JB Minireview—Lipid Signaling

J. Biochem. **131**, 781–784 (2002)

Prostaglandin Receptors: Advances in the Study of EP3 Receptor Signaling

Noriyuki Hatae, Yukihiko Sugimoto, and Atsushi Ichikawa[1]

Department of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501

Received March 14, 2002; accepted April 1, 2002

Prostaglandin (PG) E_2 produces a broad range of physiological and pharmacological actions in diverse tissues through specific receptors on plasma membranes for maintenance of local homeostasis in the body. PGE receptors are divided into four subtypes, EP1, EP2, EP3, and EP4, which have been identified and cloned. These EP receptors are members of the G-protein coupled receptor family. Among these subtypes, the EP3 receptor is unique in its ability to couple to multiple G proteins. EP3 receptor signals are primarily involved in inhibition of adenylyl cyclase *via* G_i activation, and in Ca^{2+}-mobilization through $G\beta\gamma$ from G_i. Along with G_i activation, the EP3 receptor can stimulate cAMP production *via* G_s activation. Recent evidence indicates that the EP3 receptor can augment G_s-coupled receptor-stimulated adenylyl cyclase activity, and can also be coupled to the G_{13} protein, resulting in activation of the small G protein Rho followed by morphological changes in neuronal cells. This article focuses on recent studies on the novel pathways of EP3 receptor signaling.

Key words: calcium mobilization, EP3 receptor, G_{13} protein, prostaglandin receptor.

Prostanoids comprising the prostaglandins (PGs) and thromboxanes (TXs) are potent eicosanoid lipid mediators generated by the cyclooxygenase (COX) isozymes. Prostanoids are quickly released from cells after synthesis and act as local hormones in the vicinity of their production site to maintain local homeostasis. The ability of each prostanoid to affect various biological responses is dependent on its binding to specific receptors on the plasma membrane. These prostanoid receptors are classified into five basic types, termed DP, EP, FP, IP, and TP receptors, on the basis of their sensitivities to the five primary prostanoids, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2, respectively. Furthermore, there are several receptor subtypes for PGD_2 and PGE_2. PGD_2 acts through two receptors, the DP receptor and the recently identified CRTH2 receptor (chemoattractant receptor homologous molecule expressed on Th2) (*1*). EP receptor is subdivided into four subtypes, EP1, EP2, EP3, and EP4, on the basis of their responses to various agonists and antagonists.

Prostanoid receptors are G-protein coupled, rhodopsin-type receptors with seven transmembrane domains. Knowledge accumulated from analyses on the structure and function of the prostanoid receptor molecules has been described elsewhere (*2*). The DP, EP2, EP4, IP receptors, and one isoform of the EP3 receptor can couple to G_s and thus increase intracellular cAMP concentration. The FP, IP, and TP receptors can couple to G_q, and activation of these receptors leads to an increase in intracellular calcium levels. Finally, the TP, CRTH2, and EP3 receptor can couple to G_i, causing a decrease in the cAMP levels while also mobilizing intracellular calcium. The EP1 receptor can also mobilize intracellular calcium, but activation of G proteins by the EP1 receptor has not been confirmed.

Of the prostanoid receptor molecules, the EP3 receptor has different C-terminal tail isoforms, which are generated by alternative splicing. It has been reported that the mouse EP3 receptor has three isoforms, EP3α, EP3β, and EP3γ (*3–5*), the bovine EP3 receptor has four isoforms (*6*), the rabbit EP3 receptor has five isoforms (*7, 8*), and the human EP3 receptor has seven isoforms (*9*). G_i activation mediated by the mouse EP3 receptor isoforms has been well investigated. The three mouse EP3 receptor isoforms couple to G_i with different IC_{50} values, of which EP3γ < EP3α < EP3β (*3, 4*). Regarding the agonist-dependency for G_i activation, the mouse EP3α and γ isoforms have partially constitutive G_i activity (EP3γ > EP3α), but the EP3β isoform has no constitutive G_i activity (*10, 11*). Moreover, the C-terminal tail–truncated mutant receptor, abbreviated as T-335, showed fully constitutive G_i activity (*11*). Along with G_i activity, the three isoforms and T-335 can cause agonist-dependent G_s activity (*4*). The order of potency is EP3γ > T-335 ≫ EP3α = EP3β = 0. This shows that the core of the EP3 receptor has the ability to associate with and activate G_i/G_s proteins, while the C-terminal tail of the EP3 receptor can suppress G protein activation.

Recently, novel actions of the EP3 receptor other than in G_i/G_s signaling have been identified using EP3-expressing cells and cultured neuronal cells. This review summarizes the current information regarding the EP3 receptor with a focus on its novel actions.

Ca^{2+} mobilization mediated by the EP3 receptor

Activation of the mouse EP3α, EP3β, and EP3γ receptors

[1]To whom correspondence should be addressed. Tel: +81-75-753-4527, Fax: +81-75-753-4557, E-mail: aichikaw @pharm.kyoto-u.ac.jp

Abbreviations: PG, prostaglandin; TX, thromboxane; COX, cyclooxygenase; G protein, heterotrimeric GTP-binding protein; PT, pertussis toxin.

is known to lead to intracellular Ca^{2+} mobilization in a PT-sensitive manner in CHO cells (*12*). This Ca^{2+} mobilization mediated by the EP3 receptor is conducted by the Gβγ subunits from the $G_{i/o}$ protein, since the PLCβ isoform is activated by these subunits.

We recently reported that the mouse EP3β receptor and the T-335 receptor can significantly augment G_s-coupled EP2-induced adenylyl cyclase activity, and that this augmentation is mediated by a PT-insensitive Ca^{2+} pathway (*13*). G_i-coupled receptors such as α_2 adrenoceptor (*14*) and bradykinin B_2 receptor (*15*) are also known to lead to augmentation of G_s-stimulated adenylyl cyclase in COS-7 cells. This augmentation is suspected to be *via* an increase in adenylyl cyclase type II activity by direct interaction of the Gβγ subunits released from activated $G_{i/o}$ proteins with the receptors. However, the adenylyl cyclase augmentation induced by the EP3 receptor was not attenuated by either PT treatment or expression of the PH domain of rat βARK1, which serves as a scavenger of Gβγ subunits. This result suggests that adenylyl cyclase augmentation is mediated *via* a novel signaling pathway without the involvement of Gβγ subunits released from $G_{i/o}$ proteins. In fact, the adenylyl cyclase augmentation was almost completely attenuated by pretreatment with either 1,2-bis(*o*-aminophenoxymethyl)ethane-*N,N,N′,N′*-tetraacetic acid tetra(acetoxymethyl)-ester, an intracellular Ca^{2+} chelator, or W-7, a calmodulin inhibitor. These findings suggest that the adenylyl cyclase augmentation induced by the EP3 receptor is achieved *via* a signaling pathway involving a Ca^{2+}/calmodulin reaction. Moreover, the T-335 receptor caused a similar augmentation in EP2-stimulated adenylyl cyclase activation, indicating that the C-terminal tail of the EP3β receptor is not essential for this reaction. This cross-talk between the EP3β and EP2 receptors was also reproduced by combination of the G_s-coupled luteinizing hormone (LH) receptor with the EP3β receptor in COS-7 cells. The putative EP1/EP3 agonist sulprostone significantly augmented the cAMP levels produced by LH stimulation in COS-7 cells coexpressing EP3 and LH receptors (Fig. 1). In preliminary experiments, we found that sulprostone augmented cAMP production stimulated by the EP4 agonist ONO-AE-1-329 in mouse mastocytoma P-815 cells, which mainly express the EP3 and EP4 receptors. Southhall and Vasko reported that the bovine EP3C and EP4 receptors mediate PGE_2-induced cAMP production and the sensitization of sensory neurons (*16*). Despite the extensive facts showing that G_i-coupled receptors can augment G_s-coupled receptor-stimulated adenylyl cyclase activity, it remains unknown why the G_i-coupled receptor does not preferentially interact with the G_i protein in COS-7 cells. Recent evidence suggests that many signaling molecules localize in microdomains in the plasma membrane, particularly in the caveolae. For example, the EP2 receptor does not activate adenylyl cyclase type VI, although the β-adrenergic receptor activates this adenylyl cyclase (*17*). Hence, the selective interaction of the EP3 receptor with G_s-coupled EP2-stimulated adenylyl cyclase, even in the presence of an excess of G_i protein in the plasma membrane, may be crucial for agonist-dependent augmentation of cAMP synthesis.

It has also been reported that the rabbit EP3 receptor can couple to the activation of cAMP response element (CRE)–mediated gene transcription, which is a PT-insensitive Ca^{2+} pathway in HEK293tsA201 cells (*8*). The rabbit EP3 receptor was able to elicit this activation in an agonist-dependent manner, although their EC_{50} values were 15-fold higher than that for G_i activity. This CRE activation is mediated by a Ca^{2+}-dependent kinase pathway, since activation was partially inhibited by the selective PKC inhibitor, bisindolylmaleimide I, and completely inhibited by staurosporine, a strong inhibitor of PKC, PKA, and other serine/threonine kinases. These two signals mediated by either the mouse EP3 receptor or the rabbit EP3 receptor elicited an increase in Ca^{2+} levels in a G_i-independent manner. Furthermore, the C-terminal tail–deleted receptors, T-335 being the mouse derivative and NT being the rabbit EP3 derivative, activated these PT-insensitive Ca^{2+} related pathways in an agonist-dependent manner. Since T-335 results in agonist-independent constitutive G_i activity (*11*), the C-terminal tails of the EP3 receptors have different functions in PT-sensitive G_i activity and PT-insensitive Ca^{2+} signaling. These results indicate that the conformation of the EP3 receptor may be quite different in these different signaling pathways.

It has recently been reported that EP3 receptor–mediated signals may promote a novel form of neutrophil cell death, which differs from typical apoptosis or necrosis (*18*). Incubation of neutrophils with staurosporine or H-7, which are inhibitors of PKC, prevented this EP3 receptor agonist–induced neutrophil cell death, though it remained unclear whether this neutrophil death occurs by a PT-sensitive or insensitive pathway. This study showed that the EP3 receptor promoted neutrophil cell death through the activation of PKC, indicating that Ca^{2+} signaling mediated by the EP3 receptor may play a role in various diseases.

G_{13} activity mediated by the EP3 receptor

The bovine EP3 isoform receptors (EP3A, EP3B, EP3C, EP3D) are known to couple to various G proteins. EP3A receptor can couple to G_i, EP3B and EP3C receptors to G_s and G_o, EP3D receptor to G_i, G_s, and G_q. Along with these G proteins, the bovine EP3 receptor was found to lead to the activation of G_{13} in PC12 cells (*19, 20*). The bovine EP3B receptor was able to induce neurite retraction in differenti-

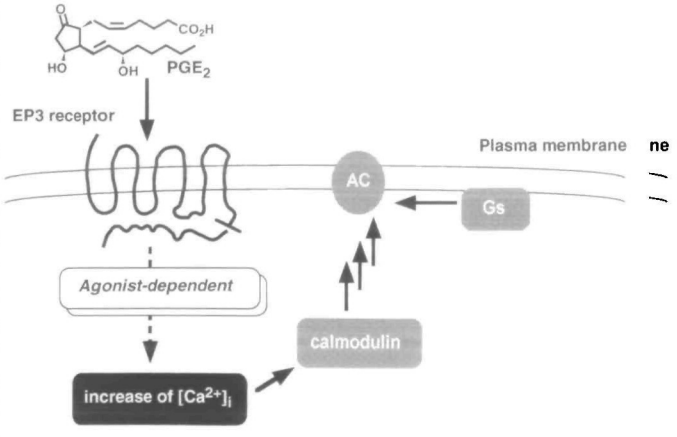

Fig. 1. **Schematic illustration of the mechanism of EP3 receptor–induced Ca^{2+}-dependent augmentation of cAMP synthesis.** The mouse EP3 receptors stimulate an increase in intracellular Ca^{2+} levels, and promote G_s-activated adenylyl cyclase (AC) through the Ca^{2+}-calmodulin pathway in an agonist-dependent and PT-insensitive manner.

ated PC12 cells in a PT-insensitive and agonist-dependen manner. *Clostridium botulinum* C3 exozyme completely i hibited EP3 receptor-induced neurite retraction when m croinjected into the PC12 cells, indicating that the morph logical effect of the EP3B receptor is dependent on Rh activity. Small GTPases of the Rho family, Rac, CDC42, an Rho, are involved in morphological changes in various cell In neuronal cells, Rac or CDC42 appears to be required f the outgrowth of neurites, while Rho is required for neuri retraction (*21*). It has been reported that G_{12}, G_{13}, and G_q induce Rho-dependent neurite retraction in nerve growt factor (NGF)-differentiated PC12 cells (*22*). The bovir EP3B receptor–induced neurite retraction was blocked b tyrphostin A25, which inhibits the G_{13} and G_q-mediate morphological changes *via* Rho. Moreover, EP3 receptor a tivation did not increase the intracellular Ca^{2+} concentration in PC12 cells, and the neuronal morphological changes induced by the EP3 receptor were not blocked by the inhibition of protein kinase C activity. These results indicate that the bovine EP3B receptor induces neurite retraction *via* a G_{13}-small GTPase Rho pathway in PC12 cells.

The mouse EP3 receptor isoforms induced the formation of stress fibers in MDCK cells (*23*). This receptor-mediated stress-fiber formation was completely inhibited by *Clostridium botulinum* C3 exozyme, indicating the involvement of Rho in the formation of stress fibers in MDCK cells. However, since the EP3 receptor–mediated stress-fiber formation was not inhibited by PT treatment, it may be mediated *via* a G_{13}-Rho pathway, as in the case of receptor-mediated neurite retraction in PC12 cells. The EP3α and EP3β receptors differed in their agonist-dependencies for stress-fiber formation: the EP3α isoform acted agonist-independently, while the EP3β isoform acted agonist-dependently. These observations indicate that the mouse EP3 isoforms differ in agonist-independent constitutive G_{13} activity, and that the carboxyl-terminal tail of the EP3 receptor can suppress G_{13} protein activation mediated by the core region of the EP3 receptor (Fig. 2).

PGE_2 is one of the major PGs synthesized in the nervous system (*24*). PGE_2 has several important functions in the nervous system, such as the generation of fever, regulation of LH-releasing hormone secretion, pain modulation, and regulation of neurotransmitter release. Furthermore, the EP3 receptor is involved in pyrogen-induced fever generation (*25*). Among the EP subtypes, the EP3 receptor is the most abundant in the brain and is specifically localized to neurons (*26*). When the brain is injured, newly synthesized PGE_2 may cause retraction of neurites of EP3 receptor-expressing neurons and mediate reorganization of damaged neuronal connections. In addition, the levels of PGE_2 are also increased in the brain upon synaptic activity or during development (*27*). PGE_2 may therefore also be involved in the refining and remodeling of initial neuronal connections through the EP3 receptors.

Conclusion

Among the PGE receptor subtypes, the EP3 receptor has been shown to mediate various physiological and pathophysiological functions. These functions are mediated through the different actions of the EP receptor subtypes, which are coupled to different G proteins, leading to the stimulation of multiple signal transduction pathways. EP3 receptor signals have been extensively studied using cells expressing a single receptor subtype. However, regular cells probably express multiple EP receptor subtypes or different hormone receptors on their plasma membranes. Hence, defining the cross-talk of multiple signaling pathways induced by the different EP receptor subtypes or hormone receptors is crucial for the biochemical and molecular biological understanding of hormone actions. Such analyses are essential for the evaluation of receptor-induced signaling pathways and receptor-induced physiological responses. In addition, these advanced studies will promote the development of the specific agonists and antagonists for clinical use against various hormone-related diseases.

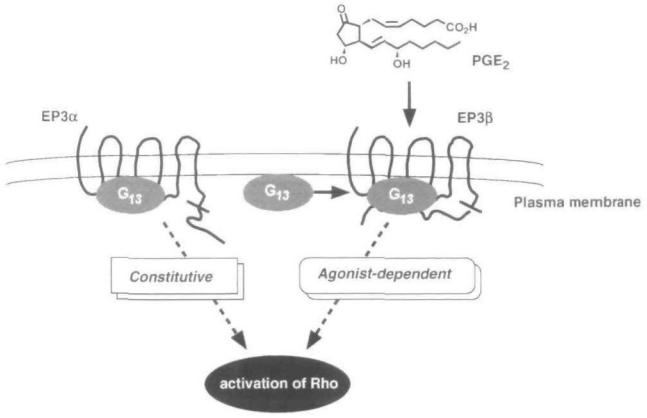

Fig. 2. **Schematic illustration of the mechanism of Rho activation induced by EP3 receptor isoform–G_{13} coupling.** The mouse EP3 receptor isoforms EP3α and EP3β constitutively and agonist-dependently activate the G_{13} protein respectively, leading to the activation of the small GTPase Rho.

REFERENCES

1. Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., Ichimasa, M., Sugamura, K., Nakamura, M., Takano, S., and Nagata, K. (2001) Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* **193**, 255–261
2. Negishi, M., Sugimoto, Y., and Ichikawa, A. (1995) Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim. Biophys. Acta* **1259**, 109–119
3. Sugimoto, Y., Negishi, M., Hayashi, Y., Namba, T., Honda, A., Watabe, A., Hirata, M., Narumiya, S., and Ichikawa, A. (1993) Two isoforms of the EP3 receptor with different carboxyl-terminal domains. *J. Biol. Chem.* **268**, 2712–2718
4. Irie, A., Sugimoto, Y., Namba, T., Harazono, A., Honda, A., Watabe, A., Negishi, M., Narumiya, S., and Ichikawa, A. (1993) Third isoform of the prostaglandin-E-receptor EP3 subtype with different C-terminal tail coupling to both stimulation and inhibition of adenylate cyclase. *Eur. J. Biochem.* **217**, 313–318
5. Negishi, M., Sugimoto, Y., Irie, A., Narumiya, S., and Ichikawa, A. (1993) Two isoforms of prostaglandin E receptor EP3 subtype. Different COOH-terminal domains determine sensitivity to agonist-induced desensitization. *J. Biol. Chem.* **268**, 9517–9521
6. Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., Ito, S., Ichikawa, A., and Narumiya, S. (1993) Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP3 determines G-protein specificity. *Nature* **365**, 166–170
7. Breyer, R.M., Emeson, R.B., Tarng, J.L., Breyer, M.D., Davis, L.S., Abromson, R.M., and Ferrenbach, S.M. (1994) Alternative

splicing generates multiple isoforms of a rabbit prostaglandin E_2 receptor. *J. Biol. Chem.* **269**, 6163–6169
8. Audoly, L.P., Ma, L., Feoktistov, I., DeFoe, S.K., Breyer, M.D., and Breyer, R.M. (1999) Prostaglandin E-prostanoid-3 receptor activation of cyclic AMP response element-mediated gene transcription. *J. Pharmacol. Exp. Ther.* **289**, 140–148
9. Adam, M., Boie, Y., Rushmore, T.H., Müller, G., Bastien, L., Mckee, K.T., Metters, K.M., and Abramovitz, M. (1994) Cloning and expression of three isoforms of the human EP3 prostanoid receptor. *FEBS Lett.* **338**, 170–174
10. Negishi, M., Hasegawa, H., and Ichikawa, A. (1996) Prostaglandin E receptor EP3γ isoform, with mostly full constitutive Gi activity and agonist-dependent Gs activity. *FEBS Lett.* **386**, 165–168
11. Hasegawa, H., Negishi, M., and Ichikawa, A. (1996) Two isoforms of the prostaglandin E receptor EP3 subtype different in agonist-independent constitutive activity. *J. Biol. Chem.* **271**, 1857–1860
12. Irie, A., Segi, E., Sugimoto, Y., Ichikawa, A., and Negishi, M. (1994) Mouse prostaglandin E receptor subtype mediates calcium signals via Gi in cDNA-transfected Chinese hamster ovary cells. *Biochem. Biophys. Res. Commun.* **204**, 303–309
13. Hatae, N., Yamaoka, K., Sugimoto, Y., Negishi, M., and Ichikawa, A. (2002) Augmentation of receptor-mediated adenylyl cyclase activity by Gi-coupled prostaglandin receptor subtype EP3 in a Gβγ subunit-independent manner. *Biochem. Biophys. Res. Commun.* **290**, 162–168
14. Fereman, A.D., Conklin, B.R., Schrader, K.A., Reed, R.R., and Bourne, H.R. (1992) Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature* **356**, 159–161
15. Hanke, S., Nürnberg, B., Groll, D.H., and Liebmann, C. (2001) Cross talk between β-adrenergic and bradykinin B_2 receptors results in cooperative regulation of cyclic AMP accumulation and mitogen-activated protein kinase activity. *Mol. Cell. Biol.* **21**, 8452–8460
16. Southhall, M.D. and Vasko, M.R. (2001) Prostaglandin receptor subtypes, EP3C and EP4, mediate the prostaglandin E_2-induced cAMP production and sensitization of sensory neurons. *J. Biol. Chem.* **276**, 16083–16091
17. Ostrom, R.S., Gregorian, C., Drenan, R.M., Xiang, Y., Regan, J.W., and Insel, P.A. (2001) Receptor number and caveolae colocalization determine receptor coupling efficiency to adenylyl cyclase. *J. Biol. Chem.* **276**, 42063–42069
18. Liu, J., Akahoshi, T., Jiang, S., Namai, R., Kitasato, H., Endo, H., Kameya, T., and Kondo, H. (2000) Induction of neutrophil death resembling neither apoptosis nor necrosis by ONO-AE-248, a selective agonist for PGE_2 receptor subtype 3. *J. Leukoc. Biol.* **68**, 187–193
19. Katoh, H., Negishi, M., and Ichikawa, A. (1996) Prostaglandin E receptor EP3 subtype induces neurite retraction via small GTPase Rho. *J. Biol. Chem.* **271**, 29780–29784
20. Aoki, J., Katoh, H., Yasui, H., Yamaguchi, Y., Nakamura, K., Hasegawa, H., Ichikawa, A., and Negishi, M. (1999) Signal transduction pathway regulating prostaglandin EP3 receptor-induced neurite retraction: requirement for two different tyrosine kinase. *Biochem. J.* **340**, 365–369
21. Jalink, K., Corven, E.J., Hengeveld, T., Morii, N., Narumiya, S., and Moolenaar, W.H. (1994) Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. *J. Cell. Biol.* **126**, 801–810
22. Katoh, H., Aoki, J., Yamaguchi, Y., Kitano, Y., Ichikawa, A., and Negishi, M. (1998) Constitutively active Galpha12, Galpha13, and Galphaq induce Rho-dependent neurite retraction through different signaling pathways. *J. Biol. Chem.* **273**, 28700–28707
23. Hasegawa, H., Negishi, M., Katoh, H., and Ichikawa, A. (1997) Two isoforms of prostaglandin EP3 receptor exhibiting constitutive activity and agonist-dependent activity in Rho-mediated stress fiber formation. *Biochem. Biophys. Res. Commun.* **234**, 631–636
24. Wolfe, L.S. (1982) Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. *J. Neurochem.* **38**, 1–14
25. Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tsuboi, K., Katsuyama, M., Ichikawa, A., Tanaka, T., Yoshida, N., and Narumiya, S. (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* **395**, 281–284
26. Sugimoto, Y., Shigemoto, R., Namba, T., Negishi, M., Mizuno, N., Narumiya, S., and Ichikawa, A. (1994) Distribution of the messenger RNA for the prostaglandin E receptor subtype EP3 in the mouse nervous system. *Neuroscience* **62**, 919–928
27. Hertting, G. and Seregi, A. (1989) Formation and function of eicosanoids in the central nervous system. *Ann. N.Y. Acad. Sci.* **559**, 84–99