The effect of tumour-bearing on 2-deoxy[U-¹⁴C]glucose uptake in normal and neoplastic tissues in the rat

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The extent to which normal and neoplastic tissues of the rat take up glucose was assessed by the 2-deoxy[U-¹⁴C]glucose tracer technique. Measurements of glucose uptake were made over 40 min in anaesthetized rats under conditions where the blood glucose concentration was constant. In fed tumour-bearing rats, the relative rates of glucose uptake per g wet wt. of tissue were tumour (100), small intestine (72), brain (61), heart (61), spleen (50), lung (42), adipose tissue (11) and muscle (8). Normal tissues of the fed tumour-bearing rats had decreased rates of glucose uptake as compared with the same tissues in fed non-tumour-bearing control rats. Blood glucose concentrations were similar in both groups, but insulin concentrations were decreased in tumour-bearing rats. Starvation decreased the rates of glucose uptake by normal tissues in both control and tumour-bearing rats, but the difference between the fed and starved states was greater in the control rats. Starvation did not decrease glucose uptake by the tumour. On an organ basis, the tumour (12–14 % of body wt.) took up 4 times more glucose than did muscle (40 % of body wt.).

INTRODUCTION

Neoplastic disease results in increased glucose turnover in the tumour-bearing host. It has been suggested that, in the presence of a decreased energy intake, the demand for glucose by glycolytic tumour cells is met by increased rates of hepatic gluconeogenesis from lactate exported from the tumour and from amino acids derived from host muscle or viscera. The increased glucose turnover has been confirmed by isotopic techniques in tumour-bearing rats (Singh *et al.*, 1980) and by the rate of glucose extraction in the tumours by using arteriovenous-difference measurements (Sauer *et al.*, 1982). However, the extent to which the tumour influences glucose uptake by other tissues has not been demonstrated directly.

Sokoloff *et al.* (1977) have described a technique using radiolabelled 2-deoxyglucose for the measurement of glucose uptake in the brain, and various adaptations of the method have been used since to obtain estimates *in vivo* of glucose uptake by other tissues (Hom *et al.*, 1984; Kraegen *et al.*, 1985; Burnol *et al.*, 1987). In this study we have used the single injection method of Hom *et al.* (1984) to compare rates of glucose uptake by normal and neoplastic tissues in a tumour-bearing rat model (Rofe *et al.*, 1986).

EXPERIMENTAL

Mammary adenocarcinomas were implanted as a cell suspension in both flanks of female Dark Agouti rats (170–200 g body wt.) as described previously (Rofe *et al.*, 1986). Rats were fed *ad libitum* (mouse M & V Cubes, Milling Industries, Adelaide, Australia; for composition see Potezny *et al.*, 1986) throughout, and then either used in the fed state (4–6 h after the end of the dark cycle) or starved for 72 h. Each rat was anaesthetized with halothane/N₂O, and cannulae were placed in the jugular vein and carotid artery. An arterial blood sample

(0.4 ml) was taken, and then a tracer amount of 2-D-deoxy-[U-¹⁴C]glucose (2 µCi/0.5 µmol in 0.5 ml of saline) was infused over 5 min via the jugular vein. Arterial blood samples (0.4 ml) were collected at 5, 10, 20, 30 and 40 min after the start of the infusion and deproteinized with 5 % (v/v) HClO₄. The rat was then killed rapidly with an intravenous injection of pentobarbitone (0.5 ml of 300 mg/ml solution), and 0.5–1.0 g portions of the various tissues were homogenized with 4 vol. of ice-cold 5 % HClO₄, by using an Ultra-Turrax homogenizer. After centrifugation (3000 g for 5 min), radioactivity in both 2-deoxy[U-¹⁴C]glucose and its 6-phosphate derivative was measured in the HClO₄ extract as described by Ferré *et al.* (1985). Glucose was measured enzymically with hexokinase/glucose-6-phosphate dehydrogenase (Slein, 1963). Plasma insulin was assayed with a commercial radioimmunoassay kit (Pharmacia Diagnostics, Uppsala, Sweden) with human insulin as standard. We found insulin values to be consistently lower for fed female Dark Agouti rats (< 10 µunits/ml) than those measured in fed male Porton rats (20 µunits/ml). However, the insulin concentrations rose appropriately in the Dark Agouti rats after an intravenous glucose challenge, so the method was accepted for comparisons within the one rat strain.

Expression of results

Accurate quantification of the rate of glucose metabolism by individual tissues is often difficult, as it requires knowledge of the tissue radioactivity due to 2-deoxy-[U-¹⁴C]glucose 6-phosphate, the blood glucose concentration, the rate of disappearance of 2-deoxy[U-¹⁴C]glucose from the plasma, and also the degree of discrimination in individual tissues between the analogues glucose and 2-deoxyglucose. The last parameter, often termed the 'lumped constant', has only been measured in brain, adipose tissue, skeletal muscle and

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mammary gland (Smith *et al.*, 1986; Burnol *et al.*, 1987), and is usually not considered in comparative studies, where the relative changes in glucose metabolism or uptake between treatment groups is the main interest.

The relative glucose uptake can be calculated by using the following expression:

$$U_g = C_b \cdot C_m^*(t) \left/ \int_0^{40} C_b \cdot dt \right.$$

where U_g is the glucose uptake ($\mu\text{mol}/\text{min}$ per g wet wt.), C_b the steady-state blood glucose concentration ($\mu\text{mol}/\text{ml}$), $C_m^*(t)$ is the tissue (2-deoxy[U- ^{14}C]glucose + 2-deoxy[U- ^{14}C]glucose 6-phosphate) (c.p.m./g wet wt.) at time t , and $\int_0^{40} C_b \cdot dt$ is the area under the blood 2-deoxy[U- ^{14}C]glucose-disappearance curve [c.p.m. $\cdot$ min $\cdot$ ml $^{-1}$]. This expression is the same as that used by Smith *et al.* (1986) to obtain a relative metabolic index, except in their expression $C_m^*(t)$ is tissue 2-deoxy[U- ^{14}C]glucose 6-phosphate at time t and the area under the plasma, rather than blood, disappearance curve was measured. For comparisons of relative glucose uptake, as distinct from metabolism, we have found that their calculation can be simplified. Tissue 2-deoxy-[U- ^{14}C]glucose 6-phosphate was measured (see Ferré *et al.*, 1985) and found to be approx. 40% of the total radioactivity in any given tissue. However, given the comparative nature of the present study, the uncertainty about the analogue effect and the variation in extracellular space in each tissue, we have chose to use the total tissue radioactivity (2-deoxy[U- ^{14}C]glucose + 2-deoxy[U- ^{14}C]glucose 6-phosphate) (c.p.m./g wet wt.) as $C_m^*(t)$ at $t = 40$ min. The area under the 2-deoxy-[U- ^{14}C]glucose-disappearance curve ($\int_0^{40} C_b \cdot dt$) was the same in the tumour-bearing and control rats and, for comparisons between groups, was omitted from the calculations. The relative glucose uptake can therefore be simply expressed as total tissue radioactivity (2-deoxy[U- ^{14}C]glucose + 2-deoxy[U- ^{14}C]glucose 6-phosphate) (c.p.m./g wet wt.) $\times$ (blood [glucose] in

treatment group/blood [glucose] in fed controls). Expressing the blood glucose as a fraction of that in the fed control group adjusts for variations in specific radioactivity in the blood and leaves the final relative glucose uptake (hereafter called 'glucose uptake') in the units of c.p.m./g fresh wt. of tissue. The results are shown as means $\pm$ S.E.M., and significant differences between the means were calculated by Student's t test.

RESULTS AND DISCUSSION

Fed state

The comparative rates of glucose uptake, expressed per g wet wt. of tissue for the different tissues in the fed control rats, are similar to the rates reported by others (Smith *et al.*, 1986), with the heart showing the highest, and muscle and adipose tissue the lowest, rates (Table 1). In fed tumour-bearing rats, the relative rates of glucose uptake per g wet wt. of tissue were tumour (100), small intestine (72), brain (61), heart (61), spleen (50), lung (42), adipose tissue (11) and muscle (8). Furthermore, tumour-bearing decreased glucose uptake in all of these normal tissues compared with that observed in the non-tumour-bearing controls. Indeed, the heart tissue of tumour-bearing rats had a rate of glucose uptake that was only 20% of that observed in the control rats. The high rate of glucose uptake by the tumour is consistent with the autoradiographic studies by Kern & Norton (1987), where uptake of radiolabelled 2-deoxyglucose was greatest in tumour tissue.

Effect of starvation

Starvation is a metabolic state associated with low plasma insulin concentrations and an increased degree of insulin-resistance (Goschke, 1977). In the present studies, glucose uptake was decreased in a number of tissues, and most markedly in the heart tissue of starved control rats (Table 1), but little affected in brain tissue. The latter result is consistent with the brain being an insulin-

Table 1. Effect of tumour-bearing on glucose uptake by tissues in fed and 72 h-starved rats

The results are shown as the radioactivity per g wet wt. of tissue as described in the Experimental section. The values are means $\pm$ S.E.M. The blood glucose concentrations (mM) were: control fed, 8.5 ± 0.4 ; tumour-bearing fed, 7.2 ± 0.3 ; control fasted, 4.0 ± 0.3 ; tumour-bearing fasted, 4.0 ± 0.4 . Significant differences in glucose uptake between individual tissues in control and tumour-bearing rats are shown as $*P < 0.05$, and significant differences between the same tissues owing to starvation are shown as $\dagger P < 0.05$. Glucose uptake by tumour tissue was significantly different ($P < 0.05$) from that of all normal tissues of the tumour-bearing rat except the small intestine.

	$10^{-3} \times$ Glucose uptake (c.p.m./g wet wt.)			
	Fed		Fasted	
	Control (n = 4)	Tumour-bearing (n = 5)	Control (n = 6)	Tumour-bearing (n = 8)
Heart	109.4 ± 13.2	$21.7 \pm 5.3^*$	$10.7 \pm 1.7\dagger$	$5.8 \pm 1.9\dagger$
Muscle	4.7 ± 0.6	2.7 ± 0.7	3.8 ± 0.5	2.9 ± 0.4
Lung	25.9 ± 2.2	$15.0 \pm 2.5^*$	$12.6 \pm 1.4\dagger$	8.3 ± 2.1
Brain	26.0 ± 0.8	22.1 ± 1.8	30.8 ± 2.2	21.8 ± 3.1
Spleen	32.5 ± 3.9	$18.2 \pm 1.7^*$	$18.6 \pm 1.4\dagger$	14.4 ± 1.7
Small intestine	38.8 ± 2.0	26.2 ± 3.8	$26.2 \pm 1.4\dagger$	16.2 ± 2.7
Adipose tissue	4.5 ± 0.6	3.5 ± 0.5	5.2 ± 0.7	3.5 ± 0.7
Tumour	—	36.2 ± 4.0	—	38.4 ± 6.7

Table 2. Comparison of 2-deoxy[U-¹⁴C]glucose uptake between various organs of control and tumour-bearing rats

The results are shown as the total radioactivity per organ, by using the data from Table 1. The relative organ weights (g/100 g body wt.; means of four to five determinations) are shown in parentheses. The organ/body wt. ratios for muscle, adipose tissue and small intestine are based on the data of Hom *et al.* (1984). In tumour-bearing rats, the organ/body wt. ratios have been corrected for the decreased carcass weight (see the text). Significant differences in glucose uptake between organs in control and tumour-bearing rats are shown as * $P < 0.05$, and significant differences between the same tissues owing to starvation are shown as † $P < 0.05$. Glucose uptake by the tumour was significantly different from all tissues ($P < 0.01$). The numbers of determinations and the source of data are as for Table 1.

	$10^{-3} \times$ Glucose uptake (c.p.m.)			
	Fed		Starved	
	Control	Tumour-bearing	Control	Tumour-bearing
Heart	61 ± 7 (0.30)	15 ± 4* (0.34)	6 ± 1† (0.34)	3 ± 1† (0.32)
Muscle	352 ± 45 (40.0)	191 ± 49* (34.6)	228 ± 30 (40.0)	177 ± 25 (35.5)
Lung	28 ± 2 (0.58)	18 ± 3* (0.57)	15 ± 2† (0.80)	10 ± 3 (0.68)
Brain	41 ± 1 (0.84)	33 ± 3* (0.73)	41 ± 3 (0.89)	32 ± 5 (0.85)
Spleen	14 ± 2 (0.23)	11 ± 1 (0.30)	7 ± 1† (0.24)	6 ± 1† (0.22)
Small intestine	248 ± 13 (3.4)	158 ± 23* (2.9)	134 ± 7† (3.4)	85 ± 14*† (3.0)
Adipose tissue	51 ± 7 (6.0)	37 ± 5 (5.2)	47 ± 6 (6.0)	33 ± 6 (5.3)
Liver	115 ± 8 (3.86)	82 ± 9* (4.08)	53 ± 19† (2.96)	50 ± 12 (3.56)
Kidney	66 ± 7 (0.77)	55 ± 11 (0.76)	36 ± 5† (0.78)	27 ± 10 (0.75)
Urine	266 ± 50 (0.10)	158 ± 19 (0.10)	—	—
Tumour	—	1006 ± 111 (13.5)	—	781 ± 136 (11.7)

insensitive tissue. Conversely, Smith *et al.* (1986) have shown that insulin treatment greatly increases the uptake of glucose by several tissues, with the heart showing the greatest response and brain the least.

In tumour-bearing rats, starvation decreased glucose uptake in nearly all normal tissues, but did not alter tumour glucose uptake (Table 1). These changes, however, were not as marked as those observed in the control rats in the fed/starved transition. This finding is attributed to the observation that glucose uptake by normal tissues in fed tumour-bearing rats was already decreased before starvation compared with the controls.

As mentioned above, the heart tissue showed the greatest response to the treatments of starvation of tumour-bearing, with glucose uptake being decreased 10-fold in the fed/starved transition in control rats, 5-fold in response to tumour-bearing in the fed state and 2-fold by tumour-bearing after starvation (Table 1). The heart has been shown to demonstrate large changes in glucose uptake in response to either insulin treatment or to cold-acclimation (i.e. increased insulin-sensitivity) (Smith *et al.*, 1986). The marked changes in glucose uptake by the heart may reflect the insulin concentration or sensitivity in the different metabolic states described here. In rodents, plasma insulin concentrations are usually normal or decreased (Goodland *et al.*, 1975; Chance *et al.*, 1986), and decrease with increasing tumour growth (Lanza-Jacoby *et al.*, 1984). We have found a similar trend in the rat tumour model used here, where insulin concentrations were 7.7 ± 1.3 μ -units/ml in controls but only 3.5 ± 1.3 μ -units/ml in tumour-bearing in the fed state ($n = 5$ rats per group), and 3.9 ± 1.0 and < 1.5 μ -units/ml ($n = 5$), respectively, for control and tumour-bearing rats after 72 h of starvation. This may explain in part the decreased glucose uptake by the heart and other tissues observed here. Blood lactate concentrations are

increased in tumour-bearing rats in both the fed and starved states (Rofo *et al.*, 1986), and this raises the issue of the extent to which the heart and other tissues use alternative substrates to glucose. In this regard, we have found that blood ketone bodies and, in particular, acetoacetate are markedly increased in 72 h-starved tumour-bearing rats (Rofo *et al.*, 1986).

Glucose uptake by each organ

When the glucose taken up by each tissue is calculated on a whole organ or tissue basis, glucose uptake by the tumour is 4 times greater than that by the muscle or small intestine (Table 2). These results again show that, whether the anaesthetized animal is fed or starved, the uptake of glucose is decreased in the normal organs of the tumour-bearing rats, compared with the appropriate controls. It should be noted that the values for muscle, adipose tissue and small intestine shown for tumour-bearing rats were calculated on the basis of carcass weight. The reason for this approach is that the weights of most of the organs in the tumour-bearing rats were either the same or, for the liver, actually increased relative to the total body weight (i.e. carcass plus tumour; Table 2). However, the carcass weight of the fed animals was only 86.5% (88.7% for starved animals) of the total body weight. It has therefore been assumed that the weight loss occurred in muscle, adipose and intestinal tissues. It is a major characteristic of tumour-bearing that the total mass of these tissues is decreased in the wasting seen in association with cancer (Stein, 1978; Lawson *et al.*, 1982). The calculated tissue radioactivity accounted for 50% of the total injected. The liver and kidney results have been included for comparison, but it is stressed that the presence of glucose 6-phosphatase in these tissues may lead to a large underestimation of glucose uptake for these tissues. Some radioactivity was excreted in the urine (Table 2),

confirming the observations of Smith *et al.* (1986). Radioactivity in the skin and bone was not measured and, as these tissues represent 30–40% of the body weight, it is assumed that these tissues may account for much of the non-detected labelled 2-deoxyglucose (Hom *et al.*, 1984).

The 2-deoxyglucose radiotracer technique does have a number of limitations. Even when allowances are made for radioactivity in the extracellular space and tissue discrimination between the analogues glucose and 2-deoxyglucose, for the uptake and phosphorylation by various tissues, it has been recommended that rates of glucose utilization between treatments should differ by at least 15% to be considered meaningful (Ferré *et al.*, 1985). The effect of anaesthesia on glucose uptake (Heath *et al.*, 1977), particularly by postural muscles (Burnol *et al.*, 1987), may also need to be considered.

However, given the magnitude of the glucose uptake by the tumour, it is unlikely that all those considerations would markedly affect the pattern of results presented here, with the possible exception of muscle. It might be expected that a comparison of glucose uptake by the tumour and muscle during anaesthesia, wakefulness and exercise might provide additional information concerning the extent of competition between muscle and tumour (see Deuster *et al.*, 1985; Issad *et al.*, 1987). Our results provide direct evidence that the tumour is a major consumer of glucose *in vivo*. Our demonstration that the tumour tissue (12–14% of body wt.) in our animal model uses more than 4 times as much glucose as does any other organ highlights the drain that the tumour exerts on the body's fuel resources. This conclusion must be tempered in the interpretation of energy requirements in tumour-bearing humans, where the tumour burden is usually less than 5% of body weight. The technique described here could be adapted to smaller mouse tumour models, and should be useful in examining treatment regimens which are aimed at decreasing glucose utilization by neoplastic tissues.

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