Volume 269, number 2, 421-424 FEBS 08811 September 1990

Interleukin-1 stimulates glucose transport in rat adipose cells

Evidence for receptor discrimination between IL-1β and IL-1α

Adrienne Garcia-Welsh, Joanna S. Schneiderman and Deborah L. Baly

Department of Nutrition, Rutgers University, New Brunswick, NJ, USA

Received 3 May 1990

The effect of interleukin 1 (IL-1) on glucose transport activity in isolated rat adipose cells was examined. IL-1β stimulated 3-*O*-methylglucose (3*O*MG) transport in a time and dose dependent manner. This effect appears to be due to increased maximal transport velocity (V_{max}) of the carrier. Addition of insulin and IL-1β resulted in an additive stimulation of transport, suggesting different mechanisms. IL-1α had no effect on glucose transport. Glu-4, a relatively inactive IL-1β analogue in most cells, stimulated glucose uptake in a time and dose dependent manner with kinetics indistinguishable from those of IL-1β.

Interleukin 1-β; Interleukin 1-β receptor; Glucose transport; Cytokine; Cachexia

1. INTRODUCTION

Cytokines play a central role in the host's response to infection. In addition to direct effects on cells of the immune system, they induce a variety of metabolic changes, including anorexia, loss of body protein and fat, changes in blood leukocyte patterns and hepatic secretory protein synthesis, trace mineral redistribution and other variations in carbohydrate and fat utilization [1]. While generally recognized as promoting host recovery, if prolonged, these host alterations may lead to severe tissue wasting and organ failure. A better understanding of the cellular effects of cytokines will provide insight into the sequence of cytokine actions that occur during the host response to infection.

Interleukin 1 (IL-1), one of several monocyte derived polypeptide hormones, is a primary mediator of the host's response to infection [2]. IL-1 induces fever, slow wave sleep, the synthesis of hepatic acute phase proteins, and the release of neutrophils, ACTH, cortisol, and insulin [1,3-6]. Cachexia, characterized by tissue wasting and negative nitrogen balance, often accompanies chronic infections including sepsis and inflammatory disease [7]. IL-1 contributes to this process at several levels, including the inhibition of lipoprotein lipase activity and synthesis [8], decreasing utilization of fat for energy. Cachexia also results in alterations in glucose homeostasis, manifested by a severe hypoglycemia [9]. Studies examining IL-1's effects on glucose metabolism are difficult to evaluate as they are either done in vivo [10-12], where the influence of other cytokines may affect the response, or use crude monokine preparations, where the contribution of IL-1 to the response may be modified [13-15].

The studies described in this communication were designed to determine the effects of IL-1 on glucose transport in isolated rat adipose cells. We present evidence that IL-1β stimulates glucose transport in intact adipose cells in a time and dose dependent manner, and demonstrate differential effects of IL-1β and IL-1α as well as Glu-4, and IL-1 analogue. Elucidation of the mechanism of action of IL-1 on glucose transport should provide further insight into the regulation of the glucose carrier.

2. EXPERIMENTAL

2.1. *Materials*

IL-1α and β, as well as the IL-1 analogue, Glu-4, were gifts from Dr. R.C. Newton from Du Pont De Nemours & Co. Crude collagenase (Type I) was purchased from Worthington (Freehold, NJ), and bovine serine albumin, fraction V, was purchased from Armour Pharmaceutical Co (Kankakee, IL). All other reagent grade biochemicals were from Sigma (St Louis, MO).

2.2. *Animals and cell preparation*

Male Sprague-Dawley rats (160-180 g) were obtained from Taconic Farms (Germantown, NY). Animals were fed laboratory chow (Ralston Purina, St. Louis, MO) ad libitum and were killed by CO_2 asphyxiation. Isolated adipocytes were obtained by collagenase digestion of the epididymal fat pad as described by Rodbell [16] and modified by Cushman and Wardzala [17]. All incubations were carried out at 37°C in a KRBH buffer, pH 7.4, containing 10 mM sodium bicarbonate, 30 mM Hepes, 5% bovine serum albumin. The exact experimental conditions are described in the figure legends. Briefly,

Correspondence address: D.L. Baly, School of Osteopathic Medicine, University of Medicine and Dentistry - New Jersey, 401 Haddon Ave., Camden, NJ 08103-1505, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

adipose cells were incubated for various lengths of time under basal conditions (no hormone added) or in the presence of either 7 nM insulin or various concentrations of IL-1β, IL-1α, or Glu-4 and 3-*O*-methylglucose uptake measured. In the studies using cycloheximide, isolated adipose cells were incubated with or without cycloheximide (10 μg/ml) for 30 min followed by an additional 2 1/2 h in the presence of 7 nM IL-1. A concentration of 10 μg/ml was chosen as this concentration was found to inhibit [^{3}H]leucine incorporation into protein 90% [18].

2.3. *Measurement of glucose transport activity*

In all experiments, glucose transport was determined by measuring the rapid uptake of ^{14}C-3-*O*-(methyl)D-glucose as described by Gliemann and Whitesell [19] and modified by Foley et al. [20]. Transport under basal and maximally stimulating insulin concentrations (7 nM) was measured routinely following a 30 min incubation period, and at the end of longer incubation times, to demonstrate normal insulin sensitivity.

3. RESULTS AND DISCUSSION

Fig. 1A shows the time course of IL-1β stimulation of 30MG uptake in isolated rat adipose cells. The maximum response, a 4-fold increase above the rate of basal transport, is achieved after 180 min, after a half time of 75 min. This slow time course of glucose transport activation contrasts strongly with the rapid activation of transport observed with insulin (half time of 2.5 min) [21]. Interestingly, the ability of IL-1β to stimulate 30MG uptake in adipose cells is more rapid than that previously reported in L-6 muscle cells [14]. However, this probably reflects the fact that the L-6 cells were treated with a crude monokine preparation which contained an unknown quantity of factors. Fig. 1B shows that IL-1β stimulates glucose transport in intact adipose cells in a dose dependent manner. The maximum response, a 4-fold increase above the basal rate of transport, required an IL-1β concentration of 7 nM. Half maximal stimulation of transport by IL-1β occurred at 0.2 nM which is a physiologically relevant dose [2,3].

The mechanism of action on IL-1β on glucose transport could be due to either an increase in the maximal transport velocity (V_{max}) or an increase in the affinity (K_m) of the glucose transporter by the cytokine. In order to distinguish between these two possibilities glucose uptake was measured at varying substrate concentrations. The kinetic data from these experiments, summarized in Table I, demonstrate that the effects of IL-1β on glucose transport are due to an increase in the maximal transport velocity. Interleukin 1 had no effect on the K_m for glucose uptake, suggesting a mechanism of action distinct from that of insulin which showed changes in both the K_m and V_{max}.

The increased V_{max} in IL-1 treated cells can be accounted for by either (i) recruitment of carriers from an intracellular pool to the plasma membrane, (ii) an absolute increase in the number of glucose carriers via an increase in de novo protein synthesis, or (iii) changes in the intrinsic activity of the carrier [18]. Given the relatively slow time course of IL-1 action on glucose transport, the involvement of de novo synthesis seems likely and we tested this by incubating adipose cells in the presence of saturating concentrations of IL-1β (7 nM) with or without cycloheximide (10 μg/ml). Cycloheximide treatment blocked the ability of IL-1β to stimulate glucose transport by 40%. 3-*O*-methylglucose uptake in cycloheximide-treated cells averaged 0.196 ± 0.026 fmol/cell/min compared to 0.313 ± 0.051 fmol/cell/min in cells incubated in the absence of cycloheximide.

The partial inhibition of IL-1β stimulation of glucose transport by cycloheximide suggests that the cytokine stimulates glucose transport activity via an increase in de novo protein synthesis. Further evidence in favor of this notion comes from experiments in which insulin and IL-1 were used to stimulate transport activity either alone or together. As can be seen in Table II, insulin increased glucose transport activity 34-fold above basal

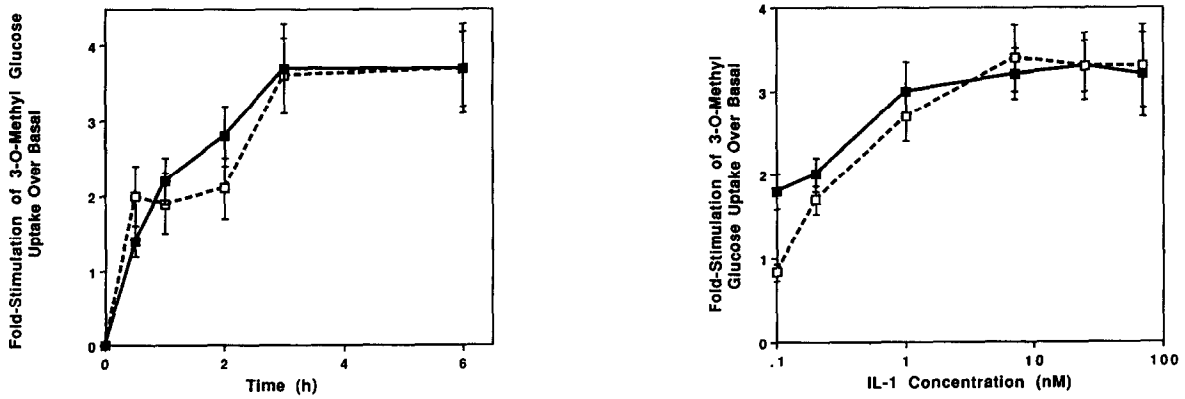

Fig. 1. Time course and concentration dependency of IL-1β and Glu-4 to stimulate Glucose Transport. (A) Isolated adipose cells were incubated in the presence of either 7 nM IL-1β [■] or Glu-4 [□] for the times indicated. (B) Isolated adipose cells were incubated for 3 h in the presence of varying concentrations (0–80 nM) of either IL-1β [■]or Glu-4 (□). 3-*O*-Methyl glucose uptake was measured as described in section 2. The values represent the fold stimulation of glucose uptake over basal levels by both IL-1β and Glu-4 from four separate experiments. Zero on the *y*-axis represents uptake under basal conditions (i.e. no stimulation). In these experiments basal and insulin stimulated 3-*O*-methyl glucose uptake was 0.100 ± 0.005 and 2.99 ± 0.310 respectively, after 30 min and 0.105 ± 0.005 and 3.86 ± 0.710 after 3 h.

Table I

Kinetic analysis of IL-1 stimulated glucose transport

	V_{max} (fmol glucose/cell/min)	K_m (mM)
Basal	7.1 ± 1.2[a]	8.8 ± 0.6[a]
IL-1	33.0 ± 11.0[b]	12.2 ± 2.6[a]
Insulin	206.0 ± 17.0[c]	3.4 ± 0.5[b]

Values represent mean ± SE from 5 separate experiments. Within a column, values with different superscripts are significantly different at the $P < 0.05$ level. Isolated adipose cells were incubated with varying concentrations of glucose (0–40 mM) under basal conditions (no additions) or in the presence of either 7 nM IL-1β or insulin. The uptake of 3-*O*-methyl glucose was determined as described in section 2. The uptake data from these experiments was analyzed by a Woolfe-Augustinsson plot (V vs V/S) [35].

Table II

Effects of insulin and IL-1β on glucose transport activity in isolated rat adipose cells

Conditions	3-*O*-Methyl glucose uptake (fmol/cell/min)	(fold stimulation)
Basal	0.10 ± 0.02	-
Insulin	3.20 ± 0.50	34.0 ± 4.0
IL-1β	0.38 ± 0.10	3.9 ± 0.2
Insulin + IL-1β	3.98 ± 0.40	40.0 ± 4.0

Values represent mean ± SE from 4 separate experiments. Fold stimulation represents the rate of glucose uptake in the presence of hormone divided by the rate of glucose uptake in the absence of hormone (basal). Isolated adipose cells were incubated under basal conditions (no treatment), and in the presence of 7 nM insulin and 7 nM IL-1β, either separately or in combination, for 3 h. 3-*O*-Methyl glucose uptake was measured at the end of the incubation period as described in section 2.

levels, IL-1β increased transport by 4-fold, and the two hormones acting together increased glucose transport activity 40-fold above basal levels. Our interpretation of these findings is that IL-1 is stimulating transport activity by increasing de novo protein synthesis while insulin, which does not affect transporter synthesis, increases transporter activity by translocating carriers from an intracellular location to the plasma membrane [17]. Thus, the effects of the two hormones, which stimulate glucose transport activity by different mechanisms, are additive.

Interestingly, when adipose cells, incubated in the presence and absence of 7 nM IL-1β, were assayed for transporter numbers by the D-glucose inhibitable cytochalasin B binding assay [17], we did not see any difference in carrier numbers between untreated and treated cells, 7 ± 2 compared to 8 ± 2 pmol/mg protein, respectively. In the same experiments, plasma membranes from insulin-treated cells had an increased number of glucose carriers, averaging 25 ± 5 pmol/mg of protein. These data suggest that the mechanism of IL-1β action on glucose transport does not involve the translocation of glucose carriers as has been shown for insulin. However, since IL-1β stimulates glucose uptake only 4-fold above basal levels, we anticipate this would result in only small changes in the distribution of glucose transporters which may not be detected given the insensitivity of the cytochalasin B binding assay.

Given the finding that IL-1β stimulates glucose uptake in adipose cells, we wondered whether Il-1α would have a similar effect. In most cells and tissues, IL-1α and IL-1β share similar biological properties despite having only a 25% sequence homology [2,3]. In contrast to the effects of IL-1β on glucose transport activity, IL-1α had no effect, even at a concentration (70 nM) ten times greater than that of IL-1β (Table III). It is unlikely that these data can be explained on the basis of variation in the quality of the cytokine preparations since both IL-1α and IL-1β were equipotent in their ability to stimulate thymocyte proliferation in the comitogenic assay for IL-1 bioactivity (data not shown) [22]. In line with our findings, several recent reports have suggested that IL-1α and IL-1β do not always share the same profile of biological activities [23–25]. Furthermore, it is now well recognized that there are at least two classes of IL-1 receptor; p80, an 80 kDa single chain protein found in T-cells, fibroblasts and many other cell types, and p68, a 68 kDa protein expressed in B-cells and macrophages [26–28]. The two classes of IL-1 receptor show distinct differences in their substrate binding site and molecular properties. Although we have not formally provided evidence of IL-1 receptors on adipose cells, the time and dose dependent nature of IL-1's ability to stimulate glucose transport suggests that the effect is most likely mediated via binding to a cell surface IL-1 receptor. Consistent with this idea, Katsuura et al. [29] have demonstrated that purified IL-1β receptor from rat brain does not recognize IL-1α.

Further evidence in support of the idea that the disparate effects of Il-1α and IL-1β on glucose transport are IL-1 receptor mediated comes from experiments with an IL-1β analog, Glu-4. This analog of

Table III

Effect of IL-1α on glucose transport activity in isolated rat adipose cells

Conditions	Concentration (nM)	3-*O*-Methyl glucose uptake (fmol/cell/min)
Basal	-	0.106 ± 0.010
IL-1β	7	0.337 ± 0.071
IL-1α	7	0.131 ± 0.030
IL-1α	70	0.112 ± 0.0651
IL-1α	110	0.254 ± 0.029

Values represent mean ± SE from 4 separate experiments. Isolated adipose cells were incubated under basal conditions (no treatment), in the presence of 7 nM IL-1β or various concentrations of IL-1α for 3 h. 3-*O*-Methyl glucose uptake was measured at the end of the incubation period. Insulin stimulated uptake at the end of the incubation was 3.39 ± 0.620 fmol/cell/min.

IL-1β has a substitution at residue four at the N-terminal end from Arg to Glu [30]. Glu-4 has been shown to have only 0.2% of the biological activity of IL-1β in EL-4 cells (p80 receptor) but 30% binding to Raji cells (p68 receptor) [26,30]. As can be seen in Fig. 1, Glu-4 stimulates glucose transport in a time and dose dependent manner. Indeed, the time course of glucose transport stimulation by 7 nM Glu-4 is identical to that of IL-1β. The data suggest that Glu-4 is equipotent with IL-1β and is a full agonist.

In conclusion, our studies demonstrate that IL-1β but not IL-1α can stimulate glucose transport in rat adipose cells. An Il-1β analogue, Glu-4, inactive in most cells, was able to fully stimulate glucose transport. Our interpretation of these data are that the differences in the ability of Il-1α and Il-1β to stimulate glucose transport are probably due to differences in their ability to bind to the adipose cell IL-1 receptor. Furthermore based on the substrate profile of the adipose cell IL-1 receptor, we speculate that it is most likely the p68 type, since this receptor discriminates between IL-1α and IL-1β and shows reasonable binding kinetics for Glu-4 which is inactive on p80 receptors [26,30]. The ability of IL-1β to stimulate glucose transport together with its ability to suppress lipoprotein lipase activity [8] suggest that this cytokine may play a critical role in regulating fuel homeostasis during chronic disease.

Acknowledgements: This work was supported by State funds and by a grant from the Juvenile Diabetes Foundation.

REFERENCES

[1] Beutler, B. and Cerami, A. (1988) Annu. Rev. Biochem. 58, 505.
[2] Dinarello, C.A. and Mier, J.W. (1986) Annu. Rev. Med. 37, 173-178.
[3] Dinarello, C.A. (1988) FASEB J. 2, 108-115.
[4] Uehara, A., Gottschall, P.E., Dahl, R.R. and Arimura, A. (1987) Endocrinology 121, 1580-1582.
[5] Besedovsky, H., Del Rey, A., Sorkin, E., Dinarello, C.A. (1986) Science 233, 652-654.
[6] Zawalich, W.S., Zawalich, K.C. (1989) Am. J. Physiol. 256, E19-E24.
[7] Beutler, B. and Cerami, A. (1988) Adv. Immunol. 42, 213-216.
[8] Beutler, B. and Cerami, A. (1985) J. Immunol. 135, 3969-3974.
[9] Filkins, J.P. (1984) in The Reticuloendothelial System: A Comprehensive Treatise, (Richard S.M. and Filkens J.P. eds) Plenum, New York, pp. 7-291.
[10] Westfall, M.V. and Sayeed, M.M. (1987) Am. J. Physiol. 253, R55-R63.
[11] Filkens, J.P. (1978) Circul. Shock 5, 347-355.
[12] Meszaros, K., Bagby, G.J., Lang, C.H., Spitzer, J.J. (1987) Am. J. Physiol. 253, E33-E39.
[13] Kawakami, M., Pekala, P.H., Lane, M.D., Cerami, A. Proc. Natl. Acad. Sci. USA 79, 912-916.
[14] Lee, M.D., Zentella, A., Pekala, P.H. and Cerami, A. (1987) Proc. Natl. Acad. Sci. 84, 2590-2594.
[15] Cornelius, P., Lee, L., Marlowe, M. and Pekala, P.H. (1989) Biochem. Biophys. Res. Comm. 165, 429-436.
[16] Rodbell, M. (1975) J. Biol. Chem. 250, 5826-5834.
[17] Cushman, S.W. and Wardzala, L.J. (1980) J. Biol. Chem. 255, 4758-4762.
[18] Baly, D.L. and Horuk, R. (1987) J. Biol. Chem. 262, 21-24.
[19] Gliemann, J. and Whitesell, R.R. (1977) Diabetologia 13, 396.
[20] Foley, J.E., Cushman, S.W. and Salans, L.B. (1980) Am. J. Physiol. 238, E180-E185.
[21] Simpson, I.A. and Cushman, S.W. (1986) Annu. Rev. Biochem. 55, 1059-1089.
[22] Gery, L., Gershon, R.K. and Waksmann, B.H. (1972) J. Exp. Med. 136, 128-132.
[23] Gottschall, P.E., Katsuura, G. and Akimura, A. (1989) Biochem. Biophys. Res. Commun. 163, 764-770.
[24] Uehara, A., Gottschall, P.E., Dahl, R.K. and Arimura, A. (1987) Biochem. Biophys. Res. Commun. 146, 1286-1290.
[25] Busbridge, N.J., Dascombe, M.J., Tilders, F.J.H., van Oers, J.W.A.M., Linton, E.A. and Rothwell, N.J (1989) Biochem. Biophys. Res. Commun. 162, 591-596.
[26] Horuk, R., Huang, J.J., Covington, M. and Newton, R.C. (1987) J. Biol. Chem. 262, 16275-16278.
[27] Bomsztyk, K., Sims, J.E., Stanton, T.H., Slack, J., McMahan, C.J., Valentine, M.A. and Dower, S.K. (1989) Proc. Natl. Acad. Sci. USA 86, 8034-8038.
[28] Chizzonite, R., Truitt, T., Kilian, P.L., Stern, A.S., Nunes, P., Parker, K.P., Kaffka, K.L., Chua, A.O., Lugg, D.L. and Gubler, U. (1989) Proc. Natl. Acad. Sci. USA 86, 8029-8033.
[29] Katsuura, P.E., Gottschall, P.E. and Akira, A. (1988) Biochem. Biophys. Res. Comm. 156, 61-67.
[30] Huang, J.J., Newton, R.C., Horuk, R., Matthew, J.B., Covington, M., Pezzella, K. and Lin, Y. (1987) FEBS Lett. 223, 294-298.