0026-895X/95/030550-09$3.00/0

MOLECULAR PHARMACOLOGY, 48:550–558 (1995).

Tumor Necrosis Factor-α and Interleukin-1α Enhance Glucose Utilization by Astrocytes: Involvement of Phospholipase A_2

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Received March 1, 1995; Accepted June 16, 1995

SUMMARY

Cytokines can be produced within the nervous system by various cell types, including astrocytes, which secrete them in response to pathological processes such as viral infections. Astrocytes are known to play an important role in the homeostasis of the nervous system, in particular, by contributing to the regulation of local energy metabolism. We report that tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α) markedly stimulate glucose uptake and phosphorylation in primary cultures of neonatal murine astrocytes, as determined with [^{3}H]-2-deoxyglucose ([^{3}H]2DG). This effect is both concentration dependent, with observed EC_{50} values of 8 ng/ml for TNF-α and 30 pg/ml for IL-1α, and time dependent, with a maximal response observed 24 hr after cytokine application. The effects of TNF-α and IL-1α on glucose uptake and phosphorylation appear to be mediated by the phospholipase A_2 signal transduction pathway. Evidence in support of this includes (i) inhibition by mepacrine, a phospholipase A_2 inhibitor, of [^{3}H]2DG uptake evoked by TNF-α and IL-1α, and (ii) stimulation of [^{3}H]arachidonic acid release by TNF-α and IL-1α. Protein kinase C activation does not appear to be involved as the specific protein kinase C inhibitor Ro 31–7549 does not abolish TNF-α- or IL-1α-induced increase in [^{3}H]2DG uptake and phosphorylation. The additional glucose imported by astrocytes on exposure to TNF-α and IL-1α is neither stored as glycogen nor released as glycolytically derived lactate, suggesting that it is processed through the tricarboxylic acid cycle or pentose phosphate pathway. These results demonstrate that TNF-α and IL-1α can fundamentally perturb the energy metabolism of astrocytes, possibly impairing their ability to provide adequate energy substrates for neurons.

A number of cytokines, including TNF (-α and -β), IL-1 and IL-6, and their cognate receptors, have been identified in the CNS (1–4). TNF-α, a cytokine with pleiotropic effects, is released from macrophages after a variety of stimuli and is cytotoxic to many cell types (5). In the CNS, microglia produce TNF-α on stimulation with LPS (1). Cultured astrocytes also generate TNF-α after stimulation with LPS or on exposure to neurotropic viruses (1, 6). High affinity binding sites for TNF-α on rat astrocytes have been identified (2).

TNF-α exerts several effects in the CNS. While serving as a mitogen for astrocytes (7), it exerts cytopathic effects on oligodendrocytes (8). The latter effect provides a possible explanation for white matter pathologies observed in HIV-infected patients. TNF-α and IL-1 can each stimulate the production of colony stimulating factor 1 by mouse astrocytes (9). Recently, in contrast to its previously described cytotoxic effects, TNF-α (as well as TNF-β) has been shown to protect neurons against glucose deprivation-induced injury and excitatory amino acid toxicity *in vitro* (10).

The major sources of IL-1α in the CNS are, as is the case for TNF-α, the invading macrophages and resident microglia (3). IL-1α is a mitogen for astrocytes and functions as an astroglial growth factor during neural development (11). Receptors for IL-1α have been localized on mouse astrocytes (4).

IL-6 is the common name given to a growth factor that had been characterized under a variety of aliases. IL-6 is a critical component of the acute-phase response and is synthesized by many cell types after stimulation with LPS or viral infection; *in vitro*, IL-6 is produced by both microglia and astrocytes (6). Although there is no direct evidence for the presence of IL-6 receptors in the CNS, IL-6 receptor mRNA has been detected in rat brain (12).

The existence of neuropsychiatric disturbances in AIDS patients has been well documented. Cognitive and visual dysfunction and deficits in motor sequencing have been de-

This work was supported by Grant MH47680 from the National Institute of Mental Health (F.E.B.).

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ABBREVIATIONS: TNF-α, tumor necrosis factor-α; IL-1α, interleukin-1α; IL-6, interleukin-6; AA, arachidonic acid; PKC, protein kinase C; PLA2, phospholipase A_2; 2DG, 2-deoxyglucose; LPS, lipopolysaccharide; AIDS, acquired immune deficiency syndrome; CNS, central nervous system; HIV, human immunodeficiency virus; VIP, vasoactive intestinal polypeptide; NA, norepinephrine; FCS, fetal calf serum; DMEM, Dulbecco's modified Eagle's medium; PDBu, phorbol-12,13-dibutyrate; PBS, phosphate-buffered saline.

scribed (13). Neuropathological postmortem analyses of brains of AIDS patients show a substantial loss of neurons in the cerebral cortex (14). Neurons, however, do not appear to be infected by the virus as viral mRNA or proteins have been detected only in nonneuronal cells, including microglia, astrocytes, and endothelial cells (15).

Various concepts have been considered to explain how HIV causes pathological changes in the CNS of AIDS patients, including HIV neurotropism, the ability of HIV to grow in endothelial cells and alter the properties of the blood-brain barrier, the toxic effects of viral proteins, autoimmune and other immunological disorders, and viral cofactors in the pathogenic pathway. Another proposed hypothesis is a neurotoxic effect of inappropriately expressed cytokines released from infected invading macrophages or resident microglia as a consequence of HIV-1 infection. Levels of IL-1β and IL-6 (16) as well as TNF-α (17) are increased in the cerebrospinal fluid of AIDS patients. Interestingly, HIV-1 has recently been shown to induce TNF-α and IL-1 in primary cultures of rat astrocytes and microglia (18). In addition to whole virus, exposure of the cultures to recombinant viral envelope proteins also induces the cytokines. Antibodies recognizing epitopes in the gp120 and gp41 moieties of the virus envelope blocked induction, stressing the specificity of the phenomenon.

Astrocytes play an important role in maintaining the neuronal microenvironment in the CNS. In addition to clearing the extracellular space of K^+, glutamate, or γ-aminobutyric acid, which accumulate as a consequence of neuronal activity, astrocytes participate in the regulation of brain energy metabolism. For example, glycogen, the largest energy reserve of the brain, is selectively localized in astrocytes, which also display a quantitatively significant uptake of glucose (19). Glycogen levels and glucose uptake by astrocytes are selectively regulated by certain neurotransmitters such as VIP, NA, adenosine, and glutamate (20–22). Thus, it is conceivable that neuropathological processes, such as HIV-1 infection, perturb the homeostatic functions of astrocytes, including those related to energy metabolism. This could lead to an impairment of neuronal vitality and may ultimately result in neurodegeneration.

Because of the foregoing and because of observations indicating the induction of TNF-α and IL-1 expression by HIV-1 in astrocytes (18), we wanted to determine whether these cytokines could affect the regulation of energy metabolism in astrocytes.

Materials and Methods

Reagents. Recombinant mouse cytokines (TNF-α, IL-1α, and IL-6) and antibodies (polyclonal rabbit anti-mouse TNF-α and monoclonal hamster anti-mouse IL-1α) were purchased from Genzyme (Cambridge, MA). Ro 31–7549, a synthetic specific inhibitor of PKC, was kindly provided by Dr. Michael H. Kroll (Veterans Affairs Medical Center, Baylor College of Medicine, and the Biomedical Engineering Laboratory, Rice University, Houston, TX) with the permission of Dr. Geoffery Lawton (Research Centre, Roche Products Ltd., Welwyn Garden City, Hertfordshire, UK). [^{3}H]2DG (specific activity, 14.9 Ci/mmol) and [^{3}H]AA (specific activity, 212 Ci/mmol) were obtained from Amersham. [^{3}H]Thymidine (specific activity, 20 Ci/mmol) was obtained from New England Nuclear. FCS was purchased from Gemini Bio-Products (Calabasas, CA). Enzymes for glycogen and lactate assays were purchased from Boehringer Mannheim (Indianapolis, IN). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). DMEM was obtained from The Scripps Research Institute Medium Center.

Cell culture. Primary cultures of cerebral cortical astrocytes were prepared from newborn C57BL/6JxSJL mice (1–2 days old) as previously described (19, 21). Briefly, forebrains were removed aseptically from the skulls, the meninges were excised carefully under a dissecting microscope, and the neocortex was dissected. The cells were dissociated by being passed through needles of decreasing gauge sizes (16 gauge, 19 gauge, and 25 gauge) 2 or 3 times with a 10-ml syringe. Trypsin was not used for tissue dissociation. The cells were seeded at a density of 10^5 cells/cm^2 on six-well plates in DMEM containing 10% FCS and 25 mM glucose in a final volume of 2 ml/well and incubated at 37° in an atmosphere containing 5% CO_2 at 95% humidity. The culture medium was renewed 3–4 days after seeding and subsequently twice per week. These conditions yielded astrocyte cultures containing >90% GFAP-immunoreactive cells (19) and <1% of MAC-1-positive (a microglial marker) cells as determined by flow cytometry (not shown).

[^{3}H]2DG uptake. Uptake experiments were conducted at cell confluence, reached after 20–25 days in culture, as previously described (19). Cytokines were dissolved in fresh culture medium and added as 1 ml/well. Twenty-four hours later (unless otherwise stated), the medium was removed, and cells were incubated for 4 hr in 2 ml of serum-free DMEM containing 5 mM instead of 25 mM glucose (DMEM5) at 37° in an atmosphere containing 5% CO_2 at 95% humidity. At the end of this 4-hr preincubation, the medium was replaced by 1 ml of DMEM5 containing [^{3}H]2DG (final concentration, 48 nM). The cells were incubated for additional 20 min. The uptake was terminated by washing the cells 3 times with 4 ml of ice-cold phosphate-buffered saline. Astrocytes were then lysed by the addition of 2 ml of 10 mM NaOH containing 0.1% Triton X-100, and a 500-μl aliquot was assayed for ^{3}H by liquid scintillation counting. The protein content was measured by the method of Bradford (23) in 100 μl of the remaining lysate. [^{3}H]2DG uptake was expressed in femtomoles/milligram of protein.

Glycogen assay. In place of the addition of [^{3}H]2DG as for uptake experiments, at the end of the 4-hr incubation in DMEM5, the cells were washed 3 times with ice-cold PBS, and 2 ml of 30 mM HCl was added. After a brief sonication, glycogen was determined enzymatically, as previously described (20). Briefly, three 100-μl aliquots were sampled. Three hundred microliters of acetate buffer (0.1 M, pH 4.65) were added to one 100-μl aliquot. Three hundred microliters of the acetate buffer containing 1% of amyloglucosidase (10 mg/ml) were added to the second aliquot. The mixtures were incubated at room temperature for 30 min. Then, we added 2 ml of Tris·HCl buffer (0.1 M, pH 8.1) containing 3.3 mM $MgCl_2$, 0.2 mM ATP, 25 μg/ml NADP, 4 μg/ml hexokinase, and 2 μg/ml glucose-6-phosphate dehydrogenase. The mixtures were incubated at room temperature for 30 min. The fluorescence of NADPH formed was read at 340/450 nm (excitation/emission) with a Model LS30 luminescence spectrometer (Perkin Elmer). The first aliquot provides the signal generated by glucose and glucose-6-phosphate, whereas the second corresponds to glycogen, glucose, and glucose-6-phosphate. The amount of glycogen is determined by the difference between the first and second aliquots. One mole of glycogen is equal to 1 mol of glycosyl unit originating from glycogen. The third 100-μl aliquot was used for protein determination. Glycogen content was expressed in nanomoles/milligram of protein.

Lactate measurement. Lactate released by astrocytes into the medium was assayed as described by Rosenberg and Rush (24). Briefly, 100-μl aliquots of the culture medium were mixed with 2 ml of a glycine buffer (0.2 M, pH 10) containing 2 mg/ml NAD and 12.5 μg/ml lactate dehydrogenase and incubated at 40° for 1 hr. After 20 min at room temperature, the amount of NADH produced was measured at 340 nm with a spectrophotometer (Perkin Elmer). Lactate content was expressed in micromoles/milligram of protein.

[^{3}H]AA release. [^{3}H]AA release was measured according to the method described by Tencé *et al.* (25). With this method, agonist-induced release of radioactivity in the extracellular medium corresponds to 1–2% of cellular ^{3}H content. Astrocyte cultures were treated with cytokines for 19 hr (unless otherwise stated) and charged with [^{3}H]AA (final concentration, 5 nM) in DMEM5 for 5 hr at 37°. Cultures were then washed 4 times at 37° with 2 ml of DMEM5 containing 1 mg/ml fatty acid-free bovine serum albumin and further incubated at 37° in 1 ml of the same medium for 15 min. At the end of the incubation, the medium was collected and centrifuged at 100 × *g* for 5 min. An aliquot of the supernatant (200 μl) was assayed for ^{3}H by liquid scintillation counting. [^{3}H]AA release was expressed in cpm/well.

Measurement of DNA synthesis with [^{3}H]thymidine incorporation. On the day of the experiment, the culture medium was replaced by fresh DMEM (without FCS). Cytokines were added for increasing periods of time, and [^{3}H]thymidine (final concentration, 10 μCi/ml) was added. Astrocytes were harvested 12 hr after the addition of [^{3}H]thymidine, and the incorporation of radioactivity was determined by counting samples on a beta plate. [^{3}H]Thymidine incorporation was expressed as cpm/well. Statistical analysis was performed with Student's *t* test.

Results

Application of TNF-α or IL-1α for 24 hr in cultured astrocytes increased [^{3}H]2DG uptake in a concentration-dependent manner, with an EC_{50} of 8 ng/ml for TNF-α and of 30 pg/ml for IL-1α (Fig. 1, A and B). The specificity of the effects of TNF-α and IL-1α was demonstrated by using specific antibodies raised against TNF-α and IL-1α. At the concentrations used, the antibodies tested alone had no influence on basal [^{3}H]2DG uptake, whereas they completely abolished the stimulating effects of the cytokines against which they were directed (Table 1). In contrast, IL-6 had a marginal stimulating effect on [^{3}H]2DG uptake (only at the highest concentration tested, 2 ng/ml) (Fig. 1C). In these experiments, cultures were exposed to the cytokines for 24 hr, followed by [^{3}H]2DG uptake measurement.

To determine the time course of the effects exerted by the cytokines on [^{3}H]2DG uptake, astrocytes were treated with maximally effective concentrations (20 ng/ml of TNF-α and 0.25 ng/ml of IL-1α) for different periods of time, followed by [^{3}H]2DG uptake determination. The stimulating effect of TNF-α on [^{3}H]2DG uptake began at 2–6 hr after application and reached maximal levels after 24 hr (Fig. 2A). A similar time course was observed for IL-1α (Fig. 2B). Cytokines had no effect when applied acutely (i.e., for 20 min). Under these conditions, [^{3}H]2DG uptake (fmol/mg protein) was 630 ± 34 for control, 680 ± 41 for TNF-α, 630 ± 17 for IL-1α, and 667 ± 18 for IL-6.

The time- and concentration-dependent effects of TNF-α and IL-1α in stimulating [^{3}H]2DG uptake were entirely protein synthesis dependent. As indicated in Fig. 3, concomitant application of 100 μM cycloheximide over 24 hr with the cytokines completely inhibited their effect on [^{3}H]2DG uptake. Cycloheximide alone decreased marginally basal [^{3}H]2DG uptake.

Previous reports have indicated that IL-1α (26) and TNF-α (27) can induce in certain cell types the expression of PLA2. Because we had previously shown that AA stimulates [^{3}H]2DG uptake by astrocytes (19), we wanted to determine whether the time- and concentration-dependent stimulations of [^{3}H]2DG uptake triggered by TNF-α and IL-1α could be

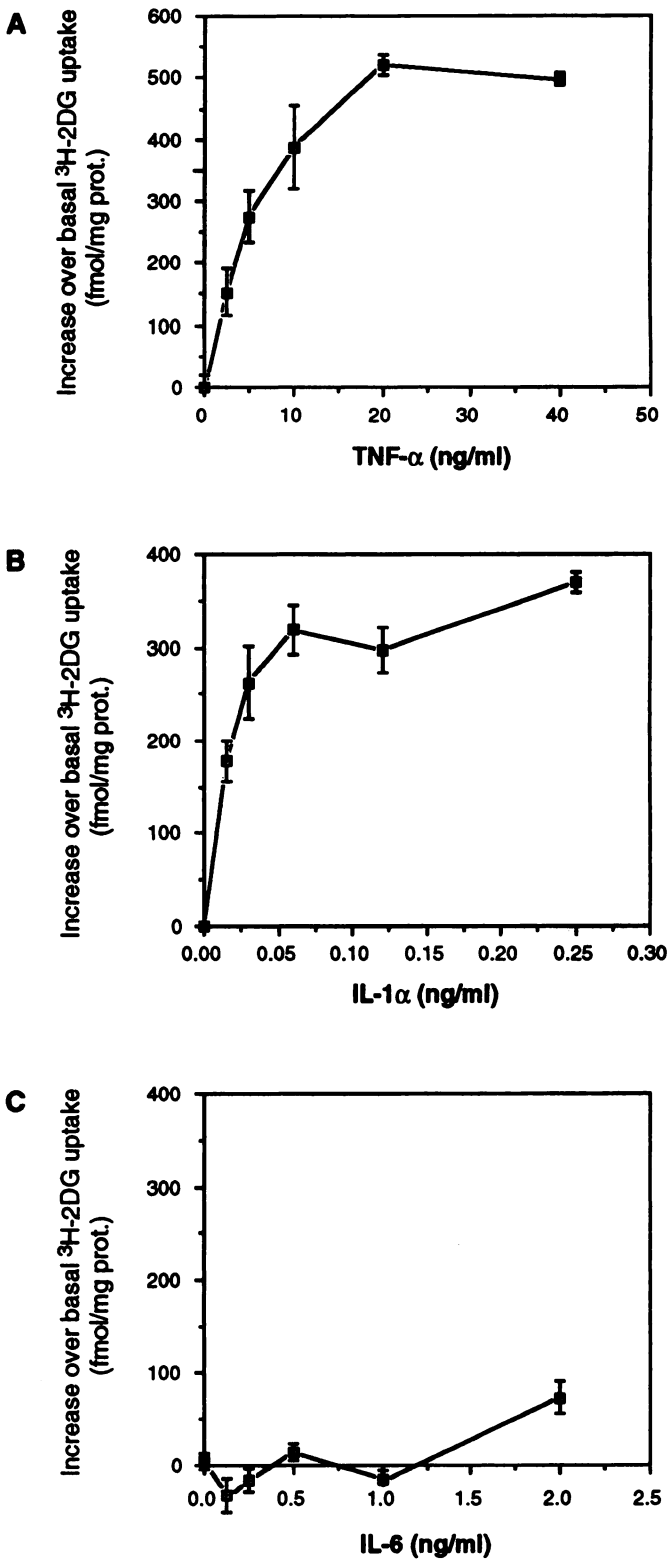

Fig. 1. Concentration-response curves of the effect of the cytokines TNF-α, IL-1α, and IL-6 on [^{3}H]2DG uptake by primary cultures of mouse cerebral cortical astrocytes. Astrocytes were incubated in the presence of increasing concentrations of TNF-α (A), IL-1α (B), and IL-6 (C) for 24 hr in DMEM. [^{3}H]2DG uptake was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. Results are expressed as absolute increases in femtomoles/milligram of protein over basal levels. Basal [^{3}H]2DG uptake was 299 ± 3 fmol/mg protein (A), 412 ± 11 fmol/mg protein (B), and 429 ± 14 fmol/mg protein (C).

TABLE 1

Neutralizing effect of antibodies to TNF-α or IL-1α on [^{3}H]2DG uptake

Antibodies were added 30 min before cytokines. After 24 hr, [^{3}H]2DG uptake was determined as described in Materials and Methods. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results.

Agents added	[^{3}H]2DG uptake
	fmol/mg protein
None	486 ± 37
TNF-α (20 ng/ml)	806 ± 61[a]
TNF-α (20 ng/ml) + anti-TNF-α antibody (10 μl/ml)	514 ± 56
Anti-TNF-α antibody (10 μl/ml)	511 ± 50
IL-1α (50 pg/ml)	650 ± 15[b]
IL-1α (50 pg/ml) + anti-IL-1α antibody (2 μg/ml)	455 ± 17
Anti-IL-1α antibody (2 μg/ml)	486 ± 16

[a] [^{3}H]2DG uptake significantly different from basal level ($p < 0.01$).
[b] [^{3}H]2DG uptake significantly different from basal level ($p < 0.05$).

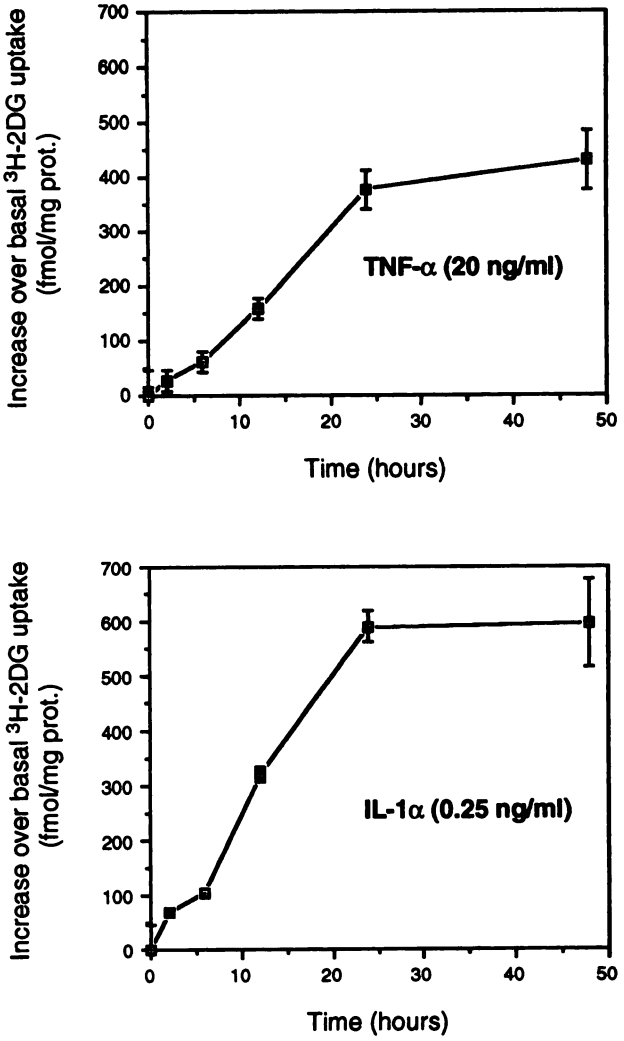

Fig. 2. Time course of cytokine-stimulated [^{3}H]2DG uptake by primary cultures of mouse cerebral cortical astrocytes. Astrocytes were incubated for various periods of time in the presence of 20 ng/ml TNF-α (A) and 1 ng/ml IL-1α (B). [^{3}H]2DG uptake was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. Results are expressed as absolute increases in femtomoles/milligram of protein over basal levels. Basal [^{3}H]2DG uptake was 433 ± 14 fmol/mg protein (A) and 429 ± 44 fmol/mg protein (B).

mediated by the induction of PLA2. As an index of such an induction, we determined the basal release of [^{3}H]AA, previously incorporated into membrane phospholipids, after a 24-hr exposure of the astrocytes to TNF-α and IL-1α. As shown in Fig. 4, basal [^{3}H]AA release from astrocytes was markedly increased after application of TNF-α and IL-1α for 24 hr. No effect was observed with IL-6 or when the cytokines were applied acutely for 15 min (data not shown). Further stressing the involvement of PLA2 induction in the effect of TNF-α and IL-1α on glucose metabolism is the inhibition elicited by mepacrine, a PLA2 inhibitor, on the cytokine-evoked [^{3}H]2DG uptake (Fig. 5). The inhibition was complete for TNF-α and partial for IL-1α .

To test whether other signal transduction pathways, in particular, PKC activation, might also be involved in the effect of the cytokines on glucose utilization, we performed a number of pharmacological manipulations that are known to inhibit PKC functionality. In initial experiments, we examined the effects of the cytokines in the presence of the nonspecific PKC inhibitor staurosporine at 100 nM. This nonspecific PKC inhibitor decreased the effect of TNF-α or IL-1α in stimulating [^{3}H]2DG uptake by astrocytes (data not shown). However, more specific manipulations failed to confirm an involvement of PKC. Thus, as shown in Fig. 6A, the specific PKC inhibitor Ro 31–7549 did not inhibit the stimulation of [^{3}H]2DG uptake evoked by TNF-α or IL-1α. In addition, preexposure of the cultures PDBu at a concentration of 1 μM, a manipulation known to down-regulate PKC activity, did not interfere with the effect of the cytokines on [^{3}H]2DG uptake (Fig. 6B). Neither Ro 31–7549 nor down-regulation of PKC with PDBu affected [^{3}H]AA release evoked by either TNF-α or IL-1α (data not shown).

Other cytokines produced by T cells were also screened for their capacity to modulate glucose utilization by astrocytes. As shown in Table 2, among the various cytokines tested, interferon-γ was the only one that promoted [^{3}H]2DG uptake. The exposure of astrocytes to LPS for 24 hr has been previously shown to induce the expression and release of certain cytokines, including TNF-α and IL-1α. This treatment markedly stimulated [^{3}H]2DG uptake (Table 2).

Another parameter in the energy metabolism of astrocytes, which is modulated by extracellular signals such as neurotransmitters, is the glycogen level. In particular, VIP and NA exert a biphasic effect on glycogen metabolism in astrocytes; within minutes of application, they promote glycogenolysis, followed between 3 and 9 hr later by a massive resynthesis that brings glycogen content to levels that are 6–10 times higher than levels before application of the neurotransmitters. This VIP- and NA-activated glycogen resynthesis process is protein synthesis dependent (21). As shown in Fig. 7A, glycogen levels increased moderately, by 10–20 nmol/mg protein, within 2 hr after medium replacement and returned to basal levels at 5–12 hr. A similar pattern was observed with TNF-α (Fig. 7B) and IL-1α (Fig. 7C), except that for TNF-α glycogen levels were significantly decreased after 12 hr compared with those measured before application of the cytokine. These results clearly indicate that cytokines do not stimulate glycogen synthesis in astrocytes. They also demonstrate that despite a marked stimulation of glucose uptake and phosphorylation, the exposure of astrocytes to TNF-α and IL-1α

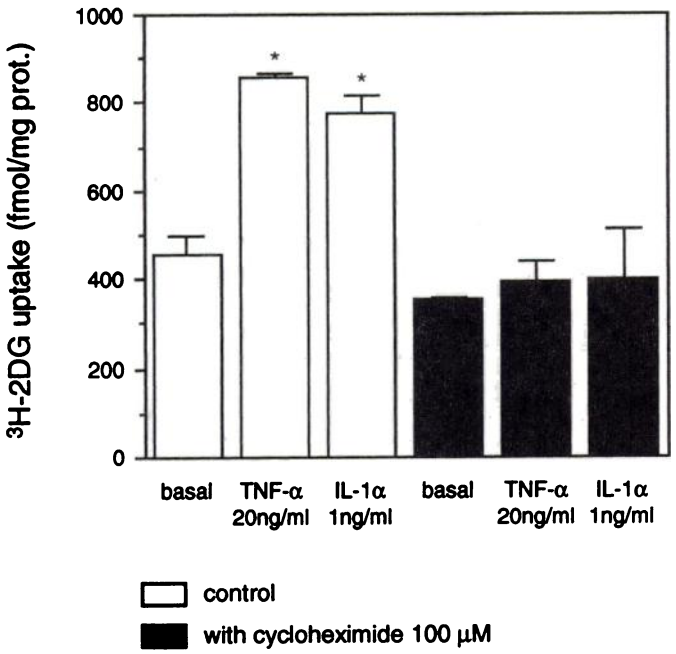

Fig. 3. Suppression by cycloheximide of the stimulation by TNF-α or IL-1α of [^{3}H]2DG uptake. Cycloheximide (100 μM) was added (■) 1 hr before the application of TNF-α or IL-1α and was maintained throughout the incubation. After 24 hr, [^{3}H]2DG uptake was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. *, [^{3}H]2DG uptake significantly different from corresponding basal level ($p < 0.01$)

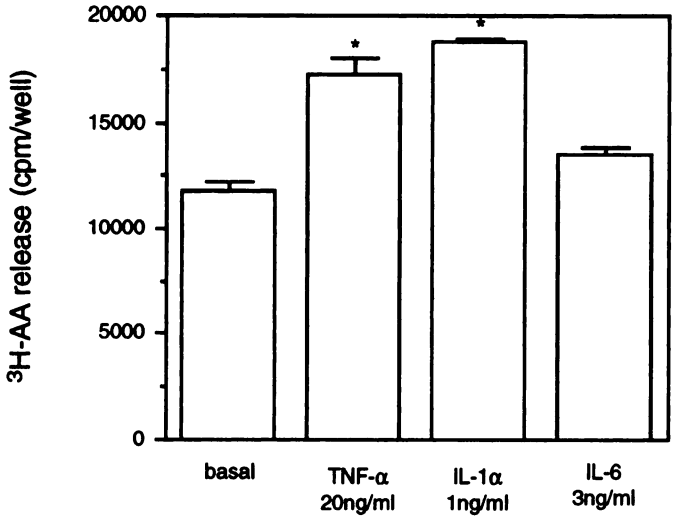

Fig. 4. Effects of cytokines on [^{3}H]AA release by astrocytes. Astrocytes were exposed for 24 hr to TNF-α (20 ng/ml), IL-1α (1 ng/ml), or IL-6 (3 ng/ml), the medium was changed, and [^{3}H]AA release was determined in the absence of cytokines for 15 min. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. *, [^{3}H]AA release significantly different from basal level ($p < 0.01$).

does not direct this metabolic substrate toward glycogen resynthesis.

Astrocytes have been shown to release considerable amounts of lactate in the extracellular space, despite the existence of an active oxidative phosphorylation in these cells (28). In this regard, astrocytes are similar to other cell types, such as erythrocytes and vascular smooth muscle cells, in

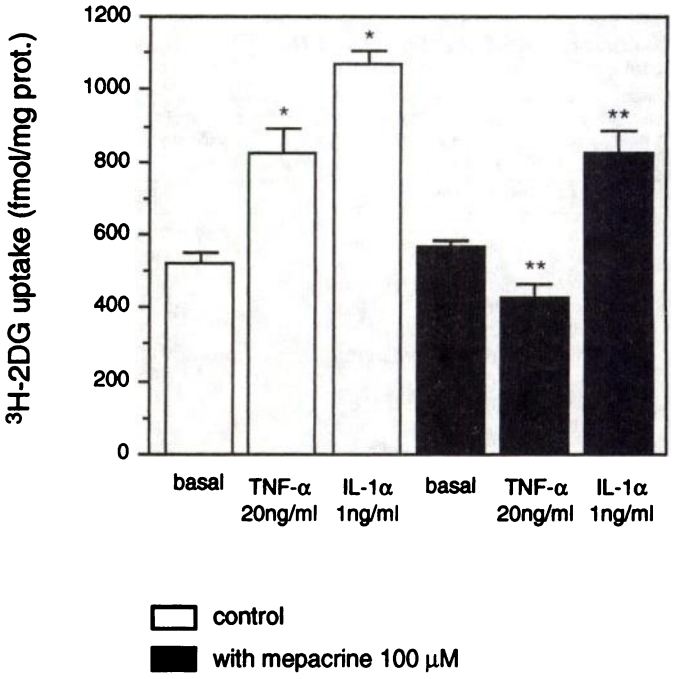

Fig. 5. Inhibition by mepacrine of [^{3}H]2DG uptake induced by TNF-α or IL-1α in astrocytes. After a 24-hr exposure to TNF-α (20 ng/ml) or IL-1α (1 ng/ml), [^{3}H]2DG uptake in the presence or absence of mepacrine (100 μM) was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. *, [^{3}H]2DG uptake significantly different from basal levels ($p < 0.01$); **, [^{3}H]2DG uptake significantly different from corresponding conditions in which mepacrine was absent ($p < 0.01$).

which aerobic glycolysis (leading to lactate production) coexists with oxidative phosphorylation. Because TNF-α and IL-1α markedly increased the basal glucose uptake of astrocytes, we wondered whether the additional imported glucose would be processed glycolytically to produce lactate. As shown in Fig. 8, even continuous application of TNF-α or IL-1α did not alter lactate release.

Finally, DNA synthesis (as reflected by [^{3}H]thymidine incorporation) and protein content were used to assess the effects of TNF-α and IL-1α on astrocyte proliferation. As shown in Fig. 9, none of the cytokines affected [^{3}H]thymidine incorporation during the first 12 hr, a time point at which both TNF-α and IL-1α markedly increased [^{3}H]2DG uptake. At 24 and 48 hr, TNF-α significantly increased[^{3}H]thymidine incorporation, whereas IL-1α still had no effect. None of the cytokines affected the protein content of the cultures 24 hr after addition.

Discussion

In the present study, we examined the effect of the cytokines TNF-α and IL-1α on various parameters of energy metabolism in mouse cerebral cortical astrocytes in culture. These two cytokines display marked effects on energy metabolism in peripheral tissues. For example, TNF-α stimulates hexose transport in aortic endothelial cells (29) as well as in hepatic nonparenchymal cells (30). The signal transduction pathways of TNF-α in nonneuronal cells appear to involve PLA2, phospholipase C, PKC, and other serine/threonine and tyrosine-specific protein kinases and ultimately lead to the induction of nuclear transcription factors such as nuclear

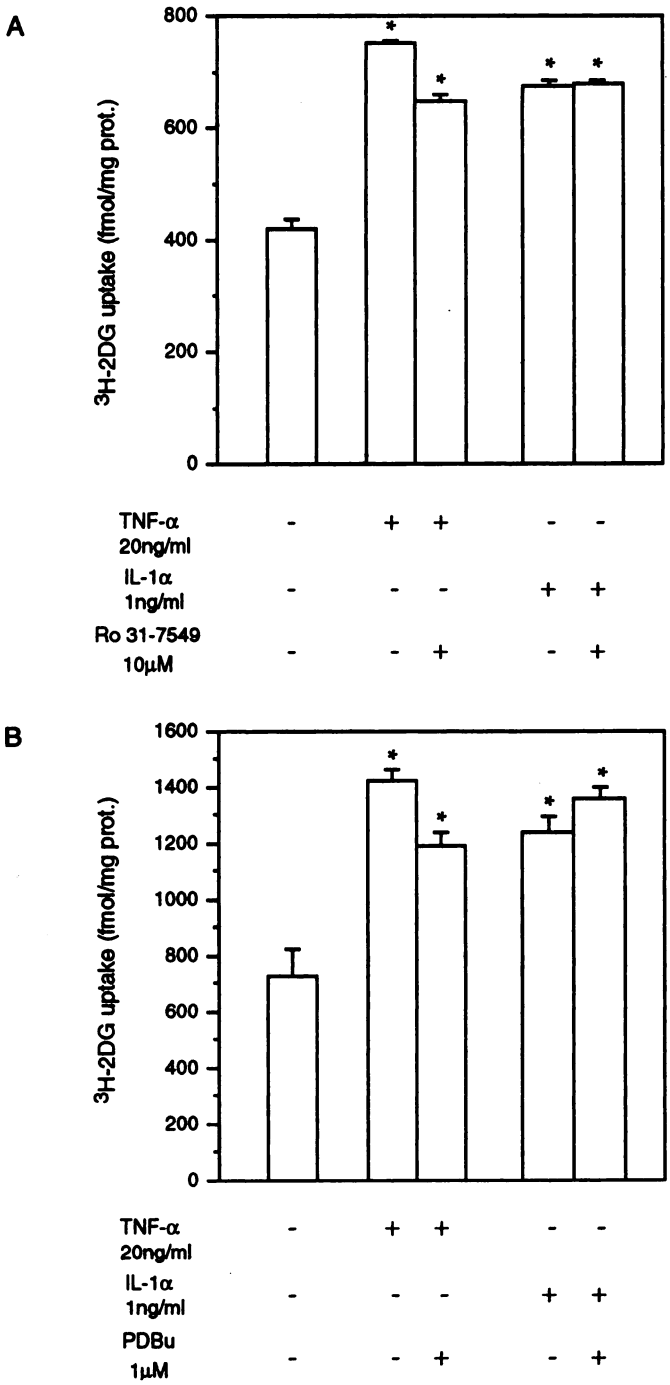

Fig. 6. Absence of involvement of PKC in the stimulation of [^{3}H]2DG uptake evoked by TNF-α or IL-1α. A, Ro 31-7549 (10 μM) was added 30 min before the cytokines and was maintained throughout the incubation. After 24 hr, [^{3}H]2DG uptake was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results and are expressed in femtomoles/milligram of protein. B, To down-regulate PKC, PDBu (1 μM) was added 3 hr before the cytokines and maintained throughout the incubation. After 24 hr, [^{3}H]2DG uptake was determined as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results and are expressed in femtomoles/milligram of protein. *, [^{3}H]2DG uptake significantly different from corresponding basal level ($p < 0.01$).

factor κB (31). The EC_{50} of TNF-α for stimulating [^{3}H]2DG uptake in astrocytes was ~8 ng/ml, corresponding to 470 pM (Fig. 1A). This value is close to 690 pM, the K_d of TNF-α

TABLE 2

Effects of cytokines predominantly produced by T cells on [^{3}H]2DG uptake

The effect of LPS is also shown. After 24 hr of incubation in the presence of various cytokines, [^{3}H]2DG uptake was determined as described in Materials and Methods. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results.

Agent added	[^{3}H]2DG uptake
	fmol/mg protein
None	725 ± 45
Interferon-γ (1000 units/ml)	1157 ± 70[a]
Transforming growth factor-β (1 ng/ml)	707 ± 47
IL-2 (20 units/ml)	618 ± 9
IL-4 (2.5%)	750 ± 75
LPS (250 ng/ml)	1610 ± 48[a]

[a] [^{3}H]2DG uptake significantly different from basal level ($p < 0.01$).

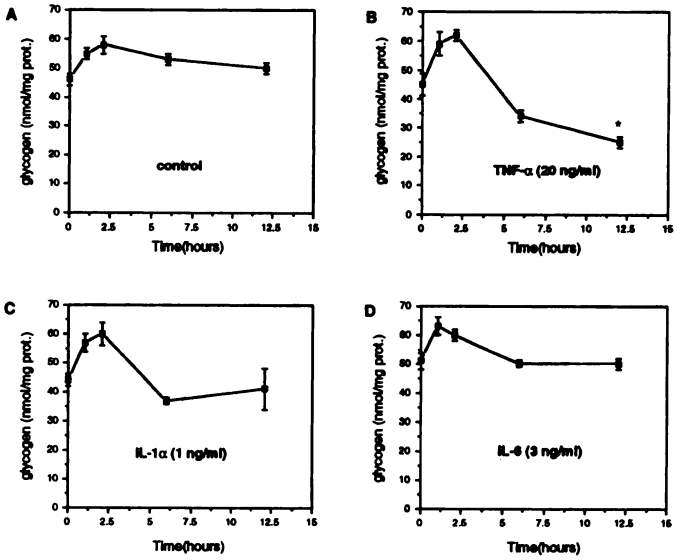

Fig. 7. Time course of effect of TNF-α (20 ng/ml), IL-1α (1 ng/ml), and IL-6 (3 ng/ml) on the glycogen content of astrocytes. Cytokines were added for various periods of time in DMEM5. At the end of the incubation, glycogen content was determined enzymatically as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results. *, Glycogen content significantly different from the control level ($p < 0.01$).

receptors in rat astrocytes in culture (2). This suggests that TNF-α is likely to stimulate [^{3}H]2DG uptake by a receptor-mediated mechanism related to that observed for other effects of TNF-α. The effects of TNF-α and IL-1α are time and protein synthesis dependent, suggesting that cytokine-mediated induction of gene expression is required for the observed metabolic effects.

The involvement of PLA2-dependent pathways in the stimulation by TNF-α-evoked [^{3}H]2DG uptake increase is supported by the observed induction of [^{3}H]AA release, with temporal profile (data not shown) similar to the effect of TNF-α on [^{3}H]2DG uptake. Also, mepacrine, a PLA2 inhibitor, completely inhibited the [^{3}H]2DG uptake increase evoked by TNF-α (Fig. 4). Recent reports have demonstrated TNF-α-mediated PLA2 activation in different cell types leading to the production of AA. For example, TNF-α and IL-1α have been shown to activate PLA2 in bovine endothelial cells (32). In addition, TNF-α and IL-1α stimulate the expression of group II PLA2 in rat cultured astrocytes (33). Our previous experimental evidence has indicated that PLA2-mediated AA

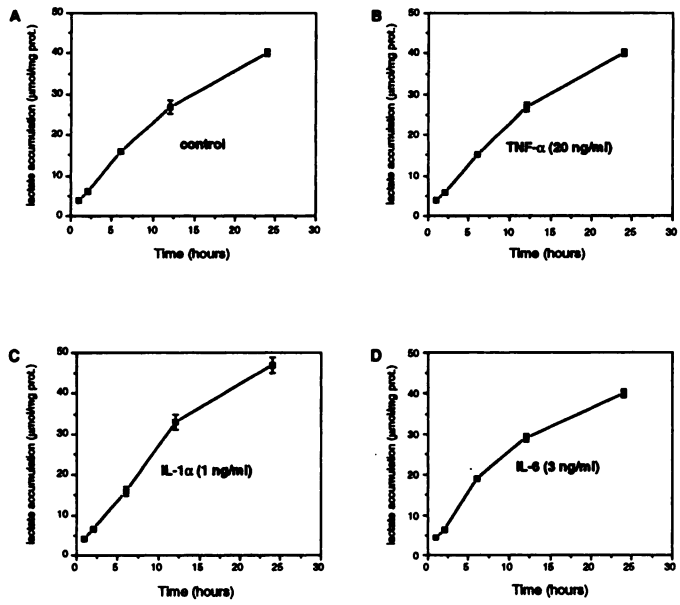

Fig. 8. Time course of the effect of cytokines on lactate release from astrocytes into the extracellular space. Astrocytes were exposed to the cytokines for increasing periods of time. At the end of the incubation, extracellular medium was collected for lactate determination as described. Results are the mean ± standard error of triplicate determinations from one experiment repeated twice with similar results.

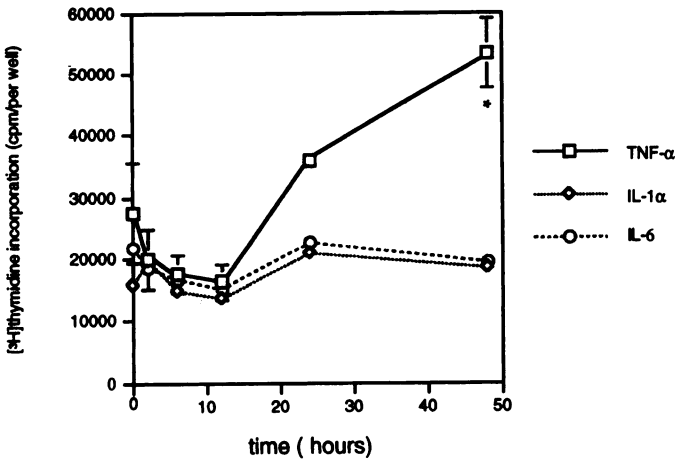

Fig. 9. Time course of DNA synthesis by astrocytes as determined by [^{3}H]thymidine after exposure to cytokines. Astrocytes were incubated in DMEM5 in the presence of cytokines for increasing periods of time; [^{3}H]thymidine (final concentration, 10 μCi/ml) was added for 12 hr. Astrocytes were then harvested and radioactivity was determined as described in Materials and Methods. Results are the mean ± standard error of triplicate determinations from one representative experiment repeated twice with similar results. *, Significantly different from control ($p < 0.05$).

release resulted in the stimulation of [^{3}H]2DG uptake in astrocytes (19). This evidence, combined with the data reported here, indicates that cytokines elevate glucose uptake through a PLA2-mediated increase in basal AA release. Induction of glucose transporter 1 is unlikely to be the mechanism of enhanced [^{3}H]2DG uptake since Northern blot analysis indicates that both IL-1α and TNF-α were without effect on glucose transporter 1 mRNA at 1.5, 6, 12, and 18 hr after application of either cytokine (data not shown).

Although phospholipase C and PLA2 appear to be parallel pathways in the signal transduction cascade triggered by TNF-α in nonneuronal cells, there are reports indicating interactions between the two pathways. For example, PKC is involved in PLA2 activation in rabbit pulmonary arterial smooth muscle cells (34). In addition, direct activation of PKC by phorbol esters has been shown to stimulate AA release in the osteoblastic MOB cell line (35). However, the absence of inhibition by the selective PKC inhibitor Ro 31–7549 seen in the present study indicates that it is unlikely that PKC is involved in TNF-α-evoked [^{3}H]2DG uptake increase. In addition, PKC down-regulation by long term (24 hr) treatment with PBDu did not modify TNF-α- or IL-1α-evoked [^{3}H]2DG uptake increase or AA liberation, further stressing the absence of PKC involvement in the effects reported in the present study.

The signal transduction pathway(s) activated by the interaction of IL-1α with its receptor are not yet fully understood. Recently, IL-1 has been shown to cause an increase in the accumulation of cytosolic PLA2 in human fibroblasts (26). In the present study, we observed that the effects of IL-1α on [^{3}H]2DG uptake are very similar to those exerted by TNF-α. Thus, a similar time course and the activation of common second-messenger pathways (PLA2 mediation) are observed, supporting the view that cytokines exhibit gross functional redundancy and overlapping actions. However, it should be noted that although IL-1α stimulated [^{3}H]2DG uptake and AA release in a manner quantitatively similar to that of TNF-α, the inhibition by mepacrine was less pronounced for the effect of IL-1α. The reasons for this difference observed experimentally are not readily apparent. There is previous experimental evidence indicating that IL-1 can induce TNF-α production by astrocytes (36). However, the [^{3}H]2DG uptake-stimulating effects of IL-1α did not appear to be mediated by TNF-α because anti-TNF-α antibodies could not abolish the effect of IL-1α on [^{3}H]2DG uptake (data not shown). The EC_{50} of IL-1α for the stimulation of [^{3}H]2DG uptake is ~1.6 pM (30 pg/ml), whereas the IC_{50} of IL-1 determined in competition binding studies carried out in mouse astrocytes is 80 pM (4). This difference in affinity could be attributed to the fact that human IL-1α was used as the ligand for competition binding experiments in mouse astrocytes.

IL-6 shares many biological actions with TNF-α and IL-1α. However, in the present study, IL-6 had no effect on [^{3}H]2DG uptake, [^{3}H]AA release, or glycogen content regulation. The concentrations of IL-6 used in this study ranged from 125 pg/ml to 2000 pg/ml, which spanned the concentrations of IL-6 (200–1000 pg/ml) found to induce B-cell differentiation (37). One possibility to account for the lack of effect of IL-6 in astrocyte energy metabolism is the lack of receptors in neural cells. This seems unlikely as there is evidence for the presence of mRNA encoding the IL-6 receptor in the CA1-CA4 region and in the dentate gyrus of the hippocampus, habenula, dorsomedial and centromedial hypothalamus, internal capsule, optic tract, and piriform cortex of the rat brain (12). A recent report indicates that in mouse astrocyte cultures, high affinity [^{3}H]γ-aminobutyric acid uptake is inhibited by 48-hr exposure to TNF-α or IL-1α but not to IL-6 (38).

There is considerable evidence indicating that glycogen is present in astrocytes and represents a highly dynamic source of energy substrates under the control of various neurotransmitters (20). One potential fate of the additional glucose taken up under the actions of TNF-α or IL-1α could have

been storage as glycogen. However, this is clearly not the case because glycogen levels are decreased as a consequence of exposure to the cytokines, in particular, TNF-α (Fig. 7).

Astrocytes have also been shown to release considerable amounts of lactate. Furthermore, in the absence of any glucose in the medium, astrocytes mobilize glycogen and release lactate rather than glucose (28). In addition, an acute increase in glucose uptake evoked by glutamate has been recently shown to be accompanied by a commensurate release of lactate (22). From the forgoing, one would expect the increased glucose uptake triggered by TNF-α or IL-1α to result in increased lactate release. Clearly, this is not the case. It should be noted that lactate is an energy-rich metabolic substrate because after conversion to pyruvate by lactate dehydrogenase, it can be fully oxidized through the tricarboxylic acid cycle and provide 18 ATP molecules. In addition, lactate is an adequate metabolic substrate for neurons as it can maintain *in vitro* synaptic activity in the absence of glucose (39).

In vivo TNF-α has been shown to increase glucose oxidation in various tissues of the rat, including liver and muscle (30). In addition to the tricarboxylic acid cycle, the pentose phosphate pathway is a metabolic pathway for glucose. It has been shown that TNF-α increases glucose utilization in Kupffer cells by enhancing the activity of the pentose phosphate pathway, resulting in increased NADPH (40). Thus, the additional glucose taken up as a consequence of the exposure of astrocytes to TNF-α or IL-1α is likely channeled through the tricarboxylic acid cycle or the pentose phosphate pathway because neither storage as glycogen nor release of lactate after glycolysis was apparent.

TNF-α stimulates astrocyte proliferation (7), and IL-1 is a potent mitogen for astroglia (11). Thus, the increased glucose utilization observed in the present study may reflect in part a proliferative effect. However, this view seems unlikely for IL-1α because the cytokine had virtually no effect on DNA synthesis reflected by [^{3}H]thymidine incorporation (Fig. 9). Total RNA and protein content (data not shown) were also unaffected, even after 24 hr incubation. In addition, the effect of IL-1α on proliferation was identical to that of IL-6, which did not influence astrocyte energy metabolism. As for TNF-α, an increase in [^{3}H]thymidine incorporation was observed only between 24 and 48 hr, whereas [^{3}H]2DG uptake had increased considerably at earlier time points (Fig. 2).

In summary, the results of the present study demonstrate that the two cytokines TNF-α and IL-1α markedly increase glucose uptake by astrocytes. Because the release of lactate, the main energy substrate produced by astrocytes, is not concomitantly increased, and the storage of glycogen, the main energy reserve of the brain, is decreased, these data indicate that the overall energy utilization of astrocytes is increased by the cytokines. This perturbation in the finely tuned energy balance of the brain could lead to inadequate energy supply to neurons, which could render them more prone to neurodegeneration, a frequent finding in AIDS dementia, a condition in which both TNF-α and IL-1 levels are increased in the brain.

ACKNOWLEDGMENTS

We heartily thank Dr. Morris J. Brinbaum (Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA) for providing the cDNA of rat brain glucose transporter 1. We also thank Dr. Michael H. Kroll (Veterans Affairs Medical Center, Baylor College of Medicine, and the Biomedical Engineering Laboratory, Rice University, Houston, TX) and Dr. Geoffery Lawton (Research Centre, Roche Products Ltd., Welwyn Garden City, Hertfordshire, UK) for providing the compound of Ro 31–7549.

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