Pergamon

Biochemical Pharmacology, Vol. 50, No. 10, pp. 1731–1733, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)02035-B

Short Communication

6-ISOPROPOXY-9-OXOXANTHENE-2-CARBOXYLIC ACID (AH 6809), A HUMAN EP_2 RECEPTOR ANTAGONIST

DAVID F. WOODWARD,*† DAVID J. PEPPERL,‡ THOMAS H. BURKEY‡ and JOHN W. REGAN‡

*Biological Sciences, Allergan, Inc., Irvine, CA 92713; and ‡Departments of Pharmacology and Toxicology, Physiology and the Program in Neuroscience, College of Pharmacy, University of Arizona, Tucson, AZ 85721, U.S.A.

(*Received* 18 *November* 1994; *accepted* 1 *June* 1995)

Abstract—On studying the interaction of various ligands with the pharmacologically defined, recombinant human EP_2 receptor (Regan *et al., Mol Pharmacol* **46**: 213–220, 1994), we discovered that the putative EP_1 receptor antagonist 6-isopropoxy-9-oxoxanthene-2-carboxylic acid (AH 6809) also has affinity for the human EP_2 receptor. Moreover, AH 6809 behaved as an EP_2 receptor antagonist and inhibited prostaglandin E_2 (PGE_2)-stimulated increases in cyclic AMP. These findings have significant implications for studies that employ AH 6809 to determine the pharmacological basis of PGE_2-induced responses in human cells and tissues.

Key words: prostaglandins; adenylyl cyclase; G-protein coupled receptor; cAMP; AH 6809

The pharmacological basis of prostanoid biology is complex and was not established until recently. The current receptor classification, which proposed that each of the primary natural prostanoids preferentially interacts with a discrete receptor, was derived according to the potency rank orders of natural prostaglandins in certain preparations and the functional selectivity of synthetic prostanoid analogs [1, 2]. Separate receptors for prostaglandins D_2, E_2, $F_{2\alpha}$, and I_2 and thromboxane A_2 were designated DP, EP, FP, IP and TP, respectively. Further subdivision of the EP receptor emerged based on the activity of the antagonists SC-19220 and AH 6809§, which selectively block the EP_1 receptor subtype [1–3].

The biochemical basis of this receptor heterogeneity has now been established by cloning the genes that encode for each of the prostanoid receptors. Heterologous expression of these recombinant receptors in cultured cell lines will now allow the pharmacology of these receptors to be studied directly without the complicating factors associated with the pharmacological interpretation of functional studies. Recently, we cloned a cDNA that encodes a novel human prostaglandin receptor that has only ~30% amino acid sequence homology to previously cloned EP receptors and which has characteristics that are consistent with the pharmacologically defined EP_2 receptor [4]. Thus, in common with the native human EP_2 receptor [5, 6], this novel receptor interacts with butaprost and recognizes other prostaglandin E analogs claimed to be EP_2 receptor selective in isolated laboratory animal tissue studies [1–3]. Upon studying the pharmacology of this human EP_2 receptor, we discovered unexpected activity associated with the purported EP_1 antagonist AH 6809.

Materials and Methods

A plasmid for expressing the recombinant human EP_2 receptor was prepared as previously described and transfected into COS-7 cells using DEAE-dextran and DMSO shock [4, 7]. Three days after transfection, the cells were harvested and used for radioligand binding studies performed under conditions identical to those reported previously [4]. Transfected cells were prepared for cAMP studies by splitting them into 24-well plates after 1 day, followed by 2 additional days of culture in DMEM containing 5% fetal bovine serum. Formation of cAMP was determined according to a protein kinase A binding assay as previously described [4, 8]. AH 6809 was prepared in solutions containing 0.1% polysorbate 80.

Results

The ability of AH 6809 and unlabeled PGE_2 to compete with 5 nM [^{3}H]PGE_2 for the recombinant human EP_2 receptor binding site is depicted in Fig. 1. AH 6809 appeared to have distinct affinity for the EP_2 receptor, and substantial competition was apparent at a concentration of 10 μM (Fig. 1).

Figure 2A shows concentration–response curves for PGE_2

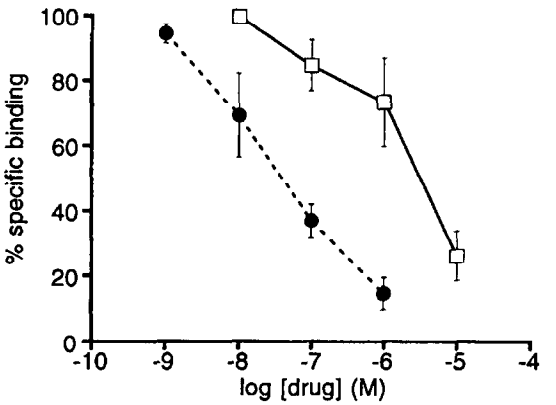

Fig. 1. Inhibition of [^{3}H]PGE_2 binding by unlabelled PGE_2 (●) and AH 6809 (□). Values are means ± SEM of three separate experiments performed in duplicate. Specific binding (represented by approximately 2000 displaceable counts per assay) for the three experiments was 49, 49 and 50%, with binding to the filter included in the calculation of non-specific binding.

† Corresponding author: Dr. David F. Woodward, Department of Biosciences (RD-2C), Allergan Inc., 2525 Dupont, Irvine, CA 92713. Tel. (714) 246-5490; FAX (714) 246-5578.

§ Abbreviations: AH 6809, 6-isopropoxy-9-oxoxanthene-2-carboxylic acid; PGE_2, prostaglandin E_2; DEAE, diethylaminoethyl; cAMP, cyclic adenosine monophosphate; and DMEM, Dulbecco's modified Eagle's medium.

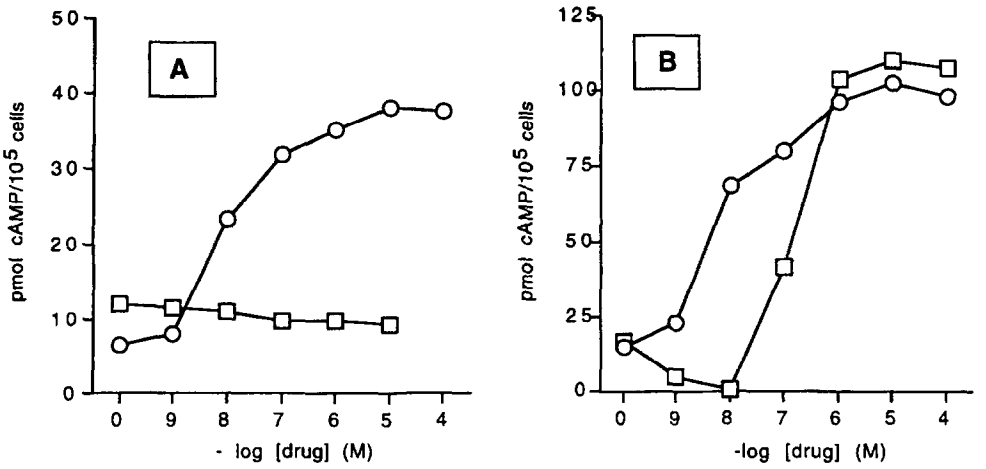

Fig. 2. Effects of PGE_2 and AH 6809 on cAMP formation in COS-7 cells transfected with the human EP_2 receptor. Panel A shows concentration–response curves for PGE_2 (○) and AH 6809 (□). Panel B shows concentration–response curves for PGE_2 in the absence (○) and presence of 10 μM AH 6809 (□). Panels A and B are separate experiments in which triplicate determinations were made for each point; the average standard deviation for each point was ~10%. The experiments were repeated two additional times with similar results. In panel A, the EC_{50} of PGE_2 was 8 nM and in panel B it was 6 nM. The EC_{50} of PGE_2 in the presence of AH 6809 was 178 nM.

and AH 6809 for the stimulation of cAMP production in COS-7 cells transiently transfected with the human EP_2 receptor. For PGE_2 there was an approximately 7-fold stimulation of cAMP formation with an EC_{50} of 8 ± 3 nM. In contrast, AH 6809 produced no increase in cAMP at concentrations up to 10 μM. In Fig. 2B, the effect of 10 μM AH 6809 on PGE_2-induced cAMP formation was examined. Consistent with competitive inhibition, AH 6809 shifted the concentration–response curve for PGE_2 approximately 30-fold to the right.

To verify that the inhibition afforded by AH 6809 was selective for cAMP formation associated with EP_2 receptor stimulation, its activity against forskolin and β_2-adrenoceptor-mediated increases in cAMP was examined in COS-7 cells. Graded concentrations of AH 6809 (10^{-8}–10^{-5} M) did not affect the increase in cAMP produced by 1 μM forskolin. Furthermore, in COS-7 cells transfected with an expression vector for the human β_2-adrenoceptor, AH 6809 did not inhibit isoproterenol-stimulated cAMP formation (data not shown).

Discussion

The ability of AH 6809 to behave as an antagonist at the prostanoid EP_1 receptor is widely regarded as an important facet of EP receptor pharmacology. Although AH 6809 exhibits weak antagonism at DP and TP receptors [3], within the EP receptor family it appears to possess a high degree of selectivity for the EP_1 receptor with no measurable activity at EP_2 [1] or EP_3 [9, 10] receptors. Thus, in laboratory animal preparations, AH 6809 has been employed successfully in characterizing EP receptor subtypes [11, 12].

In contrast to these previous findings with AH 6809, studies using recombinant human EP receptors have yielded some unexpected results. The affinity of AH 6809 for the pharmacologically defined human EP_2 receptor [4] was not dissimilar to that reported for the human EP_1 receptor [13]. AH 6809 did not stimulate the human EP_2 receptor but antagonized PGE_2-induced increases in cAMP. Other human EP receptors, such as the EP_3 receptor variants similarly transfected in COS-7 cells [14] and a murine EP receptor positively coupled to adenylate cyclase [15], do not appear to interact with AH 6809.

In summary, AH 6809 appears to behave as a competitive antagonist at both human EP_1 and EP_2 receptors. This pharmacological activity profile should be taken into consideration when using AH 6809 to determine the physiological role of EP receptors in mediating the effects of PGE_2 on human cells and tissues.

REFERENCES

1. Coleman RA, Humphrey PPA and Kennedy I, Prostanoid receptors in smooth muscle: Further evidence for a proposed classification. *Trends Auton Pharmacol* **3:** 35–49, 1982.
2. Coleman RA, Humphrey PPA, Kennedy I and Lumley P, Prostanoid receptors: The development of a working classification. *Trends Pharmacol Sci* **5:** 303–306, 1984.
3. Coleman RA, Kennedy I, Humphrey PPA, Bunce K and Lumley P, Prostanoids and their receptors. In: *Comprehensive Medicinal Chemistry* (Ed. Emmett JC), Vol. 3, pp. 643–714. Pergamon Press, New York, 1990.
4. Regan JW, Bailey TJ, Pepperl DJ, Pierce KL, Bogardus AM, Donello JE, Fairbairn CE, Kedzie KM, Woodward DF and Gil DW, Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. *Mol Pharmacol* **46:** 213–220, 1994.
5. Gardiner PJ, Characterization of prostanoid relaxant/inhibitory receptors (Ψ) using a highly selective agonist, TR 4979. *Br J Pharmacol* **87:** 45–46, 1986.
6. Senior J, Marshall K, Sangha R, Baxter GS and Clayton JK, *In vitro* characterization of prostanoid EP-receptors in the non-pregnant human myometrium. *Br J Pharmacol* **102:** 747–753, 1991.
7. Cullen BR, Use of eukaryotic expression technology in the functional analysis of cloned genes. *Methods Enzymol* **152:** 684–704, 1987.
8. Gilman AG, A binding assay for adenosine 3′,5′-cyclic monophosphate. *Proc Natl Acad Sci USA* **67:** 305–312, 1970.
9. Coleman RA, Kennedy I, Sheldrick RLG and Tolowinska IY, Further evidence for the existence of three subtypes of PGE_2-sensitive (EP-) receptors. *Br J Pharmacol* **91:** 407P, 1987.
10. Lawrence RA and Jones RL, AH 6809 distinguishes two types of direct contractile action for prostanoids on guinea-pig ileum longitudinal muscle. *Br J Pharmacol* **96:** 63P, 1989.
11. Mantelli L, Amerini S, Rubino A and Ledda F, Prejunctional prostanoid receptors on cardiac adrenergic terminals

belong to the EP_3 subtype. *Br J Pharmacol* **102:** 573–576, 1991.

12. Lawrence RA and Jones RL, Investigation of the prostaglandin E (EP-) receptor subtype mediating relaxation of the rabbit jugular vein. *Br J Pharmacol* **105:** 817–824, 1992.
13. Funk CD, Furci L, Fitzgerald GA, Grygorczyk R, Rochette C, Bayne MA, Abramovitz M, Adam M and Metters KM, Cloning and expression of a cDNA for the human prostaglandin E receptor EP_1 subtype. *J Biol Chem* **268:** 26767–26772, 1993.
14. Regan JW, Bailey TJ, Donello JE, Pierce KL, Pepperl DJ, Zhang D, Kedzie KM, Fairbairn CE, Bogardus AM, Woodward DF and Gil DW, Molecular cloning and expression of human EP_3 receptors: Evidence of three variants with differing carboxyl termini. *Br J Pharmacol* **112:** 377–385, 1994.
15. Honda A, Sugimoto Y, Namba T, Watabe A, Irie A, Negishi M, Narumiya S and Ichikawa A, Cloning and expression of a cDNA for mouse prostaglandin E receptor EP_2 subtype. *J Biol Chem* **268:** 7759–7762, 1993.