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HIV-1 Coat Glycoprotein gp120 Induces Apoptosis in Rat Brain Neocortex by Deranging the Arachidonate Cascade in Favor of Prostanoids

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Abstract: Human immunodeficiency virus type-1 coat glycoprotein gp120 causes delayed programmed cell death (apoptosis) in rat brain neocortex. Here, we investigated the possible role of the arachidonate cascade and membrane peroxidation in this process. It is shown that gp120 causes a rapid increase in the activity and expression of the arachidonate-metabolizing enzyme prostaglandin H synthase, paralleled by increased prostaglandin E_2 levels. The selective inhibitor of prostaglandin H synthase indomethacin inhibited enzyme activity, reduced prostaglandin E_2 content, and partially protected neocortex against gp120-induced apoptosis. Conversely, the activity and expression of the arachidonate-metabolizing enzyme 5-lipoxygenase decreased upon gp120 treatment, as well as the level of its product, leukotriene B_4. Treatment with gp120 also reduced membrane lipid peroxidation, and this may be implicated in the execution of programmed cell death. These results suggest that early derangement of the arachidonate cascade in favor of prostanoids may be instrumental in the execution of delayed apoptosis in the brain neocortex of rats. **Key Words:** Brain—gp120—Apoptosis—Cyclooxygenase—Lipoxygenase—Lipoperoxide.
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Disturbances in the arachidonate cascade have been proposed to play a role in human immunodeficiency virus (HIV) pathogenesis (Genis et al., 1992; Coffey et al., 1996). Arachidonic acid can be converted into biologically active compounds such as prostanoids (i.e., prostaglandins, prostacyclin, thromboxane) or leukotrienes via the cyclooxygenase (prostaglandin H synthase; PHS; EC 1.14.99.1) or the 5-lipoxygenase (arachidonate: oxygen oxidoreductase; 5-LOX; EC 1.13.11.34) pathway, respectively. Both enzymes have prominent roles in human pathophysiology (Ara and Teicher, 1996; Maccarrone et al., 1998*a*; Mayatepek et al., 1999). Recently, we reported that type-1 HIV (HIV-1) coat glycoprotein gp120 causes necrotic death in human neuroblastoma CHP100 cells (Maccarrone et al., 1998*b*). The main features of gp120-induced necrosis were activation of cyclooxygenase and lipoxygenase pathways by up-regulation of both PHS and 5-LOX activity and expression and enhanced membrane lipid peroxidation (Maccarrone et al., 1998*b*). On the other hand, HIV-1 coat glycoprotein gp120 has been shown to cause apoptosis (programmed cell death; PCD) in the brain neocortex of rats treated with this coat protein for 7 consecutive days (Bagetta et al., 1996*a*,*b*). This type of PCD may be implicated in the neuronal loss often described postmortem in the brain of patients suffering from AIDS-associated neurological syndrome (Bagetta et al., 1999). The synthesis of prostanoids (Bagetta et al., 1998) and the enhanced expression of interleukin-1β (Bagetta et al., 1999) seem to be involved in the execution of gp120-induced PCD in the rat brain; however, the underlying mechanism(s) remain(s) to be elucidated. Derangement of the arachidonate cascade seems instrumental for the execution of apoptotic program(s) induced by unrelated stimuli in different cell lines. Activation (O'Donnell et al., 1995; Aoshima et al., 1997; Maccarrone et al., 1997) or depression (Tang and Honn, 1997; Tang et al., 1997) of the lipoxygenase pathway may be involved in this process. Also, proinflammatory prostanoids may contribute to brain injury (Nogawa et al., 1998) and may

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Abbreviations used: BSA, bovine serum albumin; FLAP, 5-lipoxygenase-activating protein; GAR-AP, goat anti-rabbit polyclonal antibodies conjugated with alkaline phosphatase; HIV, human immunodeficiency virus; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 5-LOX, 5-lipoxygenase; PCD, programmed cell death; PHS, prostaglandin H synthase; RP, reverse-phase; TdT, terminal deoxynucleotidyl transferase; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling.

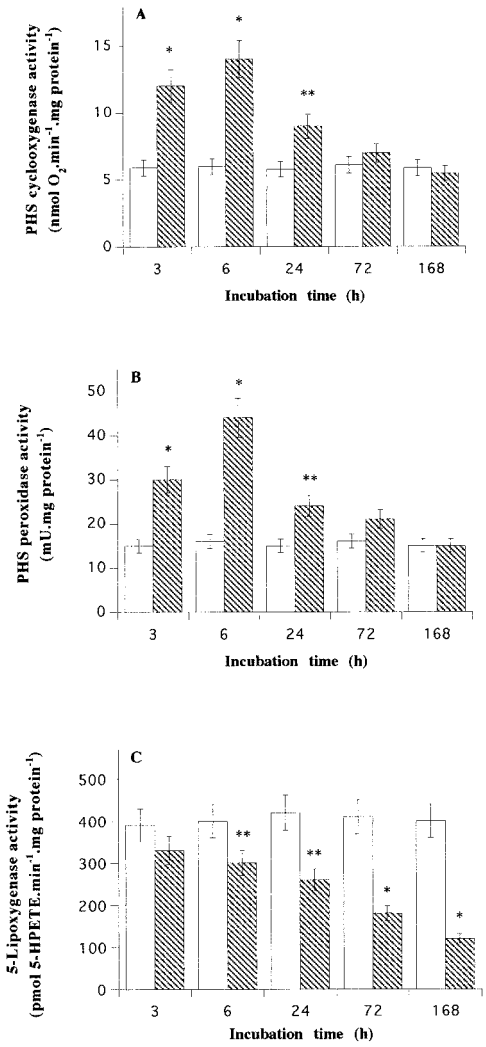

FIG. 1. Time course of PHS and 5-LOX activity in rat brain neocortex. **A** and **B:** PHS cyclooxygenase (A) and peroxidase (B) activity in the neocortex of rats after injection of BSA (open columns) or gp120 (hatched columns). **C:** 5-LOX activity in the same samples as in A and B. Vertical bars represent SD values. $^{*}p < 0.01$, $^{**}p < 0.05$ compared with BSA-treated samples (n = 6). The other differences between treated and untreated animals were not significant ($p > 0.05$).

force cells into PCD (Vierk et al., 1998; Bishop-Bailey and Hla, 1999; Rueda et al., 1999). Here, the possible roles of the arachidonate cascade and membrane lipoperoxidation in gp120-induced apoptosis in rat brain were investigated to elucidate at least in part the mechanism of HIV cytotoxicity and to shed some light on the biochemical differences between gp120-induced in vitro necrosis and in vivo apoptosis.

MATERIALS AND METHODS

Materials

All chemicals were of the purest analytical grade. Arachidonic (eicosatetraenoic) acid, ATP, hemin, phenol, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD), and indomethacin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Endotoxin-free, full-length glycosylated recombinant HIV-1 glycoprotein gp120 IIIB (from baculovirus expression system; >90% pure by sodium dodecyl sulfate–polyacrylamide gel electrophoresis) was purchased from Intracel Corp. (Cambridge, MA, U.S.A.). Anti-5-LOX and anti-5-LOX-activating protein (anti-FLAP) rabbit polyclonal antibodies were a kind gift from Dr. A. W. Ford-Hutchinson (Merck Frosst Centre for Therapeutic Research, Canada). Anti-cyclooxygenase-2 rabbit polyclonal antibodies were from Cayman Chemical Co. (Ann

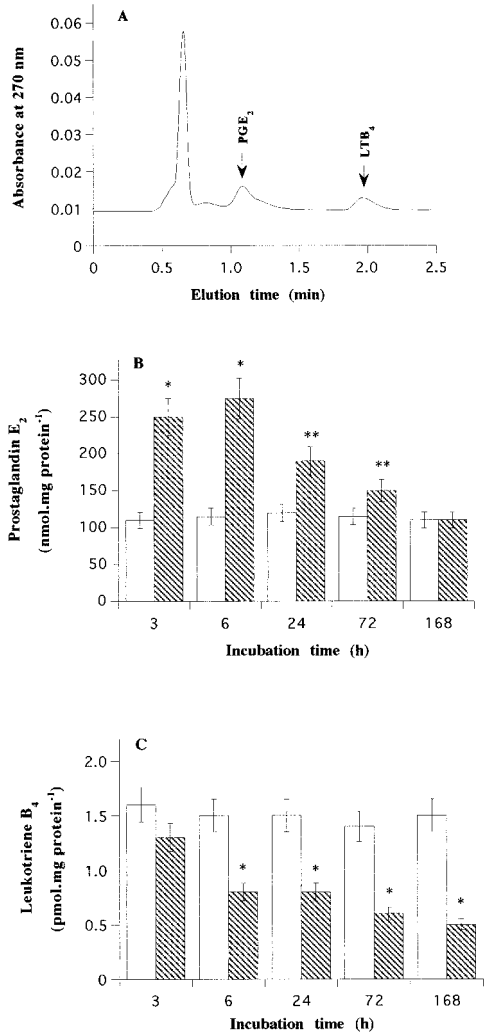

FIG. 2. Changes in prostaglandin E_2 and leukotriene B_4 in rat brain neocortex. **A:** Representative RP-HPLC chromatogram of the arachidonate metabolites prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) extracted from rat brain cortex (500 μg of proteins). Arrows show the elution times for the indicated authentic standards. **B:** Levels of prostaglandin E_2 in the neocortex of rats after injection of BSA (open columns) or gp120 (hatched columns). **C:** Levels of leukotriene B_4 in the same samples as in B. Vertical bars represent SD values. $^{*}p < 0.01$, $^{**}p < 0.05$ compared with BSA-treated samples (n = 6). The other differences between treated and untreated animals were not significant ($p > 0.05$).

TABLE 1. *Effect of gp120 on expression of PHS-2, 5-LOX, and FLAP in rat brain neocortex*

Sample	PHS-2 content at 6 h (A_{405} value)	5-LOX content at 168 h (A_{405} value)	FLAP content at 168 h (A_{405} value)
Cortex + BSA	0.354 ± 0.040 (100)	0.250 ± 0.030 (100)	0.336 ± 0.040 (100)
Cortex + gp120	0.779 ± 0.080 (220)[a]	0.125 ± 0.013 (50)[a]	0.168 ± 0.017 (50)[a]

Values in parentheses represent percentage of the BSA-treated samples.
[a]$p < 0.01$ compared with BSA-treated samples (n = 6).

Arbor, MI, U.S.A.), whereas goat anti-rabbit polyclonal antibodies conjugated with alkaline phosphatase (GAR-AP) were purchased from Bio-Rad (Richmond, CA, U.S.A.). Authentic prostaglandin E_2 and leukotriene B_4 were from Cayman Chemical Co.

Animal model

Male Wistar rats weighing 250–280 g (Morini, San Paolo D'Anza, Italy), housed in a temperature (22°C)- and humidity (65%)-controlled colony room, were anesthetized with chloral hydrate (400 mg/kg i.p.) for chronic implantation of a guide cannula (25-gauge) into one lateral cerebral ventricle (intracerebroventricularly) under stereotaxic guidance, as previously described (Bagetta et al., 1996*a*). The animals were allowed 4 days of recovery before treatment; then a single dose (100 ng) of gp120 or bovine serum albumin (BSA) as a control was administered daily for up to 7 consecutive days to each individual rat. Injections were performed with a 5-μl Hamilton syringe (1- to 2-μl volume; 1-μl/min rate) connected via a Teflon tube to an injector that exceeded by 2 mm the length of the guide cannula. In the antagonism study, daily intracerebroventricular injection of gp120 was preceded (1 h beforehand) by systemic administration of indomethacin (6.0 mg/kg i.p.). At the end of the treatment, the animals were killed, the brain was removed from the skull, and the cortex was separated, immediately immersed in liquid nitrogen, and stored at −80°C until use. The experimental procedures used met the guidelines of the Ministry of Health (G.U. no. 40, February 18, 1992) and were approved by the Animal Care Committee of the University of Rome "Tor Vergata."

Analysis of arachidonate metabolites and membrane lipid spectra

Arachidonate metabolites prostaglandin E_2 and leukotriene B_4 were extracted from brain cortex on octadecyl-SPE (solid phase extraction) columns (Baker, Deventer, The Netherlands) and were analyzed by reverse-phase (RP) HPLC on a C18 3 × 3 CR column (SGE, Austin, TX, U.S.A.) (Jakobsson et al., 1992). RP-HPLC was performed on a Perkin Elmer 1022 LC Plus liquid chromatograph (Norwalk, CT, U.S.A.) at a flow rate of 1.2 ml/min, using methanol/water/trifluoroacetic acid (70:30:0.07 by vol) as mobile phase. Chromatograms were recorded at 270 nm, assessing peak identity by comparison with authentic standards. Quantitative determinations were performed by integrating peak areas of each compound.

Membrane lipids were isolated from control and gp120-treated brain cortex as reported (Maccarrone et al., 1998*b*); then absorption spectra were recorded in the wavelength range of 200–300 nm to measure the oxidative index, that is, the $A_{234/205}$ ratio (Kühn et al., 1990). Spectra were recorded at room temperature in a UV-VIS spectrometer Lambda 18 (Perkin Elmer).

Assay of enzyme activity and expression

PHS is a dual enzyme possessing cyclooxygenase and peroxidase activity on the same protein molecule (Eling et al., 1990). Cyclooxygenase activity was measured polarographically at 30°C in 0.1 *M* potassium phosphate (pH 7.2), 1 m*M* phenol, and 75 μM arachidonic acid (Kulmacz and Wang, 1995) and was expressed as nanomoles of O_2 consumed per minute per milligram of protein. Peroxidase activity was assayed spectrophotometrically at 30°C in 0.1 *M* Tris-HCl (pH 8.1), 170 μM TMPD, 1 μM hemin, and 75 μM arachidonic acid (Gierse et al., 1995) and was expressed as milliunits per milligram of protein (1 U = amount of enzyme causing a change of 1 A_{590} U during 5-min incubation with the substrate). The activity of 5-LOX in rat brain neocortex was measured by incubating tissue homogenates for 10 min at 37°C in the presence of 1 m*M* ATP, 2 m*M* $CaCl_2$, and 40 μM arachidonic acid (Jakobsson et al., 1992). 5-LOX activity was expressed as nanomoles of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) formed per minute per milligram of protein. Protein concentration of cell extracts was determined as reported (Bradford, 1976).

ELISAs of brain cortex extracts, prepared according to Sun and Funk (1996), were performed using 25 μg of protein/well as reported (Maccarrone et al., 1998*c*). The following rabbit polyclonal antibodies were used as first antibody: anti-5-LOX (diluted 1:200), anti-FLAP (diluted 1:300), and anti-cyclooxygenase-2 (anti-PHS-2; diluted 1:400). GAR-AP were used as

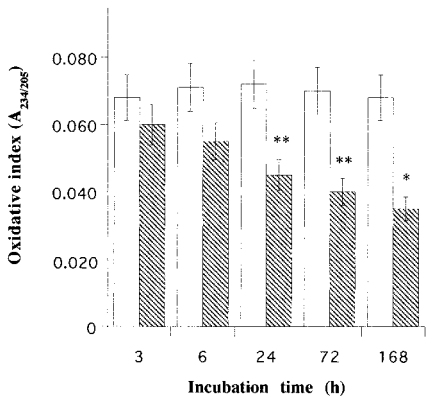

FIG. 3. Oxidative index of membrane lipids of rat brain neocortex. The oxidative index (i.e., $A_{234/205}$ ratio) was calculated after injection of BSA (open columns) or gp120 (hatched columns). Vertical bars represent SD values. *$p < 0.01$, **$p < 0.05$ compared with BSA-treated samples (n = 6). The other differences between treated and untreated animals were not significant ($p > 0.05$).

TABLE 2. *Effect of indomethacin on PHS cyclooxygenase activity, prostaglandin E_2 and leukotriene B_4 levels, and induction of PCD in brain neocortex of rats*

Parameter	Cortex + BSA		Cortex + gp120	
	−INDO	+INDO	−INDO	+INDO
PHS cyclooxygenase activity at 6 h (nmol of O_2/min/mg of protein)	5.5 ± 0.5 (100)	3.0 ± 0.3 (60)[a]	14.5 ± 1.5 (264)[b]	6.5 ± 0.7 (118)[c,d]
Prostaglandin E_2 at 6 h (nmol/mg of protein)	110 ± 10 (100)	72 ± 7 (65)[a]	275 ± 28 (250)[b]	135 ± 14 (123)[c,d]
Leukotriene B_4 at 6 h (pmol/mg of protein)	1.5 ± 0.2 (100)	2.5 ± 0.3 (167)[b]	0.7 ± 0.1 (47)[b]	1.2 ± 0.1 (80)[c,d]
PCD at 168 h (no. of TUNEL-positive cells)	0.8 ± 0.3 (100)	0.8 ± 0.3 (100)[c]	3.9 ± 0.3 (487)[b]	1.8 ± 0.5 (225)[d]

Values in parentheses represent percentage of the BSA-treated samples (controls). INDO, indomethacin (6.0 mg/kg i.p.).
[a] $p < 0.05$ compared with control −INDO.
[b] $p < 0.01$ compared with control −INDO.
[c] $p > 0.05$ compared with control −INDO.
[d] $p < 0.01$ compared with gp120-treated animals −INDO (n = 4 in all cases).

second antibody (diluted 1:2,000). Color development of the alkaline phosphatase reaction was followed at 405 nm.

Determination of PCD

Twenty-four hours after the last injection, rats were anesthetized and perfused through the left ventricle of the heart with 60 ml of heparinized saline solution followed by 200 ml of paraformaldehyde (4%) dissolved in phosphate-buffered saline. Two hours later, rat brain was removed from the skull, postfixed overnight in 4% paraformaldehyde, cryoprotected in sucrose (30%), immersed in nitrogen, and stored at −80°C until use (Bagetta et al., 1999). Cryostat brain tissue coronal sections (14 μm) were mounted on polylysine-coated slides and processed for in situ analysis of DNA fragmentation according to the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) modified method (Bagetta et al., 1995). Adjacent brain tissue sections were stained with hematoxylin and eosin. TUNEL-positive cells were counted in an area (1,161 mm^2) of the brain tissue section (n = 6/brain) corresponding to the neocortex (Paxinos and Watson, 1982). The resulting means were evaluated statistically for differences using ANOVA followed by Tukey–Kramer test for multiple comparisons.

Statistical analysis

Data reported in this article are the means ± SD of four to six independent determinations, each performed in duplicate. Statistical analysis was performed by the Student's *t* test, elaborating experimental data by means of the InStat program (GraphPad Software for Science, San Diego, CA, U.S.A.).

RESULTS

Treatment with a single intracerebroventricular dose (100 ng) of gp120 increased the cyclooxygenase activity of PHS in a time-dependent manner, reaching statistical significance compared with the BSA-injected control ($p < 0.01$; n = 6) 3 h after a single injection (Fig. 1A). A further increase up to threefold the control level was observed in the following 3 h; then PHS cyclooxygenase activity rapidly declined (Fig. 1A). Peroxidase activity of PHS had the same profile as the cyclooxygenase activity in the neocortex of gp120- or BSA-injected animals (Fig. 1B). On the other hand, Fig. 1C shows that 5-LOX activity decreased upon treatment of rats with gp120, reaching statistical significance 6 h after gp120 injection ($p < 0.05$; n = 6) and a minimum 162 h later (from 400 ± 40 to 100 ± 10 pmol/min/mg of protein). Under these experimental conditions, BSA (100 ng) did not significantly change PHS and 5-LOX activity (Fig. 1). RP-HPLC allowed separation of prostaglandin E_2 and leukotriene B_4 in rat brain extracts (Fig. 2A), yielding a pattern similar to that already reported (Jakobsson et al., 1992). Chromatographic analysis indicated that gp120 increased prostaglandin E_2, the main product of PHS in vivo (Kulmacz and Wang, 1995), by approximately threefold within 6 h after the first injection (Fig. 2B). Conversely, the intracellular levels of leukotriene B_4, the main product of 5-LOX (Ford-Hutchinson et al., 1994), decreased in the brain cortex of gp120-treated animals in a way fully analogous to that observed with 5-LOX activity, leveling off at ~30% of the control after 168 h (Fig. 2C). Again, injection of BSA did not significantly affect the intracellular concentration of either eicosanoid (Fig. 2). Consistently with the data on enzyme activity, quantitation of PHS and 5-LOX expression by ELISA showed an increase in PHS content 6 h after gp120 injection and a decrease in 5-LOX content 168 h after gp120 treatment compared with the BSA-treated controls (Table 1). Remarkably, the expression of the FLAP, which translocates 5-LOX to the nucleus (Jakobsson et al., 1995), was also decreased in gp120-treated animals to the same extent as 5-LOX (Table 1).

Intracerebroventricular injection of the HIV-1 coat glycoprotein gp120 in the neocortex led also to a significant decrease in membrane lipid peroxidation, as shown in Fig. 3 by the decrease in the oxidative index (i.e., the $A_{234/205}$ ratio) (Kühn et al., 1990). Such a decrease was significant ($p < 0.05$; n = 6) after 24 h of gp120 treatment, and at 168 h it reached 50% of the BSA-treated control value (from 0.072 ± 0.008 to 0.036 ± 0.004). BSA injection under the same experimental conditions did not significantly affect the oxidative index value (Fig. 3). It is noteworthy that the time course of the oxidative index in gp120-treated rats followed the same trend as the 5-LOX activity in the same animals (cf. Figs.

1C and 3). Indeed, the major contributors to the spectrum in the region around 230 nm are the conjugated hydroperoxides generated by lipoxygenase activity (Kühn et al., 1990; Maccarrone et al., 1998*b*).

Treatment of rats with the PHS inhibitor indomethacin (Kurumbail et al., 1996) markedly inhibited the PHS cyclooxygenase activity measured after 6 h, from 264 to 118% of the BSA-treated controls (Table 2). Consistently, the level of prostaglandin E_2 at 6 h decreased upon indomethacin administration from 250 to 123% (Table 2). In addition, indomethacin increased (~65% over the control) the levels of leukotriene B_4, both in BSA- and gp120-treated animals (Table 2). Interestingly, also the number of apoptotic cells observed after 168 h in the brain neocortex was markedly reduced by co-injection of indomethacin (6.0 mg/kg), from 487 to 225% of the BSA-treated animal values (Table 2). A typical example of the neuroprotection afforded by indomethacin is shown in Fig. 4.

DISCUSSION

The present experiments demonstrate that intracerebroventricular injection of HIV-1 coat glycoprotein gp120 causes a rapid derangement of the arachidonate cascade in favor of prostanoids in the brain neocortex of rats. The amount of gp120 used in this study (100 ng/dose) was in the same range as that detected in the brain of rat pups treated systemically with the viral protein, which also caused retardation of behavioral development and a pattern of brain damage similar to that found post mortem in the brain of AIDS patients (Hill et al., 1993). PHS is a dual enzyme, showing cyclooxygenase and peroxidase activity on the same molecule (Eling et al., 1990). Both enzyme activities of PHS were assayed, because they catalyze two different steps in prostanoid biosynthesis (Gierse et al., 1995; Kulmacz and Wang, 1995). Injection of gp120 caused a rapid increase in PHS cyclooxygenase (Fig. 1A) and peroxidase (Fig. 1B) activities, leading to a concomitant increase in the PHS product prostaglandin E_2 (Fig. 2B). Interestingly, these were early events along the pathway leading to PCD, which became evident only 7 days later (Bagetta et al., 1996*a*,*b*, 1998). At this time point, PHS activity (Fig. 1A and B) and content (not shown) and prostaglandin E_2 (Fig. 2B) had returned to the control level, suggesting that they were no longer needed to sustain the execution of the apoptotic program (Maccarrone et al., 1997). Recently, we reported immunochemistry data showing that the levels of prostaglandin E_2 were not significantly increased 7 days after treatment of rats with gp120 compared with the BSA-treated controls (Bagetta et al., 1998). In view of our present results, these data can be rationalized in terms of lack of availability of the arachidonate substrate, which is rapidly consumed by PHS activity, enhanced within 6 h. Up-regulation of PHS was linked to gp120-induced PCD by a cause–effect relationship, because the specific PHS inhibitor indomethacin (a) inhibited enzyme activity, (b) reduced prostaglandin E_2 content, and (c) minimized gp120 cytotoxicity (Table 2). The indomethacin-induced increase in leukotriene B_4, both in BSA- and in gp120-treated animals (Table 2), might be due to a larger availability of the arachidonate substrate for 5-LOX activity. gp120 significantly enhanced the expression of the type 2 isoenzyme of PHS (cyclooxygenase-2 or PHS-2), which is the most abundant form in the mammalian brain (Miettinen et al., 1997; Nakayama et al., 1998). Recently, the level of PHS-2 expression has been reported to reflect the level of activity of the excitatory glutamate-mediated neurotransmission (Nakayama et al., 1998), and spreading depression and focal brain ischemia, both implicating elevation of synaptic glutamate, have been shown to cause a rapid increase in PHS-2 expression in the brain cortex of rats (Miettinen et al., 1997). Moreover, PHS-2 inhibition prevents excitotoxic glutamate-mediated death of CA1 hippocampal neurons (Nakayama et al., 1998). Therefore, it is tempting to suggest that early alteration of the arachidonate cascade through rapid activation of PHS might trigger elevation of synaptic glutamate (Bezzi et al., 1998), leading neurons to apoptosis. In line with this concept, protection against gp120-induced PCD was afforded by competitive and noncompetitive NMDA receptor antagonists (Corasaniti et al., 1999).

As far as the down-regulation of 5-LOX is concerned, the observation that it was paralleled by a similar decrease of FLAP expression (Table 1) speaks in favor of a nuclear activity of the enzyme, because FLAP translocates 5-LOX to the nucleus (Jakobsson et al., 1995). This seems of particular interest in a process like apoptosis, which involves dramatic nuclear changes (Fadok et al., 1998; Ren and Savill, 1998). However, 5-LOX might play its role in gp120-induced PCD also at the plasma membrane level, where a reduced oxidative index (Fig. 3) was observed, an established marker of lipoxygenase-mediated lipoperoxidation (Kühn et al., 1990; Maccarrone et al., 1998*b*). HIV-1 entry into the cell occurs via a fusion mechanism (Chan and Kim, 1998; Santos et al., 1998), and lower lipoperoxidation may lead to higher membrane rigidity (Maccarrone et al., 1997, 1998*c*). This change in membrane properties might be involved in the interaction of gp120 with membrane lipids such as the primary receptor of the viral protein galactosylceramide (Delezay et al., 1997) or lipid moieties of secondary receptors (Banks et al., 1998). Moreover, leukotrienes may exert a physiological action by interacting with somatostatin-mediated signaling in the brain (Uz et al., 1997). Arachidonate products have been also found to participate in the regulation of cerebral vascular tone (Rzigalinski et al., 1999). Thus, reduction of 5-LOX, hence of the product leukotriene B_4, might play a role in these networks. Moreover, it should be recalled that leukotriene B_4 receptors have been recently found to share significant sequence homology with chemokine receptors that act as HIV-1 co-receptors (Martin et al., 1999). It is tempting to suggest that 5-LOX down-regulation might take part in gp120-induced PCD also by directly affecting the interaction of the protein with these

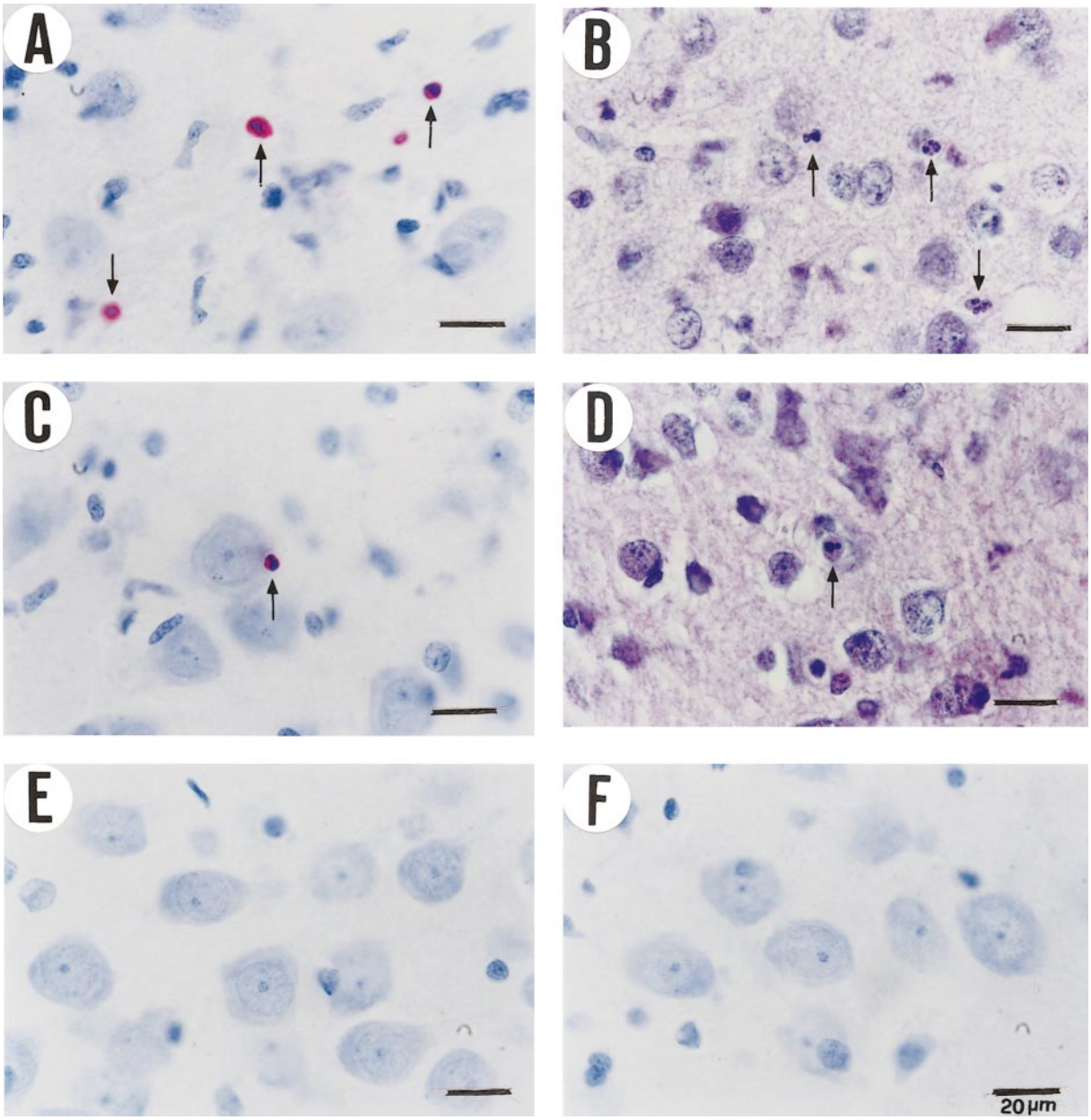

FIG. 4. Typical example of the neuroprotection afforded by systemic administration of indomethacin in a rat pretreated with gp120. In situ nick end-labeling of brain tissue hemisections (14 mm thick) of a rat treated with a single daily dose of gp120 (100 ng) given intracerebroventricularly for 7 consecutive days. Note in **A** the presence of TUNEL-positive cells (see arrows) in the brain cortex ipsilateral to the side of intracerebroventricular injection of gp120. The microphotograph in **B** shows DNA fragmentation and condensation (see arrows), typical hallmarks of apoptosis, in a tissue hemisection adjacent to the one in A but stained with hematoxylin and eosin. Systemic administration of indomethacin (6 mg/kg i.p. given 1 h before gp120) reduced the number of TUNEL-positive cells in **C** (arrow) and of cells bearing typical features of apoptosis in **D** (arrow) (hematoxylin and eosin). Lack of TUNEL-positive cells is evident in **F,** showing a tissue hemisection adjacent to the one in A and incubated in the absence of TdT for negative control (see Materials and Methods). Similarly, no TUNEL-positive cells are evident in **E,** showing a tissue hemisection obtained from a rat treated with BSA (100 ng given intracerebroventricularly once daily for 7 consecutive days) for control.

receptors. Finally, it seems noteworthy that gp120-induced apoptosis in vivo showed a marked reduction in lipoxygenase-related membrane lipoperoxidation (Fig. 3), whereas gp120-induced necrosis in vitro has been reported to enhance this process (Maccarrone et al., 1998*b*). This observation gives a first biochemical background to the difference between necrosis and apoptosis induced by the same factor, an issue as yet poorly understood (Fadok et al., 1998; Ren and Savill, 1998; Vercammen et al., 1998).

In conclusion, results reported here clearly show that the induction of PCD in rat brain neocortex by HIV-1

coat glycoprotein gp120 involves an early derangement of the arachidonate cascade in favor of prostanoids. The apoptotic program is characterized by reduced lipoperoxidation at the plasma membrane level, which reflects down-regulation of lipoxygenase.

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