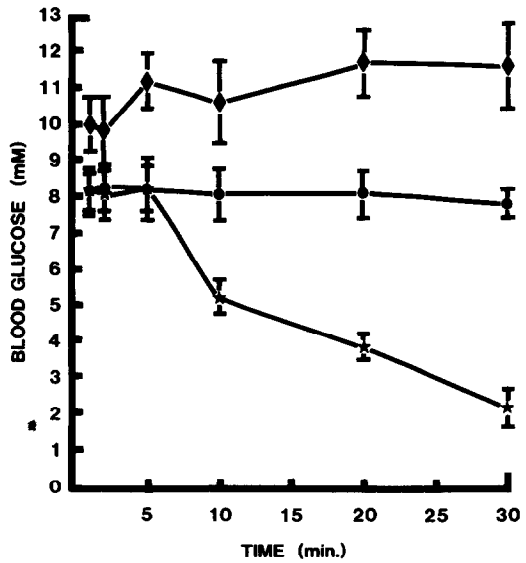

Fig.1. Blood glucose concentration of control, noradrenaline- and insulin-treated mice. For experimental details see section 2. Each group contained 8 animals and points represent the mean SE for blood samples taken at the times indicated. Control (●), noradrenaline-treated (♦), insulin-treated (★).

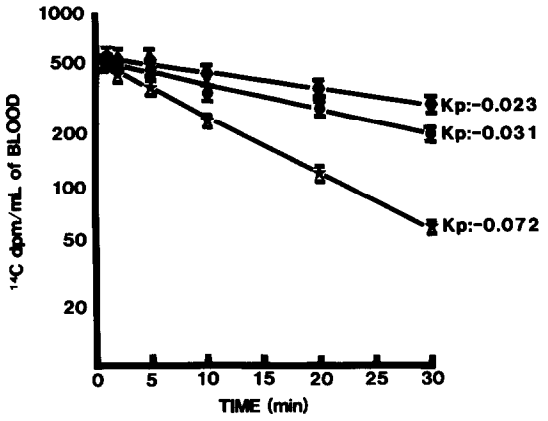

Fig.2. Concentration of 2-[1-^{14}C]deoxyglucose in the blood of control, noradrenaline- and insulin-treated mice. Each line represents a linear regression through time points on a log-linear scale to enable calculation of plasma disappearance rates of radioactivity for the 3 experimental groups. Control (●), noradrenaline-treated (♦), insulin-treated (★).

min of 2-[1-^{14}C]deoxyglucose uptake is shown in fig.1. For the mice injected with noradrenaline the blood glucose level is slightly higher than control mice, perhaps due to noradrenergic stimulation of liver glycogenolysis. However, for control and noradrenaline-treated animals the blood glucose level remains constant during the course of the experiment. In the insulin-treated mice the blood glucose level decreased after administration of insulin indicating increased glucose uptake by insulin-sensitive tissues.

The rate of disappearance of labelled deoxyglucose from the blood in the 3 experimental groups of mice is shown in fig.2. In all 3 situations the disappearance rate fits a single exponential function enabling calculation of K_p and initial plasma radioactivity (C_{p_o}) for use in determination of the deoxyglucose uptake constant (K) for any tissue [8]. The correlation coefficients for the linear regressions of each line were: control, −0.997; noradrenaline, −0.991; and insulin, −0.998 and the initial plasma radioactivity for each group was 588 840, 501 187 and 524 807 dpm/ml for control, noradrenaline- and insulin-treated mice, respectively. The same initial specific radioactivity is seen in all groups, so comparison between the tissues of different groups is possible.

Using the above data and the mathematical model described in section 2, values for the rate constant of 2-[1-^{14}C]deoxyglucose uptake (K) were calculated (table 1). In control animals the K for

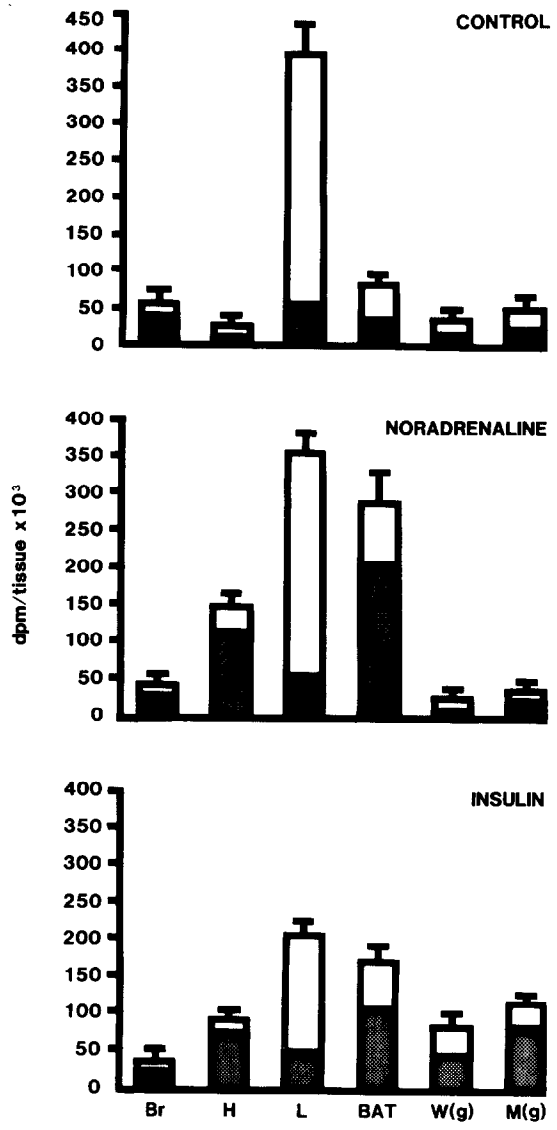

Fig.3. Relative tissue uptake of 2-[1-^{14}C]deoxyglucose in vivo of control, noradrenaline- and insulin-treated mice. Experimental details are given in section 2. The hatched area is the amount of 2-[1-^{14}C]deoxyglucose 6-phosphate. There was no significant difference between the weights of tissues from different groups. The average weights of the tissues were brain (Br), 0.407 ± 0.007; heart (H) 0.177 ± 0.004, liver (L), 1.703 ± 0.072, and BAT 0.324 ± 0.020. White adipose tissue (W) and muscle (M) results are expressed per g fresh wt.

Table 1

Calculated rate constants for 2-[1-^{14}C]deoxyglucose uptake in vivo in tissue of control, noradrenaline- and insulin-treated mice

	K		
	Control	Noradrenaline	Insulin
Brain	0.0136 ± 0.0013	0.0108 ± 0.0008	0.0146 ± 0.0010
Heart	0.0134 ± 0.0015	0.0803 ± 0.0063[a]	0.1125 ± 0.0088[a]
Liver	0.0204 ± 0.0012	0.0207 ± 0.0012	0.0215 ± 0.0014
Brown Fat	0.0165 ± 0.0012	0.0953 ± 0.0128[a]	0.0623 ± 0.0041[a]
White Fat	0.0033 ± 0.0002	0.0028 ± 0.0003	0.0130 ± 0.0019[a]
Muscle	0.0040 ± 0.0002	0.0035 ± 0.0002	0.0178 ± 0.0014[a]

K values were calculated as described in section 2. The values are the mean SE for 8 separate measurements. Significant difference from control values was determined using Student's *t*-test; [a]$P < 0.005$

BAT is similar to that of brain and heart and greater than white adipose and muscle. When mice were treated with noradrenaline the only significant changes in *K* value were in heart and BAT where a 6- and 5.7-fold increase respectively, were observed. Administration of noradrenaline increases the heart rate and the thermogenic activity of BAT and so the increased rate of deoxyglucose uptake observed is probably due to the increased energy demands of these 2 tissues. The stimulation of uptake in BAT is probably a direct effect of noradrenaline on the tissue but may also represent an increased blood flow to BAT in response to noradrenaline [17].

In mice treated with insulin there was no significant change in *K* for brain, but heart, BAT, white adipose tissue and muscle all responded with significant increases in the rate of deoxyglucose uptake. The largest increase was in heart (8.4-fold) while in BAT, white adipose tissue and muscle, the increases were 3.8-, 3.9- and 4.5-fold, respectively. Although the *K* values in white adipose tissue, muscle and BAT increased by the same amount after insulin administration, the absolute rate of glucose uptake in BAT was 3-times higher than that of white adipose tissue and muscle, indicating the large capacity of BAT for glucose uptake. Values for *K* in liver are not included since in this tissue the deoxyglucose uptake model is not valid. Liver contains glucose-6-phosphatase which could dephosphorylate 2-[1-^{14}C]deoxyglucose 6-phosphate and return it to the blood [8]. In liver, therefore, the amount of 2-[1-^{14}C]deoxyglucose 6-phosphate represents the equilibrium between the glucose phosphorylating and dephosphorylating activity of the tissue at the end of the experiment.

The total amount of labelled deoxyglucose accumulated in the tissues and the proportion of deoxyglucose in the phosphorylated form is shown in fig.3. In control animals the amount of deoxyglucose in BAT is greater than brain or heart indicating that in normal animals BAT takes up a considerable amount of glucose from the blood. When thermogenesis is stimulated by noradrenaline the deoxyglucose is increased 3.5-fold in BAT and 5-fold in heart. Under thermogenic conditions, therefore, a substantial amount of the circulating blood glucose would be taken up by BAT although liver, white adipose tissue and muscle, because of their large percentage of the body weight, would still be major tissues for glucose disposal.

When mice were injected with insulin the amount of label in BAT, heart, white adipose tissue and muscle all increased. The major tissues for glucose uptake in insulin-treated animals would be muscle and white adipose tissue, but on a g wet wt basis BAT was second to heart in deoxyglucose uptake and accumulated 4-times as much label as muscle

and white adipose tissue (not shown). The uptake in brain remained the same as in control animals confirming the previous finding that brain is essentially insulin insensitive for glucose uptake [9]. The amount of label recovered from liver of insulin-treated mice is only half that of the control animals. This decrease is probably due to the decrease in the blood glucose level since the glucose (and therefore deoxyglucose) concentration in liver is in equilibrium with blood glucose [18].

The changes in the 2-[1-^{14}C]deoxyglucose uptake of BAT and the relative amount of label in BAT compared to other insulin-sensitive tissues show that BAT, despite its small proportion of body weight, plays a significant role in glucose utilisation. In BAT, glucose metabolism leads to acetyl-CoA formation which will be oxidised or converted to lipid for storage and subsequent oxidation in BAT. Oxidation of substrates by BAT results in heat production and therefore loss of the energy equivalent of the substrate [1]. If uptake of glucose or its subsequent oxidation was defective in BAT excess energy intake might be channelled to white adipose tissue, liver and muscle where glucogen and fat production (and deposition) would result rather than heat production. Defective thermogenic mechanisms in BAT are well documented in genetically obese mice and rats [19–21] and changes in BAT have also been implicated to play a role in genetically diabetic mice [22]. The present results add evidence to the suggestion that defects in the metabolism of glucose by BAT may be important in the disorders of carbohydrate metabolism which occur in diabetes and obesity [7].

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