Prostaglandin E2 Stimulates EP2, Adenylate Cyclase, Phospholipase C, and Intracellular Calcium Release to Mediate Cyclic Adenosine Monophosphate Production in Dental Pulp Cells

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Abstract

Introduction: Prostaglandin E2 (PGE_2) plays a crucial role in pulpal inflammation and repair. However, its induction of signal transduction pathways is not clear but is crucial for future control of pulpal inflammation. **Methods:** Primary dental pulp cells were exposed to PGE_2 and 19R-OH PGE_2 (EP2 agonist) or sulprostone (EP1/EP3 agonist) for 5 to 40 minutes. Cellular cyclic adenosine monophosphate (cAMP) levels were measured using the enzyme-linked immunosorbent assay. In some experiments, cells were pretreated with SQ22536 (adenylate cyclase inhibitor), H89 (protein kinase A inhibitor), dorsomorphin (adenosine monophosphate–activated protein kinase inhibitor), U73122 (phospholipase C inhibitor), thapsigargin (inhibitor of intracellular calcium release), W7 (calmodulin antagonist), verapamil (L-type calcium channel blocker), and EGTA (extracellular calcium chelator) for 20 minutes before the addition of PGE_2. **Results:** PGE_2 and 19R-OH PGE_2 (EP2 agonist) stimulated cAMP production, whereas sulprostone (EP1/EP3 agonist) shows little effect. PGE_2-induced cAMP production was attenuated by SQ22536 and U73122 but not H89 and dorsomorphin. Intriguingly, thapsigargin and W7 prevented PGE_2-induced cAMP production, but verapamil and EGTA showed little effect. **Conclusions:** These results indicate that PGE_2-induced cAMP production is associated with EP2 receptor and adenylate cyclase activation. These events are mediated by phospholipase C, intracellular calcium release, and calcium-calmodulin signaling. These results are helpful for understanding the role of PGE_2 in pulpal inflammation and repair and possible future drug intervention. *(J Endod 2016;42:584–588)*

Key Words

Adenylate cyclase, calcium, cyclic adenosine monophosphate, prostaglandin, prostaglandin receptor, pulp cells, signal transduction

A number of irritants such as dental caries, bacterial toxins, operative restoration materials, and traumatic injuries may induce pulpal inflammation and even necrosis of the human dental pulp. Various prostanoids such as prostaglandin E2 (PGE_2), prostaglandin F2 (PGF2), and prostaglandin I2 (PGI_2) play crucial roles in these processes to regulate pulpal inflammation and repair (1–3). PGE_2 and PGI_2 may be involved in the pathogenesis of pulpal inflammation via an increase of vascular permeability in experimental pulpal inflammation of rats (1) and vascular blood flow and tissue edema (2, 3). Interleukin (IL)-1α and IL-1β, 2 proinflammatory cytokines, also induce cyclooxygenase-2 expression and prostanoids' (PGE_2 and $PGF_{2\alpha}$) production of dental pulp cells (4, 5). On the other hand, tumor necrosis factor α and IL-1α stimulated metalloproteinase-1 (MMP-1), IL-6, and tissue inhibitor metalloproteinase-1 (TIMP-1) expression of pulp cells. PGE_2 alone may also stimulate TIMP-1 expression (6) and suppress intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) expression and secretion in dental pulp cells (7, 8), suggesting the anti-inflammatory/tissue repair property of PGE_2. PGE_2 exerts its effects via the activation of 4 prostaglandin EP receptors (eg, EP1, EP2, EP3, and EP4, which linked to various downstream signal transduction pathways such as adenylate cyclase/cyclic adenosine monophosphate [cAMP]/protein kinase A [PKA] and phospholipase C (PLC)/IP3/calcium mobilization) (9–11). Recently, pulp cells have been shown to mainly express the EP2 receptor, less amounts of the EP3 and EP1 receptor, and a little amount of the EP4 receptor (5). To know the signal transduction pathways responsible for PGE_2-induced pulpal changes is critical for the development of agents against pulpal inflammation. However, the signaling pathways of PGE_2 in dental pulp cells await further clarification.

Activation of the EP2 receptor by PGE_2 or other agonists may activate adenylate cyclase in different kinds of cells (9–11). Adenylate cyclase is a membrane-bound enzyme responsible for cAMP generation and is modulated in various conditions such as psoriatic hyperproliferative epidermis and relaxation of airway smooth muscle (12, 13). Recently, cAMP/PKA/cAMP-responsive element-binding protein (CREB)

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signaling has been shown to mediate osteogenesis of dental pulp stem cells via inducing bone morphogenetic protein-2 (BMP-2), bone sialoprotein, osteocalcin, and type XXIV collagen (14). Extracellular phosphate is also shown to stimulate BMP-2 expression of dental pulp cells via cAMP/PKA/CREB signaling (15). On the contrary, activator of G-protein signaling 3 inhibits the TNF-α–induced osteogenic differentiation of dental pulp stem cells via the cAMP/PKA pathway (14). PGF2α stimulates the mitogen-activated protein kinase kinase (MEK)/extracellular signal regulated kinase (ERK)-CREB/ATF-1 signaling pathway and IL-8 production but suppresses the alkaline phosphatase activity of dental pulp cells. SQ22536, an inhibitor of adenylate cyclase, enhanced the PGF2α-induced IL-8 production (16, 17). Whether PGE_2 activates adenylate cyclase and stimulates cAMP production in dental pulp is an interesting issue that should be addressed further.

Moreover, an increase of extracellular calcium stimulates BMP-2 expression of pulp cells via L-type calcium channel and ERK signaling, which can be attenuated by PD98059 and nifedipine (18). Dental pulp cells expressed little calcium-sensing receptors (18). *In vivo* immunohistochemistry staining also shows the strong nucleus expression of CREB Ser133 phosphorylation in odontoblasts and cementoblasts as well as pulp stroma cells of human molar teeth, suggesting the involvement of CREB in mediating various signaling and pulpal functions (19). Furthermore, PGE_2 may increase intracellular calcium levels of pulp cells (5). Therefore, it is intriguing to clarify whether PGE_2 may stimulate PLC, intracellular calcium release, and extracellular calcium influx to activate calcium calmodulin and adenosine monophosphate–activated protein kinase (AMPK) to affect cAMP production of pulp cells.

Various prostanoids are important in the pulpal inflammation and repair. In this study, to clarify the role of PGE_2 in the pathogenesis of pulpal inflammation and repair, we studied the effect of PGE_2 on adenylate cyclase activity and cAMP production of pulp cells and its upstream signal transduction pathways in dental pulp cells. The results of this study can be helpful to control and treat pulpal inflammation in the future.

Materials and Methods

Materials

PGE_2 and ethylene glycol tetraacetic acid (EGTA) were purchased from Sigma (Sigma-Aldrich, St Louis, MO). Dulbecco modified Eagle medium (DMEM), fetal bovine serum, and penicillin/streptomycin were obtained from Gibco (Life Technologies, Grand Island, NY). Sulprostone, 19R-OH PGE_2, and cAMP ELISA kits were from Cayman (Cayman Chemical Company, Ann Arbor, MI). SQ22536, H89, dorsomorphin, U73122, thapsigargin, W7, and verapamil were from Tocris (Tocris Cookson Ltd, Northpoint, Avonmouth, UK).

Culture of Human Dental Pulp Cells

Human dental pulp cells were established and cultured in DMEM supplemented with penicillin/streptomycin and 10% FCS as described previously (5, 20, 21). The pulp cells in passage numbers of 3 to 8 were used for these studies. Cultured dental pulp cells were generally spindle shaped with extended cellular processes.

Effect of PGE_2, 19R-OH PGE_2, and Sulprostone on cAMP Production of Pulp Cells

Dental pulp cells were seeded at a concentration of 1×10^5 cells/well into a 24-well culture plate. After 24 hours, they were incubated for 2 hours in serum-free DMEM and then exposed to 1 μmol/L PGE_2, 19R-OH PGE_2 (EP2 receptor agonist), and sulprostone (EP1/EP3) agonist for 5, 10, 20, and 40 minutes. Medium was decanted, and cells were washed with PBS. Then, 65 μL 1 N HCl was added for collection and lysis of cells. After centrifugation, supernatant of cell lysate was used

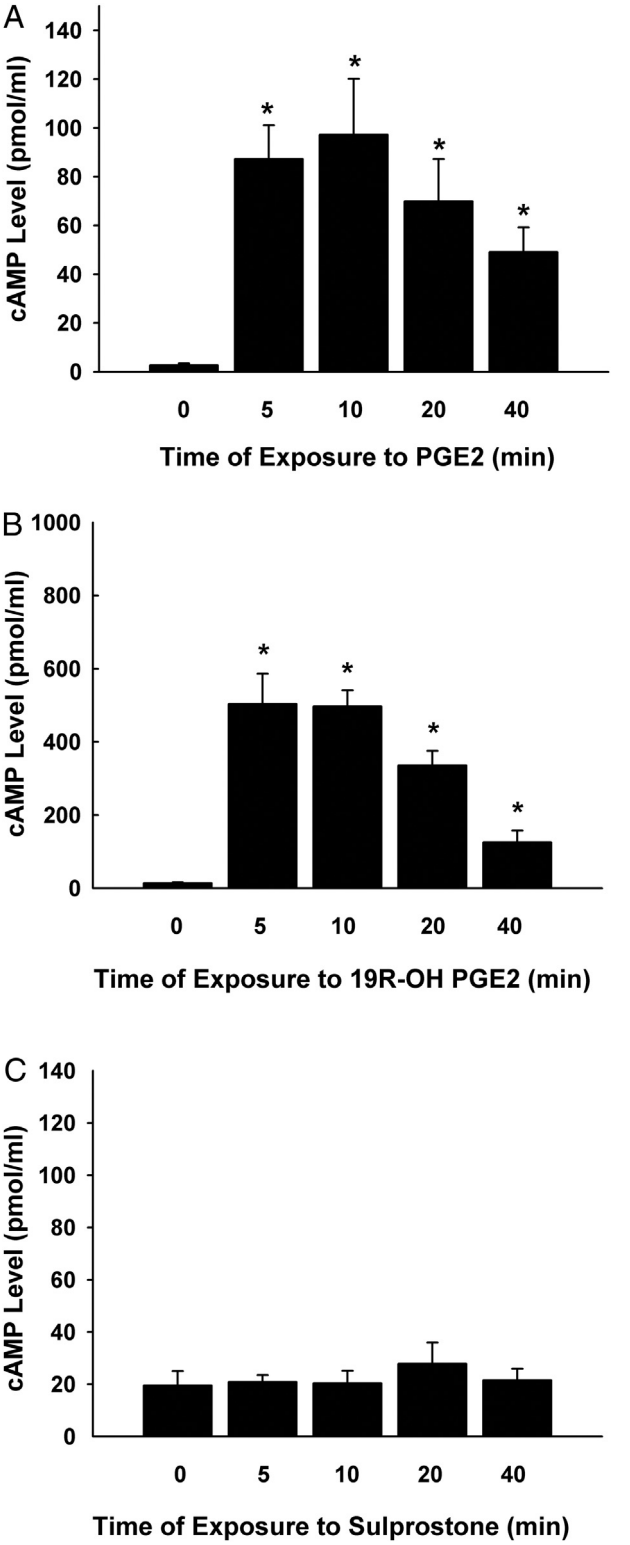

Figure 1. The effect of PGE_2, 19R-OH PGE_2, and sulprostone on cAMP levels of dental pulp cells. Dental pulp cells were exposed to (*A*) PGE_2, (*B*) 19R-OH PGE_2, and (*C*) sulprostone for 5 to 40 minutes. Cell lysates were collected and used for the measurement of cAMP levels using the enzyme-linked immunosorbent assay. Results were expressed as mean ± standard error. *A statistically significant difference when compared with the control group.

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