Platelet-activating Factor Receptor Activation

AN INITIATOR STEP IN HIV-1 NEUROPATHOGENESIS*

(Received for publication, February 12, 1998, and in revised form, April 14, 1998)

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Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system results in neuronal
apoptosis. Activated HIV-1-infected monocytes secrete high levels of the proinflammatory cytokine tumor ne-
crosis factor-α (TNF-α) and the phospholipid mediator platelet-activating factor (PAF). TNF-α and PAF are ele-
vated in the central nervous system of patients with HIV-1-associated dementia. We now demonstrate that condi-
tioned media from activated HIV-1-infected monocytes induces neuronal apoptosis, which can be prevented by
co-incubation with PAF acetylhydrolase, the enzyme that catabolizes PAF in the central nervous system. Preceding
apoptosis is a TNF-α-induced increase in neuronal ceramide levels. TNF-α-mediated neuronal apoptosis can also be
blocked by co-incubation with PAF acetylhydrolase, or a PAF receptor antagonist. Blocking pathologic activation
of PAF receptors may therefore be a pivotal step in the treatment of HIV-1-associated dementia.

There is a consensus that the neuropathogenesis of HIV-1-associated dementia (HIV-D) is initiated by productively
infected and antigenically activated brain-resident macrophages and microglia. However, there is not a good correlation between
viral burden in the central nervous system and neurologic disease, which is presumably secondary to neuronal dysfunc-
tion and death. Recently, the extent of neurologic dysfunction (i.e. dementia) has been correlated with the total number of
macrophages and activated microglia in the brain parenchyma, rather than the number of HIV-1-infected macrophages and
microglia (1). Because direct infection of neurons with HIV-1 is unlikely, these findings suggest that neuronal dysfunction and
death are mediated by soluble factors released by macrophages and microglia. We and others have demonstrated that HIV-1-
infected monocytes, when activated by antigenic stimuli in vitro or contact with neural cells in vivo, release high levels of the
proinflammatory cytokine tumor necrosis factor-α (TNF-α) and the phospholipid mediator platelet-activating factor (PAF) (2, 3).

TNF-α markedly up-regulates HIV-1 production in HIV-1-infected macrophages (4). PAF, in turn, appears to up-regulate
TNF-α synthesis in HIV-1-infected cells of monocytic lineage (5). Importantly, the number of macrophages expressing
mRNA for TNF-α is elevated in the brains of patients with HIV-D, compared with patients with HIV-1 infection but with-
out dementia (6). PAF is elevated in cerebrospinal fluid of patients with HIV-1-associated dementia and immunosuppres-
sion (7). We have previously demonstrated in both primary human neuronal cultures and in a differentiated human neu-
ronal cell line that TNF-α and PAF induce dose-dependent apoptosis (8–10). Taken together, these studies suggest that
reciprocal relationships between TNF-α and PAF exist, and that TNF-α and PAF play a major role in the pathogenesis of
HIV-D. Nevertheless, the mechanisms for TNF-α- and PAF-mediated neuronal apoptosis in HIV-D remain elusive. Because
little is known about how TNF-α and PAF interact in neuronal cultures, we investigated cellular pathways pertinent to
TNF-α- and PAF-mediated signal transduction that results in neuronal apoptosis.

One key element in this relationship may be the sphingomyelin ceramide, a probable second messenger of TNF-α. Activat-
on of monocytic p55 receptors by TNF-α is thought to result in the sphingomyelinase-mediated production of ceramide (11),
which has itself been shown to induce apoptosis (12). Consequently, we examined the temporal interrelationship of TNF-α,
ceramide, and PAF in inducing apoptotic neuronal cell death. Knowing that TNF-α induces dose-dependent apoptosis in pri-
mary human neuronal cultures and a differentiated human neuronal cell line (7–10), we first investigated whether TNF-α
induces time-dependent apoptotic cell death in primary human neuronal cultures. Next, we measured ceramide levels in a
human neuronal cell line as affected by varying lengths of TNF-α or PAF exposure. Having established both time-depend-
ent TNF-α-induced neurotoxicity and time-dependent TNF-α-
induced neuronal ceramide production, and because PAF recep-
tor antagonism can down-regulate TNF-α-mediated induction of HIV-1 in a monocytic cell line (5), we investigated whether a reciprocal relationship existed between TNF-α, cer-
amide, and PAF in the brain, i.e. if PAF receptor antagonism or
catabolism of PAF would affect TNF-α- and ceramide-mediated
toxicity of primary human neuronal cultures. Finally, to assess the relative contribution of PAF to HIV-1-induced neurotoxic-
ity, we used conditioned media from activated HIV-1-infected macrophages, co-incubated with PAF-acetylhydrolase (PAF-
AH), the principle catobolic enzyme for PAF.
**PAF Receptor-mediated Neuronal Apoptosis in HIV-1 Infection**

**Fig. 1.** TNF-α-induced neuronal cell death as a function of time. Cell death was assayed by a double stain for membrane permeability (trypan blue) and DNA fragmentation (TUNEL method, red chromagen) (13). Primary human neuronal cultures exposed to vehicle (A) or 1 ng/ml TNF-α for increasing periods of time (30 min (B), 3 h (C), and 18 h (D)) showed a time-dependent increase in neuronal apoptosis characterized primarily by DNA fragmentation and intact membranes. Representative 122× fields were photographed using Nomarski optics on an upright microscope. Experiment was replicated three times with similar results.

**EXPERIMENTAL PROCEDURES**

**Primary Neuron Cultures**—Primary human neurons were cultured from human fetal brain tissue obtained from second trimester elective therapeutic abortions in an ethical manner and in strict observation of the guidelines of the NIH and the University of Rochester. Neurons were obtained from the telencephalon with both cortical and ventricular surfaces of second trimester (13–16 weeks of gestation) human fetal brain tissue, explanted, and cultured as described previously (7). Under these conditions, cultures were comprised of 60–70% neurons, 20–30% astrocytes, ~10% macrophages and microglia, and no oligodendrocytes (7).

**Monocyte Cultures and HIV-1 Infection**—Monocytes were recovered from peripheral blood mononuclear cells of HIV- and hepatitis B-seronegative donors after leukapheresis, purified (>98%) by countercurrent centrifugal elutriation, and cultured as described previously with macrophage-colony stimulating factor (2, 3). Under these conditions, monocytes developed elongated processes and were immunoreactive for CD68; consistent with differentiation into macrophages cultures. 7–10 days after culturing with macrophage-colony stimulating factor, macrophages were exposed to HIV-1 (accession no. M60472) at a multiplicity of infection of 0.01 infectious virions/target cell as described previously (2, 3). The viral inocula were free of mycoplasma contamination (Mycoplasma Detection Kit III, Geneprobe, San Diego, CA). Under these conditions, 20–50% of the monocytes were infected 7 days after HIV-1 inoculation. This was determined by immunofluorescent and *in situ* hybridization techniques. All cultures were treated with fresh medium every 2–3 days. Reverse transcriptase activity for HIV-1 was determined in culture fluids as described previously (2, 3). Five to seven days after HIV-1 infection and during the peak of reverse transcriptase activity (1 × 10⁷ cpm/ml), cultures of HIV-1-infected and parallel cultures of uninfected monocytes were stimulated with bacterial lipopolysaccharide (10 ng/ml) (Genzyme, Cambridge, MA), cPAF, a non-hydrolyzable analog of PAF that is approximately 1/10 as potent (250 ng/ml) (Biomol, Plymouth Meeting, PA), C2 ceramide (10 μM) (Biomol, Plymouth Meeting, PA), or vehicle, then harvested in trypsin. Total cellular lipids were extracted via the Bligh and Dyer (14) method, then dried under nitrogen and resuspended in 100 μl of chloroform; 20 μl were used for phosphate measurement, and 80 μl for ceramide measurement via the dia-cyclyglycerol kinase assay, both performed as described previously (15). Ceramide mass was quantitated using external standards, normalized to total phosphate, and was measured as picomoles/nmol of phosphate.

**PAF Antagonism and Catabolism**—Primary human neuronal cultures were coincubated for 24 h with TNF-α (10 ng/ml) and/or the metabolically stable hexetazine PAF receptor antagonist WEB 2086 (10 μM); C2 ceramide (10 μM) and/or WEB 2086 (10 μM); TNF-α (10 ng/ml) and/or PAF-AH (50 μg/ml); and conditioned medium from activated HIV-1-infected macrophages (1:10, vol/vol) and/or recombinant PAF-AH (50 ng/ml). Each condition was then assayed for apoptosis as follows.

**Cell Death Assays and Quantitation**—For Fig. 1 only, a previously described (13) double stain methodology was used, in which neuronal cultures were first stained for membrane permeability (*i.e.* necrosis) with trypan blue, then fixed in 4% paraformaldehyde and stained for fragmented nuclear DNA (*i.e.* apoptosis). TUNEL method using an adaptation of the commercially available ApopTag™ kit (ONCOR, Gaithersburg, MD), modified to incorporate an alkaline phosphatase secondary antibody visualized by New Fuchsian (Dako, Carpinteria, CA). For all other figures, neuronal cultures on coverslips were fixed in 4% paraformaldehyde, and analyzed exclusively for DNA fragmentation via the TUNEL method, using the ApopTag™ kit and methodology. For immunohistochemical analyses of neuronal apoptosis, fixed neuronal coverslip cultures were examined in the following manner: digitized images of 15 microscopic fields, pooled from two identically treated coverslip cultures, were analyzed for number of apoptotic neuronal nuclei/total number of neurons (TUNEL positive/TUNEL positive and negative) per 66× field using computerized morphometry (MCID, Imaging Research, St. Catherines, Ontario, Canada). Data from each of the 15 microscopic fields are compiled and expressed as the mean percentage of apoptotic (TUNEL positive) neuronal nuclei per field ± standard error of the mean. Because gestational ages of neuronal cultures varied between 11 and 16.5 weeks at the time of explantation, TUNEL staining in control cultures varied between 2 and 18% of the total neuronal population. Tests of statistical significance between control and experimental treatments were determined by analysis of variance or paired *t* tests. Results were judged significant at *p* < 0.05.

**RESULTS**

**TNF-α-induced Time-dependent Neuronal Apoptosis**—A 1 ng/ml fixed dose of TNF-α applied to primary human neuronal cultures caused a time-dependent increase in neuronal apo-
number of candidate neurotoxins, including TNF-α. Medium from activated HIV-1-infected macrophages contains a neurotoxic. These results demonstrate that, although conditioned death to nearly control levels. PAF-AH by itself was not neurotoxic. Incubation with PAF-AH was able to reduce neuronal cell death in nearly 25% of the total population of neurons. Co-activation. The results described here imply a novel relationship between the proinflammatory cytokine TNF-α and PAF receptor activation that results in neuronal cell death. Neuronal dysfunction and death in HIV-D is thought to be due to the presence of HIV-1 gene products, increased amounts of proinflammatory cytokines, TNF-α, PAF, and other products of arachidonic acid metabolism (18). Here we establish a TNF-α-mediated neurotoxicity that increases proportionally with length of exposure. Our data further suggest that this TNF-α-induced neurotoxicity occurs via production of the second messenger ceramide and requires activation of PAF receptors, since ceramide levels were elevated in response to TNF-α exposure (Fig. 2), and PAF receptor antagonism abrogated both TNF-α- and ceramide-mediated neurotoxicity to a similar degree (Fig. 3). PAF catabolism also reduced TNF-α-mediated neurotoxicity (Fig. 4A). A comparison of the data in Figs. 1 and 2, showing maximal ceramide levels present before the majority of apoptosis has occurred, lends further support to the theory that TNF-α-induced ceramide production may serve as an initiator of apoptotic neuronal cell death. Furthermore, the findings that overexpression of the anti-apoptosis gene crmA inhibits ceramide formation in response to TNF-α (19), and protects cells from the cytotoxic action of TNF-α (8, 19), but not from ceramide-induced cytotoxicity (19), provide strong additional support for the role of ceramide as a mediator of TNF-α-induced neuronal

**DISCUSSION**

The results described here imply a novel relationship between the proinflammatory cytokine TNF-α and PAF receptor activation that results in neuronal cell death. Neuronal dysfunction and death in HIV-D is thought to be due to the presence of HIV-1 gene products, increased amounts of proinflammatory cytokines, TNF-α, PAF, and other products of arachidonic acid metabolism (18). Here we establish a TNF-α-mediated neurotoxicity that increases proportionally with length of exposure. Our data further suggest that this TNF-α-induced neurotoxicity occurs via production of the second messenger ceramide and requires activation of PAF receptors, since ceramide levels were elevated in response to TNF-α exposure (Fig. 2), and PAF receptor antagonism abrogated both TNF-α- and ceramide-mediated neurotoxicity to a similar degree (Fig. 3). PAF catabolism also reduced TNF-α-mediated neurotoxicity (Fig. 4A). A comparison of the data in Figs. 1 and 2, showing maximal ceramide levels present before the majority of apoptosis has occurred, lends further support to the theory that TNF-α-induced ceramide production may serve as an initiator of apoptotic neuronal cell death. Furthermore, the findings that overexpression of the anti-apoptosis gene crmA inhibits ceramide formation in response to TNF-α (19), and protects cells from the cytotoxic action of TNF-α (8, 19), but not from ceramide-induced cytotoxicity (19), provide strong additional support for the role of ceramide as a mediator of TNF-α-induced neuronal

**FIG. 2. Effects of TNF-α on ceramide production in human neuronal SK-N-MC cells.** Ceramide and phosphate levels were assayed following TNF-α (12 ng/ml) or cPAF (250 ng/ml) treatment for the indicated time periods. For each experimental condition, a normalized total ceramide (pmol):normalized total phosphate (nmol) ratio was obtained, then plotted as a percent difference from the normalized ceramide:phosphate ratio for the corresponding vehicle-treated time-matched control (y = 0%). Data are from a single representative experiment, replicated three times. Similar results were also obtained using 10 ng/ml TNF-α (data not shown).

Ceramide Levels—Ceramide levels increased after 2 h of TNF-α exposure and peaked by 4 h (Fig. 2). In contrast, cPAF treatment caused a decrease in ceramide production at 4 h, and an even greater decrease at 24 h. Treatment of primary human neuronal cells with C2 ceramide, a cell-permeable analog of ceramide, mimicked TNF-α-mediated neurotoxicity (Fig. 3). PAF Antagonism and Catabolism—PAF receptor antagonism by WEB 2086 (16) was able to completely reverse TNF-α-mediated neurotoxicity and by itself had no neurotoxicity (Fig. 3A). C2 ceramide-mediated neurotoxicity could also be completely reversed by co-application of WEB 2086 (Fig. 3B). Catabolism of PAF by recombinant human PAF-AH, the principle catabolic enzyme for PAF in the central nervous system (17), similarly reduced TNF-α-mediated neurotoxicity (Fig. 4A).

**FIG. 3. Effects of PAF receptor antagonism on TNF-α- and ceramide-mediated toxicity of primary human neuronal cultures.** Neuronal toxicity was assayed by immunostaining for DNA fragmentation using the TUNEL method. Digitized images of 15 microscopic fields (× 66 magnification), pooled from two identically treated coverslips, were analyzed using computerized morphometry, and data are expressed as the mean number of TUNEL-positive neurons/total number of neurons (% total) per 66× field. Error bars represent the standard error of the mean for the 15 fields. Statistical significance was determined by paired t tests. Results were judged significant if \( p < 0.01 \). Data are from a single representative experiment, replicated three times. A, TNF-α (10 ng/ml)-induced neurotoxicity was abrogated by co-incubation with the metabolically stable hetrazepine PAF receptor antagonist WEB 2086 (10 mM). B, C2 ceramide (10 mM)-induced neurotoxicity was also ameliorated by co-incubation with WEB 2086 (10 mM).
apoptosis. Further, if elevated ceramide levels do indeed lead to neurotoxic activation of PAF receptors, our finding that direct PAF receptor activation decreased neuronal ceramide levels prior to cell death (Fig. 2) suggests a potential compensatory neuroprotective mechanism against PAF receptor-mediated cell death.

**Fig. 4. Effects of PAF-AH on TNF-α- and HIV-1-induced neurotoxicity.**

Toxicity in primary human neuronal cultures was assayed by immunostaining for DNA fragmentation using the TUNEL method with a Ni-enhanced diaminobenzidine chromagen. All figures represent results from a single experiment replicated three times. A TNF-α (10 ng/ml)-induced neuronal apoptosis was reduced by co-incubation with PAF-AH (50 μg/ml). Results here were quantitated, analyzed, and presented as described in Fig. 3. B–E, 24-h incubation with conditioned medium (CM) from activated HIV-1-infected macrophages (1:10, vol/vol) induced neuronal apoptosis (C) versus medium control (B), which was markedly reduced by co-incubation with PAF-AH (50 mg/ml) (D). Co-incubation with PAF-AH vehicle did not decrease CM-induced neurotoxicity (E). Representative 132× Nomarski fields. F, the results represented photographically in B–E were quantitated, analyzed, and presented as described in Fig. 3.
The data presented here also presents the question of whether a glial cell intermediary is involved in TNF-α-mediated neurotoxicity. The answer to this question remains uncertain, but may well be species-dependent, because several important species-specific differences exist in the biological actions of TNF-α in the central nervous system. Several reports have emphasized the neuroprotective role of TNF-α in vitro and in vivo in the murine central nervous system in acute brain injury such as stroke or head trauma that may involve excitotoxicity resulting in necrotic neuronal death (20). However, in human primary neurons or neuronal cells, exposure to TNF-α results in apoptosis in most, but not all experimental systems (8–10, 21). Our confirmation here of TNF-α-induced neuronal apoptosis in vitro is consonant with findings of TUNEL-stained neuronal nuclei with chromatin condensation adjacent to focal inflammatory infiltrates of activated microglia and reactive astrocytes present in postmortem brain tissue from patients with HIV-D (22–24). TNF-α may initiate a sequence of events resulting in neuronal damage and death in a chronic setting of low neuronal inflammation, a concept that is strengthened by the observation that levels of TNF-α mRNA in brain tissue of patients with HIV-D correlate with dementia (6).

Additional studies may provide insight into how PAF receptor activation may lead to downstream neuronal death. Previous experiments from this laboratory have established that NMDA receptor channel antagonists including MK 801 and memantine substantially ameliorate PAF-induced neurotoxicity in both human and rat culture systems (7). It is unknown whether PAF can directly activate NMDA receptors with subsequent excitotoxic damage. One study has shown that PAF can increase intracellular Ca2+ in a population of rodent hippocampal neurons with NMDA receptors (25). PAF can also lead to excitotoxic neurotransmission through increased glutamate release (26–28). TNF-α, at levels present in conditioned medium from activated HIV-1-infected macrophages, decreases the Vmax by 30% for high affinity uptake in human, but not rat astrocytes (2, 3, 29). It is also possible that during conditions that promote chronic inflammation in the brain, including HIV-1 encephalitis, PAF can activate microglia to release arachidonic acid in a Ca2+-dependent manner, and arachidonic acid, in turn, can down-regulate high affinity glutamate uptake (30–32). Although the mechanisms are different, both TNF-α and PAF disrupt homeostasis for glutamatergic transmission, and thus may ultimately increase ambient glutamate to levels sufficient to cause NMDA receptor-mediated apoptosis in vulnerable neurons (33). Finally, we have previously demonstrated that levels of PAF in cerebrospinal fluid correlate well with neurologic dysfunction (i.e., dementia in adults and progressive encephalopathy in children) and immunosuppression (7). In the experiments reported here, we extend these findings to show that PAF acetylhydrolase, the catabolic enzyme for PAF in the central nervous system, can almost completely prevent the neurotoxicity induced by exposure to neurotoxins from activated HIV-1-infected macrophages. This suggests that PAF may be the principle initiator of neuronal dysfunction and death in the clinical setting of HIV-D, and further, that PAF-AH, or PAF receptor antagonists, may ameliorate or prevent the neuronal damage associated with HIV-D.

REFERENCES
1. Glassa, J. D., Fedor, H., Wesselingh, S. L., and McArthur, J. C. (1995) Ann. Neurol. 38, 755–762
2. Genis, F., Jett, M., Bernton, E. W., Boyle, T., Gelbard, H. A., Dzenko, K., Keane, R. W., Resnick, L., Miuraishi, Y., and Volsky, D. J. (1992) J. Exp. Med. 176, 1703–1718
3. Nottet, H. S., Jett, M., Flanagan, C. R., Zhai, Q. H., Persidesso, Y., Rizzino, A., Bernton, E. W., Genis, P., Baldwin, T., Schwartz, J., LaBeuz, C. J., and Gendelman, H. E. (1995) J. Immunol. 155, 3567–3581
4. Lecardi, C., Petretti, C., Bocelli, G., Testa, U., Delfin, C., Butto, S., and Belardelli, F. (1990) J. Virol. 64, 5874–5882
5. Wuytens, D., Poli, G., Bousseau, A., and Pauci, A. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2537–2541
6. Wesselingh, S. L., Power, C., Glass, J. D., Tymr, W. R., McArthur, J. C., Farber, J. M., Griffin, J. W., and Griffin, D. F. (1993) Ann. Neurol. 33, 576–582
7. Gelbard, H. A., Nottet, H. S., Swindells, S., Jett, M., Dzenko, K. A., Genis, P., White, R., Wang, L., Choi, Y. B., Zhang, D., Lipton, S. A., Tourtellotte, W. W., Epstein, L. G., and Gendelman, H. E. (1994) J. Virol. 68, 4629–4635
8. Talley, A. K., Dewhurst, S., Perry, S. W., Dallard, S. C., Gmummhuru, S., Fine, S. M., New, D., Epstein, L. G., Gendelman, H. E., and Gelbard, H. A. (1995) Mol. Cell. Biol. 15, 2359–2366
9. Dzenko, K. A., Perry, S. W., Epstein, L. G., and Gelbard, H. A. (1995) Soc. Neurosci. Abstr. 21, 561
10. Gelbard, H. A., Dzenko, K. A., DiLoreto, D., del Cerrro, C., del Cerrro, M., and Epstein, L. G. (1994) Neuroscience 51, 417–422
11. Kolesnick, R., and Golde, D. W. (1994) Cell 77, 325–328
12. Martin, S. J., Newmeyer, D. D., Mathias, S., Parson, D. M., Wang, H. G., Reed, J. C., Kolesnick, R. N., and Green, D. R. (1995) EMBO J. 14, 5191–5200
13. Perry, S. W., Epstein, L. G., and Gelbard, H. A. (1997) BioTechniques 22, 1102–1106
14. Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
15. Jayadev, S., Liu, B., Bielawska, A. E., Lee, J. Y., Natafhe, F., Puskareva, M. Y., Obeid, L. M., and Hannun, Y. A. (1995) J. Biol. Chem. 270, 2047–2052
16. Korth, R., Hiraftui, M., Keraly, C. L., Delautier, D., Bidauld, J., and Benveniste, J. (1989) Br. J. Pharmacol. 98, 653–661
17. Snyder, P. (1995) Biochem. J. 305, 689–705
18. Epstein, L. G., and Gendelman, H. E. (1993) Ann. Neurol. 33, 429–436
19. Hannun, Y. A. (1996) Science 274, 1855–1859
20. Bruce, A. J., Belling, W., Kinsey, M. S., Peschon, J., Kraemer, P. J., Carpenter, M. K., Holtsberg, F. W., and Matton, P. M. (1996) Nat. Med. 2, 788–794
21. Westmoreland, S. V., Rosen, D., and Gonzalez-Scarano, F. (1996) J. Neurosci. 2, 118–126
22. Adle-Biassette, H., Levy, Y., Colombel, M., Poron, F., Natchev, S., Kume, K., and Shimizu, T. (1992) Mol. Pharmacol. 42, 208–217
23. Gelbard, H. A., James, H. J., Sharer, L. R., Perry, S. W., Saito, Y., Kaze, A. M., Blumberg, B. M., and Epstein, L. G. (1995) Neuropharmacol. Appl. Neurobiol. 21, 218–227
24. Petito, C. K., and Roberts, B. (1995) Am. J. Pathol. 146, 1121–1130
25. Bito, H., Nakamura, M., Honda, Z., Tsumi, I., Watsudo, T., Seyama, Y., Obara, A., Kudo, Y., and Shimizu, T. (1992) Neuron 9, 265–284
26. Clark, G. D., Happel, L. T., Zorumski, C. F., and Bazan, N. G. (1992) J. Neurochem. 59, 208–217
27. Shukla, S. D. (1992) FASEB J. 6, 2296–2301
28. Wieraszko, A., Li, G., Kornecki, E., Mogan, V. M., and Ehrlich, Y. H. (1993) Neuro 10, 553–557
29. Fine, S. M., Angel, R. A., Perry, S. W., Epstein, L. G., Rothstein, J. D., Dewhurst, S., and Gelbard, H. A. (1996) J. Biol. Chem. 271, 15305–15306
30. Volterra, A., Troitt, D., Casasus, P., Tromba, C., Salvaggio, A., Melzanic, R. C., and Racagni, G. (1992) J. Neurochem. 59, 600–606
31. Volterra, A., Troitt, D., and Racagni, G. (1994) Mol. Pharmacol. 46, 986–992
32. Mori, M., Aihara, M., Kume, K., Hamamwue, M., Kohsaka, S., and Shimizu, T. (1996) J. Neurosci. 16, 3590–3600
33. Bonfoco, E., Kraine, D., Ankararona, M., Nicotera, P., and Lipton, S. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7162–7166