Online Supplemental Material for

Mandal et al. http://www.jem.org/cgi/doi/10.1084/jem.20031666

Supplemental Materials and Methods

COX-2 Promoter-Reporter (Luciferase) Assay. Transient transfection of NIH-3T3 cells (60–70% confluent) with pGL2-COX2WT and pGL2-COX2MU luciferase constructs (Singer, C. A, K.J. Baker, A. McCaffrey, D.P. AuCoin, M.A. Dechert, W.T. Gerthoffer. 2003. *Am. J. Physiol. Lung Cell Mol. Physiol.* 285:L1087–L1098; a gift from Dr. Cherie A. Singer, University of Nevada School of Medicine, Reno, NV) or vector alone were carried out using a modified CaPO4 protocol (Strategene) as described (Singer, C. A, K.J. Baker, A. McCaffrey, D.P. AuCoin, M.A. Dechert, W.T. Gerthoffer. 2003. *Am. J. Physiol. Lung Cell Mol. Physiol.* 285:L1087–L1098). After 12 h of transfection, cells were cultured in serum-free OptiMEM (Invitrogen) for 48 h and then treated with 50 mM PGD2 in the presence and absence of 1 mM rUG for 4 h. Luciferase assays were performed as reported (Singer, C. A, K.J. Baker, A. McCaffrey, D.P. AuCoin, M.A. Dechert, W.T. Gerthoffer. 2003. *Am. J. Physiol. Lung Cell Mol. Physiol.* 285:L1087–L1098) using Lumat LB9507 (EG & G Berthold) luminometer.

Molecular Modeling of UG–PGD2 Interaction. The initial coordinates for the three-dimensional structure of PGD2 were generated from its two-dimensional chemical structure using the Insight II molecular modeling program (Accelrys Inc.). The coordinates of PGD2 were energy minimized using cff91 Discover force field (Accelrys Inc.). Molecular dynamics simulation for 50 picoseconds on the energy-minimized structure of PGD2 was carried out using Discover force field. The conformations in which the two long carbon chains are favorably packed are visually selected. These conformations were docked into the central cavity of the x-ray crystal structure of the recombinant human UG dimer (Mornon, J.-P., F. Fridlansky, R. Bally, and E. Milgrom. 1980. *J. Mol. Biol.* 137:415–429; Mathews, J.H., N. Pattabiraman, K.B. Ward, G. Mantile, L. Miele, and A.B. Mukherjee. 1994. *Proteins.* 20:191–196; Callebaut, I., A. Poupon, R. Bally, J.P. Demaret, D. Housset, J. Delettre, P. Hossenlopp, and J.-P. Mornon. 2000. *Ann. N.Y. Acad. Sci.* 923:90–112; Pattabiraman, N., J.H. Matthews, K.B. Ward, G. Mantile-Selvaggi, L. Miele, and A.B. Mukherjee. 2000. *Ann. N.Y. Acad. Sci.* 923:113–127). It should be noted that UG is naturally a homodimeric protein. Since the shape of PGD2 is like a pancake, in the docking experiments two orientations related by 180 degrees rotation about the long axis of the molecule were considered. The coordinates of each complex were energy minimized as described above.

Assay for the Detection of the UG–PGD2 Complex. To determine whether PGD2 forms a complex with rUG, 25 pmoles of the rUG was incubated with 25 pmoles of 3H-PGD2 in the absence and presence of varying concentrations (2–1,000 pmoles) of cold PGD2 in 300 ml of serum-free OptiMEM medium at 4°C for 1 h. Glycerol was added to the mixture to a final concentration of 5%, and aliquots of the mixtures were resolved by electrophoresis using a nondenaturing 12% polyacrylamide gel in Tris-glycine-EDTA (pH 8.0) buffer at 4°C. The gels were dried at room temperature, and autoradiographs were obtained by using a Cyclone phosphorimager (Packard).

J. Exp. Med. © The Rockefeller University Press • 0022-1007
Volume 199 May 17, 2004
http://www.jem.org/cgi/doi/10.1084/jem.20031666