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Different responsiveness of prostaglandin D_2-sensitive systems to prostaglandin D_2 and its analogues

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1 Prostaglandin D_2 (PGD_2) and six PGD_2 analogues were used to classify responsiveness of several PGD_2-sensitive systems. The analogues used were 9β-PGD_2, 5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin (BW245C), 17-phenyl-18,19,20-trinor-PGD_2 (17-phenyl-PGD_2), PGD_2 amide, PGD_2 *N*-monomethylamide and 11-keto-15α-hydroxy-$\Delta^{5,9,12}$-prostenoic acid (9-deoxy-$\Delta^{9,12}$-PGD_2). The PGD_2-sensitive systems examined were human platelets, rat peritoneal mast cells, rabbit transverse stomach strip, guinea-pig tracheal ring chain and helical strip of the dog cerebral artery.

2 PGD_2, 9β-PGD_2 and BW245C inhibited the aggregation of human platelets, increased adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in rat mast cells and relaxed the rabbit stomach strip. The rank order of potency was BW245C > PGD_2 > 9β-PGD_2. PGD_2 amide and PGD_2 *N*-monomethylamide were inactive in the former two systems but elicited relaxant activity on the rabbit stomach strip. 17-Phenyl-PGD_2 was virtually inactive in the above three systems.

3 PGD_2 and 17-phenyl-PGD_2 contracted the guinea-pig tracheal ring chain and the helical strip of dog cerebral arteries with almost equal potency. 9β-PGD_2 and BW245C antagonized competitively the contractile action of PGD_2. PGD_2 amide and PGD_2 *N*-monomethylamide showed weak agonistic actions in the tracheal preparation.

4 9-Deoxy-$\Delta^{9,12}$-PGD_2, showing stronger growth inhibition than PGD_2 on cultured tumour cells, was inactive in human platelets, rat mast cells and guinea-pig trachea, and elicited contractile response in the rabbit stomach strip.

5 These results indicate the presence of three groups of PGD_2-sensitive systems that respond differently to PGD_2 and its analogues.

Introduction

Prostaglandin D_2 (PGD_2) is one of the prostaglandin which is ubiquitously formed and present in mammalian tissues (Nugteren & Hazelhof, 1973; Ikai *et al.*, 1984). Various actions of this prostaglandin have been reported, which include the anti-aggregatory activity on platelets of several species (Whittle *et al.*, 1978), the contraction of airway smooth muscle (Hamberg *et al.*, 1975; Schneider & Drazen, 1980), inflammatory effects (Flower *et al.*, 1976; Soter *et al.*, 1983), and the action on mast cells (Holgate *et al.*, 1980). PGD_2 also induces either contractions or relaxations in several vascular and gastrointestinal smooth muscle preparations (Horton & Jones, 1974; Whittle *et al.*, 1979; Toda, 1982a). Recently CNS effects of this prostaglandin such as hypothermia and induction of sleep have been reported in rodents (Ueno *et al.*, 1982a, b). In addition, inhibition of tumour cell proliferation by PGD_2 and its dehydration derivatives, 9-deoxy-Δ^9-PGD_2 and 9-deoxy-$\Delta^{9,12}$-PGD_2 has also been described (Fukushima *et al.*, 1982a,b; Kikawa *et al.*, 1984). However, whether these actions are mediated via a single and identical PGD_2 receptor or several PGD_2 receptors exist for each action has remained unclarified. It is also likely that PGD_2 acts on other prostaglandin or thromboxane receptors to elicit responses in some of the systems. A specific PGD_2 receptor was identified on human platelets by two groups using the radioligand receptor assay (Schafer *et al.*, 1979; Siegl *et al.*, 1979), and PGD_2 blocks aggregation of platelets possibly by stimulating adenylate

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cyclase via this receptor (Mills & Macfarlane, 1974; Feinstein *et al.*, 1983). In other tissues, the presence of PGD_2-specific receptor(s) has been suggested by the studies comparing its potency with those of other naturally occurring prostaglandins; these include arterial vascular bed of sheep (Jones, 1978), mast cells (Holgate *et al.*, 1980) and rabbit stomach (Whittle *et al.*, 1979). Recently, 5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin (BW245C) has been identified as an agonist for the PGD_2 receptor on blood platelets (Town *et al.*, 1983). In addition, a number of PGD_2 analogues such as 9β-PGD_2, 17-phenyl-PGD_2 and 9-deoxy-$\Delta^{9,12}$-PGD_2 have been synthesized chemically (Bundy *et al.*, 1983; Kikawa *et al.*, 1984).

The present study was undertaken to examine responses of several PGD_2-sensitive tissues and cells to these synthetic analogues and to classify PGD_2-sensitive systems into groups on the basis of their responsiveness. The results are discussed in relation to receptors involved and underlying mechanisms in these systems.

Methods

Experiments on isolated tissue preparations

The rabbit transverse stomach strip was prepared as described by Whittle *et al.* (1979). Japanese albino rabbits were killed by exsanguination, and a transverse segment (approximately 2 cm × 1 cm) of the middle region of the greater curvature was excised from the stomach. After washing, the gastric mucosa was peeled off and the remaining muscle layer was cut to form a strip (approximately 0.3 cm × 8 cm). The strip was suspended in a 20 ml organ bath containing Krebs-Henseleit solution (NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11 mmol l^{-1}). A combination of antagonists (atropine sulphate, 1.4×10^{-7} M; pyrilamine maleate, 2.5×10^{-7} M; methysergide maleate, 1×10^{-6} M; phentolamine mesylate, 2.6×10^{-7} M; propranolol hydrochloride, 6.8×10^{-6} M) were added to the bathing solution, which was gassed with 95% O_2:5% CO_2 at 37°C. Tension changes were recorded with an isometric transducer T7-8 (Toyo Baldwin, Tokyo, Japan) coupled to an amplifier type 7236 (San-ei Instrument, Tokyo). The strip was allowed to equilibrate for 60 to 90 min, during which period the bathing solution was replaced every 20 min. Relaxation responses to papaverine hydrochloride, 10^{-5} M, were first obtained. After washing the preparations several times and a further 40 min equilibration period, a cumulative concentration-effect curve was obtained for each PGD_2 analogue by increasing the concentration of a compound. At the end of each experiment, papaverine 10^{-5} M was added, to attain the standard relaxation. Then, the strip was washed several times and allowed to equilibrate for 30 to 40 min until the initial tension was restored. Relaxations induced by PGD_2 and PGD_2 analogues were expressed as relative values to those induced by papaverine.

The guinea-pig tracheal ring chain was prepared as follows. A guinea-pig weighing about 350g was killed by cervical dislocation, the trachea dissected out and cut transversely between the segments of cartilage. A chain was suspended in a 20 ml organ bath containing the Krebs solution and a mixture of antagonists described above. Indomethacin, when included, was added to the bathing solution at a final concentration of 3×10^{-6} M. After washing the preparation in the bathing solution several times and equilibration for 60 to 90 min, standard contractions to 30 mM KCl were first obtained and a cumulative concentration-effect curve was obtained for each compound. When antagonistic actions of BW245C and 9β-PGD_2 were tested, the preparations were incubated for 15 min with the test drugs and then a cumulative concentration-effect curve for PGD_2 was obtained. Contractions induced by PGD_2 and PGD_2 analogues were expressed as relative values to those induced by 30 mM KCl.

Experiments on helical strips of the dog cerebral (basilar and middle cerebral) artery were carried out by the procedures previously described (Toda, 1982a). The strips were fixed vertically in an organ bath containing the modified Ringer-Locke solution, which was maintained at 37 ± 0.3°C and gassed with a mixture of 95% O_2:5% CO_2. The resting tension was adjusted to 1.5g and isometric contractions were recorded via a force-displacement transducer (Nihon Koden Kogyo Co., Tokyo, Japan).

Measurement of adenosine 3':5'-cyclic monophosphate (cyclic AMP) content in rat peritoneal mast cells and rabbit stomach strip

A Wistar rat weighing 250 to 350g was killed by decapitation. After exsanguination, the skin over the abdomen was peeled off and 20 to 25 ml of calcium-free Krebs solution containing 0.1% gelatin and heparin, 0.3 mg ml^{-1}, was injected into the peritoneal cavity. The abdomen was massaged for 1 min and the peritoneal lavage was drained out; cell pellets were resuspended in calcium-free Dulbecco's phosphate-buffered saline (PBS) (Dulbecco & Vogt, 1954) in a concentration of 5×10^7 cells ml^{-1}. The cell suspension was mixed with the same volume of 40% Ficoll 400 in PBS, and 2 ml of the mixture was layered on 4 ml of 30% Ficoll-PBS solution. After centrifugation at 700 *g* for 25 min at 4°C, mast cells were recovered at the bottom of the 30% Ficoll layer. The cells were

washed once with PBS and finally suspended in Krebs solution oxygenated with 95% O_2:5% CO_2 at a concentration of 3×10^6 cells ml^{-1}. The yield of mast cells was about 5×10^5 cells per rat. Cyclic AMP accumulation in mast cells induced by PGD_2 and PGD_2 analogues was examined at 37°C in the oxygenated Krebs solution. The incubation mixture containing 1.5×10^5 mast cells and 1 mM 3-isobutyl-1-methylxanthine (IBMX) in 225 μl of Krebs was preincubated at 37°C for 15 min. Reaction was started by the addition of a compound in 25 μl of Krebs and carried out for 1 min. After the reaction was terminated by the addition of 50 μl of 50% trichloroacetic acid (TCA), the mixture was frozen and thawed, and centrifuged at 700 *g* for 5 min. The supernatant fluid was washed three times with 2 ml of water-saturated diethyl ether, and cyclic AMP in the aqueous solution was measured by radioimmunoassay as described by Honma *et al.* (1977).

Content of cyclic AMP in the rabbit stomach strip was determined as follows. Strips were suspended and incubated in an organ bath as described above. After the endogenous tension had stabilized, PGD_2 was added to the test tissues at a final concentration of 1.4 μM, and vehicle was added to the controls. After incubation for 3 min, the tissues were immediately frozen in liquid nitrogen. The frozen tissues were weighed, cut into small pieces and homogenized in 6% TCA by Polytron. Cyclic AMP content in the TCA extracts was measured as described. When the effect of IBMX was examined, strips were first incubated with 100 μM IBMX for 3 min and then subjected to PGD_2 treatment.

Aggregometry of human platelet-rich plasma

Blood was collected by venipuncture from healthy volunteers who had taken no drugs for at least two weeks, and immediately mixed with one tenth volume of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was recovered by centrifugation of citrated blood at 180 *g* for 15 min. PRP, 200 μl, was placed and stirred at 37°C in an aggregometer Hema Tracer 1 Model PAT-2M (Niko Bioscience, Tokyo). Platelet aggregation was initiated by the addition of 5 μl of ADP (200 μM). Compounds were dissolved in 50 mM Tris-HCl, pH 7.4, and added 1 min before the addition of ADP. Aggregation was followed as change in light transmission according to the method of Born (1962).

Drugs

PGD_2, 9β-PGD_2, PGD_2 amide, PGD_2 *N*-monomethylamide, 9-deoxy-$\Delta^{-9,12}$-PGD_2, PGE_2 and $PGF_{2\alpha}$ were kindly provided by Ono Pharmaceutical Co., Osaka, Japan. 17-Phenyl-PGD_2 and BW245C were gifts from the Upjohn Company and the Wellcome Research Laboratories, respectively. IBMX was obtained from Aldrich. Ficoll 400 was obtained from Pharmacia. Cyclic AMP radioimmunoassay kit was purchased from Yamasa Shoyu Co., Choshi, Japan. Detection limit of the assay was 10 fmol per tube. The intra- and interassay coefficients of variation were 5.5% and 10.5%, respectively. PGD_2 and PGD_2 analogues were stored in ethanol at a concentration of 1 mg ml^{-1} at − 20°C. For preparation of aqueous solutions, a small volume of stock ethanolic solution was evaporated under N_2 gas, and the dried residues were dissolved in Krebs solution. Purity of PGD_2 and PGD_2 analogues was examined by silica gel thin layer chromatography before use. The R_F values of PGD_2, 9β-PGD_2, PGD_2 amide, PGD_2 *N*-monomethylamide, 17-phenyl-PGD_2, 9-deoxy-$\Delta^{9,12}$-PGD_2 and BW245C were 0.19, 0.13, 0.08, 0.08, 0.19, 0.47 and 0.26, respectively, with a solvent system of benzene/ethyl acetate/acetic acid (50/50/2). After development, the compounds were visualized by exposure to iodine vapour. All the compounds gave a single spot upon this chromatography.

Statistical analyses

Results shown in the text and figures are expressed as mean ± s.e.mean. Statistical analyses were made by Tukey's method after one-way analysis of variance (Wallenstein *et al.*, 1980) (Figure 5) and by Student's *t* test.

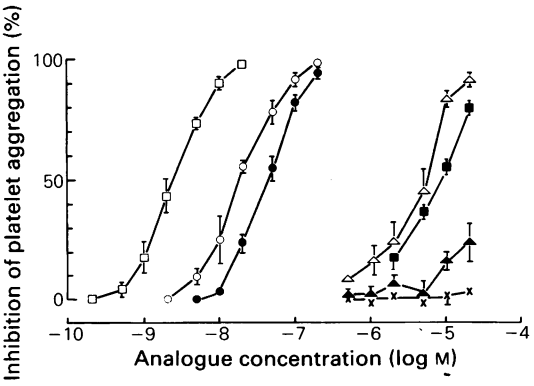

Figure 1 Concentration-inhibition curves for PGD_2 and PGD_2 analogues on ADP-induced platelet aggregation. (○) PGD_2 ($n = 5$); (●) 9β-PGD_2 ($n = 5$); (□) BW245C ($n = 5$); (■) 17-phenyl-PGD_2 ($n = 4$); (△) 9-deoxy-$\Delta^{9,12}$-PGD_2 ($n = 5$); (▲) PGD_2 amide ($n = 4$); (x) PGD_2 *N*-monomethylamide ($n = 4$). Mean values are shown. S.e.mean is shown by a vertical line when it exceeds the size of a symbol.

Results

Human platelets and rat peritoneal mast cells

The ability of PGD_2 and PGD_2 analogues to inhibit ADP-induced platelet aggregation was determined *in vitro* in human platelet-rich plasma. The concentration-effect curves are shown in Figure 1. PGD_2 inhibited platelet aggregation with an IC_{50} value of 18.6 ± 1.1 nM ($n = 7$). Among the analogues, BW245C and 9β-PGD_2 were potent agonists. BW245C was seven times more potent than PGD_2 (mean IC_{50}, 2.5 vs 18 nM). 9β-PGD_2 was about half as potent as PGD_2 (mean IC_{50}, 42 nM). 9-Deoxy-$\Delta^{9,12}$-PGD_2 and 17-phenyl-PGD_2 showed full agonist activities but they were more than two orders of magnitude weaker than PGD_2 and had mean IC_{50} values of 5.8 and 8.4 μM. Conversion of the carboxyl group to an amide almost completely abolished the anti-aggregatory activity. PGD_2 amide showed anti-aggregatory activity only above 10 μM, and PGD_2 *N*-monomethylamide was inactive up to 20 μM. Thus, the rank order of potency of PGD_2 analogues in inhibiting ADP-induced platelet aggregation was BW245C > PGD_2 > 9β-PGD_2 >> 9-deoxy-$\Delta^{9,12}$-PGD_2 > 17-phenyl-PGD_2 > PGD_2 amide >> PGD_2 *N*-monomethylamide.

The same rank order of potency among PGD_2 analogues was observed on their abilities to accumulate cyclic AMP in rat peritoneal mast cells. PGD_2 induced cyclic AMP accumulation in these cells as described (Holgate *et al.*, 1980), and in the presence of IBMX (1 mM) such accumulation reached plateau at 1 min after the addition of a prostaglandin. We, therefore, compared cyclic AMP levels in these cells 1 min after the addition of various concentrations of the analogues. Figure 2 represents the concentration-effect curves. PGD_2 stimulated cyclic AMP production with an EC_{50} value of 0.60 ± 0.12 μM, and the maximal level produced was 1988 ± 262 fmol per 10^5 cells ($n = 7$). BW245C and 9β-PGD_2 were again good agonists. BW245C was always more potent than PGD_2 in five experiments in which paired comparison of the activities was made; the equipotent molar ratio was 0.68. 9β-PGD_2 was a little less potent than PGD_2 with the molar ratio of 4.1. 9-Deoxy-$\Delta^{9,12}$-PGD_2 and 17-phenyl-PGD_2 were weak agonists. They induced cyclic AMP accumulation only above 10 μM; therefore, the full concentration-effect curves were not obtained. The equipotent molar ratios for 9-deoxy-$\Delta^{9,12}$-PGD_2 and 17-phenyl-PGD_2 were 190 and 590, respectively. PGD_2 amide and PGD_2 *N*-monomethylamide were completely inactive up to 100 μM in this system.

Rabbit transverse stomach strip

The concentration-effect curves of the rabbit stomach strip for PGD_2 and PGD_2 analogues are shown in Figure 3. PGD_2 relaxed this tissue in a concentration-dependent manner with the EC_{50} value of 0.10 ± 0.02 μM ($n = 5$). Contrary to the findings of Whittle *et al.* (1979), PGE_2 elicited contractile responses in this tissue dose-dependently at concentrations

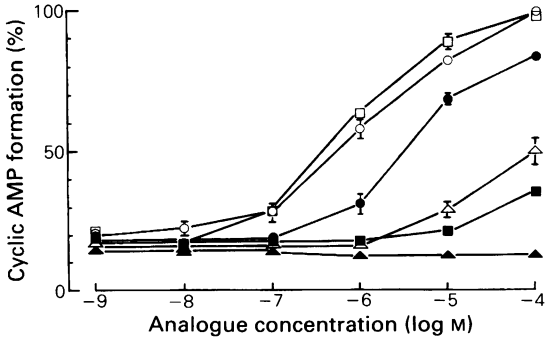

Figure 2 Concentration-dependent cyclic AMP accumulation in rat mast cells induced by PGD_2 and PGD_2 analogues. Cyclic AMP formation is expressed as a percentage of PGD_2- (100 μM) induced cyclic AMP formation in the same batches of mast cells (1988 ± 262 fmol per 10^5 cells, mean ± s.e.mean, $n = 7$). Mean values are shown. S.e.mean is shown by a vertical line when it exceeds the size of a symbol. Symbols are the same as in Figure 1. Results with PGD_2 *N*-monomethylamide are not shown, since these overlapped almost completely those of PGD_2 amide.

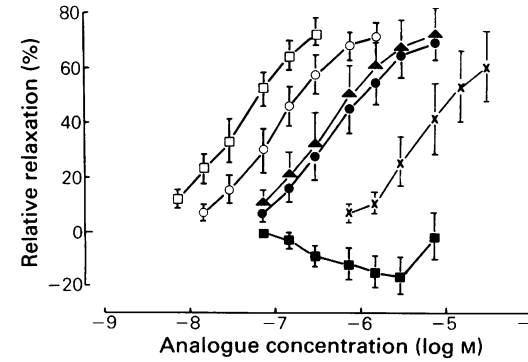

Figure 3 Concentration-relaxation curves for PGD_2 and PGD_2 analogues in rabbit stomach. Results are expressed as a percentage of the standard relaxation induced by 10^{-5} M papaverine. Mean values are shown. S.e.mean is shown by a vertical line when it exceeds the size of a symbol. Minus percentage denotes a contraction. Symbols are the same as in Figure 1. $n = 5$ for PGD_2, BW245C and 17-phenyl-PGD_2, $n = 4$ for 9β-PGD_2 and $n = 3$ for PGD_2 amide and PGD_2 *N*-monomethylamide.

above 10 nM ($n = 4$). $PGF_{2\alpha}$ also evoked dose-dependent contractions over 20 nM ($n = 2$) (data not shown). Responsiveness of this tissue to PGD_2 analogues was similar to that of human platelets and rat mast cells in that BW245C and 9β-PGD_2 were good agonists. BW245C was more potent than PGD_2; its equipotent molar ratio to PGD_2 was 0.35, and the mean EC_{50} value was 35 nM. 9β-PGD_2 also elicited full agonist activity but was about five times less potent than PGD_2. Thus, the rank order of potency is the same in three PGD_2-sensitive systems described so far; BW245C > PGD_2 > 9β-PGD_2.

Since the actions of PGD_2 on the former two systems i.e. human blood platelets and rat mast cells are both linked to elevation of the intracellular cyclic AMP (Whittle *et al.*, 1978; Holgate *et al.*, 1980), we investigated a possible link of PGD_2-induced relaxation of the rabbit stomach strip to the adenylate cyclase system. To examine whether PGD_2 actually increased the cyclic AMP level, we rapidly froze in liquid nitrogen the strips which had been maximally relaxed by PGD_2 1 μM and determined the cyclic AMP content of these strips and of control strips similarly treated but without PGD_2. In the absence of IBMX, the concentrations of cyclic AMP in the control and relaxed tissues were 0.26 ± 0.02 and 0.43 ± 0.05pmol-mg^{-1} wet tissue, respectively ($n = 4$ for each, $P < 0.05$), and in the presence of IBMX (10^{-4} M), the values were 0.79 ± 0.07 and 1.21 ± 0.03 pmol mg^{-1} wet tissue, respectively ($n = 4$ for each, $P < 0.01$). The involvement of cyclic AMP in the relaxation of this tissue was further supported by the observation that forskolin, an adenylate cyclase activator (Seamon *et al.*, 1981), added to the organ bath elicited relaxation of the tissue dose-dependently (data not shown).

The rabbit stomach strip was different from the other two systems in the following respects. First, PGD_2 amide and PGD_2 *N*-monomethylamide elicited a concentration-dependent relaxation. PGD_2 amide was as potent as 9β-PGD_2; its mean EC_{50} value was 0.70 μM. PGD_2 *N*-monomethylamide was much weaker than PGD_2 amide, and the mean EC_{50} was about 10 μM. Second, 17-phenyl-PGD_2 showed dual actions on this tissue, depending on the concentration used, and 9-deoxy-$\Delta^{9,12}$-PGD_2 elicited contractile rather than relaxant effects. 17-Phenyl-PGD_2 contracted the tissue dose-dependently at concentrations from 0.1 to 5 μM, and elicited dose-dependent relaxation over 5 μM (Figure 3). 9-Deoxy-$\Delta^{9,12}$-PGD_2 produced strong contractions of the tissue over 5 μM. Since the contractions at each concentration continued to increase for more than 30 min, a concentration-effect curve for this compound could not be obtained.

Guinea-pig trachea

Cumulative concentration-effect curves of the guinea-pig trachea for PGD_2 and PGD_2 analogues are shown in Figure 4. PGD_2 contracted this tissue; maximum contraction, 69.4 ± 5.1% of 30 mM KCl-induced contraction, was attained at 2.84 μM, and a 50% maximum response was elicited at a mean bath concentration of 0.24 ± 0.03 μM ($n = 5$). 17-Phenyl-PGD_2 was almost as potent as PGD_2. The mean EC_{50} value averaged 0.38 μM. PGD_2 amide and PGD_2 *N*-monomethylamide were also full agonists with respective mean EC_{50} values of 2.0 and 4.8 μM. 9-Deoxy-$\Delta^{9,12}$-PGD_2 elicited a weak agonist activity at concentrations higher than 2 μM. BW245C and 9β-PGD_2 did not produce any contractile response but relaxed the tissue at concentrations higher than 0.5 μM.

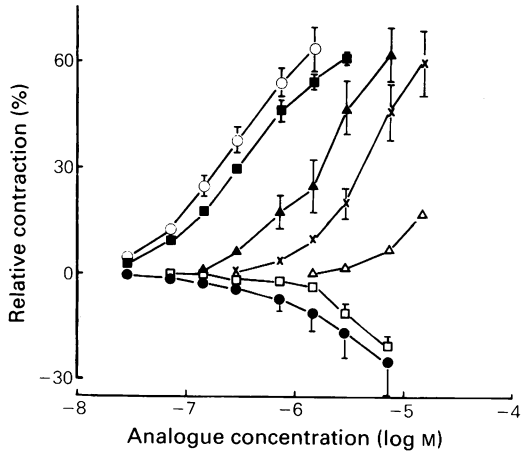

Figure 4 Concentration-contraction curves for PGD_2 and PGD_2 analogues in guinea-pig trachea. Contractions are expressed as a percentage of the KCl (30 mM)-induced maximal response in the same preparations. Mean values are shown. S.e.mean is shown by a vertical line when it exceeds the size of a symbol. Minus response denotes a relaxation. Symbols are the same as in Figure 1. $n = 5$ for PGD_2, $n = 4$ for 9β-PGD_2, BW245C, 17-phenyl-PGD_2, PGD_2 amide and PGD_2 *N*-monomethylamide, and $n = 3$ for 9-deoxy-$\Delta^{9,12}$-PGD_2.

In order to investigate antagonism of BW245C and 9β-PGD_2 to PGD_2-induced contraction, we treated the tracheal preparations with 3 μM indomethacin. The indomethacin treatment diminished the intrinsic tone of the preparations significantly. PGD_2 (2.8 μM) contracted the tissues maximally to 156 ± 8.3% of the KCl- (30 mM) induced contraction after treatment and the EC_{50} value was 0.44 ± 0.05 μM ($n = 7$). Neither BW245C nor 9β-PGD_2 evoked a response in the indomethacin-treated preparations up to concentrations of 28.4 μM. Under these conditions, we analysed the antagonism of the two compounds by obtaining cumulative concentration-response curves for PGD_2 in the presence of three (3.55, 7.1 and 14.2 μM) and four (3.55, 7.1, 14.2 and 28.4 μM) concentrations of 9β-

PGD_2 and BW245C, respectively. Both BW245C (Figure 5a) and 9β-PGD_2 (Figure 5b) caused a parallel shift to the right of the log concentration-effect curve for PGD_2. Plots of log (dose ratio − 1) versus log molar concentration were constructed. The slopes of the lines fitted by least square regression for BW245C

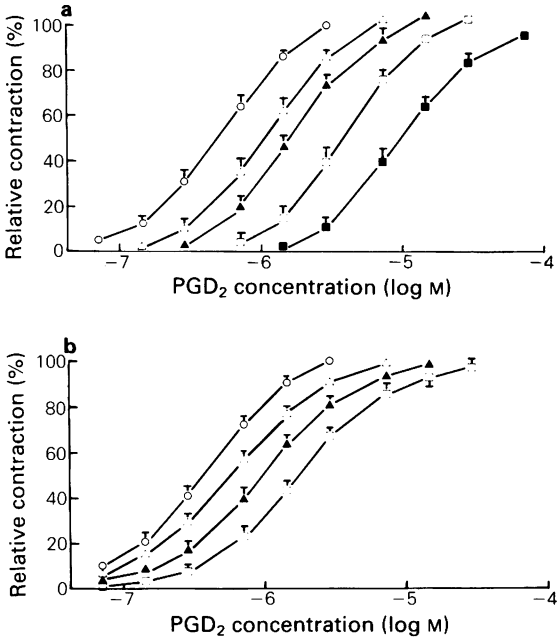

Figure 5 Concentration-contraction curves for PGD_2 in guinea-pig trachea in the absence and presence of BW245C (a) and 9β-PGD_2 (b). BW245C and 9β-PGD_2 were added at concentrations of 0 (○), 3.55 (△), 7.10 (▲), 14.2 (□) and 28.4 (■, in (a) only) μM. Contractions are expressed as a percentage of the contraction induced by PGD_2 (10^{-5} M) in the control. Experiments were carried out in the presence of indomethacin 3 μM. Pyrilamine maleate was omitted from the incubation solution. The mean ED_{50} values for PGD_2 in the control and presence of 3.55, 7.10, 14.2 and 28.4 μM BW245C were 0.49 ± 0.08, 1.07 ± 0.17, 1.63 ± 0.23, 3.75 ± 0.43, 9.30 ± 1.17 μM, respectively ($n = 4$). The F ratio obtained from the analysis of variance, 39.77, is greater than the $P = 0.005$ critical value. The values in preparations treated with 14.2 and 28.4 μM BW245C were significantly different from the control values by Tukey's method ($P < 0.01$, and $P < 0.01$, respectively). The mean ED_{50} values for PGD_2 in the control and in the presence of 3.55, 7.10 and 14.2 μM 9β-PGD_2 were 0.37 ± 0.05, 0.56 ± 0.06, 0.95 ± 0.12, 1.69 ± 0.26 μM ($n = 3$). The F ratio obtained from the analysis of variance, 18.77, is greater than $P = 0.005$ critical value. The values in 14.2 μM 9β-PGD_2-treated preparations were significantly different from the control by Tukey's method ($P < 0.01$).

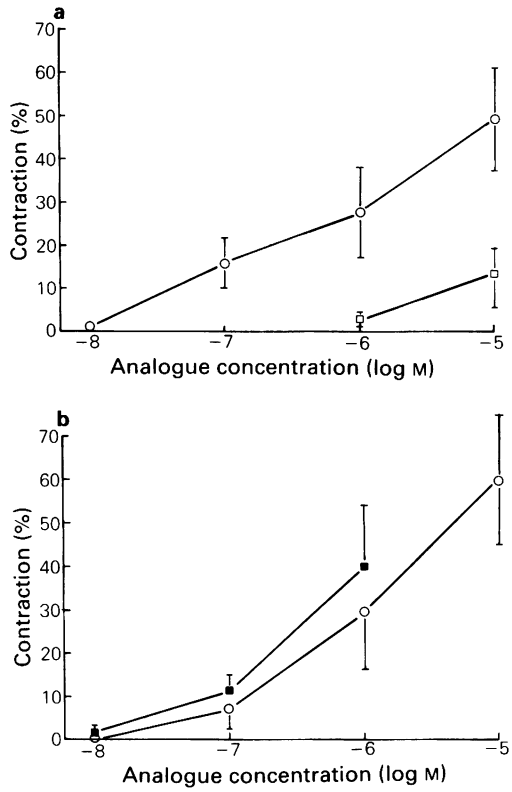

Figure 6 Concentration-contraction curves for PGD_2 and BW245C (a) or 17-phenyl-PGD_2 (b) in the dog cerebral artery strips. Results are expressed as a percentage of KCl- (30 mM) induced contraction in the same preparations. Symbols are the same as in Figure 1. $n = 7$ in (a) and $n = 5$ in (b).

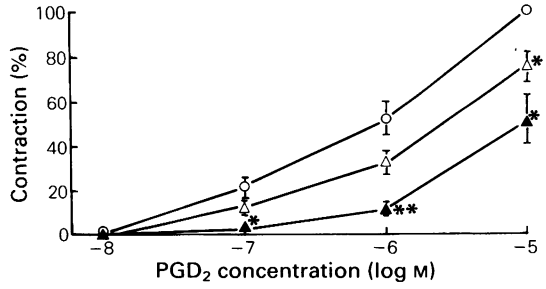

Figure 7 Modification by BW245C of the contractile response of the dog cerebral artery strips to PGD_2. Contractions induced by PGD_2 (10^{-5} M) in the control media were taken as 100%. Concentrations of BW245C were 0 (○), 1 (△) and 10 μM (▲). $n = 7$. *Significantly different from the control: $P < 0.01$; **$P < 0.001$.

and 9β-PGD_2 were 1.36 and 1.36, respectively. The pA_2 values were found to be 5.48 and 5.26, respectively. Effects of BW245C and 9β-PGD_2 were also examined on histamine-induced contraction of the tissue. These compounds reduced the histamine-induced contraction only slightly at the highest concentrations (28.4 μM BW245C and 14.2 μM 9β-PGD_2); average reductions were 14.8 ± 1.7% and 17.9 ± 0.9%, respectively.

Dog cerebral artery strips

The addition of PGD_2 in concentrations ranging from 10^{-8} to 10^{-5} M produced a dose-related contraction in helical strips of dog cerebral (basilar and middle cerebral) arteries. BW245C did not significantly alter the cerebroarterial tone in concentrations up to 10^{-6} M but contracted the arteries at 10^{-5} M (Figure 6a). 17-Phenyl PGD_2 (10^{-8} to 10^{-6} M) elicited a concentration-dependent contraction, which tended to be greater than that induced by PGD_2 (Figure 6b).

Pretreatment with BW245C (10^{-6} and 10^{-5} M) attenuated the contractile response of cerebral artery strips to PGD_2 (Figure 7), but did not affect contractions of the strips induced by 5-hydroxytryptamine (5-HT) (data not shown). BW245C in these concentrations relaxed the artery strips ($n = 3$) precontracted with PGD_2 (10^{-6} M) but in contrast, contracted the arteries ($n = 3$) precontracted with K^+ (12 to 15 mM).

Discussion

The results of the present study are summarized in Table 1. The five PGD_2-sensitive tissues and cells studied here can be classified into two groups. To one group belong human blood platelets, rat mast cells and rabbit stomach strip. They are characterized by (i) agonistic activities of two PGD_2 analogues i.e. BW245C and 9β-PGD_2; (ii) linking of PGD_2 action to activation of adenylate cyclase; and (iii) PGD_2 evoking responses in these systems as potently as or more potently than other natural prostaglandins (Whittle *et al.*, 1978; 1979; Holgate *et al.*, 1980; this study). A specific PGD_2 receptor has been identified by radioligand receptor assay on one of them, i.e. human platelets (Schafer *et al.*, 1979; Siegl *et al.*, 1979) and BW245C has been shown to act on that receptor (Town *et al.*, 1983). It, therefore, seems likely that responses of the other two tissues are mediated by the same type of PGD_2 receptor. However, there were some differences in responsiveness to other PGD_2 analogues between the former two systems and the rabbit stomach strip. One difference was that platelets and mast cells did not respond to PGD_2 amide and PGD_2 *N*-monomethylamide, whereas the rabbit stomach strip responded to them with relaxation. Therefore, PGD_2 receptors in human platelets, rat mast cells and in the rabbit stomach strip do not appear to share all the same properties. The other

Table 1 Activities of prostaglandin D_2 analogues on human platelets, rat peritoneal mast cells, isolated preparations of rabbit stomach, guinea-pig trachea and dog cerebral artery, and murine L-1210 leukaemia cells

	Equipotent molar ratios (standard agonist, PGD_2 = 1.0)					
	PGD_2	*BW245C*	*9β-PGD_2*	*17-phenyl PGD_2*	*PGD_2-amide*	*9-deoxy-$\Delta^{9,12}$-PGD_2*
Inhibition of human platelet aggregation	1.0 (18 nM)	0.14	1.9	>400	>2,000	>300
Cyclic AMP elevation in rat mast cells	1.0 (0.60 μM)	0.68	4.1	>500	>500	190
Relaxation of rabbit stomach strip	1.0 (0.10 μM)	0.35	4.8	Contraction	3.9	Contraction
Contraction of guinea-pig trachea	1.0 (0.24 μM)	No effect relaxation	No effect relaxation	1.4	8.5	150
Contraction of dog cerebral artery	1.0 (1.1 μM)	>100	NT	0.46	NT	NT
Growth inhibition of L-1210 cells	1.0 (6.9 μM)	No effect	NT	NT	NT	0.3

NT not tested.
Results with PGD_2 monomethylamide are omitted from the table.
Values on growth inhibition of L-1210 cells were obtained from Fukushima *et al.* (1982b) and Kikawa *et al.* (1984).

group of PGD_2-sensitive tissues consists of the guinea-pig trachea and dog cerebral artery. These tissues are characterized as follows; (i) PGD_2 and 17-phenyl-PGD_2 produce contractions with almost equal potency and (ii) BW245C and 9β-PGD_2 produce no response or only a slight contractions but antagonize the contractile action of PGD_2. It is evident that these contractions by PGD_2 are mediated via prostaglandin receptor(s) for the following two reasons. First, Toda (1982a, b) previously found that polyphlorethin and diphlorethin phosphates (prostaglandin antagonists) inhibit the contractile responses to PGD_2, $PGF_{2\alpha}$, PGE_2 and carbocyclic thromboxane A_2 ($cTXA_2$) of dog cerebral artery but not the response to other vasoconstrictors such as K^+ and 5-HT. Second, in the present study we have found that BW245C as well as 9β-PGD_2 effectively antagonizes the contractile responses of the tissues to PGD_2 but attenuates very little such responses to histamine or 5-HT. However, the above results does not necessarily imply that the receptor(s) involved is specific for PGD_2. Since $PGF_{2\alpha}$ and $cTXA_2$ constrict a helical strip of the dog cerebral artery more potently than PGD_2 (Toda, 1982a, b), it is likely that PGD_2 acts on the receptors of $PGF_{2\alpha}$ and/or thromboxane to elicit contraction. Jones *et al.* (1982) also postulated from their studies using EP-045 (a presumed thromboxane antagonist) that PGD_2 acts as a partial agonist on both contractile PGE_2 and thromboxane receptors to elicit contraction in the guinea-pig trachea. On the other hand, Feigen & Chapnick (1979) suggested separate contractile receptors for PGD_2 and $PGF_{2\alpha}$ in the canine mesenteric vascular bed and Jones (1978) suggested a potent contractile PGD_2 receptor in the sheep arterial vascular bed. Whether the responses we observed were mediated via interaction of PGD_2 on other prostaglandin and thromboxane receptors or via action on a PGD_2-specific contractile receptor(s) cannot be determined, unless highly selective antagonists against actions of each of the prostaglandin and thromboxane are introduced.

Another system which is sensitive to PGD_2 analogues but has not been analyzed in the present study is cultured tumour cells. Inhibition of cultured tumour cell growth by PGD_2 and its analogues has been reported by several groups on more than 20 tumour cell lines (Narumiya *et al.*, 1985). 9-Deoxy-$\Delta^{9,12}$-PGD_2 is the most potent PGD_2 analogue and has a growth inhibitory activity several times stronger than PGD_2 itself (Kikawa *et al.*, 1984). Interestingly, this compound is a poor agonist of PGD_2 in all of the four PGD_2-sensitive systems examined in this study. On the other hand, BW245C which showed significant actions on the rabbit stomach, human platelets and rat mast cells (Table 1) has been reported to lack such growth-inhibitory activity (Fukushima *et al.*, 1982b). Thus, the growth inhibition by PGD_2 analogues does not appear to be mediated via mechanisms mediating PGD_2 responses in the tissues and cells used in the present study, and cultured tumour cells may belong to another group of PGD_2-sensitive systems.

In conclusion, the present study suggests the presence of three group of PGD_2-sensitive systems which respond differently to synthetic PGD_2 analogues. These PGD_2 analogues can be used to modulate selectively these PGD_2-sensitive systems.

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