Astrocytes represent the most abundant cell type of the adult nervous system. Under normal conditions, astrocytes participate in neuronal feeding and detoxification. However, following brain injury, local increases in inflammatory cytokines trigger a reactive phenotype in astrocytes during which these cells produce their own inflammatory cytokines and neurotoxic free radicals. Indeed, progression of this inflammatory reaction is responsible for most neurological damage associated with brain trauma. Insulin-like growth factor-I (IGF-I) protects neurons against a variety of brain pathologies associated with glial overproduction of proinflammatory cytokines. Here, we demonstrate that in astrocyte cultures IGF-I regulates NFκB, a transcription factor known to play a key role in the inflammatory reaction. IGF-I induces a site-specific dephosphorylation of IκBα (phospho-Ser32) in astrocytes. Moreover, IGF-I-mediated dephosphorylation of IκBα protects this molecule from tumor necrosis factor α (TNFα)-stimulated degradation; therefore, IGF-I also inhibits the nuclear translocation of NFκB (p65) induced by TNFα exposure. Finally, we show that dephosphorylation of IκBα by IGF-I pathways requires activation of calcineurin. Activation of this phosphatase is independent of phosphatidylinositol 3-kinase (PI3-kinase) and Akt, is a survival factor for cerebellar granule neurons (4, 5). In vivo, IGF-I protects neurons against a variety of brain insults typically associated with overproduction of proinflammatory cytokines such as stroke, brain trauma, and multiple sclerosis (6, 7). Moreover, IGF-I therapy dramatically reduces reactive astrocytosis following neuronal damage in the cerebellum (8), presumably by limiting the glial reaction and the progression of inflammation. Thus, it is likely that the benefits obtained by IGF-I treatment in these pathological conditions results from its direct action on neuronal survival and an inhibition of the glial inflammatory reaction. Expression of multiple genes implicated in inflammation, including proinflammatory cytokines and their receptors, is under the transcriptional control of the transcription factor nuclear factor κB (NFκB) (9). In resting cells NFκB (formed by a dimer of p50 and p65 subunits) is located in the cytoplasm where it is associated with its inhibitory subunit, IκB. Proinflammatory cytokines such as TNFα or IL-1 induce site-specific serine phosphorylation of IκB through a signaling cascade involving the serine kinases NFκB-inducing kinase and IκB kinase (IKK). Serine phosphorylation of IκB induces its ubiquitination and degradation by the 26 S proteasome. Loss of IκB from the complex exposes the nuclear location sequence of nuclear factor κB (α or β).

The abbreviations used are: TNFα, tumor necrosis factor α; IL, interleukin; IGF-I, insulin-like growth factor I; PI3-kinase, phosphatidylinositol 3-kinase; NFκB, nuclear factor κB; IκB, nuclear factor κB inhibitory subunit; IKK, IκB kinase; PP2A, protein phosphatase 2A; PP2B, protein phosphatase 2B; PP1, protein phosphatase 1; GFAP, glial fibrillary acidic protein; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; BAPTA-AM, 1,2-bis(2-aminoethoxy)ethane-N,N,N′,N′-tetraacetic acid; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
NFκB resulting in translocation of this molecule to the nucleus where it binds to specific DNA motifs (10). Recently, an alternative mechanism has been reported to regulate NFκB translocation. In Jurkat T cells, pervanadate stimulates tyrosine phosphorylation of IkB, promoting its association with the Src homology 2 (SH2) domains of p85, one of the PI3-kinase regulatory subunits. The binding of p85 to IkB displaces NFκB which is then translocated to the nucleus (11). However, no growth factor or cytokine has yet been reported to utilize this mechanism.

In the present work, we demonstrate that NFκB nuclear translocation in astrocytes is modulated by opposing signals that regulate serine phosphorylation and dephosphorylation of IkB. Furthermore, we show that IGF-I activates a phosphatase activity that dephosphorylates the basal and TNFα-induced phosphorylation (Ser32) of IkB. Finally, using specific phosphatase inhibitors, we demonstrate that calcineurin, but not PP2A or PP1, mediates IGF-I-induced dephosphorylation of IkB through a PI3-kinase and MAPK independent mechanism that requires intracellular calcium signaling. In view of these results, we hypothesize that the neuroprotective effects of IGF-I are based upon its known capacity to promote neuronal survival and on the novel concept that this factor reduces glial inflammatory response by inhibiting NFκB activation as induced by proinflammatory cytokines.

EXPERIMENTAL PROCEDURES

Antibodies and Inhibitors—Phosphospecific antibodies that recognize the activated forms of IkBα (phospho-Ser32 of IkBα), Akt (phospho-Ser473 of Akt), and MAPK (phospho-Thr202/Tyr204 of p44/42 MAPK) were purchased from New England Biolabs. Antibodies specific for IkBα (SC371) and NFκB (SC109) were from Santa Cruz Biotechnology Inc. A monoclonal antibody against glial fibrillary acidic protein (GFAP) was from Sigma, and Alexa-conjugated anti-mouse and anti-rabbit secondary antibodies were from Molecular Probes. The PI3-kinase inhibitor LY294002, the MEK inhibitor PD98059, the cell-permeable calcium chelator BAPTA-AM, okadaic acid, and cyclosporin A were all purchased from Calbiochem.

Cell Culture—Primary astroglial cultures were prepared as described previously (12). Briefly, P3 brains were dissected under aseptic conditions and immersed in ice cold Earle’s balanced salt solution (Life Technologies, Inc.). Cerebellum, brain stem, and all meningeal membranes were carefully removed and discarded. The tissue was cut into 1-mm pieces using thin tweezers. Tissue fragments were washed once with fresh Earle’s balanced salt solution and incubated with 2 ml of 1% trypsin-EDTA dissociation solution (Life Technologies, Inc.) for 20 min at 37 °C. 100 units/ml DNase I was added, and the tissue was dispersed with a fire-polished Pasteur pipette. Cell preparations were then centrifuged from 800 rpm for 10 min and plated in Dulbecco’s modified Eagle’s medium-F12 containing 10% fetal calf serum.

Immunohistochemistry—Cells were plated on 20-mm coverslips and cultured for 10 days as described above. Cells were then serum-starved for 4 h and immediately following different treatments, fixed with cold 4% paraformaldehyde. Cells were washed three times with phosphate-buffered saline containing 0.1% Triton X-100 (PBT) and incubated with the antibodies (a rabbit polyclonal anti-NFκB and a mouse monoclonal anti-GFAP) diluted 1:1000 in PBT containing 0.1% bovine serum albumin overnight at 4 °C. Cells were then washed three times in PBT and incubated for 1 h at room temperature with the secondary antibodies: an anti-rabbit IgG-Alexa 488 (green) or anti-mouse IgG-Alexa 594 (red). Subsequently, coverslips were washed three times with PBT, rinsed in water, and mounted with mowiol. Cell counting and pictures were performed in a Leica microscope using a green-red double filter.

Immunoprecipitation and Western Blotting—Immunoprecipitations were performed as described previously (13). Ten-day-old astroglial cultures were serum-starved for 4 h and immediately after treatments, lysed with 150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 1 μg/ml leupeptin, 1 μg/ml phenylmethanesulfonyl fluoride, using 1 ml of buffer per 10-cm dish. Insoluble material was removed by centrifugation, and supernatants were incubated overnight with the antibodies. Immunocomplexes were collected with Protein A-Sepharose 6MB (Amersham Pharmacia Biotech) for 1 h at 4 °C. Immunoprecipitates were washed three times in homogenization buffer before separation by SDS-polyacrylamide gel electrophoresis and trans-

FIG. 1. IGF-I induces site-specific serine dephosphorylation of IkBα. Serum-starved astroglial cultures were stimulated with 100 nM IGF-I for 5, 10, and 30 min. The clarified lysates were immunoprecipitated with antibodies against IkBα or anti-IkBα antibodies. The blots were developed with the ECL system. The graph represents the results of three different experiments (mean ± S.E.) (** p < 0.01; *** p < 0.005). Values were as determined by analysis of variance test.

RESULTS AND DISCUSSION

IGF-I Induces Transitory Dephosphorylation of Serine in IkBα—IGF-I has been reported to exert antipapoptotic and neuroprotective effects in different primary neuronal cultures and neuronal cell lines (5, 6). Moreover, in the GT1–7 neuronal line, IGF-I-mediated neuroprotection against oxidative stress is associated with activation of NFκB (14). However, in glial cells where NFκB mediates the action of proinflammatory cytokines (15, 16), IGF-I reduces glial inflammation after brain injury (8). Based on this apparent biological contradiction, we postulated that IGF-I might differentially regulate the NFκB pathway in astrocytes as compared with neurons. Thus, to study the effects of IGF-I on NFκB activation in glial cells, confluent 10-day-old, astroglial cultures were deprived of serum for 4 h and stimulated with 100 nM IGF-I for 5, 15, and 30 min. Cell lysates were immunoprecipitated with antibodies against IkBα. The immunocomplexes were collected with Protein A-Sepharose, split, and separated by two parallel SDS-polyacrylamide gel electrophoresis gels. The resulting nitrocellulose membranes were probed with either phosphospecific anti-IkBα (phospho-Ser32) or anti-IkBα antibodies. Western blotting revealed that in astrocyte cultures IGF-I did not stimulate site-specific serine phosphorylation of IkBα; on the contrary, IGF-I induced a pronounced but transitory dephosphorylation of phospho-Ser32 IkBα (Fig. 1). This result sharply contrasts the effect of IGF-I in neurons where it induces IkB degradation presumably based on serine phosphorylation of this molecule (14). As discussed above, serine phosphorylation of IkBα initiates a cascade of events that leads to IkB degradation and the consequent translocation of NFκB to the nucleus where it activates transcription of downstream genes. NFκB is strongly activated by proinflammatory cytokines like TNFα, a key suspect in the development of some neurodegenerative diseases and in neurological damage after brain lesions (17, 18). Moreover, it is well documented that IGF-I functions as a neuroprotective factor following cellular insults, both in vitro and in vivo (14, 19). Thus, we...
reasoned that IGF-I-induced dephosphorylation of Ser\(^{32}\) of IκB\(\alpha\) might ultimately prevent NFκB activation triggered by the cytokines released in response to brain injury. To address this question, we next studied the effects of IGF-I on TNFα-induced activation of IκB\(\alpha\).

**IGF-I Abolishes TNFα-induced Serine Phosphorylation of IκBα and Reduces Its Degradation**—TNFα conveys proinflammatory and proapoptotic effects in different tissues and cell cultures where it induces NFκB activity through a site-specific (Ser\(^{32}\)) phosphorylation of IκB\(\alpha\) (10). Thus, to study the potential of IGF-I to modulate TNFα-induced NFκB activation, we evaluated the effect of this factor on the TNFα-activated serine phosphorylation of IκB\(\alpha\). Serum-starved astroglial cultures were treated with IGF-I, TNFα, or both factors for 5 or 15 min. Cultures were lysed and immunoprecipitated with anti-IκB\(\alpha\) and then blotted with anti-phosphospecific IκB\(\alpha\) or anti-IκB\(\alpha\). Similar to observations in other cell systems (10), TNFα stimulated IκB\(\alpha\) serine phosphorylation by almost 2-fold in astrocytes (Fig. 2A). Interestingly, IGF-I significantly decreased not only the basal phosphorylation of Ser\(^{32}\) in IκB\(\alpha\) but also that induced by TNFα (Fig. 2A). Consistent with the dephosphorylation kinetics presented in Fig. 1, IGF-I reduced the TNFα—stimulated IκB\(\alpha\) serine phosphorylation to below control levels following 5 min of treatment, with the effect being less robust after 15 min. Stimulation of IκB\(\alpha\) serine phosphorylation by TNFα induces its degradation and subsequent translocation of NFκB to the nucleus. In the cultures employed for this study, TNFα-induced degradation of IκB\(\alpha\) was apparent after 15 min of treatment (Fig. 2A). After 1 h of treatment, TNFα reduced IκB\(\alpha\) to below detectable levels (Fig. 2B). However, IGF-I fully protected IκB\(\alpha\) from TNFα-induced degradation at 15 min (Fig. 2A) and partially at 1 h of treatment (Fig. 2B). Interestingly, after 1 h, prevention of degradation by IGF-I was not accompanied by a decrease in serine phosphorylation of IκB\(\alpha\) as detected at 15 min. On the contrary, long term exposure to both IGF-I and TNFα coincided with an accumulation of serine-phosphorylated IκB\(\alpha\) (Fig. 2B). These results suggest a two-step mechanism by which the initial dephosphorylation evoked by IGF-I prevents the entrance of phosphoserine-IκB\(\alpha\) into the degradation pathway, indicating that regulation of degradation is not solely dependent on the serine phosphorylation status of IκB\(\alpha\).

Previous studies have shown that pervanadate stimulates association of IκB\(\alpha\) with p85 PI3-kinase and thus protects it from degradation (11). Thus, we examined the ability of IGF-I to promote formation of such complexes in astrocytes. In parallel with the protection of IκB\(\alpha\) from degradation, IGF-I stimulated the association of IκB\(\alpha\) to p85 (Fig. 2C) in our studies. Thus, it is likely that association of IκB\(\alpha\) with p85 contributes to the protective effects elicited by IGF-I upon the degradation of this molecule. However, it is also reported (11) that p85 binding to IκB\(\alpha\) displaces NFκB, which is then translocated to the nucleus. Thus, the fact that IGF-I induced IκB\(\alpha\) association to p85 but also inhibited NFκB translocation to the nucleus in astrocytes (shown in Fig. 3) suggests that this factor utilizes additional mechanisms to prevent the nuclear translocation of the released NFκB in our cell system.

**TNFα-induced Nuclear Translocation of NFκB Is Inhibited by IGF-I**—Site-specific serine phosphorylation of IκB\(\alpha\) triggers the cascade of events that ultimately lead to NFκB translocation to the nucleus. Since we observed that TNFα-mediated serine phosphorylation of IκB\(\alpha\) was significantly reduced by IGF-I treatment, we next evaluated whether the nuclear translocation of NFκB was also modulated by this growth factor. For this purpose, serum-starved astroglial cultures were treated with TNFα or with TNFα plus IGF-I for 15 or 30 min. After fixation, cells were double-stained with NFκB (p65) and the astrocyte marker GFAP. Using a red-green double filter, we detected TNFα—induced nuclear translocation of NFκB in some cells following 15 min of treatment (not shown), but the translocation was more uniform after 30 min (Fig. 3). Interestingly, nuclear localization of NFκB induced by TNFα treatment was greatly reduced by addition of IGF-I (Fig. 3). Additionally, TNFα stimulated NFκB translocation to the nucleus only in GFAP-positive cells, whereas the small percentage of cells not stained with GFAP (5–10% of the cells are typically not labeled with GFAP in these cultures) did not respond to TNFα although they show abundant cytoplasmic NFκB staining (Fig.
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3). Our results demonstrate that the IGF-I-mediated prevention of IκB degradation in TNFα-treated cells correlates with a reduction of NFκB nuclear translocation. The observation that NFκB is not translocated to the nucleus in response to TNFα in GFAP negative cells supports the idea that the actions of proinflammatory cytokines may be cell type-dependent. For example, in neurons TNFα induces apoptosis (4), whereas in astrocytes this cytokine induces the expression of proinflammatory molecules (20).

Calcineurin (PP2B) Mediates IGF-I-induced Phospho-IκBα Ser Dephosphorylation—The balance between kinase-phosphatase activities represents one of the most powerful control mechanisms in cell signaling and has been described in the regulation of many signal transduction pathways (21). Several factors have been reported to enhance phosphorylation of IκB at Ser62 (10, 22). However, our results are the first report of a factor that stimulates IκB Ser62 dephosphorylation. To examine the molecular nature of the phosphatase implicated in this process, the effect of IGF-I on IκB serine phosphorylation was studied in the presence of several specific phosphatase inhibitors. Okadaic acid was used at 0.5 nM to specifically inhibit PP2A and at 5 nM to inhibit PP2A and PP1. Cyclosporin was used at 500 nM to inhibit calcineurin (PP2B). The astroglial cultures were pretreated for 30 min with the phosphatase inhibitors. Cells were then stimulated with IGF-I for 5 min and lysed. Lysates were immunoprecipitated with anti-IκBα antibodies and blotted with anti-phosphospecific IκB or anti-IκBα. Parallel gels containing total cell lysates were blotted with anti-phosphospecific Akt and anti-phosphospecific MAPK to monitor the possible effects of phosphatase inhibitors on IGF-I signaling. Okadaic acid, at either 0.5 or 5 nM, did not suppress IGF-I-induced IκB Ser dephosphorylation (Fig. 4A). However, when used at 5 nM it increased basal IκB Ser phosphorylation by 2.5-fold. Interestingly, MAPK but not Akt phosphorylation was also increased by inhibition of PP1 (Fig. 4A). These results indicate that although PP1 is not the phosphatase activated by IGF-I it may have a role in the serine phosphorylation status of IκB. The fact that PP1 inhibition increased both IκBα and MAPK Ser phosphorylation suggests an action of this phosphatase on a common upstream kinase such as mitogen-activated protein kinase/ERK kinase kinase 1, 2, and 3 (MEKK1, -2, -3) (23, 24) rather than a direct action on IκBα and MAPK.

In contrast, IGF-I-stimulated dephosphorylation of IκBα serine residues was abolished when calcineurin was specifically inhibited with cyclosporin (Fig. 4B). Moreover, consistent with its substrate specificity for calcineurin (25), cyclosporin did not alter the basal or IGF-I-stimulated levels of Akt and MAPK activation (Fig. 4B). The fact that cyclosporin treatment did not modify basal IκBα serine phosphorylation indicates that, in contrast to PP1, calcineurin has a very low basal activity. Thus, stimulation of this phosphatase by IGF-I represents a very powerful mechanism to control NFκB-mediated inflammation progression. Given this potential mechanism of regulation, it would be interesting to determine whether IκBα dephosphorylation, mediated by calcineurin or any other phosphatase, is a common mechanism used by IGF-I and anti-inflammatory cytokines such as IL-4 or IL-6. IGF-I shares components of signaling cascades, including the tyrosine phosphorylation of insulin receptor substrate proteins, with these anti-inflammatory cytokines (26).

IGF-I-induced IκBα Dephosphorylation Does Not Require MEK or PI3-Kinase Activation—Following activation of the IGF-I receptor, this kinase recruits and phosphorylates tyrosine residues in several signaling molecules. Phosphorylation of Src homologous and collagen and the insulin receptor substrate proteins activates MAPK and PI3-kinase pathways (26). Although other signaling molecules are engaged by the IGF-I receptor (27), most of the cellular actions of IGF-I depend on activation of these two pathways. Moreover, e-Jun N-terminal kinase, activated by inflammatory cytokines and cellular stress, has been reported to be inhibited by IGF-I. Although the precise mechanisms by which IGF-I suppresses e-Jun N-terminal kinase activity are not known, a central role of PI3-kinase and Akt in this effect has been well established (28). To assess the role of MAPK and PI3-kinase signaling pathways in IGF-I-induced IκBα dephosphorylation, we studied the effect of IGF-I on IκBα Ser phosphorylation in the presence of LY294002 and PD098059, compounds that specifically block PI3-kinase and MAPKK (MEK), respectively (Fig. 5A). By immunoblotting, levels of P-MAPK and P-Akt were evaluated to monitor activation of PI3-kinase and MAPK pathways. As ex-
expected the inhibitors specifically abolished IGF stimulation of both these pathways; however, the IGF-I-mediated dephosphorylation of IκBa was not affected by these agents. Interestingly, PD098059 alone induced a reduction of IκBa serine phosphorylation and produced an additive effect when used together with IGF-I, suggesting independent mechanisms for these pathways. These results demonstrate that IGF-I activation of calcineurin is not mediated by p38 kinase or MEK. In an effort to further explore the mechanism by which IGF-I stimulates calcineurin pathways, we next tested the effects of SB203580 and U-73122, pharmacological inhibitors that specifically block p38 and phospholipase Cγ, respectively. Unfortunately, inhibition of either p38 or phospholipase Cγ activity had no effect on IGF-I-induced IκBa dephosphorylation (data not shown). Calcineurin has been shown recently to mediate IGF-I-dependent skeletal muscle hypertrophy in myocyte cultures (29, 30). In these cells, IGF-I activates calcineurin by increasing intracellular Ca2⁺ through a PI3-kinase/MEK independent mechanism. In muscle, activated calcineurin dephosphorylates the transcription factor NT-ATc1 which is then translocated to the nucleus (30), a mechanism that very much resembles the IGF-I-induced IκBa dephosphorylation reported in the present work. To explore the possibility that, as seen in muscle cells, IGF-I could be activating calcineurin by increas-

**FIG. 4.** IGF-I-induced serine dephosphorylation of IκBa is calcineurin-dependent and is PP2A or PP1 independent. A, starved astroglial cultures were treated with 0.5 nM okadaic acid (OK1) to specifically inhibit PP2A or 5 nM (OK2) to inhibit PP2A and PP1. Cultures were pretreated for 30 min with the phosphatase inhibitors and with 100 nM IGF-I (I) for 5 min, immunoprecipitated with anti-IκBa antibodies, and blotted with phosphospecific anti-IκB (P-IκB) and anti-IκBa. Parallel gels containing total cell lysate were blotted with anti-P-Akt and anti-P-MAPK. The bar graph represents ± S.E. of three independent experiments *, p < 0.05. B, in an experiment similar to the one shown in A, astroglial cultures were pretreated for 45 min with 500 nM cyclosporin to specifically inhibit calcineurin (Cs) and with 100 nM IGF-I (I) for 5 min. The blots are as in A. The graph represents ± S.E. of four independent experiments, *, p < 0.01.

**FIG. 5.** IGF-I-induced IκBa site-specific serine dephosphorylation requires intracellular calcium signaling but not MEK or PI3-kinase activation. A, starved astroglial cultures were treated with 20 μM LY294002 (LY) or 25 μM PD098059 (PD) to specifically block PI3-kinase and MEK, respectively. Cells were pretreated with the inhibitors for 30 min and with 100 nM IGF-I (I) for 5 min, lysed, and immunoprecipitated with anti-IκBa antibodies. Immunocomplexes were blotted with anti-phosphospecific IκBa and anti-IκBa. Parallel gels loaded with total cell lysates were blotted with anti-PI3-kinase Akt and anti-phosphospecific MAPK to monitor the efficiency of the inhibitors. The graph represents the mean ± S.E. of three independent experiments. *, p < 0.005. B, in an experiment similar to the one shown in A, astroglial cultures were pretreated for 30 min with 100 μM BAPTA-AM (BA) to specifically chelate intracellular calcium and with 100 nM IGF-I (I) for 5 min. The blots are as in A. The graph represents ± S.E. of three independent experiments, *, p < 0.005.
ing intracellular Ca\(^{2+}\), we tested the effects of BAPTA-AM, an intracellular calcium chelator, on IGF-I action in our astrocyte cultures (Fig. 5B). Chelation of intracellular calcium with BAPTA-AM had the same effect as the pharmacological inhibition of calcineurin with cyclosporin; stimulation with IGF-I failed to mediate serine dephosphorylation of IkB without affecting the normal IGF-I activation of MAPK and Akt. These results suggest that IGF-I stimulation of IkB dephosphorylation relies on a mechanism that involves regulation of intracellular calcium levels. Consistent with this, phosphatase activity of calcineurin is dependent upon calcium and calmodulin (31). Elevation of intracellular calcium is required in many systems for cell growth and expression of several cytokine genes. Although some recent reports propose a direct regulation of a Ca\(^{2+}\) channel by the IGF-I receptor (32), there is no clear evidence to support this model in glial cells. Nevertheless, our current working hypothesis is that IGF-I alters intracellular calcium levels, thereby regulating the activity of calcineurin and its dephosphorylation of IkB. Thus, ongoing studies in our laboratory are directed at defining the precise mechanism by which IGF-I regulates calcineurin activity and the dephosphorylation of IkB. Based on these recent observations, our future efforts will also include studies of the effects of IGF-I on intracellular calcium in astrocytes.

Also noteworthy from these studies was the reduction in basal serine phosphorylation of IkBz when MEK was inhibited with PD98059. This observation suggests that IkBz itself or one of its upstream kinases might be a target of MEK. MEK has been reported to participate in IkBz Ser phosphorylation. Different signaling pathways have been implicated in the phosphorylation of IkBz in various cell systems. In HeLa cells, site-specific phosphorylation of IkBz through the activation of its upstream kinases I\(\kappa\)K\(\alpha\) and I\(\kappa\)K\(\beta\) can be achieved by different pathways, whereas TNF\(\alpha\) activates I\(\kappa\)K\(\alpha\) and I\(\kappa\)K\(\beta\) by stimulating PI3-kinase and its downstream Ser/Thr kinase Akt (22). In this same cell system, I\(\kappa\)K\(\alpha\) and I\(\kappa\)K\(\beta\) can also be activated by MAPK/ERK pathways (23, 24). It is also important to note the possible roles that Akt may be playing in NF\(\kappa\)B activation. In human fibroblasts (33), Akt induces a robust NF\(\kappa\)B activation by stimulating IkBz Ser phosphorylation. However, here we demonstrate that in astrocytes, although it is strongly activated by IGF-I, Akt seems not to participate in IkBz Ser phosphorylation status since no modification of IkBz phosphorylation levels were observed when Akt activation is blocked by PI3-kinase inhibitors.

In conclusion, we have identified a novel mechanism by which IGF-I modulates the inflammatory pathways of TNF\(\alpha\) in astrocyte cultures. Given the central importance of inflammation processes in brain trauma and neurodegenerative diseases, our findings, which connect the IGF-I signaling pathway with NF\(\kappa\)B function, may direct the development of new treatments to reduce the neurological damage associated with inflammation.

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