

# Not a mouse stirring: deletion of the EP2 and love's labor's lost

Commentary

See related article  
this issue, pages 1539–1545.

Sandra Austin and Garret A. FitzGerald

Center for Experimental Therapeutics, University of Pennsylvania, Philadelphia, Pennsylvania, 19104, USA

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has long been implicated as a proinflammatory mediator; indeed, inhibition of PGE<sub>2</sub> formation is thought to underlie the actions of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective inhibitors of the PGG/PGH synthase-2 enzyme, commonly termed cyclooxygenase-2 (COX-2) (1, 2). PGs activate specific G protein-coupled receptors; there are 4 distinct PGE receptors (EPs) (3). Targeted disruption of each of these subtypes and both COX isozymes has been accomplished. Given the anti-inflammatory efficacy of COX-2 inhibitors in clinical trials (4), it is surprising that neither the COX-2 nor EP knockouts have yet exhibited an anti-inflammatory phenotype. By contrast, targeted disruption of a phospholipase with particular affinity for arachidonic acid (cPLA<sub>2</sub>), COX-1, and the prostacyclin (PGI<sub>2</sub>) receptor (IP) modulates inflammatory responses in the mouse (5–7). The EP knockouts are more informative about the role of PGE<sub>2</sub> in the fibrile response: deletion of the EP3 subtype confers resistance to endogenous and exogenous pyrogens (8). Remodeling of the ductus arteriosus fails to occur after birth in EP4-deficient mice, resulting in death of the neonatal animals (9, 10).

Two groups have now reported on targeted disruption of the EP2 (11, 12). In the case of blood pressure, the potent effects on vascular tone in vitro of other COX products, thromboxane (Tx), A<sub>2</sub> and PGI<sub>2</sub>, might have forecast alterations in Tx receptor (TP) and IP knockouts. However, in both cases, baseline blood pressure is normal (7, 13). IP-deficient mice are, however, prone to developing hypertension on salt loading (14). Both Kennedy et al. (11) and Tilley et al. (12) have reported that mice lacking the EP2 (like the IP, the EP2 is coupled to adenylate cyclase activation) also exhibit salt-sensitive hypertension. The mechanism by which these eicosanoids modulate the vascular response to salt loading is unknown, as is the origin of their formation. Sodium retention has been reported anecdotally

with both conventional NSAIDs and COX-2 inhibitors. Chronic dosing with COX-2 inhibitors causes sodium retention – but this is transient and not associated with a rise in systemic blood pressure in healthy individuals (15). Kennedy et al. (11) reported elevated resting blood pressure, which is more pronounced in female mice, while Tilley et al. (12) reported systemic hypotension. Perhaps this distinction reflects differences in the genetic background of these mice (C57BL/6 versus 129/SvEv) or, less likely, the different approaches to gene targeting. More recently, Audoly et al. (16) have reported that deletion of the EP4, which is also coupled to adenylate cyclase, also results in salt-sensitive hypertension, but only in males. By contrast, deletion of the EP1, which is coupled to phospholipase C activation, results in resting systolic hypotension, an effect most evident in males (17). Thus, the effects of PGE<sub>2</sub> on blood pressure are likely to reflect a complex interplay between receptor subtype expression, sex steroids, and other modifying factors in the genetic background. The mice in the current study are also likely to prove useful in elucidating the role of the EP2 in additional systems, such as maturation and differentiation of B and T lymphocytes (18, 19).

In contrast to their studies on resting blood pressure, both groups had similar findings with respect to the importance of PGE<sub>2</sub> in fertility. The number of successful pregnancies achieved by female mice lacking the EP2 is reduced, because the majority of ova released from the corpora lutea fail to become fertilized in vivo. EP2-deficient males are reproductively competent. Prior to these observations, targeted disruption of cPLA<sub>2</sub> (5), COX-1 (6), and COX-2 (20, 21) suggested the importance of PGs, which were originally discovered in semen, in reproduction. Female mice deficient in COX-2 suffer from multiple defects in early pregnancy, including impaired implantation, decidualization, and fertilization (22). There is coordinate expression of cPLA<sub>2</sub>, COX-

2, and PGI synthase at the site of implantation, and PGI<sub>2</sub> analogues can rescue the implantation defect in COX-2-deficient mice (22). However, mice lacking IP have no apparent reproductive defect. Interestingly, peroxisome proliferator-activated receptor  $\delta$  (PPAR $\delta$ ), which can be activated by PGI<sub>2</sub> analogues in vitro (23), is also upregulated at the site of implantation; it will be interesting to examine the reproductive competence of mice lacking this putative nuclear receptor for PGI<sub>2</sub>. Mice lacking COX-1 also have reproductive difficulties: although pregnancy develops normally to term, the mice fail to undergo parturition (24). Analogues of PGF<sub>2 $\alpha$</sub>  have been used to induce labor and are recognized as powerful myometrial stimulants. Mice lacking the PGF<sub>2 $\alpha$</sub>  receptor (FP) also fail to enter labor at term. In the absence of PGF<sub>2 $\alpha$</sub>  or FP, persistent generation of progesterone prevents the onset of labor. PGF<sub>2 $\alpha$</sub>  appears to work upstream of the oxytocin receptor to induce luteolysis by a mechanism independent of its uterotonic action (25).

Impaired fertility in EP2-deficient females is not a defect intrinsic to the ovum (ova are fertilized efficiently in vitro), nor can it be due to a problem with implantation (wild-type blastocysts implant normally in the knockout uterus). Thus, PGE<sub>2</sub> may, in some way, contribute to the microenvironment in which fertilization takes place in vivo. While this is a novel suggestion in the case of eicosanoids, it appears to be true of platelet-activating factor (PAF), another bioactive lipid mediator. PAF, like PGs, is present in semen and appears to be important for sperm motility and maturation (26). Furthermore, it is produced by both the embryo and the endometrium in early pregnancy, and may facilitate implantation. Addition of exogenous PAF may enhance the likelihood of success of in vitro fertilization (27).

In summary, PGs, and probably other bioactive lipids, appear to play a coordinate role in the reproductive process: PGI<sub>2</sub> at implantation, PGE<sub>2</sub> in fertiliza-

tion, and  $\text{PGF}_{2\alpha}$  in the induction of labor. Additional roles may at present be obscured by redundancies in the system. For example, myometrial TPs, FPs, EP1s, and EP3s may all mediate uterotonic effects of their ligands (28, 29). Both FP and EP agonists are used in the induction of labor, and NSAIDs may prevent preterm labor (30). A refined understanding of the role of  $\text{PGE}_2$  in inflammation, blood pressure homeostasis, and reproduction may reveal new therapeutic opportunities for EP subtype-specific agonists and antagonists. Also, given the marked induction of COX-2 at the site of the implanting blastocyst and in decidual cells (18), elucidation of the effects of selective COX-2 inhibitors on the reproductive process in humans would seem timely.

1. Seibert, K., et al. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc. Natl. Acad. Sci. USA*. **91**:12013–12017.
2. Anderson, G.D., et al. 1996. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J. Clin. Invest.* **97**:2672–2679.
3. Coleman, R., Smith, W., and Narumiya, S. 1994. International Union of Pharmacology classification of prostanoid receptors: properties, distribution and structure of the receptors and their subtypes. *Pharmacol. Rev.* **46**:205–229.
4. Simon, L.S., et al. 1998. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor. *Arthritis Rheum.* **41**:1591–1602.
5. Bonventre, J., et al. 1997. Reduced fertility and postischemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature*. **390**:622–625.
6. Langenbach, R., et al. 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*. **83**:483–492.
7. Murata, T., et al. 1997. Altered pain perception and inflammatory response in mice lacking the prostacyclin receptor. *Nature*. **388**:678–682.
8. Ushikubi, F., et al. 1998. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature*. **395**:281–284.
9. Nguyen, M., et al. 1997. The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature*. **390**:78–81.
10. Segi, E., et al. 1998. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem. Biophys. Res. Commun.* **246**:7–12.
11. Kennedy, C.R.J., et al. 1999. Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. *Nat. Med.* **5**:217–220.
12. Tilley, S.L., et al. 1999. Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E<sub>2</sub> receptor. *J. Clin. Invest.* **103**:1539–1545.
13. Thomas, D.W., et al. 1998. Coagulation defects and altered haemodynamic responses in mice lacking receptors for thromboxane A<sub>2</sub>. *J. Clin. Invest.* **102**:1994–2001.
14. Yahata, K., et al. 1998. Blood pressure, renal function, salt handling and renin release in prostacyclin receptor knockout mice. *J. Am. Soc. Nephrol.* **9**:417A. (Abstr.)
15. Catella-Lawson, F., et al. 1999. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J. Pharmacol. Exp. Ther.* **289**:735–741.
16. Audoly, L.P., Nguyen, M.T., Koller, B.H., and Coffman, T.M. 1999. The prostaglandin LP4 receptor subtype is an essential component of blood pressure regulation in males, but not females. A study using knockout mice. *FASEB J.* **13**:A1549. (Abstr.)
17. Audoly, L.P., et al. 1999. Mice lacking the prostaglandin E<sub>2</sub> EP1 receptor subtype have hypotension, hyperreninemia and altered responses to angiotensin II. *FASEB J.* **13**:A1549. (Abstr.)
18. Rocca, B., Spain, L., Pure, E., Patrono, C., and FitzGerald, G.A. 1999. Distinct and coordinated roles of prostaglandin H-synthases 1 and 2 in T-cell development. *J. Clin. Invest.* **103**:1469–1477.
19. Fedyk, E.R., and Phipps, R.P. 1996. Prostaglandin E<sub>2</sub> receptors of the EP<sub>2</sub> and EP<sub>4</sub> subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. *Proc. Natl. Acad. Sci. USA*. **93**:10978–10983.
20. Morham, S.G., et al. 1995. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell*. **83**:473–482.
21. Dinchuk, J.E., et al. 1995. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature*. **378**:406–409.
22. Lim, H., et al. 1997. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell*. **91**:197–208.
23. Forman, B.M., Chen, J., and Evans, R.M. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors  $\alpha$  and  $\delta$ . *Proc. Natl. Acad. Sci. USA*. **94**:4312–4317.
24. Gross, G.A., et al. 1998. Opposing actions of prostaglandins and oxytocin determine the onset of murine labor. *Proc. Natl. Acad. Sci. USA*. **95**:11875–11879.
25. Sugimoto, Y., et al. 1997. Failure of parturition in mice lacking the prostaglandin F receptor. *Science*. **277**:681–683.
26. Krausz, C., Gervasi, G., Forti, G., and Baldi, E. 1994. Effect of platelet activating factor on motility and acrosome reaction of human spermatozoa. *Hum. Reprod.* **9**:471–476.
27. Minhas, B.S., et al. 1996. Platelet activating factor and conception. *Am. J. Reprod. Immunol.* **35**:267–271.
28. Swanson, M.L., et al. 1992. The expression of thromboxane A<sub>2</sub> synthase and thromboxane A<sub>2</sub> receptor gene in human uterus. *Biol. Reprod.* **47**:105–117.
29. Wilhelmsson, L., Wikland, M., and Wiquist, N. 1981.  $\text{PGH}_2$ ,  $\text{TXA}_2$  and  $\text{PGI}_2$  have potent and differentiate actions on human uterine contractility. *Prostaglandins*. **21**:277–286.
30. Dawood, M.Y. 1993. Non-steroidal anti-inflammatory drugs and reproduction. *Am. J. Obstet. Gynecol.* **169**:1255–1265.