*Br. J. Pharmacol.* (1990), **99**, 13–14 **SHORT COMMUNICATION** 

# Evaluation of ZK110841 and AH6809, an agonist and an antagonist of prostaglandin DP-receptors on human platelets, with a $PGD_2$-responsive cell line from bovine embryonic trachea

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ZK110841 and AH6809, an agonist and an antagonist of prostaglandin DP-receptors on human platelets, were evaluated with a fibroblastic cell line from bovine embryonic trachea (EBTr) which specifically responds to prostaglandin $D_2$ ($PGD_2$). ZK110841, equipotent to $PGD_2$, elevated the adenosine 3':5'-cyclic monophosphate (cyclic AMP) level in EBTr cells dose-dependently, being more than 100 fold over the basal level at 1 $\mu$M. The half-maximally effective concentration of $PGD_2$ or ZK110841 was 10–30 nM. Increasing concentrations ($10^{-7}$–$10^{-4}$ M) of AH6809 (which possesses negligible intrinsic agonist activity) produced parallel shifts to the right of dose-response curves to $PGD_2$ and ZK110841. Schild plots of these data were linear (correlation close to unity) and $pA_2$ values against $PGD_2$ and ZK110841 were calculated to be 6.36 and 6.57, respectively, indicating that AH6809 is a simple and competitive antagonist. These results demonstrate that ZK110841, AH6809 and EBTr cells will be useful for characterization of DP-receptors.

**Introduction** A general classification for prostaglandin receptors has been proposed by Kennedy and colleagues (1982). This classification was originally based on the discontinuity of potency orders of the naturally occurring agonists in different tissues and has been reinforced by agonist and antagonist information. Prostaglandin $D_2$ ($PGD_2$) is formed in virtually all mammalian tissues and has diverse biological effects (Ito *et al.*, 1989). $PGD_2$ is well known to inhibit human platelet aggregation through a specific $PGD_2$ receptor (DP-receptor). Recently, ZK110841 (9-deoxy-9$\beta$-chloro-16,17,18,19,20-pentanor-15-cyclohexyl-$PGF_{2\alpha}$, Thierauch *et al.*, 1988) and AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid, Keery & Lumley, 1988) were reported to be an agonist and an antagonist of DP-receptors on human platelets, respectively. However, some derivatives of 9-deoxy-9$\beta$-halogen-$PGF_{2\alpha}$ show mixed $PGD_2$/$PGE_2$-characters (Thierauch *et al.*, 1988) and AH6809 was initially reported to antagonize the actions of $PGE_2$ in smooth muscle preparations (Coleman *et al.*, 1985). The classification of prostaglandin receptors has been hampered by the lack of selective agonists and antagonists. Furthermore, in the case of DP-receptors the evaluation of their characteristics is compromised, as although human platelets are commonly used for studying DP-receptor-mediated effects, in addition to DP-receptors they also contain IP- and TP-receptors. Indeed, to date no preparation containing only DP-receptors has been available. Quite recently, Sugama *et al.* (1989) in our laboratory found that EBTr cells, a fibroblastic cell line derived from bovine embryonic trachea, specifically respond to $PGD_2$ among prostaglandins by elevating the intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) level. In the present study, we have examined the effect of ZK110841 and AH6809 upon the cyclic AMP formation in EBTr cells.

**Methods** EBTr cells (ATCC CCL 44) were grown to confluence ($6 \times 10^4$ cells/well) in 24-well culture plates. Cyclic AMP formation in EBTr cells was measured in the presence of 3-isobutyl-1-methylxanthine, a cyclic AMP phosphodiesterase inhibitor, by radioimmunoassay with an Amersham cyclic AMP assay kit as described previously (Sugama *et al.*, 1989). Apparent $pA_2$ values for AH6809 against $PGD_2$ and ZK118041 were determined by the method of Arunlakshana & Schild (1959). Authentic prostaglandins, ZK110841 and AH6809 were kindly provided by Ono Pharmaceuticals (Osaka, Japan), Schering (Berlin, F.R.G.) and Glaxo (Hertfordshire, U.K.), respectively.

**Results** As shown in Figure 1a, $PGD_2$ elevated the cyclic AMP level more than 200 fold over the basal level in EBTr cells ($0.690 \pm 0.03$ pmol/well, $n = 6$), and the level reached a plateau at 10 $\mu$M. While $PGE_1$, $PGE_2$, sodium $PGI_2$, and $PGF_{2\alpha}$ were much less potent than $PGD_2$, ZK110841 was equipotent with $PGD_2$ in elevating the cyclic AMP level in EBTr cells. The dose-response curves for $PGD_2$ and ZK110841 were almost the same and the half-maximally effective concentration was estimated to be 10–30 nM. AH6809 itself had little effect on the cyclic AMP formation at concentrations up to 300 $\mu$M, but AH6809 ($10^{-7}$–$10^{-4}$ M) produced parallel and concentration-dependent rightward shifts in the dose-response curve to $PGD_2$ or ZK110841. AH6809 at 300 $\mu$M almost completely antagonized the effect of $PGD_2$ or ZK110841 ($10^{-10}$–$10^{-5}$ M) on cyclic AMP formation. A Schild plot analysis was carried out based on these data, by which dose-ratios were calculated at a response intersecting all curves obtained with $10^{-7}$–$10^{-4}$ M AH6809 (representing 67% inhibition of cyclic AMP formation). As shown in Figure 1b, Schild plot regression lines revealed linear relationships, and the slopes were close to unity. The $pA_2$ values against $PGD_2$ and ZK110841 were 6.36 and 6.57, respectively. These results indicate that ZK110841 is a $PGD_2$ mimetic and that AH6809 behaves as a simple and competitive antagonist of $PGD_2$ and ZK110841 in EBTr cells.

**Discussion** While PGE receptors are detected in almost all tissues of the body, little information is available on DP-receptors except for human platelets and rat brain synaptic membranes (Robertson, 1986; Ito *et al.*, 1989). Therefore, human platelets have been almost exclusively employed for the study of structure-activity relationship of $PGD_2$ analogues. However, the interpretation of results obtained with platelets is often complicated and speculative, and conclusions are uncertain because $PGD_2$ can bind to different prostaglandin receptors at higher concentrations. $PGD_2$ reportedly could interact at the TX-receptor on guinea-pig platelets (Hamid-Bloomfield & Whittle, 1986) and $PGD_2$ showed a pro-aggregatory action on human platelets in the presence of BW A868C, a novel potent DP-receptor antagonist (Giles *et al.*, 1989). AH6809 employed here was also reported to antagonize the aggregatory effect of U-46619, a TX-receptor agonist, at higher concentrations. Since the slope of the Schild regression was less than unity in platelets, probably because AH6809 bound to plasma extensively, it was not concluded that the interaction of AH6809 and $PGD_2$ was competitive

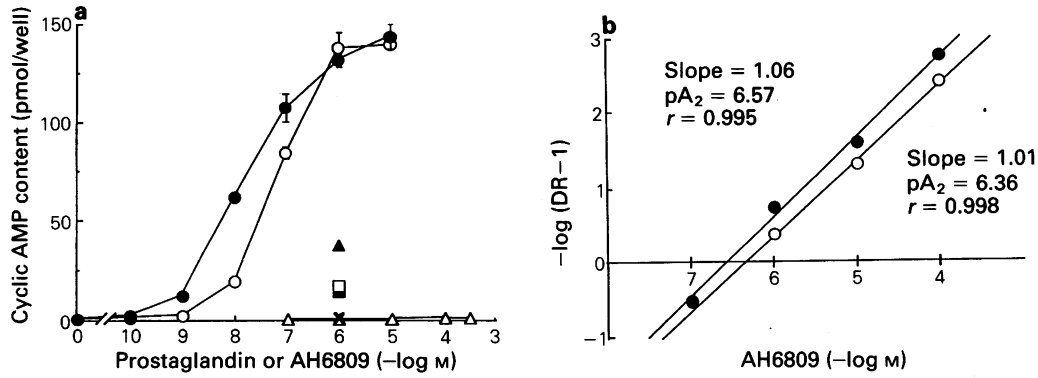


**Figure 1** (a) Dose-response curves for the effect of prostaglandin $D_2$ ($PGD_2$), ZK110841, and AH6809 on the cyclic AMP formation in EBTr cells. Confluent cells ($6 \times 10^4$ cells/well) were incubated at 37°C for 15 min with various concentrations of $PGD_2$ (○), ZK110841 (●) or AH6809 (△) or with 1 μM $PGE_1$ (▲), $PGE_2$ (□), $PGF_{2\alpha}$ (■) or sodium $PGI_2$ (×) in the presence of 0.5 mM 3-isobutyl-1-methylxanthine. The value shown by each point is the mean of three determinations with s.e.mean indicated by vertical bars. (b) Schild plots for the antagonism of responses to $PGD_2$ (○) and ZK110841 (●) by AH6809. DR, dose-ratio, i.e., the ratio of the concentration of the agonist required to give 67% reduction of the cyclic AMP level in the presence of a given concentration of AH6809 to that without AH6809. DR value for $PGD_2$ was 1 at $10^{-7}$ M AH6809. The best fit to the line and the correlation coefficient ($r$) were calculated by linear least squares regression.

(Keery & Lumley, 1988). The present study clearly demonstrates that AH6809 is a competitive antagonist for the action of $PGD_2$ in EBTr cells. The $pA_2$ value of 6.36 against $PGD_2$ is in agreement with the rough estimate obtained with resuspended platelets (Keery & Lumley, 1988). The present study also indicates that ZK110841 is a DP-receptor agonist. Because BW245C, a well-known potent DP-receptor agonist, is converted into a 1:1 mixture with its much less biologically acitve epimer in an aqueous solution (Brockwell *et al.*, 1981), the chemically stable ZK110841 may gain an advantage over BW245C when their long-term effects, such as on cell growth, are examined (Ito *et al.*, 1989). Since EBTr cells respond to $PGD_2$ specifically at concentrations as low as 10 nM, and are a cell line commercially available, they will provide a good system for the characterization of $PGD_2$ analogues and DP-receptors.

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