Prostanoid receptors: ontogeny and implications in vascular physiology

D. HAMISH WRIGHT,1 DANIEL ABRAN,2 MOUSUMI BHATTACHARYA,1 XIN HOU,3 SYLVIE G. BERNIER,3 ASMÄÄ BOUAYAD,3 JEAN-CLAUDE FOURON,3 ALEJANDRO VAZQUEZ-TELLO,3 MARTIN H. BEAUCHAMP,3 RONALD I. CLYMAN,4 KRISHNA PERI,2 DAYA R. VARMA,1 AND SYLVAIN CHEMTOB1,3

1Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G-1Y6; 2Theratechnologies, St. Laurent, Quebec H4S-2A4; and 3Departments of Pediatrics, Ophthalmology, and Pharmacology, Research Center of Hôpital Ste. Justine, Montreal, Quebec H3T-1C5, Canada; and 4University of California, San Francisco, California 94143

Wright, D. Hamish, Daniel Abran, Mousumi Bhattacharya, Xin Hou, Sylvie G. Bernier, Asmaâ Bouayad, Jean-Claude Fouron, Alejandro Vazquez-Tello, Martin H. Beauchamp, Ronald I. Clyman, Krishna Peri, Daya R. Varma, and Sylvain Chemtob. Prostanoid receptors: ontogeny and implications in vascular physiology. Am J Physiol Regulatory Integrative Comp Physiol 281: R1343–R1360, 2001.—Prostanoids exert significant effects on circulatory beds. They play a role in the response of the vasculature to adjustments in perfusion pressure and oxygen and carbon dioxide tension, and they mediate the actions of numerous factors. The role of prostanoids in governing circulation of the perinate is suggested to surpass that in the adult. Prostanoids are abundantly generated in the perinate. They have been implicated in autoregulation of blood flow as studied in brain and eyes. Prostaglandins are also dominant regulators of ductus arteriosus tone. The effects of these autacoids are mediated through specific G protein-coupled receptors. In addition to the pharmacological characterization of the prostanoid receptors, important advances in understanding the biology of these receptors have been made in the last decade. Their cloning and the development of animals with disrupted genes of these receptors have been very informative. The involvement of prostanoid receptors in the developing subject, especially on brain and ocular vasculature and on ductus arteriosus, has also begun to be investigated; the expression of these receptors changes with development. Some but not all of the ontogenic changes in these receptors are attributed to homologous regulation. Interestingly, in the process of elucidating their effects, functional perinuclear prostaglandin E2 receptors have been uncovered. This article reviews prostanoid receptors and addresses implications on the developing subject with attention to vascular physiology.

G protein-coupled receptors; brain vasculature; eye vasculature; ductus arteriosus; newborn

PROSTANOIDS are important autacoids that exert diverse physiological and pathophysiological effects in various systems. These involve modulation of neuronal activ-
various conditions. Notably, prostaglandins are implicated in implantation of the fertilized egg (211), parturition (162, 200), breathing (124), control of brain and ocular circulation (38, 85, 122), and the regulation of ductus arteriosus tone (191). Of relevance, increased neonatal levels of prostanoids (103, 129, 134) have been suggested to contribute to the genesis of intraventricular cerebral hemorrhage (133, 220), patent ductus arteriosus (191), and possibly retinopathy of prematurity (73, 146).

There are five physiologically major prostanoids that are well characterized, prostaglandin (PG) E\(_2\), PGF\(_{2\alpha}\), PGD\(_2\), PGI\(_2\), and thromboxane (Tx) A\(_2\); other prostanoid-like compounds, namely isoprostanes, also exert biological effects through receptor sites that are not yet clearly identified (179–181), and these will not be covered in this review. PGE\(_2\), PGF\(_{2\alpha}\), PGD\(_2\), PGI\(_2\), and TxA\(_2\) produce their effects by acting on distinct G protein-coupled receptors (GPCRs). In the last two decades the pharmacological profile of specific agents and the cloning of GPCRs to prostanoids, along with the disruption of the genes encoding them, have provided great insight on the biology of prostanoid receptors and functions. A number of comprehensive reviews on the subject have been published (30, 53, 76, 147, 173). Lately, several studies have reported on the ontogenic profile and developmental regulation of prostanoid receptors. This review focuses on the implications of prostanoid receptors on the developing subject with particular attention to vascular physiology.

**FORMATION OF PROSTANOIDS**

Prostaglandins and TxA\(_2\), collectively named prostanoids, are mainly derived from arachidonic acid. Cyclooxygenase (COX) is the enzyme responsible for the committed step in the conversion of arachidonic acid to the bioactive prostanoids. There are two separate genes encoding COX proteins, COX-1 and COX-2. Both isozymes of COX catalyze the same reactions and share similar enzyme kinetics (18, 116), and their crystal structures are essentially superimposable (195, 218). The metabolism of the COX product PGH\(_2\) is the first committed step in the production of prostanoids. Thereafter, each primary bioactive prostanoid is synthesized by way of individual enzymes. The catalysis of PGH\(_2\) to PGE\(_2\) occurs mostly via PGE synthase (PGES); PGE\(_2\) can also be formed nonenzymatically from endoperoxides (183). There exists possibly two cytosolic glutathione (GSH)-dependent human isoforms purified from cerebrum (158) and two microsomal forms, of which one is GSH dependent and the other possibly independent (226). The molecular identity of PGES has more recently been determined (102, 136, 206); it appears that a GSH-dependent microsomal form is mainly coupled to COX-2 and the cytosolic one to COX-1 (136, 206). There are currently three known PGD synthases (PGDS): 1) GSH-independent PGDS (GSH-I-PGDS) (also called brain-type PGDS) (190), 2) GSH-dependent PGDS (GSH-D-PGDS) (also called spleen-type PGDS) (41), and 3) GSH S-transferase (GST) (214); in addition, serum albumin catalyzes this conversion as well (45). PGF synthase (PGFS) is a dual functioning enzyme that requires NADPH, which catalyzes the reduction of PGH\(_2\) to PGF\(_{2\alpha}\), and of PGD\(_2\) to 9\(,11\)-PGF\(_2\) (a stereoisomer of PGF\(_{2\alpha}\)) (224) at two distinct sites. Concurrent but independent investigations into the mechanisms of PG\(_{2\alpha}\) (229) and TxA\(_2\) (86, 215) synthesis led to the suggestion that the enzymes responsible for catalysis are analogous to P-450 monoxygenases. Subsequent purification and cDNA cloning of the PG\(_{2\alpha}\) (56, 79) and TxA\(_2\) (159, 188) isomerases verified these predictions. Neither synthase has greater than 16% sequence identity to any other P-450 enzymes or to each other such that each constitutes its own subfamily, designated CYP8 and CYP5 for PG\(_{2\alpha}\) synthase and TxA\(_2\) synthase, respectively. Of physiological relevance, because of inherent characteristics and reductive cofactor requirements (GSH and NADPH) for PG\(_{2\alpha}\), PGE\(_2\), PGD\(_2\), and PGF\(_{2\alpha}\) synthases, TxA\(_2\) synthesis is preferentially preserved over that of the other prostanoids during oxidative processes (3, 6, 89, 182, 207, 223, 227).

High levels of prostaglandins, especially PGE\(_2\), are detected in the blood and brain of the neonate (103, 129, 134). In retina, both increased COX-1 and COX-2 activities contribute to the augmented production of neonatal prostaglandins (81). In the brain, however, this mostly arises from increased expression and activity of the COX-2 pathway in brain vasculature, as opposed to adult brain, where prostanoid formation is catalyzed mainly by COX-1 (167). The rapid drop in prostaglandin levels in brain within the first 48–72 h after birth (103) is associated with a relative decrease in COX-2 expression, which seems to increase again thereafter (165). In the ductus arteriosus both COX-1 and COX-2 are involved in prostaglandin generation, but these enzymes play distinct roles (45, 49, 75, 203). COX-1 dominates throughout gestation in PGE\(_2\) formation and regulation of ductal tone, whereas COX-2 is induced at term (49, 75), possibly by increasing oxygen tension perhaps via endothelin formation; in addition, because COX-2 is activatable by endotoxins, this enzyme may contribute more importantly to ductal tone in the prematurely born subject (49).

**EFFECTS OF PROSTANOIDS ON CEREBRAL AND OCULAR VASCULAR TISSUE AND DUCTUS ARTERIOSUS**

Prostanoids produce significant effects on circulation. They mediate vasomotor response to asphyxia, ischemia, hypercapnia, and hypotension, as well as both systemic and pulmonary hypertension (35–37, 66, 80, 91, 118, 120, 122, 141, 170, 228). The vasomotor effects of prostanoids vary not only by virtue of their nature but also as a function of the type of tissue and their development. PGD\(_2\) and PGI\(_2\) are for the most part vasorelaxants, PGF\(_{2\alpha}\) and TxA\(_2\) are constrictors, and the effects of PGE\(_2\) depend on the type of tissue, which takes into account the receptor and receptor coupling; as will be discussed later, these seemingly
opposing effects of PGE$_2$ are due to the existence of multiple receptor subtypes with different signal transduction pathways that are often coexpressed.

The developmental profile of prostaglandin-mediated actions has been thoroughly investigated, mostly in the ductus arteriosus, as well as in the vascular beds of the brain and eye. On retinal and brain pial microvasculature and main arteries, PGE$_2$ seems to be an important vasodilator in the neonate (88, 119) and, as a result, probably contributes to the lower cerebral and retinal vascular resistance characteristic of fetal life. Prostanoids have been implicated in cerebral and retinal blood flow autoregulation in the adult and newborn subject (34, 36, 38, 85, 169). PGE$_2$, along with PGI$_2$ and PGF$_2\alpha$, is released from brain and ocular vasculature in response to acute rises in systemic blood pressure (34–36). Because PGF$_2\alpha$ (and PGE$_2$ on parenchymal vessels) causes minimal constriction in newborns compared with adults (7, 88, 125), the balance of prostaglandin action is shifted onto PGI$_2$-elicited [and possibly PGD$_2$-elicited (5)] relaxation, which in turn curtails the upper limit of cerebral blood flow autoregulation. Accordingly, inhibition of COX and more specifically of COX-2 in the newborn unveils an otherwise appropriate autoregulatory response (34, 36, 81, 126).

PGI$_2$ is readily released in response to conditions that evoke adaptive responses, such as during acute hypercapnia, hypotension, and ischemia, and exerts a major role in increasing blood flow to the central nervous system (66, 118, 119, 121, 166, 197, 198). However, it is of interest that in response to prolonged hypercapnia, PGE$_2$ [and not PGI$_2$ or nitric oxide (NO); Refs. 32, 99, 121, 122, 231] may become the main regulator of vascular tone, but this effect is mediated indirectly through induction of endothelial NO synthase (eNOS; Ref. 141), consistent with a role for PGE$_2$ in governing the expression of this enzyme (62). Along these lines, PGE$_2$, by acting through its EP$_3$ receptor, exerts a dominant role in controlling the increased constitutive NOS expression in brain and microvasculature of the newly born subject (62, 63); in choroid, however, it is PGD$_2$, via the DP receptor, that seems to govern increased neonatal eNOS expression (61). Thus the high levels of prostaglandins during birth transition regulate constitutive NOS expression, which in turn contributes to the relatively limited cerebral and ocular autoregulatory ability of the neonate (61, 83). Contrary to PGE$_2$ and PGF$_2\alpha$, the effects of PGI$_2$ on brain vasculature do not differ as a function of age (88). This age-dependent action of PGI$_2$ varies, however, according to the tissue, such that in retina PGI$_2$ is a less effective relaxant in the newborn than in the adult (7), whereas in choroid the reverse is observed (5). Along the same lines, the relaxant effects of PGD$_2$ are greater in the immature than the mature subject (5).

TXA$_2$ is largely responsible for the delayed effects of asphyxia-ischemic insults, as observed in retinal, choroidal (3, 6, 37), pulmonary (207), and placental (223) circulatory beds. These compromising actions of TXA$_2$ are further facilitated during oxidant stresses, especially in the immature subject (3, 6, 97) relatively devoid of antioxidants (55, 208); correspondingly, during oxidative stresses, TXA$_2$ is generated more abundantly in the newborn than in the adult (6, 97). In this context TXA$_2$ has been found to mediate the effects of major stable products of peroxidation, the isoprostanes (97, 114). Furthermore, not only is TXA$_2$ production greater in the immature than in the mature subject, but also TXA$_2$-induced constriction is more pronounced in fetus and newborn than in adult (7, 97). Moreover, an effect previously unidentified for TXA$_2$ acting via TP was recently unveiled, specifically, neuromicrovascular degeneration (Fig. 1) resulting from endothelial cell death, which contributes to the vasoobliteration in models of retinopathy of prematurity (22).

In ductus arteriosus PGE$_2$ is the major prostaglandin that affects tone (48). Although the ductus generates more PGI$_2$ than PGE$_2$, PGE$_2$ is markedly more potent in relaxing this vessel (46). The other prostanoids play small, if any, physiological roles (191, 192,

\[\text{Fig. 1. Role of thromboxane A}_2 (\text{TXA}_2) \text{ mimetic on retinal microvascular degeneration. TXA}_2 \text{ mimetic U-46619 (20 pmol; estimated ocular concentration 0.5 \mu M) was injected (0.4 \mu l; eye volume } 
\]
The effects of PGE\textsubscript{2} on ductal tone are also developmentally regulated with loss of responsiveness in the immediate neonate compared with the fetus (1, 47). Physiological mechanisms contributing to decreased actions of circulating PGE\textsubscript{2} include loss of the placenta, which is the major source of circulating PGE\textsubscript{2} in the fetus (210), an increase in pulmonary blood flow at birth, because the lungs are a major site of prostaglandin catabolism (213), and a decrease in relaxant PGE\textsubscript{2} receptors (23, 29). Increased oxygen tension and PGE\textsubscript{2} oppose each other’s actions in controlling ductal tone (191); in addition, elevated oxygen tension also inhibits PG\textsubscript{I}2 synthase, which decreases formation of ductal PG\textsubscript{I}2, thus promoting contraction of the ductus arteriosus (192, 193).

**MOLECULAR CHARACTERISTICS OF PROSTANOID RECEPTORS**

As discussed above, prostanoids exert their effects through their respective receptors. Changes in prostanoid actions as a function of development take into account receptor density and coupling. Before proceeding with a description of the ontogeny of prostaglandin receptors we will review the pharmacology, molecular biology, and signaling of these receptors.

The earliest prostanoid receptor classification system was based on the differential effects of PGE and PGF analogs on three isolated tissues (guinea pig uterus, human myometrium, and rabbit jejunum) (171). Subsequent reports supported directly (72) or indirectly (12) the existence of multiple receptor types, and Kennedy et al. (108) proposed a prostanoid receptor classification. Using a comparison of the rank orders of agonist potency in a range of smooth muscle preparations and prior evidence in the literature, these investigators hypothesized the existence of distinct receptors for each of the bioactive prostanoids. Under the proposed classification, the receptors for PG\textsubscript{D}2, PGE\textsubscript{2}, PG\textsubscript{F}\textsubscript{2\alpha}, PGL\textsubscript{2}, and TX\textsubscript{A}2 are denoted DP, EP, FP, IP, and TP, respectively. Studies also identified PGE-sensitive tissues that exhibited different activities to specific agonists and antagonists, prompting a further division of the EP receptors into EP\textsubscript{1}, EP\textsubscript{2}, EP\textsubscript{3}, and EP\textsubscript{4} (52, 53). In addition, a newly identified receptor that binds PG\textsubscript{D}2, named chemoattractant receptor-homologous molecule on TH2 cells because of its properties, has been uncovered in T helper type 2 cells, eosinophils, and basophils (92); however, this seven-transmembrane PD\textsubscript{2} receptor does not contain characteristic molecular signatures of prostanooid receptors but rather of chemokine receptors (139).

The cloning of the prostanoid receptors identified further heterogeneity, which arises as a result of alternative mRNA splicing. The molecular mechanism of alternative mRNA splicing involves the formation of multiple mRNA and protein products from a single gene. Specifically, splice variants have been identified for the EP\textsubscript{1}, EP\textsubscript{3}, FP, and TP receptor homologs of various species. There are currently nine known subtypes of the human EP\textsubscript{3} receptor: EP\textsubscript{3-1a}, EP\textsubscript{3-1b}, EP\textsubscript{3-II}, EP\textsubscript{3-III}, EP\textsubscript{3-IV}, EP\textsubscript{3-V}, EP\textsubscript{3-VI}, EP\textsubscript{3-e}, and EP\textsubscript{3-f} (8, 111, 176). Mouse (100, 152, 199), rat (204), bovine (145), and rabbit (31) counterparts have also been identified for some of these EP\textsubscript{3} subtypes; subtypes of the EP\textsubscript{3} receptors are distinguished by variances in the tail of the carboxyl-terminal portion. Human subtypes also exist for the TP receptor (174), specifically TP\textsubscript{a} and TP\textsubscript{b}, which, as for EP\textsubscript{3} subtypes, vary by the tail of their carboxyl-terminal end. Two splice variants have been identified for each of the rat EP\textsubscript{1} (160) and ovine FP (172) receptors. These have been designated as EP\textsubscript{1} and EP\textsubscript{1-variant} and as FP\textsubscript{A} and FP\textsubscript{B}, respectively; variants of EP\textsubscript{1}, FP, and TP also differ between each other by their carboxyl-terminal portions. Human homologs to EP\textsubscript{1} and FP subtypes have not been identified.

The eight known types of prostanoid receptors are each encoded by an individual gene. Phylogenetic analyses indicate that receptors sharing a common signaling pathway have higher sequence homology than receptors sharing a common prostanoid as their preferential ligand (28, 177, 212). The effects of prostanoid receptors on smooth muscle reflect this relationship. Thus EP\textsubscript{3}, EP\textsubscript{4}, DP, and IP induce smooth muscle relaxation and are more closely related to each other than to the other prostanoid receptors. Similarly, EP\textsubscript{1}, FP, and TP receptors cause smooth muscle contraction and form another group based on sequence homology. The EP\textsubscript{3} receptors also usually stimulate smooth muscle contraction and define a third group. The signal transduction pathways underlying these mechanisms of prostanoid action are shared within these groups and will be explained in a section below. On the basis of these phylogenetic analyses, it has been suggested that the COX pathway may have evolved from PGE\textsubscript{2} and an ancestral EP receptor (147). The evolution of the different EP receptor types from this ancestral prostanoid receptor would have linked PGE\textsubscript{2} to different signal transduction pathways. The receptors for the other prostanoids would have then evolved by gene duplication of these different EP receptor subtypes.

Alternative splicing of the exon encoding the seventh transmembrane domain occurs at a position approximately 9–12 amino acids into the carboxy terminus of the EP\textsubscript{3}, FP, and TP receptors of various species. The rat EP\textsubscript{1} receptor is also subject to alternative splicing but instead diverges midway into the sixth transmembrane domain. The variant form (rEP\textsubscript{1-variant}) contains none of the amino acids that are highly conserved within the seventh transmembrane domain of the other prostanoid receptors. Generally, prostanoid receptor isoforms exhibit similar ligand binding but differ in their signaling pathways, their sensitivity to agonist-induced desensitization, and their tendency toward constitutive activity, as will be discussed. Whereas there is homology between the EP\textsubscript{3} receptor isoforms of different species, the human and mouse TP receptor isoforms demonstrate no homology. This may be indicative of other TP isoforms (147). The receptors that are subject to alternative splicing (EP\textsubscript{1}, EP\textsubscript{3}, FP, and TP) are phylogenetically related, perhaps suggesting the evolutionary conservation of the sequence(s)
involved in this process. The rEP₁, EP₂, EP₃, FP, and TP₆, splice variants are all generated by the failure to utilize a potential splice site (173). The splicing out of various introns and the use of downstream exons generate the other alternatively spliced forms of EP₆. The regulation of the process of alternative splicing with respect to the prostanoid receptors has yet to be studied.

Prostanoid receptors are rhodopsin type, containing seven transmembrane domains, an extracellular amino terminus, and an intracellular carboxy terminus; accordingly, three intracellular and three extracellular loops are found. The three-dimensional crystal structure of a membrane-contained mammalian GPCR, only accomplished specifically for rhodopsin so far, confirms this topology (163). The evidence suggesting that prostanoid receptors are rhodopsin-type receptors precedes their molecular cloning and was based on coupling of the TP receptor to G proteins (17) and its sensitivity to agonist-induced desensitization (138). The purification of the TP receptor from human platelets (216) allowed its partial protein sequence to be identified, leading to the isolation of a cDNA for TP (93). Homology screening based on this sequence has established recombinant clones from various species for the eight individual prostanoid receptors previously defined pharmacologically. There are 28 amino acid residues conserved within all prostanoid receptor sequences, and 8 of these are shared with other GPCRs. These residues are believed to be particularly important in receptor structure and/or function. For instance, an Asp residue in the second transmembrane domain of various GPCRs is involved in ligand binding and signal transduction (184), although the role of this residue has not been studied directly in any prostanoid receptors. Two conserved Cys residues (1 in each of the 1st and 2nd extracellular loops) are suggested to form a disulfide bond and contribute to the stabilization of GPCRs in the membrane (59). In the rabbit EP₃ receptor, an Ala residue substituted for the Cys residue in the second extracellular loop had no effect on binding (14). This is in contrast to studies of the human TP receptor, where substitution of Ser for the analogous Cys completely abolished ligand binding (39, 54). These studies also showed a similar effect by mutating the Cys of the first extracellular loop, which corroborates evidence demonstrating a loss of agonist binding to TP after chemical perturbation of these Cys residues (with dithiothreitol or sulfhydryl alklylation) (60).

There are several prostanoid receptor-specific motifs, including sequences in the second extracellular loop (G-R-Y-X-Q-X-P-G-T/S-W-C-F) as well as in the third and seventh transmembrane domain (M-X-F-F-G-L-X-X-L-L-X-X-A-M-A-X-E-R and L-X-A-X-R-X-A-S/T-X-N-Q-I-L-D-P-W-V-Y-I-L, respectively). These conserved regions are thought to play fundamental roles in the structure of the prostanoid binding domains. An Arg in the seventh transmembrane domain conserved between all prostanoid receptors was proposed to be the binding site for the carboxy moiety of the prostanoids (14, 98); the conserved motif in the second extracellular loop may also function in this regard (14).

Residues are also conserved among prostanoid receptors for signal transduction. An Arg in the first intracellular loop is conserved between all prostanoid receptors. A mutation of this residue to Leu in the TP receptor was associated with a bleeding disorder (94). As well as containing conserved residues, prostanoid receptors share several other characteristics with other members of the GPCR family. They enclose consensus sites for N-glycosylation of Asn residues (Asn-X-Ser/Thr) in their extracellular domains. The amount of glycosylation can be significant as demonstrated when purified TP receptors of molecular mass ~57 kDa were shifted to their predicted molecular mass (based on primary structure) of ~37 kDa on treatment with N-glycanase (131). The glycosylation sites are requisite for ligand binding at the human TP receptor (40), and mutation of the Asn residues or deletion of their carbohydrate moieties abolishes binding. In addition, it has recently been found that N-glycosylation of the EP₆ receptor is required for localization to the plasma membrane but not for appropriate folding of the protein (26).

RECEPTOR SIGNALING

Early studies of the second messengers downstream of the prostanoids focused on cyclic nucleotides (53). For instance, PGE₂ and PGF₂α, were reported to stimulate cAMP (33) and cGMP (64), respectively. Since then, other signal transduction pathways have been suggested by the observation of prostanoid-mediated activation of second messengers such as free Ca²⁺ and inositol phosphate (147). The molecular cloning of the prostanoid receptors has facilitated observation of their coupling to heterotrimeric G proteins. These heterotrimeric G proteins are composed of three structural subunits designated α, β, and γ, of which numerous subtypes exist for each (78). Functionally, G proteins comprise two subunits, since receptor activation provokes the dissociation of the Ga-subunit from a complex of the Gβγ-subunits. Both the Ga- and the Gβγ-subunits can act as effectors in signal transduction (44, 178).

Prostanoid receptors sharing a common signaling pathway have higher sequence homology than receptors sharing a common prostanoid as their preferential ligand (28, 177, 212). Thus three clusters of related receptors have been defined: 1) DP, IP, EP₂, and EP₆; 2) EP₂, FP, and TP; and 3) EP₆. Prostanoid receptors in group 1 are linked to heterotrimeric G proteins that are composed of a Ga-subunit that stimulates adenylate cyclase (designated Gα) to produce cAMP. An increase in intracellular cAMP concentration is observed after stimulation of the recombinant human DP (28), IP (27, 105, 142), EP₂ (177), and EP₆ (11, 20) receptors, in addition to their species homologs. The results obtained with recombinant receptors corroborated those obtained previously in isolated tissues. For instance, PGD₂, PGE₂, and PGI-responsive receptors cause the stimulation of cAMP production in platelets (230) and in vasculature (2, 5, 81). However, the recombinant
human IP receptor can also mediate inositol phosphate production and increases in free Ca\(^{2+}\) levels by coupling with Go\(_q\) (144). Likewise, EP\(_2\), EP\(_4\), and DP receptors in choroid do not couple to adenylate cyclase but rather to eNOS (2, 5); this may be evoked by G\(\beta\gamma\) action on phosphatidylinositol 3-kinase (44), which in turn activates, sequentially, protein kinase B (PKB) (219) and eNOS (58, 71). Of interest, ontogenic differences in the expression of EP\(_2\), EP\(_4\), and DP in ocular vasculature do not explain the greater relaxation evoked by their stimulation in the young subject; rather, it is the greater expression of the eNOS per se that is responsible for their augmented vasomotor actions (2, 5, 84).

Prostanoid receptors in group 2 couple to increases in intracellular free Ca\(^{2+}\) through the activation by Go\(_q\) of phospholipase C, with subsequent inositol phosphate liberation. This pathway has been demonstrated for FP using anti-Go\(_q\) antibodies (101), which corroborates earlier results demonstrating inositol phosphate turnover in isolated luteal cells on PGF\(_{2\alpha}\) administration (175). In the case of TP, Go\(_q\) activation is the primary effector pathway (189) as shown during stimulation of native TP receptors in platelets (13). However, the previously described TP receptor splice variants TP\(_{\alpha}\) and TP\(_{\beta}\) also can signal through G\(_{\alpha}\) and G\(_{\alpha}\) to inhibit and stimulate adenylate cyclase, respectively (95). EP\(_1\) preferentially couples to Go\(_q\) (53). An increase in inositol phosphate after its stimulation in brain and ocular vasculature is clearly observed (4, 128).

The EP\(_3\) subtypes constitute group 3 of the prostanoid receptor family and employ as their primary effector pathway the inhibition of adenylate cyclase through the G\(_{\alpha}\)-family (149). However, the molecular cloning of the bovine EP\(_3\) receptor splice variants demonstrates the array of second messengers to which these receptors are coupled. Four subtypes of bovine EP\(_3\) have been cloned (designated A, B, C, and D), and all show identical agonist binding properties (145). However, EP\(_{3A}\) acts through G\(_{\alpha}\) to inhibit adenylate cyclase, EP\(_{3B}\) and EP\(_{3C}\) signal through G\(_{\alpha}\) to activate adenylate cyclase, and EP\(_{3D}\) is coupled to G\(_{\alpha}\), G\(_{\alpha}\), and Go\(_q\), resulting in the inhibition and activation of adenylate cyclase as well as the activation of phospholipase C. On the other hand, nuclear EP\(_{3b}\) receptors seem to be G protein dependent but not coupled to adenylate cyclase or phospholipase C activation (25). A novel type G protein regulation has also been reported for the EP\(_{3b}\) and EP\(_{3c}\) receptors. In addition to their stimulatory effects on G\(_{\alpha}\), they are thought to negatively regulate G protein activity by specifically inhibiting the GTPase activity of G\(_{\alpha}\), a member of the G\(_{\alpha}\)-family (150). Along the same lines, EP\(_{3D}\)-induced ductus arteriosus relaxation is pertussis toxin, NO, and endothelium insensitive but dependent on ATP-sensitive potassium channel activation (29); the mechanisms remain to be elucidated, although direct receptor-channel interaction is a possibility (130). The EP\(_3\) receptor subtypes may also differ in their levels of constitutive activity, the agonist-independent activity of the receptor. This evidence comes from studies of the mouse isoforms of the EP\(_3\) receptor (designated \(\alpha\), \(\beta\), and \(\gamma\) ). EP\(_{3b}\) is not constitutively active, whereas EP\(_{3\alpha}\) is, with respect to the G\(_{\alpha}\)-mediated inhibition of adenylate cyclase (148); the demonstration of levels of activity similar to EP\(_{3b}\) on treatment of EP\(_{3\alpha}\)-transfected cells with pertussis toxin (which inactivates G\(_{\alpha}\)) confirms that the EP\(_{3\alpha}\)-G\(_{\alpha}\) is constitutively active.

PROSTANOID RECEPTOR DISTRIBUTION AND DEVELOPMENTAL CHANGES

Among the PGE\(_2\) receptors, EP\(_3\) and EP\(_4\) are the most widely distributed and are detected in nearly every tissue (53, 147). EP\(_1\) and EP\(_2\), on the other hand, have a limited distribution. EP\(_1\) is present mostly on vascular and nonvascular smooth muscle (38, 53), and EP\(_3\) is present mostly in lung, placenta, endometrium, renal tubules, brain synaptosomes, and heart (127, 177), as well as on vascular and nonvascular smooth muscle (7, 117). FP receptors are found in corpus luteum, iris sphincter muscle, trabecular meshwork, and vascular smooth muscle (53, 147). IP and TP receptors are primarily localized in platelets and vascular smooth muscle; IP is also present in dorsal root ganglia. DP is the least abundant prostanoid receptor and is primarily found in retina; DP is also present in leptomeninges and choroid plexus (53, 147) and is weakly expressed in gut and lungs.

Studies on plasma membrane of brain microvessels revealed a two- to threefold lower density of EP and FP receptors in newborn compared with adult (128). In both porcine newborn and adult brain intraparenchymal microvessels, ~80% of PGE\(_2\) receptors are of the EP\(_1\) subtype and the remainder are EP\(_3\) (128); EP\(_2\) and EP\(_4\) are undetectable. Accordingly, stimulation of EP\(_1\), EP\(_3\), and FP evokes less constriction of intraparenchymal brain vessels in the newborn than in the adult (125). Similar functional observations have been made on retinal and choroidal vessels (2, 5, 7). However, these ocular vascular beds exhibit a different profile of EP\(_3\) and EP\(_4\) expression in the developing subject, such that in retinal microvessels EP\(_4\) is not found, whereas EP\(_3\) density is greater in adult than in newborn, and in choroid all EP receptors are expressed more in the mature subject with the exception of EP\(_2\), which is equivalently expressed in newborn and adult. The density of EP\(_2\) and EP\(_4\), however, does not readily translate functionally in choroid, where EP\(_2\)- and EP\(_4\)-induced relaxation is significantly greater in younger subjects (2). The same applies to DP, which exhibits a similar density in newborn and adult brain, retinal, and choroidal vessels but markedly greater relaxation in the immature subject (Ref. 2; personal observations). The developmental profile of IP and TP receptor expression on vascular beds has not been determined; functional assays using specific agonists have been described above. A summary of prostanoid receptor differences in newborn and adult brain and ocular vascular beds is presented in Table 1.
Although PGE2 plays a cardinal role in fetal ductal tone, the types of EP receptors implicated have only recently been identified. EP2, EP3D (the only EP3 detected in ductus; Ref. 29), and EP4 receptors have been detected in fetal porcine and ovine ductus arteriosus (0.75 gestation) (23, 29); EP1 was not detected. EP4 has been suggested to confer the principal relaxant function induced by PGE2 (192). These ex vivo findings were substantiated in vivo in fetal sheep (23, 29); EP1 was not detected. EP4 has been observed in the immediate newborn along with preservation of proliferative effects mediated through their interactions. Several lines of evidence suggest that prostanooids may exert effects via intracellular receptors in the vicinity of their formation. This inference has recently been tested and corroborated. DP and all known subtypes of EP receptors were detected in nuclear membrane fractions using binding studies, where their relative distribution differed from that in plasma membrane. In addition, the presence of EP1, EP3D, and EP4 on perinuclear membranes was identified on native endothelial cells by immunocytochemical techniques employing confocal as well as electron microscopy (24, 25).

Prostanoids function in an autocrine, intracrine, and/or paracrine manner. The wide tissue distribution of prostanoids and their receptors underscores the various effects mediated through their interactions. Several lines of evidence suggest that prostanooids may act not only on cell-surface receptors, but also intracellularly at receptors other than peroxysome proliferator-activated receptors (90, 110, 168), which mediate actions of metabolites of PGD2 (mostly PGJ2 and 15-deoxy-Δ12,14-PGJ2) but not of PGE2 and PGF2α (110, 232). The enzymes responsible for prostaglandin synthesis, cytosolic phospholipase A2 (PLA2, which releases arachidonic acid) and COX-1 and -2, have been found to be located at the nuclear membrane, and these can also translocate to this site in response to stimuli (135, 185, 196). A membrane-associated transporter that facilitates influx of prostanoids has been identified (186). A number of other observations pointed toward possible intracellular sites of action for major prostanoids. For instance, a robust increase in eNOS expression was observed after stimulation of EP3 despite a marked paucity of plasma membranal EP3 receptors in newborn microvessels (62). Similarly, the total absence of EP2 receptors in plasma membrane preparations of the newborn brain was inconsistent with the neuroprotective effects of EP2 stimulation in the neonate (140). Thus it was proposed that prostanoids may exert effects via intracellular receptors in the vicinity of their formation. This inference has recently been tested and corroborated. DP and all known subtypes of EP receptors were detected in nuclear membrane fractions using binding studies, where their relative distribution differed from that in plasma membrane. In addition, the presence of EP1, EP3D, and EP4 on perinuclear membranes was identified on native endothelial cells by immunocytochemical techniques employing confocal as well as electron microscopy (24, 25).

The mechanisms responsible for the perinatal-related decrease in PGE2 responsiveness of ductus arteriosus have recently been investigated. It has been observed that PGE2 receptor density decreased by threefold in the immediate newborn compared with the fetus (0.75 gestation). As indicated above, EP2, EP3D, and EP4 receptors are present in fetus ductus in equivalent densities, whereas only EP2 is found in the immediate postnatal ductus (23, 29). Surprisingly, but of relevance, EP3 stimulation in ductus arteriosus was associated with relaxation in both fetal rabbits and lambs (29, 194). Thus a loss of EP3D and EP4 receptors in the immediate newborn along with preservation of EP2 density and function was consistent with a decreased response of the newborn ductus to PGE2; hence EP2 appears to mediate the vasorelaxant effects of PGE2 in full-term neonatal ductus arteriosus.

Prostanoids function in an autocrine, intracrine, and/or paracrine manner. The wide tissue distribution of prostanoids and their receptors underscores the various effects mediated through their interactions. Several lines of evidence suggest that prostanoids may act not only on cell-surface receptors, but also intracellularly at receptors other than peroxysome proliferator-activated receptors (90, 110, 168), which mediate actions of metabolites of PGD2 (mostly PGJ2 and 15-deoxy-Δ12,14-PGJ2) but not of PGE2 and PGF2α (110, 232). The enzymes responsible for prostaglandin synthesis, cytosolic phospholipase A2 (PLA2, which releases arachidonic acid) and COX-1 and -2, have been found to be located at the nuclear membrane, and these can also translocate to this site in response to stimuli (135, 185, 196). A membrane-associated transporter that facilitates influx of prostanoids has been identified (186). A number of other observations pointed toward possible intracellular sites of action for major prostanoids. For instance, a robust increase in eNOS expression was observed after stimulation of EP3 despite a marked paucity of plasma membranal EP3 receptors in newborn microvessels (62). Similarly, the total absence of EP2 receptors in plasma membrane preparations of the newborn brain was inconsistent with the neuroprotective effects of EP2 stimulation in the neonate (140). Thus it was proposed that prostanoids may exert effects via intracellular receptors in the vicinity of their formation. This inference has recently been tested and corroborated. DP and all known subtypes of EP receptors were detected in nuclear membrane fractions using binding studies, where their relative distribution differed from that in plasma membrane. In addition, the presence of EP1, EP3D, and EP4 on perinuclear membranes was identified on native endothelial cells by immunocytochemical techniques employing confocal as well as electron microscopy (24, 25).

The mechanisms responsible for the perinatal-related decrease in PGE2 responsiveness of ductus arteriosus have recently been investigated. It has been observed that PGE2 receptor density decreased by threefold in the immediate newborn compared with the fetus (0.75 gestation). As indicated above, EP2, EP3D, and EP4 receptors are present in fetus ductus in equivalent densities, whereas only EP2 is found in the immediate postnatal ductus (23, 29). Surprisingly, but of relevance, EP3 stimulation in ductus arteriosus was associated with relaxation in both fetal rabbits and lambs (29, 194). Thus a loss of EP3D and EP4 receptors in the immediate newborn along with preservation of EP2 density and function was consistent with a decreased response of the newborn ductus to PGE2; hence EP2 appears to mediate the vasorelaxant effects of PGE2 in full-term neonatal ductus arteriosus.

### Table 1. Relative differences in expression of prostanoid receptors between newborn and adult brain and ocular vasculature

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>A—&gt;NB</td>
<td>ND</td>
<td>A—&gt;NB</td>
<td>ND</td>
<td>A—&gt;NB</td>
<td>NB—A*</td>
<td>NB—A*</td>
<td>NB—A*</td>
</tr>
<tr>
<td>Retina</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>ND</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
</tr>
<tr>
<td>Choroid</td>
<td>A—&gt;NB</td>
<td>NB—A</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>A—&gt;NB</td>
<td>NB—A</td>
<td>NB—A</td>
<td>A—&gt;NB</td>
</tr>
</tbody>
</table>

Relative differences in prostanoid receptor expression are based on Refs. 2, 4–6, 81, 128, and personal observations. Developmental comparisons for IP and TP are based on functional assays (5, 7, 97). *Based on personal observations. A, NB, and ND refer to adult, newborn, and not detectable, respectively.
nuclear EP3 receptors is presented in Fig. 4. These findings set forth new perspectives in the biology of prostanoid receptors.

**PROSTANOID RECEPTOR REGULATION: PHYSIOLOGICAL IMPLICATIONS IN THE DEVELOPING SUBJECT**

Prostanoid receptor expression regulation is not yet clearly elucidated. Different factors act on the cis-acting regulatory elements of their respective genes (21, 69, 106, 111, 156, 157). The 5'-flanking region and first intron of many of these receptor genes have basal promoter motifs (such as a TATA box), as well as several responsive motifs, including those for proinflammatory agents (such as nuclear factor-κB). Although many different motifs have been identified, less information is available regarding the actual regulation of receptor gene expression. The studies to date suggest that species or cell-type differences may play a role. For instance, the TP receptor contains a phorbol ester response element, and TP receptor expression can be stimulated in human erythroleukemia (HEL) cells (143). However, despite the presence of response elements for glucocorticoids and interleukin (IL)-6 in the TP gene, these factors are insufficient to induce TP expression in HEL cells (109). They can induce TP expression in rat cultured vascular smooth muscle cells (202). In vivo regulation of prostanoid receptor expression has not yet been studied directly, however.

Mechanisms that regulate translation also partake in governing receptor expression, but these remain poorly defined. Despite the high homology between the prostanoid receptors of various species, there are differences in the translation initiation sites of some receptor types that affect the amino-terminal extracellular domain of the receptor (147). For instance, the human, bovine, and rabbit EP3 receptor is 20 amino acid residues longer than the rat and mouse homologs. The human IP receptor is 30 amino acids shorter than the mouse and rat homologs. In contrast, the human
Many reports identify the importance of heterologous desensitization in the regulation of prostanoid receptor activity, and a role for homologous desensitization has only recently been suggested.

Both short-term (5 min) and long-term (24 h) desensitization of the mouse EP1 receptor involve PKC, and the desensitization is observed as a suppression of the agonist-mediated dose response and a reduction in EP1 mRNA levels, respectively (104). The mouse EP2 receptor undergoes long-term agonist-induced desensitization in the form of receptor downregulation after a 12-h exposure to PGE2 but is insensitive to short-term desensitization (30 min) (155). Short-term (1 h) desensitization of human EP3 was observed on PKC activation (233). Splice variants of a given species homolog of EP3 differ in their sensitivity to desensitization. The mouse EP3(α) exhibits sequestration after short-term (30 min) PGE2 exposure and downregulation on long-term (24 h) PGE2 exposure, while mouse EP3(β) does not undergo agonist-induced desensitization (152, 153). Similarly, the human EP3-II demonstrates slow persistent desensitization in contrast to the rapid transient desensitization exhibited by the EP3-III and EP3-IV receptors (10). Desensitization of EP4 is perhaps the most intensively studied to date of the EP receptors. The mouse EP4 receptor is sensitive to both short-term (30 min) and long-term (12 h) desensitization (155). The rapid agonist-induced desensitization of human EP4 is independent of second messenger kinases and is instead regulated by GRKs (19, 153).

Even though the desensitization of the IP receptor has been studied for the native receptor in a single cell type (neuroblastoma-maglioma cell hybrid, NG108–15), the results are contradictory. Long-term (17 h) desensitization has been observed as receptor sequestration and downregulation with no change in agonist affinity (113), where the kinases involved appear to depend on the agonist used (112). In contrast, another group has identified that long-term desensitization is specifically accompanied by a concurrent downregulation of the G(αq)-subunit (9). PKC is observed to mediate short-term (45 min) desensitization of the native FP receptor in bovine iris sphincter (201). Short-term desensitization in vivo of the FP receptor also occurs in the ovine corpus luteum, where its temporal nature has been suggested to influence the oxytocin-mediated pulsatile release of PGF2α (115). The TP receptor has been shown to undergo desensitization, specifically as short-term (10 min) agonist-dependent phosphorylation, which could be blocked by antagonist (161). The desensitization of the phospholipase C effector pathway downstream of TP has also been shown in human platelets (65). A recent report demonstrates that the TP splice variants exhibit different sensitivities to desensitization; TPβ is susceptible to agonist-induced desensitization in a GRK-dependent manner, while TPγ does not desensitize (164).

In contrast to the other prostanoid receptors, there are no direct reports of desensitization of the DP receptor. Because prostanoids, especially prostaglandins and to a much lesser extent TxA2, are increased during the birth transition period (6, 103, 125, 134), and because
the density of many prostanoid receptors is decreased during this time (see Table 1), homologous developmental regulation of the prostanoid receptors has been invoked. A reduction in prostanoid levels in newborn brain, retina, and choroid to those of the adult, by titrating the dose of the COX inhibitor ibuprofen (24–48 h) (81), led to a concomitant increase in \(^\text{[H]}\text{PGE}_2\) and \(^\text{[H]}\text{PGF}_2\alpha\) binding in vascular tissue (2, 81, 125, 126). This increase in prostaglandin receptors could be specifically prevented by \(\text{PGE}_2\) or \(\text{PGF}_2\alpha\), antagonists, suggesting a cause-effect relationship between the binding and the prostaglandin levels in the immediate newborn period (125, 129); receptor-coupled mechanisms seemed unaffected. However, this homologous upregulation was not uniformly observed for all prostanoid receptors. The constriction-evoking \(\text{EP}_1\), \(\text{EP}_3\), and \(\text{FP}\) exhibited a homologous regulation by the high \(\text{PGE}_2\) and \(\text{PGF}_2\alpha\) tissue levels during the birth transition period, and densities of these receptors increased to adult values when prostaglandin levels were reduced to those of the adult. In contrast, the densities of the relaxation-inducing \(\text{EP}_2\), \(\text{EP}_4\), and \(\text{DP}\) receptors in brain and ocular vasculatures were unaffected by prostaglandin synthase inhibition (2, 5, 81); likewise, \(\text{IP}\) and \(\text{TP}\) receptor-associated functions were also unaltered (5). The absent modulation in TP-induced constriction may be related to the minor changes in \(\text{TXA}_2\) levels in the neonate, but this does not apply to \(\text{EP}_2\), \(\text{EP}_4\), \(\text{IP}\), and \(\text{DP}\) receptors where \(\text{PGE}_2\), \(\text{PGI}_2\), and \(\text{PGD}_2\) significantly change (5). Hence, changes in prostanoid receptor expression during the birth transition period can only in part be attributed to a homologous regulation by their ligands. The findings also disclose a possibly physiologically relevant pattern whereby high perinatal levels of prostaglandins lead to a downregulation of receptors associated with vasoconstriction without affecting those associated with relaxation, favoring altogether a vasorelaxation. An insufficient ability to limit blood flow and oxygen delivery to brain and retina in a premature subject may participate in the development of intraventricular cerebral hemorrhage and retinopathy of prematurity (85). However, in the term neonate this excess vasorelaxation may be regarded as beneficial, especially at the end of parturition when frequent episodes of hypoxia occur secondarily to intensifying uterine contractions (43, 57). A graphic scheme depicting the modulation of contractile prostaglandin receptors by endogenous prostanoids in the immediate neonate is presented in Fig. 5.

**PROSTANOID RECEPTOR GENE DISRUPTION**

Targeted gene ablation or disruption has provided important information on the role of prostanoid receptors, especially because of the lack of suitable antagonists for this receptor family. The disruption of all of the prostanoid receptor genes has now been reported.

There are three individual reports of the genetic disruption of the \(\text{EP}_1\) receptor gene (\(\text{EP}_1^{-/-}\)) (16, 217, 225). A gender-specific effect on blood pressure homeostasis was observed. Surprisingly and as yet not understood, male but not female \(\text{EP}_1^{-/-}\) mice exhibited less \(\text{PGE}_2\)-induced hypotension relative to wild-type mice (16), suggesting that in males most of the vasodepressor action of \(\text{PGE}_2\) is mediated by \(\text{EP}_1\). A decrease in the number of preneoplastic lesions in \(\text{EP}_1^{-/-}\) mice relative to wild-type mice was found using a mouse model of colon cancer (225).

Of all of the prostanoid receptors, the ablation of the \(\text{EP}_2\) receptor gene (\(\text{EP}_2^{-/-}\)) has been studied most intensely (15, 96, 107, 211). Contradictory effects of the \(\text{EP}_2^{-/-}\) phenotype are apparent for blood pressure homeostasis. Both hypertension (107) and hypotension (211) are reported for \(\text{EP}_2^{-/-}\) mice relative to wild type; the hypertension is consistent with the vasorelaxant effects of \(\text{EP}_2\) stimulation, while the hypotension possibly relates to an effect of \(\text{EP}_2\) on activation of the renin-angiotensin system (211). The hypotensive responses of \(\text{PGE}_2\) are markedly attenuated in female but not male \(\text{EP}_2^{-/-}\) mice (15), suggesting a major role for \(\text{EP}_2\) in vasodepressor responses of \(\text{PGE}_2\) in females. Salt-sensitive hypertension is observed in \(\text{EP}_2^{-/-}\) mice, suggesting a role for \(\text{EP}_2\) in the regulation of sodium in the kidney (107, 211). The \(\text{EP}_2\) receptor plays an important role in reproduction since female \(\text{EP}_2^{-/-}\) mice exhibit a reduced litter size, a decrease in ovulation number, and a reduced fertilization rate (96, 107, 211). An \(\text{EP}_2\)-dependent role was demonstrated in the expansion of the follicular granulosa cells surrounding the oocyte, which is a process known as cumulus expansion. These results suggest that the incomplete cumulus expansion observed in the absence of \(\text{EP}_2\) contributes to the reduced ovulation and the failure of fertilization (96).
The knockout of the EP3 receptor gene (EP3−/−) has also been reported (68, 205, 217). EP3 is clearly involved in PGE2-induced pyrexia because EP3−/− mice fail to mount a febrile response to exogenous (i.e., lipopolysaccharide) and endogenous (i.e., IL-1β) pyrogens (217). PGE2 also functions through the EP3 receptor to concentrate urine; however, these effects are deemed unessential for the normal regulation of urinary osmolality (68). The EP3−/− mouse was also reported to be unable to secrete duodenal bicarbonate on luminal perfusion with PGE2 relative to wild type (205). Because these EP3−/− mice subsequently exhibit susceptibility to acid-induced injury, this receptor may function in maintaining mucosal integrity. EP3 is also implicated in vasopressor responses, but this role is also gender specific; male but not female EP3−/− mice exhibited more PGE2-induced hypotension relative to wild-type mice (16), suggesting a pressor response attributed to EP3 in males.

Individual reports of the effects of ablation of the EP4 receptor gene (EP4−/−) concur that it has various functions in the vascular system (15, 16, 154, 187). The principal observation in EP4−/− mice is their inability to close the ductus arteriosus immediately after birth; this causes pulmonary edema and death within 72 h of birth of nearly 100% of animals (154, 187). Interestingly, the ductus is unresponsive to COX inhibition, suggesting that other mechanisms are responsible for its patency; a role for NO in ductal patency is possible given its contribution in this regard (50, 70). When this mouse strain was bred on a mixed genetic background, ~20% of EP4−/− survived (16); in female EP4−/− mice hypotensive response to PGE2 was attenuated, suggesting as for EP2 a major role for EP4 in vasodepressor responses of PGE2 in females (15). The gender-related, pressor-mediated effects of EP receptors reflect sexual dimorphism of blood pressure regulation, but the mechanisms are not clear.

FP-knockout (FP−/−) mice have been generated and studied for their reproductive function (200). Female FP−/− mice are fertile, carry their litters to term, but fail to undergo parturition. In mice this is largely because progesterone levels remain elevated because PGF2α is responsible for the luteolysis that subsequently reduces progesterone, which otherwise maintains uterine quiescence and gestation. PGF2α is also responsible for upregulating the uterine oxytocin receptors that facilitate parturition (74); this also contributes to the failure of initiating labor in FP−/− mice.

Ablation of the gene for the DP receptor (132) suggests a role for DP in allergic asthma. DP−/− mice demonstrated no difference compared with wild-type mice in total IgE after sensitization to ovalbumin. However, a marked decrease was observed in the content of TH2 cytokines (IL-4, IL-5, and IL-13) but not TH1 cytokines (such as interferon-γ) found in the bronchial alveolar lavage fluid. Infiltrating lymphocytes into the lung are thought to be the source of the TH2 cytokines, which in turn provide the signal for the pulmonary infiltration of eosinophils. Only marginal recruitment of both these cell types was demonstrated in DP−/− with respect to the wild-type mice. Also, DP−/− mice exhibited compromised airway hyperreactivity responses to acetylcholine and fewer mucus-containing cells in the airway epithelium relative to wild-type mice.

The susceptibility of IP−/− mice to thrombosis was increased relative to wild type; IP−/− mouse platelets and vascular smooth muscle were unresponsive to IP agonists (137). In addition, the pain and inflammation responses of IP−/− mice in multiple models were comparable to those observed in wild-type mice treated with COX inhibitors (indomethacin); the role of IP in pain is consistent with its distribution in dorsal root ganglia, but its participation in inflammation was unexpected.

Targeted disruption of the TP receptor gene (TP−/−) confirms a role for TP in vascular responses and hemostasis (209). The hemodynamic collapse observed on arachidonic acid infusion into wild-type mice is absent in TP−/− mice. Platelet aggregation in response to collagen and TP agonists is also impaired in TP−/− mice, causing a prolonged bleeding time.

CONCLUSION

The cloning of prostanoid receptors and development of animals with disrupted genes of these receptors have largely advanced our understanding of the functions of prostanoid receptors. Until recently the physiological role of prostanoid action was for the most part determined using COX inhibitor aspirin-like drugs. Our understanding of the involvement of specific prostanoid receptors in pathophysiological conditions of the developing subject remains fragmentary. The involvement of PGE2 in ductus arteriosus patency is clearly demonstrated (191). A role for prostanoids in the pathogenesis of intraventricular cerebral hemorrhage (133, 220) and possibly in retinopathy of prematurity (73, 146) has been suggested. However, identification of the specific prostanoid targets requires knowledge of the receptors involved. We presented in this review evidence for a possible role for prostanoid receptors in the conditions mentioned. However, the dynamic changes in prostanoid receptors during progression of serious disorders of a vascular nature in the developing subject mentioned above are only now beginning to be elucidated. For instance, a major contribution for TP in the vasoobliteration that precedes the neovascularization of retinopathy of prematurity has been identified, disclosing a new function for TP in inducing neuroretinal microvascular endothelial cell death (see Fig. 1) (22). Along the same lines, specific prostaglandin receptors involved in ductal patency have recently been identified (23, 29); these findings provide the basis for potential use of more-selective EP receptor ligands to control ductal patency and potentially diminish side effects associated with PGE (to maintain ductus open in infants with certain congenital heart malformations) and COX inhibitors (to close the ductus arteriosus). Finally, the discovery of functional nuclear prostanoid receptors proposes new avenues for
intracellular actions of these eicosanoids. The development of highly selective agonists and antagonists (not available for many receptors) of the prostanoid receptors should further widen our understanding of prostanoid physiology as well as lead to novel and more specific therapeutics.

**Perspectives**

The transition period from intra- to extrauterine life is associated with marked effects on the circulatory system of the perinate. Overall, the vasculature, especially of major organ/systems such as the central nervous system, needs to dilate, particularly at the end of parturition when strong uterine contractions reduce oxygen delivery to the fetus and cause brain compression, which can further reduce brain oxygenation. Prostanoids exert a major influence on these perinatal vascular changes, and in particular the dramatic and very transient increases in PGE$_2$ levels during labor (103). This occurs in concert with an apparent desensitization of contractile prostanoid receptors. However, this excess vasodilation and associated relative lack of cerebral blood flow autoregulation can predispose the stressed preterm infant to intraventricular brain hemorrhage (221). By extending this reasoning, it is conceivable that the resultant increased tissue oxygenation in the phase of incompletely developed antioxidant systems (55, 208) can favor peroxidation and toxicity to the retinal vasculature partly mediated by TxA$_2$ with opposing actions to PGE$_2$ and PGI$_2$ (5, 6, 7, 22), which could predispose to the development of retinopathy of prematurity (82). At the level of the ductus arteriosus, the precipitous drop in circulating PGE$_2$ that follows birth results in loss of dilation overwhelmed by oxygen-induced constriction. Failure of ductal closure in the immature preterm subject is likely contributed to by sustained high levels of prostaglandins; knowledge of the type of receptor on which PGE$_2$ acts in the ductus arteriosus may be beneficial by reducing adverse effects of currently utilized COX inhibitors. A better understanding of the mechanisms of actions of prostanoids mediated by extra- and intracellular receptors, in concert with other regulatory components of the circulatory system, will heighten our comprehension of vascular physiological and pathophysiological changes that occur in the developing subject.

We thank the Canadian Institutes of Health Research, the Fonds de la Recherche en Santé du Québec, the Heart and Stroke Foundation of Quebec, the March of Dimes Birth Defects Foundation, and the United Cerebral Palsy Foundation for support of part of the work presented here.

D. H. Wright is a recipient of a Medical Research Council-Pharmaceutical Manufacturer’s Association of Canada Health Program Award. A. Bouayad, M. Beauchamp, and S. Bernier are recipients of studentships/fellowships from the Research Center of Hôpital Ste. Justine. S. Chemtob is a recipient of a Canadian Institutes of Health Research Scientist Award and is an awardee of a Canada Research Chair (Perinatology).

Present address for D. H. Wright: Merck, PO Box 2000, Rahway, NJ 07065.

**REFERENCES**


