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NF-κB and IκBα Expression Following Traumatic Brain Injury to the Immature Rat Brain

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NF-κB is one of the most important modulators of stress and inflammatory gene expression in the nervous system. In the adult brain, NF-κB upregulation has been demonstrated in neurons and glial cells in response to experimental injury and neuropathological disorders, where it has been related to both neurodegenerative and neuroprotective activities. Accordingly, the aim of this study was to evaluate the cellular and temporal patterns of NF-κB activation and the expression of its endogenous inhibitor IκBα following traumatic brain injury (TBI) during the early postnatal weeks, when the brain presents elevated levels of plasticity and neuroprotection. Our results showed that cortical trauma to the 9-day-old rat brain induced a very fast upregulation of NF-κB, which was maximal within the first 24 hours after injury. NF-κB was mainly observed in neuronal cells of the degenerating cortex as well as in astrocytes located in the corpus callosum adjacent to the injury, where a pulse-like pattern of microglial NF-κB activation was also found. In addition, astrocytes of the corpus callosum, and microglial cells to a lower extent, also showed de novo expression of IκBα within the time of NF-κB activation. This study suggests an important role of NF-κB activation in the early mechanisms of neuronal death or survival, as well as in the development of the glial and inflammatory responses following traumatic injury to the immature rat brain.

Key words: astrocyte; microglia; glial response; developing brain; transcription factor; inflammation

Focal traumatic brain injury (TBI) induces primary neuronal degeneration in the site of injury and initiates a series of cellular events that lead to protracted secondary damage in the surrounding tissue and distally connected areas. In base to experimental studies performed in rodents, it is now known that secondary damage is exacerbated by the inflammatory response, commonly mediated and magnified by the upregulation or de novo expression of different genes. Several studies have shown that the transcription factor nuclear factor kappa B (NF-κB) is one of the most important modulators of inflammatory gene expression in the nervous system as well as in non-neural tissues (Baldwin, 1996; Grilli and Memo, 1999). In normal conditions, NF-κB is found in the cytoplasm in a latent form bound to its endogenous inhibitor IκBα. Upon activation by lesion-induced oxidative stress, neurotrophins, glutamate, depolarisation, bacterial endotoxin or cytokines, IκBα is phosphorylated, ubiquitinated, and degraded by the proteasome system. Free NF-κB is able then to translocate to the nucleus, where it binds to the κB consensus sequence and induces the expression of NF-κB-regulated genes. Whereas in neurons, activation of NF-κB has been generally involved in synaptic plasticity, neuronal function, development, and survival (O'Neill and Kaltschmidt, 1997; Mattson et al., 2000), in glial cells, NF-κB has been mainly related to the inflammatory response (Kaltschmidt et al., 1994; Bales et al., 1998) through induction of expression of pro-inflammatory genes such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), cyclooxygenase-2 (Cox-2), inducible nitric oxide synthase (iNOS), and adhesion molecules (VCAM-1 and ICAM-1; Baldwin, 1996; Grilli and Memo, 1999).

In this sense, in the last decade several studies have proposed a role of NF-κB activation in the degenerative and inflammatory response occurring after TBI in the adult brain (Yang et al., 1995; Nonaka et al., 1999). However, little is known about the involvement of this transcription factor in the evolution of TBI-induced inflammatory response and remodelling processes in the immature rat brain. Previous studies have demonstrated that postnatal cortical brain damage is associated with enhanced plasticity resulting in better lesion outcome and sparing of neural connections (Kolb et al., 1994, 1996); therefore, the study of NF-κB and IκBα expression along the evolution of TBI in the immature brain may provide

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some insights into the importance of this signal transduction pathway in the degenerative/regenerative events that underlie postnatal brain lesion outcome. In this regard, the aim of the present study was to evaluate the spatial and temporal pattern of NF-κB and IκBα following an aspiration lesion in the postnatal rat neocortex.

MATERIALS AND METHODS

Aspiration Lesions

Long Evans black-hooded 9-day-old rats (P9; Iffa-Credo, Lyon, France) of both sexes were used in this study. Under ether anaesthesia, each pup was placed in a stereotactic frame adapted for newborns (Kopf, Tujunga, CA), and the skull was opened using a trephine. The forelimb area (FL) of the sensorimotor cortex (2 mm lateral from Bregma) was removed by using a Pasteur pipette connected to a vacuum pump. After suture, the pups were placed in an incubator and maintained at 36°C for 2 hours before being returned to their mother. A total of 36 animals were used in this study. Four lesioned animals, two sham-operated and two control animals were used for each of the six postlesion (PL) survival times. This experimental procedure was approved by the Ethical Commission of the Autonomous University of Barcelona. All efforts were made to minimise animal suffering in every step.

Fixation and Histology

At 4 hours, 10 hours, 1 day, 3 days, 5 days, and 7 days PL, animals were anaesthetised by ether inhalation and perfused intracardially for 10 minutes with 4% paraformaldehyde in 0.1 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.4). Brains were removed and kept in the same fixative for 4 hours at 4°C, cryoprotected in a 30% sucrose solution in 0.1 M phosphate buffer (pH 7.4) and frozen with dry CO_2. Frozen coronal sections (30 μm) were obtained and stored free-floating in De Olmos antifreeze solution (Warr et al., 1981). Alternate cryostat sections (20 μm) were mounted onto gelatin-coated slides and stained with toluidine blue for routine histological examination. Free-floating cryostat sections were processed for the immunohistochemical and histochemical techniques described below.

Immunohistochemistry for NF-κB and IκBα

After endogenous peroxidase blocking with 2% H_2O_2, 70% methanol in 0.05 M Tris-buffered saline (TBS; pH 7.4), cryostat sections were treated with 10% fetal calf serum (FCS) in TBS with 1% Triton X-100 (TBS-T) for 30 minutes, and incubated with the primary antibody diluted in TBS-T containing 10% FCS. As primary antibody, either of the following was used: rabbit anti-p65 subunit of NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA; sc-109; 1:100), incubated at 4°C for 36 hours, or mouse anti-IκBα (Santa Cruz Biotechnology, sc-1643; 1:100) incubated at 4°C for 12 hours. After washing with TBS-T, sections were incubated at room temperature for 1 hour with the corresponding secondary biotinylated antibody, an anti-rabbit donkey antibody (Amersham, RPN-1004, U.K.; 1:200) for NF-κB and anti-mouse sheep antibody (Amersham, RPN-1001, U.K.; 1:200) for IκBα. Sections were rinsed in TBS-T and incubated for 1 hour at room temperature with avidin-peroxidase (Dako, P364, Denmark; 1:400). After rinsing again in TBS-T, the peroxidase reaction product was visualised by incubating the sections in 100 ml of Tris buffer (TB; pH 7.4) containing 50 mg diaminobenzidine (DAB) and 33 μl of hydrogen peroxide (30%). As negative controls, sections were incubated in media lacking primary antibodies. Finally, sections were mounted onto gelatin-coated slides, dehydrated in increasing concentrations of ethanol, cleared in xylene, and coverslipped in DPX mounting medium (Bancroft and Stevens, 1996).

Quantitative Analysis of Immunohistochemical Staining

Sections processed for the demonstration of NF-κB and IκBα were quantified as previously described (Acarin et al., 1997, 1999a, b). Briefly, sections were digitised by a video camera mounted on a Leitz microscope and interfaced to a Macintosh computer. National Institute of Health Image software was used to quantify the immunocytochemical staining. The quantification measure, referred to as the "reactivity grade," was defined as the ratio between density values of a specific area of 0.24 mm^2 in the hemisphere ipsilateral to the aspiration lesion versus the same area in the contralateral control hemisphere (measured areas are detailed in the corresponding figures). Researcher was "blinded" to sample identity. A "reactivity grade" was obtained for each marker and for each survival time. Data were obtained analysing three to four sections in each of the two control animals and the four lesioned animals for each survival time. Statistical analysis was performed using Statview 4.5 software. Analysis of variance (ANOVA) with Fisher's PLSD post-hoc comparisons were used.

Double Immunohistochemistry for NF-κB or IκBα and Glial Markers

We used double staining techniques for the simultaneous visualisation of NF-κB or IκBα and glial populations. Double techniques were carried out using glial fibrillary acidic protein (GFAP) as an astroglial cell marker, and tomato lectin histochemistry for the demonstration of microglial cells. Free-floating sections were immunostained for NF-κB or IκBα as described above, but using indocarbocyanine (Cy3)-conjugated anti-rabbit IgG (Amersham, PA-43004; 1:1,000) as secondary antibody for NF-κB immunostaining, and Cy3-conjugated avidin (Amersham, PA-43000; 1:1000) for IκBα labelling. Immnunoreactive sections were processed for GFAP immunohistochemistry by using the primary antibody anti-GFAP (Dako, Z-0334, Denmark; 1:1,800), biotinylated an anti-rabbit donkey antibody (Amersham, RPN-1004, U.K.; 1:200) as the secondary antibody, and Cy2-conjugated avidin (Amersham, PA-42000; 1:1,000). Sections for double staining with tomato (*Lycopersicon esculentum*) lectin histochemistry were incubated with the biotinylated tomato lectin (Sigma, St. louis, MO, L-9389) diluted to 6 μg/ml and treated with Cy2-conjugated avidin (Amersham, PA-42000; 1:1,000). Double-stained sections were analysed using a LEICA TCS 4D confocal microscope.

RESULTS

Histopathology and Glial Response

As previously reported (Sanz et al., 2001), aspiration of cortical tissue generated a cavity filled with cellular debris and blood. Between 4 hours and 1 day PL, the

lesion cavity was limited by a neurodegenerative area (NA), 500- to 700-μm wide, formed by dying neurons and blood cells and surrounded by cortical tissue that had lost its characteristic laminated pattern (Fig. 1C). From 3 days PL, the NA was progressively reduced, forming a glial scar limiting the cavity by 5 days PL.

The aspiration lesion caused an important glial response in the NA and surrounding cortex as well as in the adjacent corpus callosum. The microglial reactivity was first discerned at 4–10 hours PL within the NA bordering the cavity. This response became maximal at 1–3 days PL, when a pronounced increase in the number of cells displaying reactive ramified and amoeboid morphologies was observed both in the damaged cortex and the adjacent corpus callosum (Fig. 1L). From that timepoint, the microglial response clearly diminished and reactive microglia/macrophages were exclusively observed within the glial scar lining the cavity. The astroglial response became evident at 10 hours PL, when hypertrophied astrocytes, showing increased GFAP labelling, were first observed in the damaged cortex as well as in the corpus callosum (Fig. 1K). In both areas, maximal astroglial reactivity was achieved at 3–5 days PL and decreased at longer time points, when reactive astrocytes concentrated in the glial scar.

In addition to cortical damage, the aspiration lesion caused axotomy-induced retrograde and anterograde injury in the ipsilaterally connected thalamic nuclei. Thalamic damage was characterised by degeneration of neuronal cells in the ipsilateral ventrobasal complex (VB) at 7 days PL, accompanied by a reduced and mildly protracted glial reaction both in the VB complex as well as in the centrolateral (CL) nucleus. Thalamic reactive ramified and ameboid microglial cells were observed from 10 hours PL, peaked at day 3 PL, and diminished later on, achieving the typical resting morphology by 7 days PL. Thalamic astroglial reactivity was not evident until 1 day PL and reached maximal cell hypertrophy and GFAP labelling between 3 and 5 days PL, decreasing afterwards. By 7 days PL, remaining astroglial reactivity was only observed in the inferior one-third of the VB complex (see Sanz et al., 2001 for details).

NF-κB and IκBα in Control and Sham-Operated Animals

No differences in the pattern of NF-κB immunoreactivity were observed between control and sham-operated groups. In these animals, cytoplasmic NF-κB labelling was seen in neuronal cells located in thalamic and hypothalamic nuclei, hippocampal pyramidal neurons, and cortical layers II, IV–VI (Fig. 1A). Additionally, glial cells in the corpus callosum (Fig. 1B), identified as astrocytes by their double immunostaining with GFAP (Fig. 4A), also showed cytoplasmatic NF-κB. In contrast, no immunoreactivity for IκBα was observed in either control or in sham-operated animals at any survival time studied (Fig. 4F, H).

NF-κB in Lesioned Animals

As the cellular pattern and time course of NF-κB labelling in the NA versus the adjacent corpus callosum differed, it has been quantified (Fig. 2A,B) and described separately.

In the NA surrounding the lesion cavity, a significative increase in NF-κB immunoreactivity was observed from 4 hours until 1 day PL (Fig. 2A), when NF-κB labelling was mainly observed in neuronal cells (Fig. 1D). At longer survival times, although it was not quantitatively significant (Fig. 2A), strongly hypertrophied fibrous astrocytes forming the glial scar also showed NF-κB labelling (Fig. 4E).

In the adjacent corpus callosum, sometimes extending to neighbouring cortical layer VI, increased glial NF-κB labelling was noticed from the first survival time analysed (Figs. 1E,F, 2B) and it was maintained until 1 day PL (Figs. 1G,H, 2B). At 4–10 hours PL, NF-κB immunoreactivity was mainly observed in the nucleus of astroglial cells (Figs. 1E,F, 4B), although at 4 hours PL, a small subpopulation of amoeboid microglial cells also showed NF-κB immunoreactivity (Fig. 4C). Remarkably, no NF-κB-positive microglial cells were noticed at any other survival time studied. At 1 day PL, NF-κB labelling was primarily found in the cytoplasm of hypertrophied astroglial cells (Figs. 1G,H, 4D), but some of these also exhibited NF-κB-positive nuclei. In this white matter tract, NF-κB immunostaining was clearly diminished from 3 days PL.

Finally, no changes in NF-κB labelling were observed in the ipsilateral damaged thalamic nuclei at any time analysed.

IκBα in Lesioned Animals

No IκBα immunoreactivity was noticed at any of the studied survival times in the NA and subsequently formed glial scar. In contrast, de novo expression of IκBα was observed in the damaged corpus callosum from 4 hours until 1 day PL (Fig. 3). IκBα immunoreactivity was observed as a vesicular labelling in the cytoplasm of glial cells (Fig. 1I–J) that were mainly identified as astrocytes by their double staining with GFAP (Fig. 4G). Moreover, scattered ameboid IκBα-positive cells identified as microglial cells by their double staining with tomato lectin were also found, both in the corpus callosum (Fig. 4I) and in the neighbouring subependymal plate. Finally, no IκBα expression was observed in the secondarily affected thalamus at any of the studied survival times.

DISCUSSION

In this study, we have reported that cortical traumatic injury induces an immediate increase in cortical neuronal NF-κB labelling in the area surrounding the cavity and in glial cells of the adjacent corpus callosum, mainly reactive astrocytes, which also show transient IκBα expression. In contrast, no NF-κB immunolabelling was observed in the distally connected thalamic nuclei, although neuronal degeneration and glial response do occur.

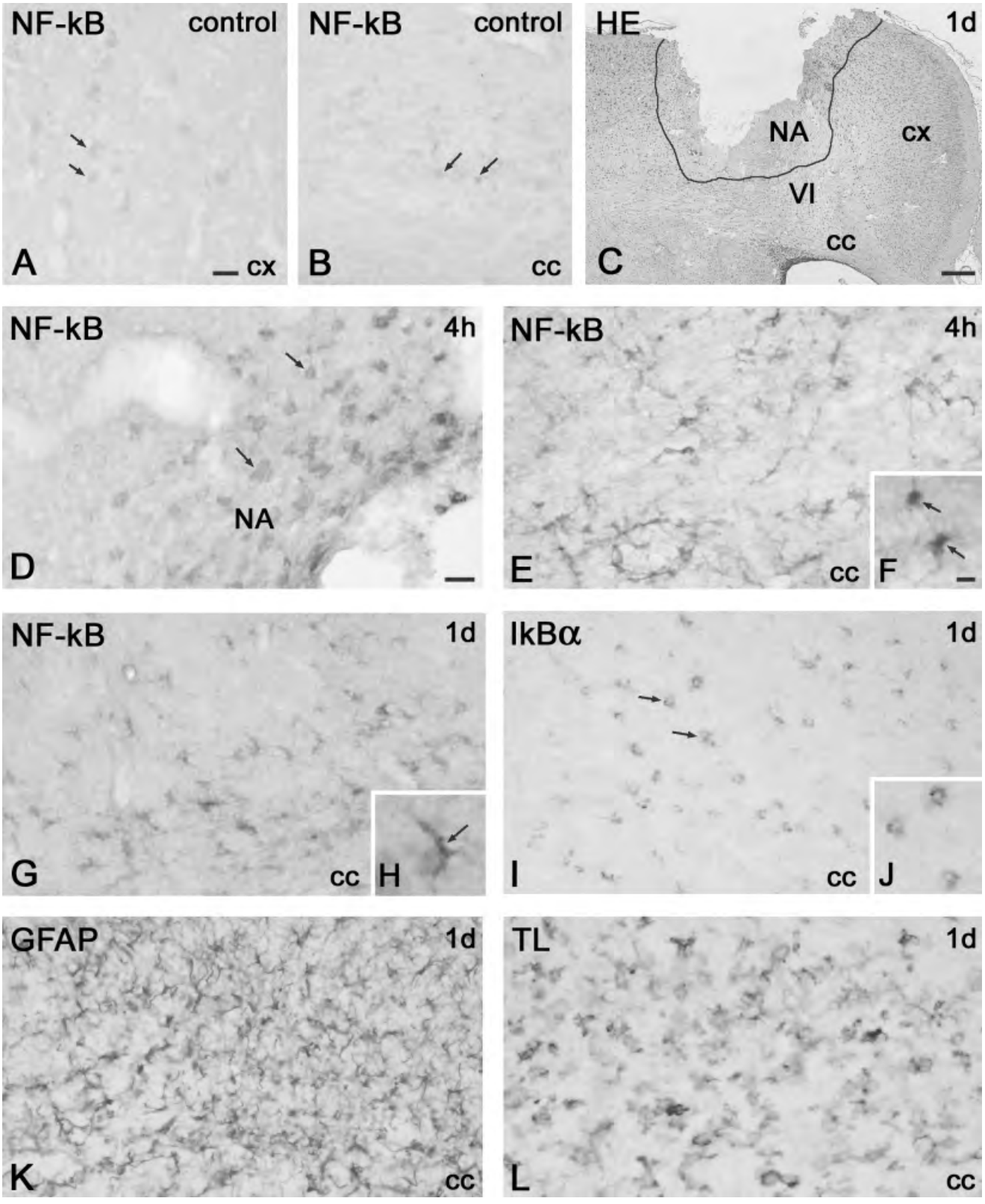
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Figure 1.

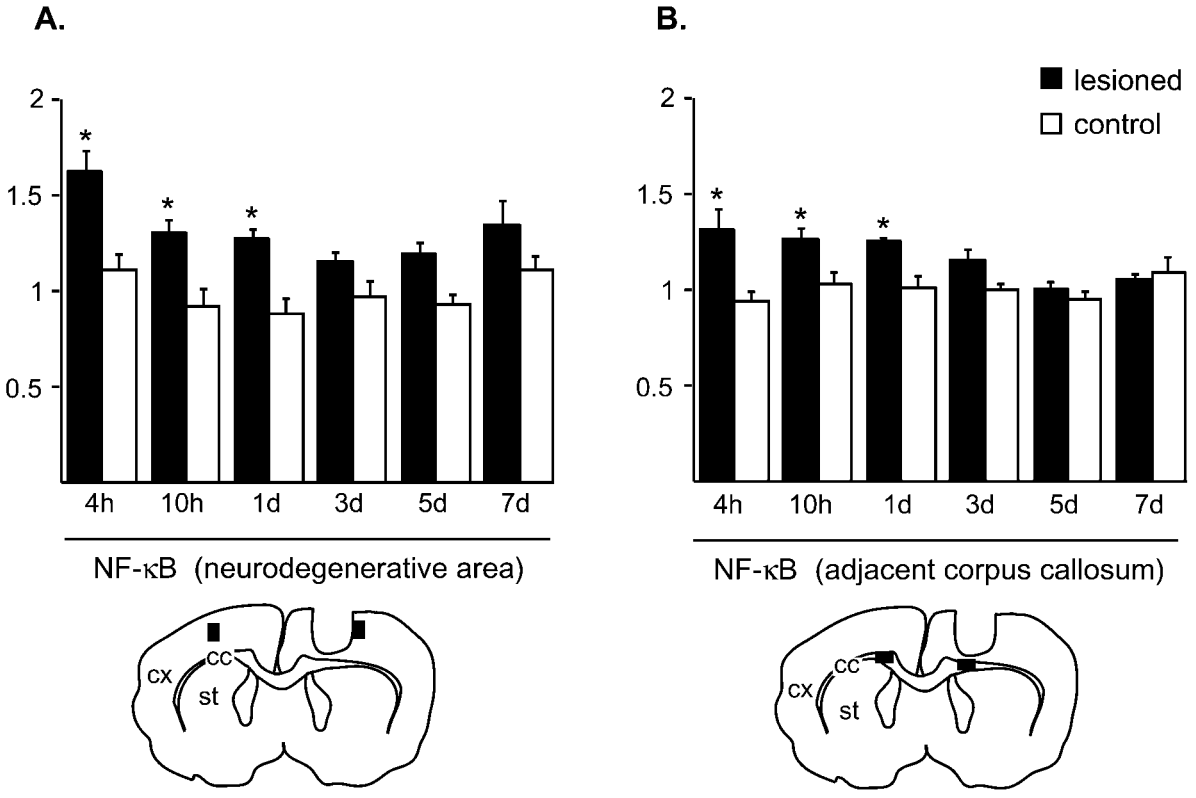

Fig. 2. Quantification of NF-κB immunoreactivity in the neurodegenerating cortical area (**A**) and in the adjacent corpus callosum (**B**) of unlesioned controls (white columns) and lesioned animals (black columns). Data are presented as the mean values of "reactivity grades" ± S.E.M. Significant values are obtained comparing with nonlesioned littermates of each survival time ($*P < 0.05$). Black squares in camera lucida drawings indicate the area analysed (cc, corpus callosum; cx, cortex; st, striatum). NF-κB is significantly increased in lesioned animals from 4 hours until 1 day postlesion, both in the neurodegenerating area (A) and the adjacent corpus callosum (B).

Neuronal NF-κB Activation

In agreement with previous studies both in the adult and the postnatal brain (Acarin et al., 1998, 2000b; Gabriel et al., 1999), neuronal cells in cortical layers and other brain areas showed constitutive NF-κB, which has been involved in synaptic plasticity, neuronal function, and development (O'Neill and Kaltschmidt, 1997; Mattson et al., 2000). Increased activation of constitutive neuronal NF-κB after injury is a common feature in different pathological conditions including trauma, excitotoxicity and ischemia, both in the postnatal and adult brain (Acarin et al., 1998, 2000b; Gabriel et al., 1999; Nonaka et al., 1999), pointing to the activation of this transcription factor as a sensitive marker of neuronal stress. However, the precise role of NF-κB activation in damaged neurons is still unknown. Several studies have proposed that activation of NF-κB could mediate neuronal cell death (Qin et al., 1998; Nakai et al., 2000), but other reports suggest that NF-κB may represent a signalling pathway contributing to protect neurons against damage (Mattson, 1998; Mattson et al., 2000). Candidate mechanisms that may underlie the protective role of NF-κB in neurons include the anti-apoptotic effects mediated by triggering an endogenous caspase inhibitory system, and by inducing the expression of the antioxidant enzyme manganese superoxide dismutase (MnSOD), as well as the calcium-binding protein calbindin D28k (Chu et al., 1997; Mattson et al., 1997; Kaltschmidt et al., 1999; Yu et al., 1999). Thus, modula-

Fig. 1. Micrographs showing hematoxilin-eosin (HE) staining, NF-κB, IκBα, and glial fibrillary acidic protein (GFAP) immunohistochemistry and tomato lectin (TL) histochemistry in the cortex (cx) or corpus callosum (cc) of unlesioned controls (**A,B**) and at 4 hours (**D–F**) and 1 day (**C, G–L**) after the aspiration lesion. Very mild NF-κB immunostaining is observed in the cortex (arrows in A) and the corpus callosum (arrows in B) of control animals. In lesioned animals, the cortical aspiration lesion generated a cavity surrounded by a neurodegenerating area (NA, limited by the black line) just above the corpus callosum (cc). At 4 hours postlesion, increased NF-κB immunoreactivity is found in neuronal cells located in the NA (arrows in D) as well as in glial cells of the cc (E), which show nuclear labelling (arrows in F). Glial NF-κB staining is maintained in the cc at 1 day postlesion (G), although labelling becomes mainly cytoplasmatic (arrow in H). IκBα immunoreactivity is seen in glial cells of the cc at 1 day postlesion (arrows in I), appearing as vesicular cytoplasmatic staining (J). During the time of glial NF-κB and IκBα labelling, hypertrophied GFAP-positive astrocytes (K) and TL-positive reactive microglia/macrophages (L) are found in the cc. Scale bars = 25 μm for A,B,D,E,G,I,K,L,; 200 μm for C; 10 μm for F, H, J.

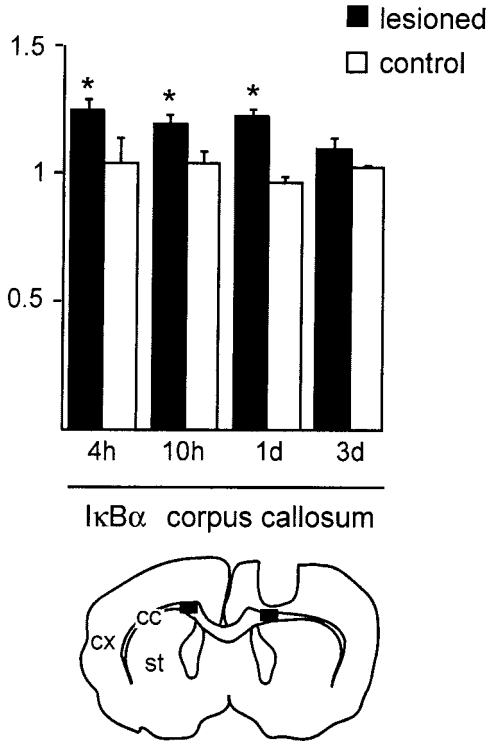

Fig. 3. Quantification of IκBα immunoreactivity in the corpus callosum adjacent to the damaged cortex of unlesioned controls (white columns) and lesioned animals (black columns). Data are presented as the mean values of "reactivity grades" ± S.E.M. Significant values are obtained comparing with nonlesioned littermates of each survival time ($\star P < 0.04$). Black squares in camera lucida drawings indicate the area analysed (cc, corpus callosum; cx, cortex; st, striatum). IκBα is significantly increased in lesioned animals from 4 hours until 1 day postlesion.

tion of the expression of proteins involved in the regulation of cellular antioxidant pathways and calcium homeostasis could provide the mechanisms whereby NF-κB intends to protect against apoptotic cell death. Thus, although it is largely assumed that several types of neural damage is accompanied by NF-κB activation, the objective of this response remains speculative (Lipton, 1997); both the developmental stage and the cellular milieu determined by other simultaneously occurring events are possible influences that may control the balance between NF-κB-induced cell death or survival.

Several studies have described NF-κB activation after several types of traumatic injury in the adult brain; however, to our knowledge, this is the first report on NF-κB induction following postnatal traumatic injury. Interestingly, the better outcome of brain damage during the second week of postnatal life in the rat, attributed to enhanced cortical plasticity and sparing of neural connections (Kolb et al., 1994, 1996), correlates with a faster induction of neuronal NF-κB, occurring within the first 24 hours postinjury. In contrast, trauma to the adult cortex or spinal cord induces neuronal NF-κB activation between 1 and 7 days postinjury, but not at earlier time points (Bethea et al., 1998; Nonaka et al., 1999).

Furthermore, it should be noted that besides inducing or preventing apoptosis of the cell in which is activated, NF-κB may indirectly lead to neurodegeneration by promoting expression of inflammatory mediators that exacerbate tissue damage. Actually, inflammation is now recognised as a key factor in the development of brain damage following neuropathological conditions (Stoll et al., 1998).

Glial NF-κB Activation and IκBα Expression

Cortical traumatic injury induced NF-κB activation mainly in astroglial cells, preceding cell hypertrophy and GFAP overexpression, and being maintained in the long-lasting astrogliosis forming the glial scar. Moreover, a pulse of NF-κB induction was observed in a small subset of reactive amoeboid microglia/macrophages. Of note, different studies in the adult brain have reported predominant and lasting microglial NF-κB in several neuropathological conditions such as ischemia or demyelination (Kaltschmidt et al., 1994; Bonetti et al., 1999; Gabriel et al., 1999). Possibly, the specific brain developmental stage may participate in the modulation of microglial NF-κB activation following injury, as previous studies in our laboratory have shown that excitotoxic damage to the postnatal rat brain also induces a pulse-like activation of microglial NF-κB (Acarin et al., 1998, 2000b).

In contrast to NF-κB activation in neuronal cells, glial NF-κB is commonly related to the induction of inflammatory genes modulated by this transcription factor and produced by reactive glia after brain injury. Among others, NF-κB modulates the expression of the astroglial cytoskeletal protein GFAP, several pro-inflammatory cytokines, major histocompatibility complexes, adhesion molecules, and inflammation-related enzymes such as iNOS and Cox-2 (for review see Baldwin, 1996; O'Neill and Kaltschmidt, 1997; Bauerle, 1998). In this regard, we have recently demonstrated that inhibition of glial NF-κB by pharmacological agents is accompanied by an attenuation of the microglial and astroglial responses and a significative reduction in lesion volume following excitotoxic injury to the postnatal brain (Acarin et al. 2001), thus pointing to glial NF-κB as a key participant in the exacerbation of brain damage after postnatal injury.

Furthermore, it should be noted that although the whole thickness of the cortex was lesioned, astrocytes from the adjacent corpus callosum were the main cell type showing NF-κB activation. This may be explained by the presence of ameboid microglia in the rat corpus callosum during the first postnatal weeks of age (Dalmau et al., 1997), which are well-known sources of pro-inflammatory cytokines and reactive oxygen species in response to injury (Morganti-Kossmann and Kossmann, 1995), and could account for the activation of astroglial NF-κB observed in this white matter tract. Additionally, these corpus callosum reactive astrocytes are the main cell type showing expression of IκBα, the NF-κB endogenous inhibitor. IκBα is one of the NF-κB target genes; therefore, the increase in IκBα we have observed after TBI may represent newly synthesised IκBα, serving as a marker of NF-κB-mediated gene expression. As IκBα is the major repressor of NF-κB

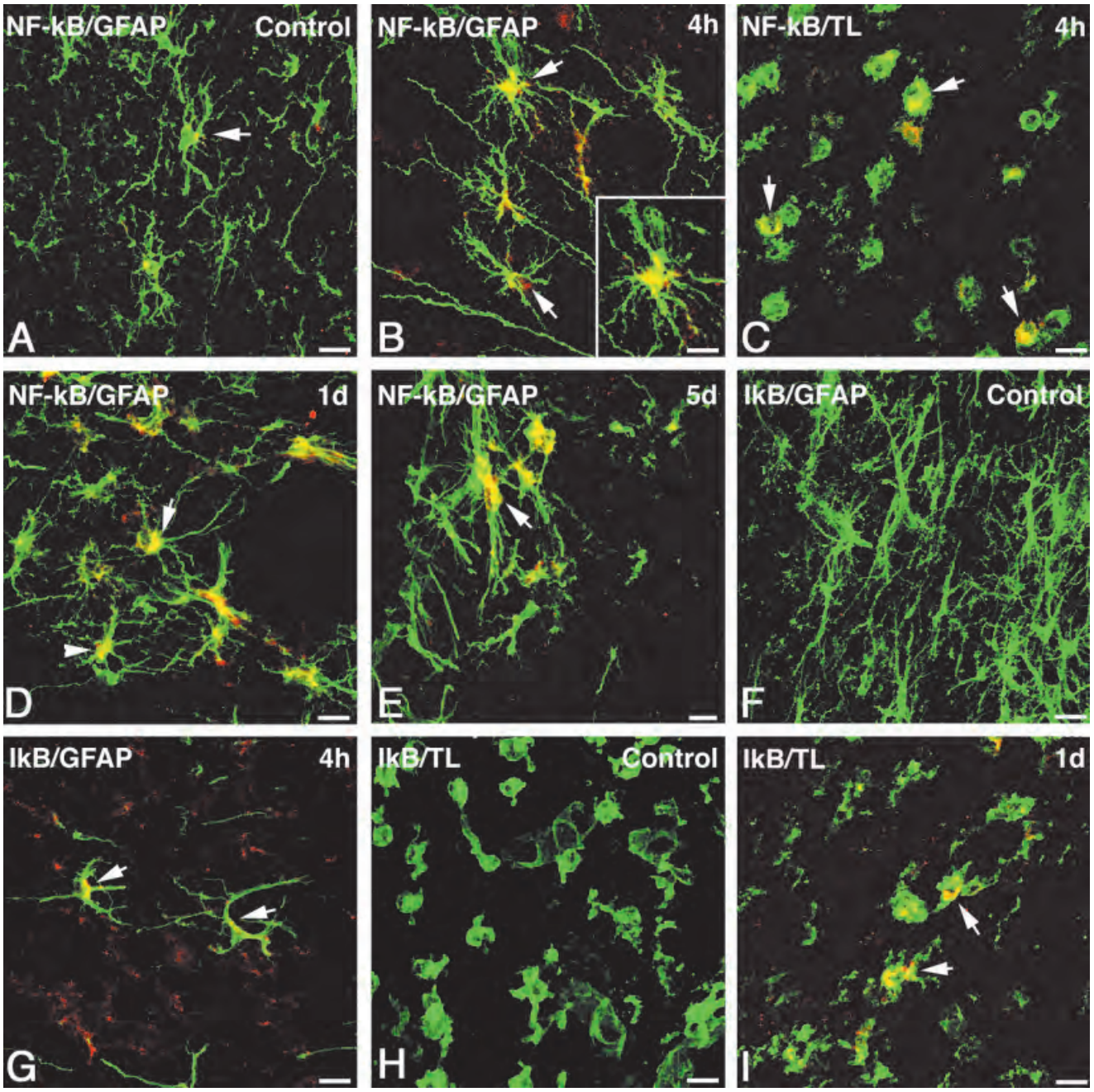

Fig. 4. Identification of NF-κB (A–E) and IκBα (F–I) positive glial cells following cortical damage. NF-κB and IκBα are labelled in red, whereas the glial markers, tomato lectin (TL) for microglia and GFAP for astrocytes, are labelled in green. Yellow areas show colocalisation. Control animals show basal cytoplasmic NF-κB staining in astrocytes located in the corpus callosum (**A**). At 4 hr postlesion (PL; **B**), astroglial NF-κB is found in the nucleus (arrows and **inset**), and some amoeboid microglial cells also showed strong NF-κB immunoreactivity (**C**). At 1 day PL (**D**), astrocytes are strongly positive for NF-κB, and showed both cytoplasmic (arrow) and nuclear (arrowhead) labelling, and at 5 days PL (**E**), NF-κB-immunoreactive astrocytes are observed at the glial scar. No expression of IκBα is observed in control or sham-operated animals, either in astroglial (**F**) or in microglial cells (**H**). Following the lesion, an increase in IκBα immunoreactivity is observed in astrocytes from the corpus callosum (**G**) and microglial cells located in the corpus callosum (**I**) or in the subependymal plate. Scale bars = 16.7 μm; 9.3 μm for inset in B.

(Simeonidis et al., 1999), its upregulation may serve as a negative feed-back loop to modulate NF-κB activity (Baldwin, 1996), implying that the lesion-induced increase of IκBα in astrocytes could be involved in the autocrine modulation of the NF-κB pathway. Interestingly, a recent study has shown that NF-κB binding to the IκBα promoter is extremely fast, being observed immediately after NF-κB entry to the nucleus, and being removed rapidly,

less than 30 minutes after stimulation (Saccani et al., 2001). Thus, it is not strange that IκBα expression in the corpus callosum lasts only for 24 hours, just as long as NF-κB is activated (see Results). Finally, it should be noticed that IκBα was only expressed in glial cells located in the corpus callosum, whereas no IκBα was observed in glial cells forming the glial scar, even when they showed high levels of NF-κB. The reason for this difference in IκBα expression is presently unknown, although functional or physiological differences between white matter and glial scar reactive glial cells, may explain this divergence.

Absence of NF-κB Activation in the Secondarily Affected Thalamus

Although neuronal loss accompanied by microglial and astroglial reactivity were observed in the distally connected thalamus, NF-κB immunoreactivity in this area remained unchanged during all survival times studied. These results are in agreement with earlier studies reporting neither NF-κB activation (Acarin et al., 2000b) nor expression of NF-κB inducers (Holmin et al., 1997; Acarin et al., 2000a) in the corresponding thalamic nuclei following experimental cortical contusion or excitotoxic damage, suggesting that events occurring in the secondarily affected thalamus take place in the absence of an NF-κB-mediated inflammatory response.

It is likely that the lack of both tissue loss and leukocyte recruitment in the affected thalamus provides a much less disturbed environment that induces an attenuated glial response in the presence of low or undetectable levels of proinflammatory mediators, therefore not providing appropriate stimuli in sufficient amounts for NF-κB activation.

In conclusion, the present study demonstrates that following a cortical aspiration lesion in the postnatal rat brain, NF-κB is very rapidly induced, mainly in neuronal cells located in the degenerating area and in astrocytes of the adjacent corpus callosum. Furthermore, white matter reactive astrocytes also show de novo expression of IκBα during the time of NF-κB activation. This study may suggest an important role of this transcription factor pathway in neuronal death/survival mechanisms, as well as in the development of the glial and inflammatory response after TBI in the immature rat brain.

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