Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 1-6

TUMOR NECROSIS FACTOR INCREASES IN VIVO GLUCOSE UTILIZATION OF MACROPHAGE-RICH TISSUES

Károly Mészáros, Charles H. Lang, Gregory J. Bagby, and John J. Spitzer

Department of Physiology, Louisiana State University Medical Center, New Orleans, LA 70112

Received September 28, 1987

SUMMARY: Glucose utilization of different tissues was investigated in vivo by the 2-deoxyglucose tracer technique. After infusion of a non-lethal dose of recombinant human TNF-α (150 μg/kg) to rats, glucose utilization was increased by 80-100% in spleen, liver, kidney, by 60% in skin and by 30-40% in lung and ileum. The largest increase (150%) was observed in the diaphragm. There was no significant change in glucose utilization by skeletal muscles, testis and brain. These data show that TNF exerts metabolic effects on macrophage-rich tissues, and suggest that enhanced secretion of TNF may be one of the important factors in eliciting the metabolic changes in sepsis and endotoxicosis.

Tumor necrosis factor is a monokine of diverse biologic actions (reviewed in ref. 1). Bacterial endotoxin is a potent stimulator of macrophage TNF secretion (2). Many of the drastic effects of lethal endotoxin treatment have been reproduced by administration of toxic doses of TNF (upto 1.8 mg/kg rat; ref. 3). Recently Bagby et al. (4) have reported metabolic effects using non-lethal doses of TNF. The blood pressure, heart rate and plasma insulin concentration were normal after TNF infusion. Glucagon, epinephrine and norepinephrine concentrations were moderately increased (2-3 fold). Body temperature was significantly elevated (0.9° C). Plasma lactate was increased about 2-fold, and the turnover rate of plasma glucose was increased by 24% (4). Using similar experimental conditions, the present study was undertaken to determine which tissues were responsible for the increase in whole-body glucose utilization, and to compare the changes with those observed previously in response to endotoxin (5,6). We employed the 2-deoxyglucose tracer method as a sensitive indicator of glucose utilization by different tissues in living, conscious rats.

0006-291X/87 $1.50

MATERIALS AND METHODS

Male Sprague-Dawley rats (280-320 g, Charles River) were used. The day preceding an experiment arterial and venous catheters were implanted. Then the animals were caged individually without food but with water ad libitum.

Human recombinant TNF-α ($5x10^7$ U/mg; kindly provided by Genentech, Inc.) was dissolved in saline (15 μg/ml), and was infused at a rate of 50 μg/kg/h for 3 h (about $2.2x10^6$U per rat). Animals infused with TNF had a normal appearance, and did not show any signs of sickness or fatigue. Time-matched control rats were infused with an equal volume of saline.

Tracer amounts of 2-deoxy-D-[2,6-^{3}H]glucose (3 μCi/100 g bw; Amersham, 46.8 Ci/mmol) were injected as a bolus immediately after the termination of the TNF infusion. The decay of radioactivity in blood and plasma glucose concentration were monitored for 40 min (5). Rats were then sacrificed and the accumulation of phosphorylated metabolites of 2-deoxyglucose measured in selected tissues (5,6). Glucose utilization was calculated according to the following equation:

$$R_g = \frac{C_m^*(T)}{LC \int_0^T \frac{C_p^*}{C_p}\, dt}$$

where R_g is tissue glucose metabolic rate (nmol/g/min); $C_m^*(T)$ is the concentration of phosphorylated metabolites of 2-deoxyglucose in the tissue (dpm/g) at t=40 min; C_p is plasma glucose (nmol/ml); C_p^* is the concentration of radioactive 2-deoxyglucose in the plasma (dpm/ml; equal to concentration in whole blood); LC (lumped constant) is a dimensionless correction factor for discrimination against 2-deoxyglucose in glucose metabolic pathways (7). Since the increase of plasma glucose concentration was small and very slow in the TNF-infused rats (Fig.1A), the term for the equilibration between plasma and brain glucose concentration (which is very fast) was not applied (8). For LC we used the previously determined value of 0.5 (ref. 6). LC does not change appreciably in the observed range of plasma glucose concentrations (9). We assumed that the rate of glucose utilization within each tissue was constant during the labeling period.

In vitro glucose utilization was investigated as follows. Intact epitrochlearis muscle was removed from normal rats and incubated in Krebs-Ringer bicarbonate buffer (5). Spleen cells from normal rats were isolated and incubated in phosphate buffered saline solution (6). Alveolar macrophages were obtained by bronchial lavage with saline (10) and incubated in a similar manner. The glucose utilization rates were determined in the presence of 5 mM glucose by measuring the formation of ^3H_2O from the tracer D-[2-^{3}H]glucose (5).

Statistical significance was calculated by the Student's *t* test.

RESULTS AND DISCUSSION

Glucose utilization of different tissues was investigated in vivo by the 2-deoxyglucose tracer method, which has been most extensively applied for the study of the brain. The glucose metabolic rate (R_g) in control brain was 677 nmol/g/min, in agreement

Table 1. Tissue glucose metabolic rate (R_g) after TNF infusion

	Control	TNF		Control	TNF
Spleen	270±35	479±18**	Brain	677±19	694±17
Liver	27± 3	52± 3**	Testis	214± 9	205± 4
Kidney	123±16	255±17**	Blood	16± 3	18± 3
Skin	66± 7	108± 8**	Gastrocnemius	55± 7	54± 9
Lung	215±19	310±26*	Quadriceps m. white portion	27± 2	32± 1
Ileum	321±36	431±31*	red portion	48± 3	64± 8
Diaphragm	141±22	360±49**	Abdominal m.	28± 3	33± 1

R_g values are expressed as nmol glucose/g/min, means ± SE, n=6. Rats were infused with saline or TNF, then injected with 2-deoxyglucose tracer. R_g was calculated from the concentration of the phosphorylated metabolites of 2-deoxyglucose accumulating in each tissue (see Materials and Methods). * and ** indicate significant difference as compared to the control group, $p<0.05$ and $p<0.005$, respectively.

with earlier data obtained by various methods (11,12), and it did not change after TNF infusion (Table 1). The measured values for the accumulation of phosphorylated metabolites of 2-deoxyglucose in the brains of control and TNF-infused rats were 185,000±13,000 and 147,000±3,000 dpm/g wet tissue, respectively (mean±SE, n=6). This difference (21%, $p<0.05$) was due to the higher plasma glucose concentration and faster decay of the label in the blood of TNF-treated rats (Fig.1), resulting in a lower integrated tracer/glucose ratio (424±12 dpm/nmol vs. the control value of 544±36 dpm/nmol; $p<0.05$). Hence the calculated glucose utilization rates for the brains of control and TNF-treated rats (677 vs. 694 nmol/g/min) did not differ significantly (Table 1).

The 2-deoxyglucose technique has also been applied successfully to gain information on glucose uptake by other tissues (5,6). Table 1 shows that after TNF infusion the glucose metabolic rate was enhanced by 80-100% in the spleen, liver and kidney. In skin Rg was elevated by 62%. The elevation was somewhat smaller in the lung and ileum (about 40%). The largest increase was seen in the diaphragm (150%). No significant change was observed in (whole) blood, various skeletal muscles, testis and brain.

The effect of TNF infusion on the glucose utilization rate of different tissues shows some resemblance to the pattern observed 3h after the administration of 100 µg/100 g endotoxin (6), though in the present experiments the changes were more moderate, and they affected a smaller number of organs. Under both experimental

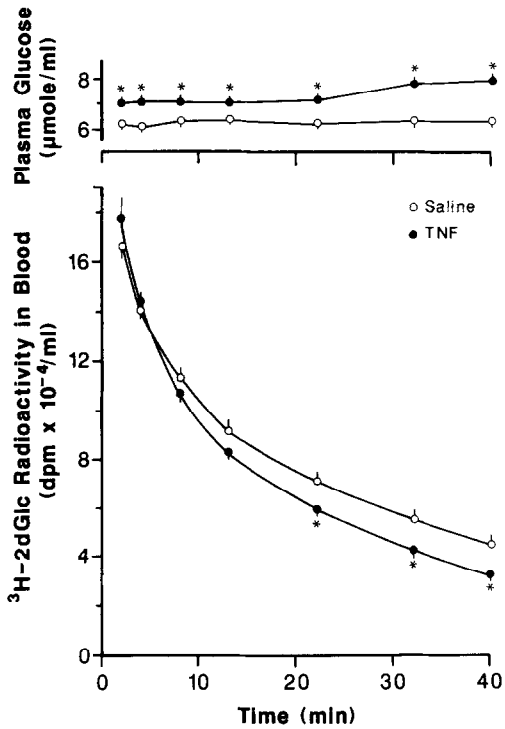

Fig. 1. Plasma glucose concentration and the disapppearance of radioactive 2-deoxyglucose (^{3}H-2dGlc) from the blood. After 3h of infusion with TNF or saline, the tracer was injected as a bolus, and serial blood samples were withdrawn. Symbols indicate means ± SE, n=6. *, difference from control satatistically significant, p<0,05.

circumstances a pronounced increase was found in macrophage-rich tissues like the spleen, liver, kidney, lung and skin. While different skeletal muscles exhibited a significant increase after endotoxin (5), no such change was observed after TNF in the present experiments, except in the diaphragm. Brain and testis (protected by blood-tissue barriers) were not affected by either of these treatments.

The glucose utilization rates of whole organs and organ systems are shown in Fig. 2. We calculated that the total glucose utilization by these organs (comprising 81% of the whole body mass) increased by 28% after TNF infusion. This compared well with the 24% increase in whole-body glucose turnover, determined previously by the infusion of 6-[^{3}H]glucose after a similar treatment with TNF (4). In Fig. 2, the numbers in parentheses indicate the percent contribution of individual organs to the total increase. The magnitude of their contribution depends on the increase of R_g as well as on the size of the particular organ.

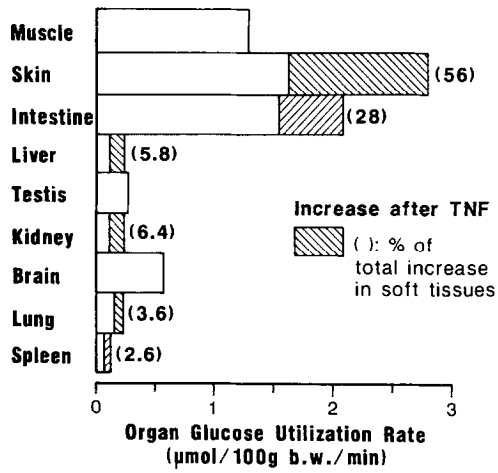

Fig. 2, Glucose utilization rates of different organs of the rat 3h after infusion with saline or TNF. Open bar indicates glucose uptake by the respective organ in control animals. Open bar plus hatched bar represents glucose uptake in TNF-treated rats. Number in parentheses indicates the contribution (%) of the respective organ to the total increase after TNF (which is the difference between the sums of glucose utilization in the soft tissues of TNF-treated and control rats). Estimations were based on organ weights (6) and R_g (Table 1). The gastrocnemius muscle, containing a mixed-type fiber population, was assumed to represent the total muscle mass (5).

Thus, the contribution of spleen, liver and kidney to the total increase was modest, although their metabolic rates increased most markedly. In turn, two large tissues, i.e. the skin and ileum (26% and 4.8% of body weight, respectively; ref. 6) were responsible for more than 80% of the increase in total glucose utilization after TNF infusion. A pattern similar to that shown in Fig. 2 has been found previously in endotoxicosis with the difference that skeletal muscle also contributed significantly to the total increase (5,6).

To investigate whether TNF could directly stimulate glucose metabolism we performed additional in vitro experiments, since it has been reported that TNF enhanced the glucose uptake of cultured L6 myotubes (14). We were unable to detect any significant effect of TNF on the glucose utilization of the isolated epitrochlearis muscle, spleen cells or alveolar macrophages under the present experimental conditions (Table 2). The reason for the difference between the in vivo and in vitro findings is not clear at this time; however, other hormones and mediators elicited by TNF are likely to be involved in vivo.

In conclusion, TNF administered in vivo induced a marked increase in the glucose utilization of several tissues. The tissue

Table 2. In vitro glucose utilization in the presence of TNF

	Unit	Control	TNF
Epitrochlearis muscle	nmol/g/min		
without insulin		48 ± 4	51 ± 5
200 μU/ml insulin		69 ± 5	65 ± 4
Spleen cells	nmol/10^7cells/h	44 ± 2	43 ± 2
Alveolar macrophages	nmol/10^6cells/h	37 ± 3	39 ± 1

Values are means ± SE, n=6. The incubation media (cf. Methods) contained 5 mM glucose, and 1 μg/ml TNF as indicated. After 150 min, 2-[^{3}H]glucose (0.2 μCi/ml) was added, and the incubation was continued for 60 min. Glucose utilization during the last hour was determined by measuring the amount of ^3H_2O formed.

pattern resembled that observed previously after endotoxin administration (5,6), with the notable difference that skeletal muscle did not respond to TNF under these experimental conditions. Though its precise mechanism of action awaits further elucidation, a role of TNF as a mediator of the metabolic changes in endotoxicosis and possibly sepsis is consistent with the present results.

ACKNOWLEDGMENTS: The excellent technical assistance of June T. Bechtel is gratefully acknowledged. This work was supported by National Institutes of Health Grant GM-32654.

REFERENCES

1. Beutler, B. and Cerami, A. (1986) Nature, 320, 584-588.
2. Beutler, B., Mahoney, J., Le Trang, N., Pekala, P. and Cerami, A. (1985) J. Exp. Med. 161, 984-995.
3. Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, I.W., Hariri, R.J., Fahey, T.J., III, Zentella, A., Albert, J.D., Shires, G.T. and Cerami, A. (1986) Science, 234, 470-474.
4. Bagby, G.J., Lang, C.H., Wilson, L.A., Thompson, J.J. and Spitzer, J.J. (1987) Fed. Proc. 46, p.790. (Abstract)
5. Mészáros, K., Bagby, G.J., Lang, C.H. and Spitzer, J.J. (1987) Am. J. Physiol. 253, 33-39.
6. Mészáros, K., Bagby, G.J., Lang, C.H. and Spitzer, J.J. (1987) J. Biol. Chem. 262, 10965-10970.
7. Patlak, C.S. (1981) J. Cereb. Blood Flow Metab. 1, 129-131.
8. Savaki, H.E., Davidsen, L., Smith, C. and Sokoloff, L. (1980) J. Neurochem. 35, 495-502.
9. Pettigrew, K.D., Sokoloff, S. and Patlak, C.S. (1983) J. Cereb. Blood Flow Metab. Vol.3, Suppl. 1, 89-90.
10. Brain, J.D. and Frank, N.D. (1968) J. Appl. Physiol. 25, 63-69.
11. Siesjo, B. (1978) Brain Energy Metabolism, pp. 107-109, John Wiley and Sons, New York.
13. Nelson, T., Lucignani, G., Goochee, J., Crane, M. and Sokoloff, L. (1986) J. Neurochem. 46, 905-919.
14. Lee, M.D., Zentella, A., Pekala, P.H. and Cerami, A. (1987) Proc. Natl. Acad. Sci. USA, 84, 2590-2594.