Sustained Induction of Prostaglandin Endoperoxide Synthase-2 by
Seizures in Hippocampus

INHIBITION BY A PLATELET-ACTIVATING FACTOR ANTAGONIST*

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Prostaglandin G/H synthase-2 and zif-268 mRNA expression is transiently induced in rat brain by kainic acid (KA)-induced seizures and by a single electroconvulsive shock. Induction of both genes by KA shows neuroanatomical specificity in the order hippocampus > cerebral cortex > striatum > brain stem > cerebellum. Nuclear run-on and Western blotting shows that both genes are transcriptionally activated, and that kainic acid up-regulation of prostaglandin G/H synthase-2 mRNA expression in hippocampus matches increased protein levels. Whereas the magnitude of hippocampal zif-268 mRNA induction is similar in both seizure models, peak induction of prostaglandin G/H synthase-2 mRNA is 7-fold greater in the kainic acid model than in the electroconvulsive shock model and is much more prolonged. Pretreatment of animals by intracerebroventricular injection with the intracellular platelet-activating factor receptor antagonist BN 50730 strongly attenuates kainic acid and electroconvulsive shock induction of prostaglandin G/H synthase-2 expression. The drug partially inhibits electroconvulsive shock induction of zif-268, but is relatively ineffective against kainic acid-induced zif-268 expression. Seizure-induced expression of both genes involves platelet-activating factor, but the mechanisms of induction must be otherwise distinct. The selectively elevated induction of hippocampal prostaglandin G/H synthase-2 by kainic acid correlates with a neuroanatomical region in which the agonist induces neuronal damage.

Seizure activity in the brain initiates complex pathways of signal transduction and cell-to-cell communication. Whereas a single seizure usually has little or no long-term effects on the brain, repeated and uncontrolled seizures can cause delayed neuronal death and synaptic reorganization. Activation of phospholipases and accumulation of bioactive lipids takes place early after seizures (1) primarily in synaptic endings (2). Arachidonic acid and its oxygenated metabolites and platelet-activating factor are important classes of bioactive lipids generated during seizure activity (3, 4) because they are known to have modulatory effects on synaptic transmission and neuronal plasticity (5–7). Additionally, PAF is also an activator of receptor-mediated immediate early gene expression in the brain and neuronal cells (8, 9). Thus, excitable membrane-derived bioactive lipids may have both acute and long-term effects on neuronal activity.

PAF exerts its biological actions through a rhodopsin-type receptor (10). In neurons, this receptor mediates the presynaptic effects of PAF on excitatory neurotransmitter release (6), long-term potentiation (7, 11), and memory formation (12, 13). A high affinity PAF binding site has been found in microsomal membranes isolated from hippocampus and cerebral cortex (9, 14). This site has distinct ligand binding kinetics and sensitivity to PAF receptor antagonists and may represent either an intracellular form of the plasma membrane receptor or a novel PAF receptor subtype. The intracellular PAF-binding site may be involved in the induction of immediate-early transcription factors in the hippocampus during electrically induced seizures (9). Additionally, PAF activates the expression of transfected prostaglandin G/H synthase-2 (PGS-2, cyclooxygenase, COX-2) promoter constructs, and this effect is blocked by a PAF antagonist (BN 50730) selective for the intracellular site (15). PGS catalyzes the first committed step in the conversion of arachidonic acid to prostaglandins and thromboxanes. The PGS-2 isoform is normally expressed in most tissues only as a rapid and transient response to mitogenic and inflammatory stimuli, and is encoded by an immediate-early gene (16). Brain, however, is one of the few anatomical sites in which a basal level of PGS-2 expression has been demonstrated (17). This expression is localized to selected groups of neurons and is regulated by synaptic activity and, more specifically, by activation of the N-methyl-D-aspartate class of glutamate receptors (17, 18).

In this study, we tested the hypothesis that PAF is involved in the seizure-induced accumulation of PGS-2 in the brain and is thus linked to the metabolism of free arachidonic acid generated during seizures. We present evidence that PGS-2 transcriptional activity and protein expression is up-regulated in a single electrically-induced seizure (ECS) and in multiple, kainic acid (KA)-induced seizures, and that PAF is involved in this mechanism. In addition, we show that induction of PGS-2, but not the transcription factor immediate-early gene zif-268, is more sensitive to the severe KA-induced seizures than ECS in a brain region, the hippocampus, selectively vulnerable to damage in this model. Partial accounts of these results have appeared in abstract form (19).

EXPERIMENTAL PROCEDURES

Animals—Rats (Albino male Wistar, 250–320 g) from Charles River Breeding Laboratories, Wilmington, MA, were housed in the LSUMC

prostaglandin G/H synthase-2, cyclooxygenase, EC 1.14.99.1, COX-2, TIS-10; PGS-1, prostaglandin G/H synthase-1; KA, kainic acid; ECS, electroconvulsive shock; icv, intracerebroventricular; ip, intraperitoneal; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
animal facility for at least 24 h before experiments were performed. Ka- Seizures—Drugs and Compounds—-treated groups received 10 mg/kg KA in saline by ip injection (150 µL). Controls received saline alone. At 0, 1, 2, 3, 6, 12, and 24 h after injection, animals were anesthetized with ether and decapitated. In a second series of experiments, rats were anesthetized with ether and received intracerebroventricular (icv) injections of the PAF receptor antagonist BN 50730 in dimethyl sulfoxide (Me2SO) (2 µl/ventricle, 100 µg/kg) or Me2SO alone as a control. 15 min later, KA was injected ip (6 mg/kg). Animals were anesthetized with ether and decapitated at 0, 6, 12 h after KA treatment.

Single Electroconvulsive Shock (ECS)—Two platinum needle electrodes were implanted 1 cm apart under the scalp of the rats. A train of pulses was delivered at 100 V, 0.5-ms pulse duration, frequency 150 pulses/s for 10 min. Rats were kept on ice until pulse delivery. The time to the first behavior was calculated by counting 2-s time-lapse frames of film. The time to succumb to the ECS was determined by the time required for the animal to be completely flaccid. The time to resumption of convulsions was determined by the first occurrence of a convulsion following the ECS. Controls were anesthetized with ether and received saline alone. Animals were then killed by ether anesthesia followed by decapitation at 0, 1, 2, 6, and 12 h after ECS. Other rats under ether anesthesia were pretreated with BN 50730 in Me2SO (2 µl/ventricle, 100 µg/kg, icv) 15 min before ECS. Animals were then killed by ether anesthesia followed by decapitation at 0, 1, 2, 6, and 12 h after ECS.

Sampling of Brain Regions—After decapitation, the brain was rapidly excised, and hippocampus, brain cortex, brain stem, striatum, and cerebellum were rapidly dissected on a cold-cold dissection board. Tissues were homogenized with a Polytron type homogenizer in different buffers according to the assays to be performed. For RNA extraction, buffer A (4 mM guanidine thiocyanate, 25 mM sodium citrate, 0.5% n-lauroyl sarcosine, and 0.1 M β-mercaptoethanol, pH 7.0, 4 °C) was used. For Western blots, the sample was incubated with buffer B (50 mM HEPES, 5.5 mM EDTA, 100 mM NaCl, 0.2% I-Block (Tropix, Bedford, MA), 0.1% Tween 20, pH 7.4) overnight at 4 °C on an oscillating platform, then incubated 3 h at 25 °C with the primary antibody (mouse anti-PGHS-1 (Oxford Biochemical Research, Oxford, MI) dilution 1:2500; monoclonal anti-PGHS-2 (Transduction Laboratories, Lexington, KY), dilution 1:2500). Membranes were washed in buffer (55 mM Na2HPO4, 17 mM NaH2PO4, 66 mM NaCl, 0.1% Tween 20, pH 7.4), then incubated with the secondary antibody (goat anti-mouse IgG-conjugated alkaline phosphatase or goat anti-rabbit IgG-conjugated alkaline phosphatase, 1:10,000, Tropix, for 30 min at 25 °C in a rotary oven. For detection, membranes were equilibrated in assay buffer (0.1 M diethanolamide, 1 mM MgCl2, pH 10) 2× for 2 min each time, incubated in 5 ml of assay buffer containing 0.25 mM CSPD (Western-lightTM, Tropix) and 1:20 Nitro-Block (Western-lightTM, Tropix) for 1 h, then rapidly exposed to x-ray film (Hyperfilm-ECL, Amersham International) or a PhosphorImager plate.

RESULTS

KA- and ECS-induced Seizures Elevate PGS-2 and zif-268 mRNA Levels in Brain—The abundance in the brain of several immediately-early gene transcripts shows a rapid and transient increase in response to electrically and chemically induced seizures (22, 23). We had previously shown that a single ECS induced zif-268 mRNA expression in the rat hippocampus and cerebral cortex, and this induction was sensitive to the intracellular PAF receptor antagonist BN 50730 (9). We were, therefore, interested in determining if PGS-2 is regulated by a similar mechanism in the ECS model, and if both are regulated in a similar manner in the KA-induced seizure model.

KA-induced seizures elevated mRNA levels of both PGS-2 and zif-268. However, there were differences in the magnitude and duration of the responses, and differences in the levels of response of the various neuroanatomical regions (Fig. 1). Induction of PGS-2 mRNA 2 h after treatment was highest in the hippocampus (35-fold), followed by the cerebral cortex (8-fold). Small changes were observed in the brain stem and striatum, whereas there was no change in cerebellum. Similar to PGS-2, the largest induction of zif-268 mRNA was in hippocampus (5.5-fold), followed by cerebral cortex (4.8-fold). Again, smaller changes were found in brain stem and striatum and no change in cerebellum.

Hippocampal PGS-2, but Not zif-268 mRNA Accumulation Is Much Greater in KA- versus ECS-induced Seizures—The time courses of the KA-induced up-regulation of PGS-2 and zif-268 mRNA in hippocampus were distinct from each other and different from the time courses of expression in the ECS model. In both models, the peak of PGS-2 mRNA induction was later than zif-268. In KA-treated animals, PGS-2 reached a 71-fold increase within 3 h whereas zif-268 increased only 2.5-fold (Fig. 2A). At 24 h, the PGS-2 mRNA levels were still above baseline (data not shown). With the ECS model, PGS-2 mRNA levels increased 5.2-fold at 12 h (Fig. 2A). At 24 h, the PGS-2 mRNA levels were still above baseline (data not shown). With the ECS model, PGS-2 mRNA levels increased 5.2-fold at 12 h (Fig. 2A). At 24 h, the PGS-2 mRNA levels were still above baseline (data not shown).
mRNA increased 10-fold over controls at 2 h, and zif-268 increased 8.5-fold after 1 h. Additionally, levels of PGS-2 mRNA returned to basal values by 24 h. The delayed induction of PGS-2 relative to transcription factor immediate-early genes is a well-described phenomenon of many in vivo and cell culture systems. The differences between the two seizure models are the most significant observations. When the peak induction of zif-268 mRNA levels were similar in magnitude in both models, PGS-2 induction was seven times greater in KA- than in ECS-induced seizures.

Changes in PGS-2 and zif-268 Expression during Seizures Involve Transcriptional Regulation—The changes in mRNA abundances during KA- and ECS-induced seizures may be due to a number of seizure-induced changes in hippocampus, including enhanced transcriptional activity, increased availability of factors that prolong mRNA stability, and/or changes in translational modulation. To determine if transcriptional activation is a component of PGS-2 and zif-268 induction in the seizure models, nuclear run-on transcription assays were performed. The profiles of PGS-2 transcriptional activation qualitatively match the changes found in mRNA abundance (Fig. 2B), suggesting that transcriptional activation is a major factor in PGS-2 induction. The relative induction of PGS-2 transcriptional activation was, however, less than the relative increase in mRNA levels. The methodologies used to obtain these two sets of values are very different and may not be readily comparable. Therefore, we can only conclude that the in vitro transcription profile is analogous to that of relative mRNA abundance. Similar comparative patterns can be observed between in vitro transcription and mRNA abundance for zif-268.

We also monitored transcriptional activation of PGS-1, the constitutively expressed isoform of PGS. As expected, there was no significant induction up to 6 h; however, there was a small increase in KA-injected animals after 12 h. ECS did not trigger any activation.

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FIG. 1. Neuroanatomical distribution of PGS-2 mRNA (closed bars) and zif-268 mRNA (open bars) up-regulation in rat brain after KA administration. Experimental animals (+) received a single ip injection of KA, whereas controls (−) received saline. RNA was isolated 2 h after injection, and PGS-2, zif-268, and GAPDH mRNAs were detected by Northern analysis. (n = 6, error bars ± S.D.).

FIG. 2. Time course of changes in relative mRNA abundance and transcriptional activity in hippocampus after KA treatment or a single ECS. A, relative abundance of PGS-2 and zif-268 mRNAs compared to GAPDH mRNA, as assessed by Northern analysis. (n = 9–12 for each time point from 3 separate experiments, error bars ± S.D.). B, transcriptional activity of PGS-2, PGS-1, and zif-268 genes assessed by nuclear run-on transcription (n = 3–4 from 3 separate experiments). Data are normalized to transcriptional activity of GAPDH.

FIG. 3. Time course of PGS-2 and PGS-1 protein accumulation in rat hippocampus after KA treatment. A, representative Western blots of hippocampal protein extracts using PGS-2 and PGS-1 antibodies. The positive control for PGS-2 (ϕ) is from lipopolysaccharide-induced macrophages. B, quantification of Western blots to assess relative induction of PGS-2 and PGS-1 protein in experimental (KA-treated) versus control (saline-treated) (n = 8–12 from 3 separate experiments, error bars ± S.D.).
levels at 6 h. The time course profile showed a shoulder that may represent a smaller, overlapping peak at 12 h. PGS-1 protein levels showed no significant changes over the 24-h time course. There was, however, a small increase in PGS-1 protein levels after KA injection, which mirrored that seen in the run-on transcription experiments. Thus far, the significance of this remains to be explored.

**BN 50730 Inhibits Seizure-induced PGS-2 mRNA and Protein Expression in Hippocampus and Cerebral Cortex**—The intracellular PAF receptor antagonist BN 50730 was more effective in reducing seizure-induced early gene expression, when administered intracerebroventricularly (icv) as compared with the ip route (9). Therefore, in the present study, BN 50730 was given icv 15 min before KA treatment. Fig. 4 shows that BN 50730 reduced the seizure-induced accumulation of PGS-2 mRNA in hippocampus and cerebral cortex by about 90% at 2 h, but was much less effective against *zif*-268 mRNA accumulation. This was matched by a similar attenuation of PGS-2 protein accumulation in the hippocampus (Fig. 5). Under the same conditions, expression of PGS-1 protein was unaffected. Using the ECS model, when rats were pretreated by icv with BN 50730 15 min before stimulation, PGS-2 and *zif*-268 mRNA accumulation in rat hippocampus was strongly inhibited with the inhibition for both PGS-2 and *zif*-268 peaking 1 h after injection (Fig. 6). The decreased effectiveness of the drug after this time presumably represents a combination of diffusion or transport away from the site of action and/or metabolic inactivation.

The dose of KA used in the experiments involving pretreatment with BN 50730 was reduced from 10 mg/kg for the other experiments to 6 mg/kg. The higher dose of kainic acid in BN 50730-treated animals produced extremely severe seizures and high mortality. We propose that the solvent for BN 50730 (Me₂SO) increased the permeability of the blood-brain barrier to KA. Only around 1% of KA delivered ip normally reaches the brain (24), and, thus, any increase in blood-brain barrier permeability could easily lead to overdosage.

**DISCUSSION**

The data presented here implicate PAF-stimulated signal transduction pathways as major components of the seizure-induced expression of PGS-2 in brain. We also confirm previous studies demonstrating seizure-induced increases in PGS-2
mRNA levels in brain (17, 25, 26). In addition, we show that accumulation of PGS-2 mRNA correlates with transcriptional activation of the gene and accumulation of PGS-2 protein. Furthermore, when the effects of ECS were compared with KA-induced seizures, we demonstrate that PGS-2, but not the zinc-finger transcription factor zif-268, is subject to additional hippocampal up-regulation in the more severe seizure model.

The inhibitory effect of BN 50730 on seizure-induced PGS-2 and zif-268 implies that they share a common requirement for stimulation of the intracellular form of the PAF receptor in the mechanisms by which both genes are induced under these conditions. BN 50730 is a competitive antagonist of PAF, binding to an intracellular site, but not to synaptic membranes (9). Nonetheless, the different kinetics of induction and the selective increase in response of hippocampal PGS-2 induction in KA- versus ECS-induced seizures suggest that other components of the induction mechanisms are distinct. BN 50730 almost totally abolishes KA-induced PGS-2 mRNA and protein accumulation. Therefore, it is likely that PGS-2 expression responds either to increased levels of PAF and/or with an increased sensitivity of the PAF receptor. PAF induces mouse PGS-2 promoter-driven luciferase activity transfected in neuroblastoma cells (NG 108–15 or SH-SY5Y) and in NIH 3T3 cells, and BN 50730 inhibits this effect (15). Increased PAF content was detected in brain during seizures (4); however, it is difficult to assess the actual increases of PAF that might occur intracellularly in the brain due to the presence of a wide variety of PAF acetylhydrolases (27, 28). zif-268 expression is less sensitive than PGS-2 expression to BN 50730 inhibition in the ECS model, does not show an augmented response in hippocampus in the KA model, and, in the latter model, shows little response to BN 50730. This suggests that either zif-268 is induced by different mechanisms in the two seizure models or that ECS is sufficient to maximally stimulate expression. The induction of PGS-2 in hippocampus by KA is elevated and prolonged in neuroanatomical regions known to exhibit selective neuronal apoptosis in response to this agonist (29).

PGS-2 expression in the normal rat brain is localized to the post-synaptic structures of discrete groups of neurons in the forebrain and is enriched in the hippocampus and cerebral cortex (18, 30). The onset of expression in the developing rat correlates with the early postnatal period when synaptic remodeling is most active. Even when expression is up-regulated by ECS, the mRNA is still localized to neurons and is not detected in glial or vascular cells (17). Hence, the up-regulation of PGS-2 expression in response to KA is probably due to additional stimulation of the neuronal pathway by the multiple KA-induced seizures. This would suggest an atypical role for the prostaglandins generated by PGS-2 activity. PGS-2 activation is generally thought to be associated with inflammatory events, whereas the constitutive PGS-1 isoform is thought to be responsible for physiological production of prostanooids. It should be added, however, that this strict definition of the roles of the two isoforms is being reassessed (91), particularly in the light of the phenotypes exhibited by knockout mice for the two PGS isoforms (32–34). PGS-1 knockout mice have few phenotypic abnormalities, whereas PGS-2 knockout mice have severe renal abnormalities. Thus PGS-2 is required for specific aspects of normal development. Furthermore, testing of various inflammatory models on these animals suggest that both PGS isoforms participate in inflammatory events. At this time, the neurological responses of these mice have not been reported.

The major endogenous inflammatory cells of the brain are glia and microglia. Accordingly, these cell types express relatively large amounts of such components of the inflammatory cascade as secretory and cytosolic PLA2 and the PAF receptor (35–37). Prostaglandins synthesized through PGS-2 activation in neurons are unlikely, to be directly involved in the inflammatory response. They may, however, be involved in functions related to synaptic transmission and neuronal plasticity. Prostaglandins D2, E2, and F2a have been known for some time to have modulatory effects on neurotransmission (5), and, more recently, prostacyclin has been found to facilitate excitatory neurotransmission in the hippocampus through a novel receptor subtype (38). Beyond this common property, however, the different prostanooids have diverse and, in some cases, antagonistic effects on the central nervous system. It is important, therefore, to know which species of prostanooids are being synthesized via neuronal PGS-2 both in physiological and pathophysiological states. Temporal changes in the patterns of prostanooids synthesized during seizures using the pilocarpine model of epileptogenesis show production of different prostanooid species at different stages during status epilepticus and the formation of recurrent seizures (39).

The events in which PAF activates PGS-2 expression during seizures sets into motion pathways which can either elicit neuroprotection or lead to neuronal damage. PAF is not stored in cells but is rapidly synthesized in response to stimuli. In addition, it is a short-lived molecule, being rapidly degraded by a specific PAF acetylhydrolase (28). Nonetheless, by inducing PGS-2, this transient signal can have long-term effects on neuronal function. The localization of PGS-2 protein in the nuclear envelope and perinuclear endoplasmic reticulum (40) suggests that, in addition to paracrine effects on the cell-surface prostaglandin receptors, the prostanooids produced through PGS-2 activation may have effects at the nucleus. Thus, PAF might be eliciting cascades of gene expression in addition to those potentially initiated by activation of transcription factor genes such as zif-268. To fully understand the significance of seizure-induced PGS-2 expression in hippocampus, the types and functions of PGS-2 derived prostanooids synthesized in response to seizures will have to be better defined. With this information, the consequences of BN 50730 inhibition of PGS-2 expression can be more fully assessed.

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