Short communication

Effects of centrally administered prostaglandin E₃ and thromboxane A₃ on plasma noradrenaline and adrenaline in rats: comparison with prostaglandin E₂ and thromboxane A₂

Takahiro Shimizu*, Kunihiro Yokotani

*Department of Pharmacology, School of Medicine, Kochi University, Nankoku, Kochi
783-8505, Japan*

* Corresponding author. Tel./fax: +81-88-880-2328
E-mail address: shimizu@kochi-u.ac.jp (T. Shimizu)

Abstract

Previously, we reported the involvement of brain ω -6 prostanoids, especially prostaglandin E_2 and thromboxane A_2 , in the activation of central sympatho-adrenomedullary outflow in rats. ω -3 Prostanoids, including prostaglandin E_3 and thromboxane A_3 , are believed to be less bioactive than ω -6 prostanoids, although studies on the functions of ω -3 prostanoids in the central nervous system have not been reported. In the present study, therefore, we compared the effects of centrally administered ω -3 prostanoids, prostaglandin E_3 and thromboxane A_3 , with those of ω -6 prostanoids, prostaglandin E_2 and thromboxane A_2 , on the plasma catecholamines in anesthetized rats. Intracerebroventricularly (i.c.v.) administered prostaglandin E_2 (0.15, 0.3 and 1.5 nmol/animal) and prostaglandin E_3 (0.3 and 3 nmol/animal) predominantly elevated plasma noradrenaline but not adrenaline, but the latter was less efficient than the former. On the other hand, U-46619 (an analog of thromboxane A_2) (30, 100 and 300 nmol/animal, i.c.v.) and Δ^{17} -U-46619 (an analog of thromboxane A_3) (100 and 300 nmol/animal, i.c.v.) both elevated plasma catecholamines (adrenaline >> noradrenaline) to the same degree. These results suggest that centrally administered prostaglandin E_3 is less effective than prostaglandin E_2 to elevate plasma noradrenaline, and that thromboxane A_3 is almost as equipotent as thromboxane A_2 to elevate plasma catecholamines in rats.

Keywords: Prostaglandin E₂; Prostaglandin E₃; Thromboxane A₂; Thromboxane A₃;
Brain; Noradrenaline; Adrenaline

1. Introduction

Prostanoids (prostaglandins and thromboxanes) are bioactive lipids derived from membrane polyunsaturated fatty acid (PUFA). Cyclooxygenase is the key enzyme in prostanoids synthesis from arachidonic acid, a common ω -6 PUFA. On the other hand, ω -3 PUFA (eicosapentaenoic acid and docosahexaenoic acid) found primarily in fish oils can function as substrates for cyclooxygenase (Jump, 2002). When the availability of ω -3 PUFA is increased by fish oil ingestion, the formation of ω -3 PUFA-derived prostanoids (ω -3 prostanoids) such as prostaglandin E_3 is increased but the corresponding ω -6 PUFA-derived prostanoids (ω -6 prostanoids) such as prostaglandin E_2 is reduced (Fischer and Weber, 1984; Fischer et al., 1988; Knapp, 1990). Although similar in structure and stability (Hansen, 1983), ω -3 prostanoids have been considered to be less bioactive than ω -6 prostanoids. For instance, prostaglandin E_3 , unlike prostaglandin E_2 , is not mitogenic to NIH 3T3 fibroblasts and substantially less efficient than prostaglandin E_2 in interleukin-6 synthesis in RAW 264.7 macrophages (Bagga et al., 2003). Prostaglandin $F_{3\alpha}$ is less protective than prostaglandin $F_{2\alpha}$ against gastric mucosal injury in rats (Faust et al., 1989) and prostaglandin I_3 is less potent than prostaglandin I_2 in inhibition of platelet aggregation in rabbits (Kobzar et al., 2001). However, studies directly comparing the functions of ω -6 versus ω -3 prostanoids in the central nervous system have not been reported, although ω -3 PUFA deficiency has been shown to be associated with memory loss and diminished cognitive function (Salem et al., 2001).

It has been demonstrated that ω -6 prostanoids act as a neurotransmitter and/or neuromodulator in the brain's actions on the cardiovascular function (Wood et al., 1993; Zhang et al., 2003) and regulation of hormone secretion (Bernardini et al., 1989; Reimsnider and Wood, 2006). We previously reported that centrally administered arachidonic acid, a source of ω -6 prostanoids, elevated plasma levels of noradrenaline and adrenaline by a cyclooxygenase-dependent mechanism in rats (Yokotani et al., 2000). Furthermore, centrally administered ω -6 prostanoid prostaglandin E₂ (but not prostaglandin D₂, I₂ and F_{2alpha}) elevated plasma noradrenaline in rats (Yokotani et al., 1995, 2005; Murakami et al., 2002) and thromboxane A₂ mimetic elevated plasma catecholamines (adrenaline >> noradrenaline) in rats (Murakami et al., 2002). These lines of evidence suggest that centrally administered ω -6 prostanoids, especially prostaglandin E₂ and thromboxane A₂, activate the central sympatho-adrenomedullary outflow in rats. In the present study, therefore, we compared the effects of centrally administered ω -3 prostanoids (prostaglandin E₃ and thromboxane A₃) and ω -6 prostanoids (prostaglandin E₂ and thromboxane A₂) on the plasma catecholamines using urethane-anesthetized rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 350 g were maintained in an air-conditioned room at 22-24°C under a constant day-night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water *ad libitum*. Under urethane anesthesia (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 ml/h) and the femoral artery was cannulated for collecting blood samples. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for intracerebroventricular administration of test substances using a stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP -0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas (Paxinos and Watson, 1986). Three hours were allowed to elapse before the application of reagents.

Prostaglandin E₂ and prostaglandin E₃ were dissolved in 99% ethanol and stored at -20°C. These stock solutions were diluted with saline whenever we used them and the final concentration of ethanol was adjusted to 0.5%. These diluted solutions were slowly injected into the right lateral ventricle in a volume of 10 µl/animal using a 25-µl Hamilton syringe. U-46619 and Δ^{17} -U-46619 dissolved in 100% *N,N*-dimethylformamide (DMF) were intracerebroventricularly (i.c.v.) administered in a

volume of 2.5 μ l/animal using a 10- μ l Hamilton syringe. Each animal received only one dose of these reagents or vehicles.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Kochi University.

2.2. *Measurement of plasma catecholamines*

Blood samples (250 μ l) were collected through an arterial catheter and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically with high performance liquid chromatography (HPLC) (Shimizu et al., 2004). Briefly, after centrifugation (1,500 g for 10 min, at 4°C), the plasma (100 μ l) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of twice deionized water, 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA and 1 ng of 3,4-dihydroxybenzylamine as an internal standard. The tube was shaken for 10 min and the alumina was washed three times with 4 ml of ice-cold twice deionized water. Then, catecholamines adsorbed onto the alumina were eluted with 300 μ l of 4% acetic acid containing 0.1 mM disodium EDTA. A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France) and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with HPLC. Analytical conditions were as follows: detector, +450 mV potential against an Ag/AgCl reference electrode; column, Eicompac CA-50DS, 2.1 x 150 mm (Eicom);

mobile phase, 0.1 M NaH_2PO_4 - Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l disodium EDTA, 0.75 g/l sodium 1-octanesulfonate and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 μl . The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. By this assay, coefficients of variation for intra- and inter-assay were 3.0 and 3.7%, respectively, and 0.5 pg of noradrenaline and adrenaline was accurately determined.

2.3. *Treatment of data and statistics*

All values are expressed as the means $\pm$ S.E.M. of the net changes above the respective basal values. The data were analyzed by repeated-measure analysis of variance (ANOVA), followed by *post-hoc* analysis with the Bonferroni method. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. *Compounds*

The following drugs were used: prostaglandin E_2 (Sigma Aldrich Fine Chemicals, St. Louis, MO, U.S.A.); prostaglandin E_3 , U-46619 (9,11-dideoxy-9 α , 11 α -methanoepoxy-prosta-5Z,13E-dien-1-oic acid) and Δ^{17} -U-46619 (9,11-dideoxy-9 α , 11 α -methanoepoxy-prosta-5Z,13E,17Z-trien-1-oic acid) (Cayman Chemical, Ann Arbor, MI, U.S.A.). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of centrally administered prostaglandin E₂ and prostaglandin E₃ on the plasma levels of catecholamines

Blood sampling for 5 times during 60 min had no effect on the basal plasma levels of either noradrenaline or adrenaline (Fig. 1A and B). Treatment with vehicle (10 μ l of 0.5% ethanol in saline, i.c.v.) also had no effect on the basal plasma levels of catecholamines (Fig. 1A and B). In preliminary studies, we monitored systemic blood pressure in our animal model since a large increase in peripheral noradrenaline is well known to alter systemic blood pressure. The blood pressure was largely unaffected by the blood sampling (data not shown). Ten minutes after administration of prostaglandin E₂ (0.3 nmol/animal, i.c.v.), mean arterial blood pressure was increased from 76 ± 6 to 106 ± 10 mmHg (n=4).

Prostaglandin E₂ [0.15, 0.3 and 1.5 nmol (53, 106 and 529 ng)/animal, i.c.v.] dose-dependently elevated the plasma level of noradrenaline (Fig. 1A). The responses in each dose of prostaglandin E₂ reached a maximum 10 min after administration of the reagent and then declined towards their basal levels (Fig. 1A). On the other hand, prostaglandin E₂ (0.15, 0.3 and 1.5 nmol/animal, i.c.v.) slightly, but not significantly, elevated the plasma level of adrenaline (Fig. 1A).

I.c.v. administered prostaglandin E₃ [0.3 nmol (105 ng)/animal] had little effect on the plasma level of noradrenaline, but a large dose of the reagent [3 nmol (1.1 μ g)/animal, i.c.v.] significantly elevated the plasma level of noradrenaline (Fig. 1B). The response also reached a maximum 10 min after administration of the reagent and

then declined toward the basal levels (Fig. 1B). On the other hand, prostaglandin E₃ (0.3 and 3 nmol/animal, i.c.v.) slightly elevated the plasma level of adrenaline, but a maximal response was obtained by a dose of 0.3 nmol/animal (Fig. 1B).

3.2. Effects of centrally administered U-46619 (an analog of thromboxane A₂) and Δ^{17} -U-46619 (an analog of thromboxane A₃) on the plasma levels of catecholamines

Treatment with vehicle (2.5 μ l of DMF, i.c.v.) also had no effect on the basal plasma levels of catecholamines (Fig. 2A and B). U-46619 [30, 100 and 300 nmol (11, 35 and 105 μ g)/animal, i.c.v.] significantly elevated the plasma levels of noradrenaline and adrenaline (adrenaline >> noradrenaline); maximal noradrenaline responses were obtained by a dose of 100 nmol/animal (i.c.v.) and maximal adrenaline responses were obtained by a dose of 300 nmol/animal (i.c.v.), respectively (Fig. 2A). The noradrenaline and adrenaline responses reached a maximum at 5 and 10 min after administration of U-46619, respectively, and then declined towards basal levels (Fig. 2A).

I.c.v. administered Δ^{17} -U-46619 [100 nmol (35 μ g)/animal] had little effect on the plasma levels of noradrenaline and adrenaline, but the reagent significantly elevated the plasma levels of both catecholamines (adrenaline >> noradrenaline) with a large dose [300 nmol (105 μ g)/animal, i.c.v.] (Fig. 2B). These responses reached a maximum 10 min after administration of the reagent and then declined towards basal levels (Fig. 2B).

4. Discussion

So far four types of prostanoid receptors (EP₁, EP₂, EP₃ and EP₄) have been identified to couple with prostaglandin E₂ (Breyer et al., 2001). Prostanoid EP₃ receptor mRNA is highly expressed in brain regions such as the hippocampus, preoptic area and hypothalamus (Sugimoto et al., 1994; Vasilache et al., 2007). Previously, we reported the involvement of brain prostanoid EP₃ receptors in the centrally administered prostaglandin E₂-induced elevation of plasma noradrenaline in rats (Yokotani et al., 1995). Recently, prostanoid EP₃ receptors have been shown to bind with less affinity and lower efficiency to prostaglandin E₃ than prostaglandin E₂ (Wada et al., 2007). In the present experiment, centrally administered prostaglandin E₂ (0.15, 0.3 and 1.5 nmol/animal) effectively elevated plasma noradrenaline in a dose-dependent manner, while prostaglandin E₃ effectively elevated plasma noradrenaline only at a larger dose (3 nmol/animal). Both prostaglandin E₂ and E₃ had little effect on plasma adrenaline. These results suggest that centrally administered prostaglandin E₃ is less effective than prostaglandin E₂ to elevate plasma noradrenaline in rats.

Messenger RNA of thromboxane receptors (prostanoid TP receptors) has been reported to be present in the brain (Namba et al., 1992; Gao et al., 1997). Previously, we reported that centrally administered thromboxane A₂ mimetic induced the elevation of plasma catecholamines (adrenaline >> noradrenaline) by activation of the brain prostanoid TP receptors in rats (Murakami et al., 2002). In the present experiment, we used two analogs of thromboxane A₂ and A₃, U-46619 and Δ^{17} -U-46619 (Abramovitz et al., 2000), because of the instability of thromboxanes ($t_{1/2}$ for thromboxane A₂, ~30 s). Centrally administered U-46619 (100 and 300 nmol/animal) effectively elevated

plasma levels of both catecholamines (adrenaline >> noradrenaline) in rats. On the other hand, centrally administered Δ^{17} -U-46619 significantly elevated plasma levels of both catecholamines (adrenaline >> noradrenaline) only at a larger dose (300 nmol/animal), at this dose Δ^{17} -U-46619-induced responses were almost the same as those induced by U-46619. Recently, it has been shown that both U-46619 and Δ^{17} -U-46619 activate human platelet aggregation via TP receptors and the EC₅₀ values for them are similar (650 and 850 nM, respectively) (Wada et al., 2007). These lines of evidence suggest that centrally administered thromboxane A₃ is almost equipotent for thromboxane A₂ to elevate plasma catecholamines in rats.

Dietary supplements of fish oils rich in ω -3 PUFA have been reported to increase the levels of ω -3 PUFA (docosahexaenoic acid) in the hippocampus and forebrain of aged rats (Puskás et al., 2003; Dyllal et al., 2007). Interestingly, these supplements have been shown to drastically reduce the levels of arachidonic acid in the hippocampus (Puskás et al., 2003). Recently, we reported the possibility that brain phosphatidylinositol-derived arachidonic acid is involved in the centrally administered histamine-induced elevation of plasma catecholamines (Shimizu et al., 2007). Dietary ω -3 PUFA has been shown to increase the incorporation of ω -3 PUFA (docosahexaenoic acid) in the brain structural phosphatidylinositol and to reduce blood pressure in hypertensive rats (de Wilde et al., 2003). These observations suggest a possibility that supplemented ω -3 PUFA competes with arachidonic acid at the level of incorporation into membrane phospholipids and at the level of substrate for cyclooxygenase, thereby increasing the synthesis of ω -3 prostanoids in the brain. Additionally, it has been reported that sympathetic outflow is reduced by ω -3 PUFA as

suggested by decreased plasma level of noradrenaline after increased dietary intake of fish or fish oils in humans or rats, respectively (Singer et al., 1983; Hashimoto et al., 1999). Taken together with the results of the present study, ω -3 PUFA supplementation may attenuate the activation of central sympatho-adrenomedullary outflow by increasing the synthesis of ω -3 prostanoids such as prostaglandin E₃ in the brain.

In summary, we demonstrated here that (1) centrally administered prostaglandin E₃ is less effective than prostaglandin E₂ on elevation of plasma noradrenaline, and (2) centrally administered thromboxane A₃ is almost as effective as thromboxane A₂ on elevation of plasma catecholamines (adrenaline >> adrenaline) in rats.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 20590702) from the Japan Society for the Promotion of Science, a Grant from The Smoking Research Foundation in Japan, The Kochi University President's Discretionary Grant and The Head of the Kochi Medical School Hospital's Discretionary Grant.

References

- Abramovitz, M., Adam, M., Boie, Y., Carrière, M., Denis, D., Godbout, C., Lamontagne, S., Rochette, C., Sawyer, N., Tremblay, N.M., Belley, M., Gallant, M., Dufresne, C., Gareau, Y., Ruel, R., Juteau, H., Labelle, M., Ouimet, N., Metters, K.M., 2000. The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochem. Biophys. Acta.* 1483, 285-293.
- Anton, A.H., Sayre, D.F., 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.* 138, 360-375.
- Bagga, D., Wang, L., Farias-Eisner, R., Glaspy, J.A., Reddy, S.T., 2003. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1751-1756.
- Bernardini, R., Chiarenza, A., Calogera, A.E., Gold, P.W., Chrousos, G.P., 1989. Arachidonic acid metabolites modulate rat hypothalamic corticotropin-releasing hormone secretion in vitro. *Neuroendocrinology* 50, 708-715.
- Breyer, R.M., Bagdassarian, C.K., Myers, S.A., Breyer, M.D., 2001. Prostanoid receptors: subtypes and signaling. *Annu. Rev. Pharmacol. Toxicol.* 41, 661-690.
- de Wilde, M.C., Hogyes, E., Kiliaan, A.J., Farkas, T., Luiten, P.G., Farkas, E., 2003. Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats. *Brain Res.* 988, 9-19.

- Dyall, S.C., Michael, G.J., Whelpton, R., Scott, A.G., Michael-Titus, A.T., 2007. Dietary enrichment with omega-3 polyunsaturated fatty acids reverses age-related decreases in the GluR2 and NR2B glutamate receptor subunits in rat forebrain. *Neurobiol Aging* 28, 424-439.
- Faust, T.W., Lee, E., Redfern, J.S., Feldman, M., 1989. Effect of prostaglandin F_{3alpha} on gastric mucosal injury by ethanol in rats: comparison with prostaglandin F_{2alpha}. *Prostaglandins* 37, 493-504.
- Fischer, S., Weber, P.C., 1984. Prostaglandin I₃ is formed in vivo in man after dietary eicosapentaenoic acid. *Nature* 307, 165-168.
- Fischer, S., von Schacky, C., Schweer, H., 1988. Prostaglandins E₃ and F_{3alpha} are excreted in human urine after ingestion of n-3 polyunsaturated fatty acids. *Biochem. Biophys. Acta.* 963, 501-508.
- Gao, H., Peng, B., Welch, W.J., Wilcox, C.S., 1997. Central thromboxane receptors: mRNA expression and mediation of pressor responses. *Am. J. Physiol.* 272, R1493-R1500.
- Hansen, H.S., 1983. Dietary essential fatty acids and in vivo prostaglandin production in mammals. *World Rev. Nutr. Diet* 42, 102-134.
- Hashimoto, M., Shinozuka, K., Gamoh, S., Tanabe, Y., Hossain, M.S., Kwon, Y.M., Hata, N., Misawa, Y., Kunitomo, M., Masumura, S., 1999. The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. *J. Nutr.* 129, 70-76.
- Jump, D.B., 2002. The biochemistry of n-3 polyunsaturated fatty acids. *J. Biol. Chem.* 277, 8755-8758.

- Knapp, H.R., 1990. Prostaglandins in human semen during fish oil ingestion: evidence for in vivo cyclooxygenase inhibition and appearance of novel trienoic compounds. *Prostaglandins* 39, 407-423.
- Kobzar, G., Mardla, V., Järving, I., Samel, N., 2001. Comparison of anti-aggregatory effects of PGI₂, PGI₃ and iloprost on human and rabbit platelets. *Cell Physiol. Biochem.* 11, 279-284.
- Murakami, Y., Okada, S., Nishihara, M., Yokotani, K., 2002. Roles of brain prostaglandin E₂ and thromboxane A₂ in the activation of the central sympatho-adrenomedullary outflow in rats. *Eur. J. Pharmacol.* 452, 289-294.
- Namba, T., Sugimoto, Y., Hirata, M., Hayashi, Y., Honda, A., Watabe, A., Negishi, M., Ichikawa, A., Narumiya, S., 1992. Mouse thromboxane A₂ receptor: cDNA cloning, expression and northern blot analysis. *Biochem. Biophys. Res. Commun.* 184, 1197-1203.
- Paxinos, G., Watson, C., 1986. In: Paxinos, G., Watson, C. (Eds.), *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Boston.
- Puskás, L.G., Kitajka, K., Nyakas, C., Barcelo-Coblijn, G., Farkas, T., 2003. Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1580-1585.
- Reimsnider, S., Wood, C.E., 2006. Differential modulation of ovine fetal ACTH secretion by PGHS-1 and PGHS-2. *Neuroendocrinology* 83, 4-11.
- Salem, N. Jr., Litman, B., Kim, H.Y., Gawrisch, K., 2001. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids* 36, 945-959.

- Shimizu, T., Okada, S., Yamaguchi-Shima, N., Yokotani, K., 2004. Brain phospholipase C-diacylglycerol lipase pathway is involved in vasopressin-induced release of noradrenaline and adrenaline from adrenal medulla in rats. *Eur. J. Pharmacol.* 499, 99-105.
- Shimizu, T., Yamaguchi, N., Okada, S., Lu, L., Sasaki, T., Yokotani, K., 2007. Roles of brain phosphatidylinositol-specific phospholipase C and diacylglycerol lipase in centrally administered histamine-induced adrenomedullary outflow in rats. *Eur. J. Pharmacol.* 571, 138-144..
- Singer, P., Jaeger, W., Wirth, M., Voigt, S., Naumann, E., Zimontkowski, S., Hajdu, I., Goedicke, W., 1983. Lipid and blood pressure-lowering effect of mackerel diet in man. *Atherosclerosis* 49, 99-108.
- Sugimoto, Y., Shigemoto, R., Namba, T., Negishi, M., Mizuno, N., Narumiya, S., Ichikawa, A., 1994. Distribution of the messenger RNA for the prostaglandin E receptor subtype EP₃ in the mouse nervous system. *Neuroscience* 62, 919-928.
- Vasilache, A.M., Andersson, J., Nilsberth, C., 2007. Expression of PGE₂ EP₃ receptor subtypes in the mouse preoptic region. *Neurosci. Lett.* 423, 179-183.
- Wada, M., DeLong, C.J., Hong, Y.H., Rieke, C.J., Song, I., Sidhu, R.S., Yuan, C., Warnock, M., Schmaier, A.H., Yokoyama, C., Smyth, E.M., Wilson, S.J., FitzGerald, G.A., Garavito, R.M., Sui de X., Regan, J.W., Smith, W.L., 2007. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. *J. Biol. Chem.* 282, 22254-22266.
- Wood, C.E., Cudd, T.A., Kane, C., Enkelke, K., 1993. Fetal ACTH and blood pressure responses to thromboxane mimetic U-46619. *Am. J. Physiol.* 265, R858-R862.

- Yokotani, K., Nishihara, M., Murakami, Y., Hasegawa, T., Okuma, Y., Osumi, Y., 1995. Elevation of plasma noradrenaline levels in urethane-anaesthetized rats by activation of central prostanoid EP₃ receptors. *Br. J. Pharmacol.* 115, 672-676.
- Yokotani, K., Wang, M., Murakami, Y., Okada, S., Hirata, M., 2000. Brain phospholipase A₂-arachidonic acid cascade is involved in the activation of central sympatho-adrenomedullary outflow in rats. *Eur. J. Pharmacol.* 398, 341-347.
- Yokotani, K., Okada, S., Nakamura, K., Yamaguchi-Shima, N., Shimizu, T., Arai, J., Wakiguchi, H., Yokotani, K., 2005. Brain prostanoid TP receptor-mediated adrenal noradrenaline secretion and EP₃ receptor-mediated sympathetic noradrenaline release in rats. *Eur. J. Pharmacol.* 512, 29-35.
- Zhang, Z.H., Wei, S.G., Francis, J., Felder, R.B., 2003. Cardiovascular and renal sympathetic activation by blood-borne TNF-alpha in rat: the role of central prostaglandins. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R916-R927.

Legends to figures

Fig. 1. Effects of centrally administered prostaglandin E₂ and prostaglandin E₃ on the plasma levels of catecholamines. Δ Noradrenaline and Δ Adrenaline: increments of noradrenaline and adrenaline above the basal level. Each point represents the mean $\pm$ S.E.M. (A) Arrow indicates the administration of vehicle (0.5% ethanol in saline 10 μ l/animal, i.c.v.) or prostaglandin E₂ (0.15, 0.3 and 1.5 nmol/animal, i.c.v.).

*Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(3,19)=13.12$, $P<0.017$; at 10 min, $F(3,19)=26.39$, $P<0.017$; at 30 min, $F(3,16)=5.09$, $P<0.017$; at 60 min, $F(3,19)=3.48$, $P<0.017$]. (B) Arrow indicates the administration of vehicle or prostaglandin E₃ (0.3 and 3 nmol/animal, i.c.v.). The vehicle-treated group is the same as that in (A). *Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(2,14)=3.38$, $P<0.025$; at 10 min, $F(2,15)=6.43$, $P<0.025$; at 30 min, $F(2,15)=10.77$, $P<0.025$; at 60 min, $F(2,14)=8.02$, $P<0.025$: adrenaline; at 5 min, $F(2,15)=5.17$, $P<0.025$; at 10 min, $F(2,15)=5.27$, $P<0.025$]. The actual values for noradrenaline and adrenaline at 0 min were 261 ± 18 and 288 ± 27 pg/ml ($n=36$), respectively.

Fig. 2. Effects of centrally administered U-46619 (an analog of thromboxane A₂) and Δ^{17} -U-46619 (an analog of thromboxane A₃) on the plasma levels of catecholamines. Each point represents the mean $\pm$ S.E.M. Other conditions are the same as those of Fig. 1. (A) Arrow indicates the administration of vehicle (100% DMF 2.5 μ l/animal, i.c.v.) or U-46619 (30, 100 and 300 nmol/animal, i.c.v.). *Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 5 min,

$F(3,12)=7.20$, $P<0.017$; at 10 min, $F(3,12)=5.04$, $P<0.017$: adrenaline; at 5 min, $F(3,12)=8.67$, $P<0.017$; at 10 min, $F(3,12)=9.88$, $P<0.017$; at 30 min, $F(3,12)=10.41$, $P<0.017$]. (B) Arrow indicates the administration of vehicle or Δ^{17} -U-46619 (100 and 300 nmol/animal, i.c.v.). The vehicle-treated group is the same as that in (A).

*Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 10 min, $F(2,10)=7.31$, $P<0.025$: adrenaline; at 10 min, $F(2,10)=6.43$, $P<0.025$; at 30 min, $F(2,10)=14.17$, $P<0.025$]. The actual values for noradrenaline and adrenaline at 0 min were 263 ± 21 and 231 ± 29 pg/ml ($n=25$), respectively.

Figure-1

Figure-2

A

B