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REVIEW —*Current Perspective*—

Roles of Prostanoids Revealed From Studies Using Mice Lacking Specific Prostanoid Receptors

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ABSTRACT—The actions of prostanoids in various physiological and pathophysiological conditions have been being examined using mice lacking different prostanoid receptors. Prostaglandin (PG) I_2 worked not only as a mediator of inflammation but also as an antithrombotic agent. $PGF_{2\alpha}$ was found to be an essential inducer of labor. Several important actions of PGE_2 are exerted via each of the four PGE_2 receptor subtypes: EP_1, EP_2, EP_3 and EP_4. PGE_2 participated in colon carcinogenesis via the EP_1. PGE_2 also participates in ovulation and fertilization and contributes to the control of blood pressure under high-salt intake via the EP_2. PGE_2 worked as a mediator of febrile responses to both endogenous and exogenous pyrogens and as a regulator of bicarbonate secretion induced by acid-stimulation in the duodenum via the EP_3. It regulated the closure of ductus arteriosus and showed bone resorbing action via the EP_4. PGD_2 was found to be a mediator of allergic asthma. These studies have revealed important roles of prostanoids, some of which had not previously been known.

Keywords: Prostanoid, Prostaglandin, Thromboxane, Prostanoid receptor, Knock-out mouse

Prostanoids, the prostaglandins (PGs) and thromboxanes (TXs), are the cyclooxygenase metabolites of arachidonic acids and exert a range of actions in the body. These actions are mediated by their respective receptors expressed in the target cells, which are generally present in the vicinity of prostanoid production. The receptors include the DP, EP, FP, IP and TP receptors for PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2, respectively. Moreover, there are four subtypes of the EPs: EP_1, EP_2, EP_3 and EP_4 (1–4). Recently, mice lacking each type and subtype of the prostanoid receptor have been produced and are being examined to clarify the roles of the prostanoids in various physiological and pathophysiological conditions. In this article, we summarize the roles of the prostanoids revealed by these studies to date.

Roles of PGI_2 in inflammation and thrombosis

Prostanoids are known to be an important mediator of inflammation. At inflammation sites, PGE_2 and PGI_2 synergize with other inflammatory mediators, such as histamine and bradykinin, to induce an increase in vascular permeability and hyperalgesia (5, 6), although which of these prostaglandins mediates the effects in vivo has not been fully elucidated. While PGI_2 is a potent inhibitor of platelet activation and shows a protective effect on vascular endothelial cells, the roles of PGI_2 in thrombosis and/or atherosclerosis have not been fully determined. To clarify these issues, the gene encoding the IP receptor was disrupted (7). Mice lacking the IP ($IP^{-/-}$ mice) showed decreased paw edema compared with wild-type mice when injected with carrageenan. Indomethacin treatment decreased this edema by approx. 50% in wild-type mice, which was comparable to the decrease found in $IP^{-/-}$ mice. The role of PGI_2 in inflammatory pain was examined using the acetic acid-induced writhing test. $IP^{-/-}$ mice showed markedly decreased responses compared with wild-type mice, and their responses were again comparable to those observed in wild-type mice treated with indomethacin. These results suggest that PGI_2 is the main prostanoid mediating the increase of vascular permeability and generating inflamma-

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tory pain, at least in these systems. However, PGE_2 and/or PGD_2 would participate in increased vascular permeability and pain transmission under different inflammatory conditions as has been suggested (1). The anti-thrombotic effect of PGI_2 was examined using a thrombosis model in which vascular endothelial cells were damaged by the topical application of $FeCl_3$ around the carotid artery. $IP^{-/-}$ mice showed a markedly enhanced thrombotic tendency, suggesting an important anti-thrombotic role of PGI_2 in thrombosis following endothelial damage. This may partially explain the anti-atherosclerotic role of PGI_2, because atherosclerotic lesions begin with endothelial damage.

Roles of $PGF_{2\alpha}$ in parturition

$PGF_{2\alpha}$ is known to be a potent constrictor of the uterus and a luteolytic factor (8). However, its role in parturition has not been determined. While female $FP^{-/-}$ mice showed a normal estrous cycle, ovulation, fertilization and implantation, they failed to perform parturition due to the absence of labor (9). This failure was observed in homozygous female mice regardless of the paternal genotype. Moreover, fetuses could be rescued by cesarean operation at the expected term and developed normally, indicating that the absence of labor was of maternal origin. Because uterine sensitivity to oxytocin increases markedly at term due to the upregulation of oxytocin receptors in the uterus, oxytocin has been proposed to be a key regulator of parturition (10, 11). In $FP^{-/-}$ mice, uterine sensitivity to oxytocin remained low, and expression of oxytocin receptor mRNA did not change at term. On the other hand, parturition is preceded by a decline in plasma concentration of progesterone, leading to an increase in myometrial contractility (12, 13). While the plasma concentration of progesterone decreased progressively at day 19–21 in wild-type mice, it remained at a high level during this period in $FP^{-/-}$ mice. These results suggest that failure of parturition in $FP^{-/-}$ mice may be derived from persistent production of progesterone. In accordance with this, the ovariectomized $FP^{-/-}$ female at term delivered pups alive after 24 h, and uterine expression of oxytocin receptor mRNA was induced within 12 h. These results clearly showed that $PGF_{2\alpha}$ works upstream of oxytocin to induce luteolysis and that its uterotonic action in myometrium is not essential for parturition.

Role of PGE_2 in closure of ductus arteriosus and bone resorption

More than 95% of $EP_4^{-/-}$ neonates became pale and lethargic approx. 24 h after birth and died within 72 h (14, 15). Histological examination of dying $EP_4^{-/-}$ neonates showed marked congestion in the pulmonary capillaries and disorganized and collapsed alveolar structures, suggesting left-sided heart failure. Further examination revealed patent ductus arteriosus in $EP_4^{-/-}$ neonates. The ductus arteriosus, which connects the main pulmonary artery and the descending aorta, shunt the pulmonary blood flow to the aorta during the fetal period (16) and closes just after birth in accordance with the start of respiration. In fact, PGE_2 relaxes via the EP_4 receptor the ductus arteriosus (17), in which the mRNA was abundantly expressed (15). Moreover, less than 5% of the $EP_4^{-/-}$ neonates, those which survived and grew normally, had closed ductus arteriosus. These results suggest that left-sided heart failure caused by patent ductus arteriosus may be a major cause of neonatal death of $EP_4^{-/-}$ mice. However, this patency of ductus arteriosus in $EP_4^{-/-}$ neonates did not coincide with the relaxant actions mediated by the EP_4. The reason for this discrepancy is unknown and remains an interesting issue to be clarified.

Among the prostanoids produced by osteoblasts, PGE_2 is a major product and its synthesis is regulated by several cytokines. While PGE_2 primarily stimulates bone resorption in vitro, it stimulates both bone formation and resorption in vivo (18–20). Miyaura et al. and Sakuma et al. recently examined which subtypes of the EPs participate in this bone resorption to PGE_2 using mice lacking each of these subtypes (21, 22). PGE_2 concentration-dependently released Ca^{2+} into the medium of calvarial or long born cultures and induced osteoclast formation detected by staining for tartrate-resistant acid phosphatase. This bone-resorbing activity of PGE_2 was deficient specifically in cultures from $EP_4^{-/-}$ mice, suggesting the mediation of this action by the EP_4. However, the roles of PGE_2 in both bone resorption and formation in vivo have not been fully elucidated; they are expected to be clarified in the near future using mice lacking each of the EP subtypes.

Roles of PGE_2 in fever generation and bicarbonate secretion in the duodenum

Fever is elicited by exogenous pyrogens such as lipopolysaccharide (LPS) and by non-infectious inflammatory insults. Both stimulate the production of inflammatory cytokines, which work as endogenous pyrogens acting on the brain (23). These cytokines include interleukin (IL)-1β, IL-6, IL-8, TNFα and macrophage inflammatory protein (MIP)-1β. Because aspirin-like drugs suppress fever, prostanoids have been implicated in fever generation (24). Among the prostanoids, PGE_2 was proposed as a central mediator of fever (25), but this has been much debated (23, 26–29). Moreover, the receptor subtype that mediates this action of PGE_2 has not been determined, although some investigators suggested the participation of the EP_1 using SC19220, an EP_1 antagonist, as a tool, which, however, was revealed not to be able to discriminate the four subtypes of the EP receptor of rodents (30). To address these issues, mice lacking each subtype of EPs were produced

(31). While PGE_2, when injected intracerebroventricularly, induced a febrile response in wild-type, $EP_1^{-/-}$, $EP_2^{-/-}$ and $EP_4^{-/-}$ mice all to a similar extent, $EP_3^{-/-}$ mice failed to respond to PGE_2. $EP_3^{-/-}$ mice also failed to respond to IL-1β injected intravenously or intracerebroventricularly, indicating that EP_3 mediates the IL-1β-Induced febrile response irrespective of the route of administration of IL-1β. Fever generated by LPS, an exogenous pyrogen, cannot be accounted for by the action of IL-1β alone (32, 33). For example, LPS induces the production of other endogenous pyrogens, including MIP-1β and IL-8, which were suggested to induce fever in a prostaglandin-independent manner (34, 35). Therefore, attemps were made to determine whether the EP_3 also contributes to LPS-induced fever. The responsiveness of peritoneal macrophage to LPS was examined first by determining the production of IL-1β and IL-6 because the prostanoids were reported to participate in the regulation of cytokine production of macrophages. Macrophages from $EP_3^{-/-}$ mice produced the two cytokines in amounts similar to those observed with macrophages from the wild-type mice, indicating that the initial step of the response to LPS may not be impaired in $EP_3^{-/-}$ mice. The febrile response to LPS was then examined. LPS injected intravenously induced a fever in wild-type mice, whereas it failed to induce a fever in $EP_3^{-/-}$ mice, suggesting that the EP_3 is essential in the febrile response to LPS. It was also confirmed that $EP_3^{-/-}$ mice show a normal febrile response when subjected to a restraint stress, suggesting that these mice maintain the efferent pathway to fever generation intact.

In the gastrointestinal tract, PGE_2 is a major prostanoid and shows a variety of actions (36). Among these actions, duodenal bicarbonate secretion is essential for maintaining mucosal integrity, because secreted bicarbonate protects the mucosa from acid-induced injury. While bicarbonate secretion is under control of neuronal regulation, endogenous PGE_2 produced by acid stimulation plays an important role in local maintenance of the surface pH gradient (37 – 39). However, physiological roles of PGE_2 in duodenal bicarbonate secretion and the receptor subtype(s) mediating the effect of PGE_2 have not been determined. Takeuchi et al. recently addressed this issue using $EP_1^{-/-}$ and $EP_3^{-/-}$ mice (40); duodenal bicarbonate secretion increased in response to mucosal acidification to a similar extent in wild-type and $EP_1^{-/-}$ mice, although the duodenal mucosa of $EP_3^{-/-}$ mice failed to respond at all to the stimulus. Taken together with the result that indomethacin completely suppressed the response in wild-type mice, PGE_2 would appear to play a major role via the EP_3 receptor in acid-induced bicarbonate secretion. In accordance with this result, acid-induced mucosal injury was markedly augmented in $EP_3^{-/-}$ mice compared with that in wild-type mice and was almost similar to that in wild-type mice pretreated with indomethacin, suggesting an important protective role of PGE_2 in acid-induced mucosal injury.

Roles of PGE_2 in reproduction and salt-sensitive hypertension

Prostanoids are implicated in various aspects of reproduction, including ovulation, fertilization, implantation, decidualization and parturition. For example, PGE_2 and $PGF_{2\alpha}$ were reported to participate in luteinizing hormone-induced ovulation (41), and $PGF_{2\alpha}$ is a potent luteolytic agent as described above. Moreover, mice lacking the cyclooxygenase-2 showed multiple reproductive failures in early pregnancy (42, 43), and mice lacking cytosolic phospholipase A_2 showed abnormal parturition similar to that found in $FP^{-/-}$ mice (44). Female $EP_2^{-/-}$ mice have reduced litter size (45 – 47). Hizaki et al. analyzed the mechanism of this reproductive failure in detail (45). The average number of embryos at day 19 of pregnancy in $EP_2^{-/-}$ mice was 1.5, whereas that in wild-type mice was 7.1. There was no abnormality of implantation in $EP_2^{-/-}$ mice, when examined by intrauterine transfer of wild-type blastocysts. However, $EP_2^{-/-}$ females had slightly impaired ovulation and severely impaired fertilization in both natural ovulation and super-ovulation. Cumulus cells, which surround the oocytes, start to synthesize hyaluronic acid and to expand when triggered by the preovulatory surge of gonadotropins (48). This cumulus expansion facilitates ovulation through the ruptured follicle wall, capture of cumulus-oocyte complex by oviductal fimbria, and fertilization. In cumuli oophori from wild-type females, PGE_2, FSH and dibutyryl cAMP elicited expansion in vitro. However, in cumuli oophori from $EP_2^{-/-}$ females, only PGE_2 failed to elicit expansion. Moreover, abortive cumulus expansion was found in $EP_2^{-/-}$ females in vivo. These results demonstrated that PGE_2 via the EP_2 contributes to cumulus expansion and that this contribution was indispensable for successful fertilization in the oviduct, where cumulus-oocyte complexes were already free from the influence of FSH.

Vascular responsiveness and blood pressure of $EP_2^{-/-}$ mice was examined (46). Bolus administration of PGE_2 produced a transient hypotensive effect in wild-type mice, whereas it elicited a transient hypertensive response in $EP_2^{-/-}$ mice, suggesting that the vasodilatory action of PGE_2 was mediated by the EP_2 and that the hypertensive response in $EP_2^{-/-}$ mice may be induced by unopposed hypertensive actions mediated by the EP_1 and/or EP_3. While baseline blood pressure in $EP_2^{-/-}$ mice was slightly higher only in females compared with that in wild-type females, marked elevation of systolic blood pressure was observed in $EP_2^{-/-}$ mice after they were fed the high-salt diet. Systolic blood pressure did not change in wild-type mice when fed the high-salt diet. Urinary PGE_2 production

was increased within 2 days of beginning the high-salt diet to a similar extent in wild-type and $EP_2^{-/-}$ mice, suggesting that the high-salt diet stimulates the production of PGE_2 in the body. These results show that unopposed vasoconstriction via the EP_1 and/or EP_3 receptor in $EP_2^{-/-}$ mice could contribute to the observed hypertension, particularly when PGE_2 production was increased with a high-salt diet.

Roles of PGD_2 in allergic asthma

PGD_2 is released in large amounts by activated mast cells in response to antigen challenge (49) and has been proposed as a marker of mast cell activation in asthma (50). To clarify the role of PGD_2 in allergic asthma, Matsuoka et al. disrupted the gene encoding the DP (51). Mice were sensitized with intraperitoneal injections of ovalbumin followed by exposures to aerosolized ovalbumin. The serum concentrations of both total and ovalbumin-specific IgE were markedly increased in response to the antigen challenge to a similar extent in wild-type and $DP^{-/-}$ mice. In wild-type mice, total cell number in BAL fluid increased markedly compared with that of saline-treated control mice (22.2×10^5 versus 2.5×10^5), and the cells consisted of eosinophils (approx. 80%), lymphocytes (approx. 10%) and macrophages (approx. 10%) after the sensitization. However, in $DP^{-/-}$ mice, only marginal increases in the numbers of eosinophils and lymphocytes were observed, suggesting the failure in chemoattraction of these cells. Moreover, airway hyperreactivity to acetylcholine was induced by the sensitization in wild-type mice, whereas it was not induced in $DP^{-/-}$ mice. On the other hand, Th2 cytokines play an essential role in the pathogenesis of allergic asthma (52, 53). While, in wild-type mice, antigen challenge induced marked increases in the concentrations of Th2 cytokines such as IL-4, IL-5 and IL-13 in BAL fluid, these increases were significantly lower in $DP^{-/-}$ mice compared with those in wild-type mice. In contrast, no difference was observed in the increase in the concentration of interferon-γ, a Th1 cytokine, between wild-type and $DP^{-/-}$ mice. In accordance with these findings, Th2 cytokine-responsive cells, lymphocytes and eosinophils, extensively infiltrated in the bronchial submucosa and around the blood vessels of the lungs in antigen-challenged wild-type mice, and little cell infiltration was observed in the lungs of antigen-challenged $DP^{-/-}$ mice. Immunohistochemical examination using a specific antibody to the DP revealed the expression of the DP in the cells of bronchioles and alveoli in wild-type mice, which was markedly up-regulated by airway exposure to the antigen. Immunoelectron microscopy identified the cell types expressing the DP, which mainly included ciliated and non-ciliated epithelial cells in the bronchioles and type II alveolar epithelial cells. These results suggest that PGD_2 produced in response to allergic challenge acts on the DP to stimulate the production of cytokines and chemokines, which lead to the recruitment of lymphocytes to the site of antigen challenge.

Roles of PGE_2 in colon carcinogenesis

Epidemiological studies have revealed a significant decrease in the death rates from colon cancer in individuals who have taken aspirin for prolonged periods (54). Non-steroidal anti-inflammatory drugs (NSAIDs) also inhibited chemically induced colon carcinogenesis (55), and they were found effective for the treatment of patients with familial adenomatous polyposis (56). While mutations in the APC gene were found in cancer cells from more than 80% of patients with sporadic colon cancer, disruption of the COX-2 gene in APC knockout mice markedly reduced the number and size of intestinal polyps (57), suggesting that prostanoids formed by the action of COX-2 participate in colon carcinogenesis. Watanabe et al. tested azoxymethane-induced colon carcinogenesis in $EP_1^{-/-}$ and $EP_3^{-/-}$ mice (58). The number of azoxymethane-induced aberrant crypt foci (ACF) in the colon of $EP_1^{-/-}$ mice decreased by approx. 40% relative to that for wild-type mice. In contrast, there was no such difference between $EP_3^{-/-}$ and wild-type mice. They further examined the effect of ONO-8711, a selective EP_1 antagonist, on colon carcinogenesis and found that it reduced by approx. 30% the number of ACF developed in the colon of azoxymethane-treated wild-type mice. ONO-8711 also reduced the number of intestinal polyps of Min mice by approx. 40%. The suppression potential of ONO-8711 is comparable to that of NSAIDs and COX-2 inhibitors on azoxymethane-induced ACF formation and intestinal polyp formation in Min mice. These results suggest that PGE_2 via the EP_1 mediates carcinogenic changes in the colon, and that selective EP_1 antagonists may be useful as chemopreventive agents for colon cancer.

Roles of TXA_2 in hemostasis and immunity

TXA_2 is well known as a potent stimulator of platelets and as a strong constrictor of vasculature. As is expected, $TP^{-/-}$ mice showed remarkably prolonged bleeding time, showing an essential role of TXA_2 in hemostasis (59). Some investigators have reported that the TP in smooth muscles cells of blood vessel has pharmacologically different characteristics from that of the TP in platelets. However, both blood vessel and platelets from the $TP^{-/-}$ mice failed to respond to U46619, a TP agonist, suggesting that the same products from the single known TP gene are expressed in both tissues. On the other hand, the TP was reported to be abundantly expressed on the T cells in the thymus and spleen, and TXA_2 was suggested to participate in the regulation of T cell functions (60). This role of TXA_2 will be clarified in the near future using mice lacking the TP.

Table 1. Roles of prostanoids revealed by studies using mice lacking specific prostaniod receptors

Prostanoids	Receptors	Roles	Reference Nos.
PGD_2	DP	A mediator of allergic asthtma	(51)
PGE_2	EP_1	Augmentation of colon carcinogenesis	(58)
	EP_2	Ovulation and fertilization	(45, 46)
		Salt-sensitive hypertention	(46, 47)
	EP_3	A mediator of febrile responses to pyrogens	(31)
		Acid-induced bicarbonate secretion in the duodenum	(38)
		Urinary concentration	(61)
	EP_4	Closure of ductus arteriosus	(14, 15)
		Bone resorption	(21, 22)
$PGF_{2\alpha}$	FP	An essential inducer of labor	(9)
PGI_2	IP	Antithrombotic function, a mediator of inflammation	(7)
TXA_2	TP	Hemostsis	(59)

Conclusions

Several important physiological and pathophysiological roles of prostanoids have been revealed by studies using mice lacking specific prostanoid receptors as summarized in Table 1. However, a number of known actions of prostanoids have still not been fully elucidated, especially regarding their roles in vivo. As presented in this article, knock-out mouse studies would contribute further to clarify these and any yet unknown roles of prostanoids and may lead to the development of novel drugs that could selectively regulate each function of the prostanoids.

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