The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist

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1   BW A868C, a novel compound, behaved as a simple competitive antagonist in a human washed platelet aggregation assay. Anti-aggregatory concentration-effect curves to BW 245C were displaced in a parallel manner. The shifts accorded with a Schild plot slope of unity and a pK_B of 9.26.

2   Inhibition of platelet aggregation by prostaglandin D_2 (PGD_2) was antagonized with a similar potency, as were the relaxation effects of BW 245C and PGD_2 in the rabbit jugular vein. BW A868C can, therefore, be classified as a DP-receptor antagonist.

3   Actions of BW A868C at other prostaglandin receptors (IP, EP_1, EP_2, TP and FP) were excluded at concentrations up to 1,000 times higher than the DP-receptor affinity.

4   Analyses of BW 245C- and PGD_2-mediated effects were complicated by additional agonist receptor interactions which were revealed by BW A868C. In rabbit jugular vein a resistant phase of agonism was detectable, indicating that both agonists exerted effects through another receptor (possibly EP_2). Also, PGD_2, in addition to its anti-aggregatory effect on platelets, demonstrated a pro-aggregatory action in the presence of BW A868C.

5   The contractile effects of PGD_2 in guinea-pig tracheal strips were resistant to $10\,\mu\text{M}$ BW A868C indicating that they were not mediated through DP-receptors.

6   To our knowledge this is the first account of a well-classified competitive antagonist at the DP-receptor. Its potency and selectivity make it an important new tool in prostanoid receptor classification and identification.

Introduction

The classification of prostaglandin receptors has in general been hampered by the lack of selective agonists and antagonists. It has been proposed (Kennedy *et al.*, 1982; Coleman *et al.*, 1984) that distinct receptors types exist for each of the naturally occurring prostaglandins: a thromboxane, TP-receptor; a prostacyclin, IP-receptor; a prostaglandin D_2, DP-receptor; a prostaglandin $F_{2\alpha}$, FP-receptor and at least two prostaglandin E_2 receptors, EP_1 and EP_2; more recently the same workers have invoked the presence of a third E type receptor, EP_3 (Coleman *et al.*, 1987b,c). This scheme was based originally on the discontinuity of potency orders of the naturally occurring agonists in different tissues. However, as its proponents explain (Coleman *et al.*, 1984), identi-

fication of a particular receptor type on this basis is compromised by the multiplicity of actions that these prostaglandins exhibit. Although this classification scheme was based in part on antagonist information, SC-19220 and AH19437 were claimed to antagonize selectively EP_1- and thromboxane-receptors respectively, selective antagonists for the majority of the putative receptor types remained to be discovered.

In the case of the claimed DP-receptor, several antagonists have been reported, among them NO164 (MacIntyre & Gordon, 1977), diphloretin phosphate (Westwick & Webb, 1978), desacetyl-1-nantradol (Horne, 1984), and AH6809 (Keery & Lumley, 1985), but none of them possess the selectivity or potency necessary for use in the quantitative classification of receptors.

In this paper, we describe the pharmacological actions of BW A868C, (3-benzyl-5-(6-carboxyhexyl)-
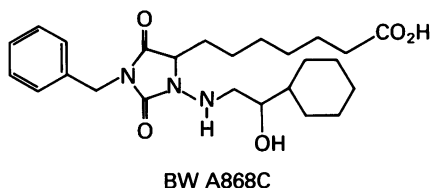
Figure 1 Chemical structure of BW A868C: The synthesis and chemical properties of BW A868C will be described in a separate publication.

1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin) a novel, 3-benzyl substituted hydantoin (Figure 1), in human washed platelets and rabbit vasculature. We demonstrate that this compound behaves as a highly potent, simple competitive antagonist at the DP-receptor in platelets and vasculature with negligible actions at the other claimed prostaglandin receptors.

Methods

Washed human platelets

Washed platelet suspensions were used rather than platelet-rich plasma in order to avoid possible errors in the analysis of drug action caused by plasma-protein binding. Suspensions were prepared according to a modified version of the method of Vargas *et al.* (1982).

Blood was drawn from healthy male and female volunteers who had not taken any drugs known to affect platelet function for at least two weeeks. Blood was collected from the antecubital vein into (3.15% w/v) sodium citrate in proportion 9:1 v/v. The blood was centrifuged for 20 min at 225 g and the resulting supernatant platelet-rich plasma was collected. PGI_2 (0.3 $\mu g\,ml^{-1}$) was added before further centrigugation at 760 g for 10 min. The platelet-poor plasma supernatant was then discarded and the platelet pellet gently resuspended in calcium-free Tyrode buffer pH 7.4 of the following composition (mM): NaCl 136.89, $NaHCO_3$ 11.90, KCl 2.68, NaH_2PO_4 0.42, $MgCl_2$ 1.05, glucose 5.55, warmed to 37°C. PGI_2 (0.3 $\mu g\,ml^{-1}$) was added again and a final centrifugation, 400 g for 10 min, was performed. The Tyrode buffer was then removed and the platelets resuspended in a volume of fresh buffer sufficient to give a platelet concentration of 2 × $10^8\,ml^{-1}$.

The suspension was stored at 4°C for 2 h. Before use the following additions were made: Ca^{2+} 1mM, indomethacin 5 $\mu g\,ml^{-1}$, fibrinogen 400 $\mu g\,ml^{-1}$. Indomethacin was used in all assays to prevent production of cyclo-oxygenase products which could compromise analysis of exogenous drug effects. Aggregation responses were measured in 0.5 ml ali-

quots of the suspension maintained at 37°C and stirred with a metal bar at 900 r.p.m. in 300 BD-S Payton dual channel aggregometers and recorded on Gould BS 272 pen recorders.

Rabbit jugular veins

External jugular veins were removed from male New Zealand White rabbits (2.4–3.0 kg) killed by injection of phenobarbitone sodium (Sagatal, 60 $mg\,kg^{-1}$) into a marginal ear vein. The vessels were cannulated, which caused the removal of the endothelium, and then cleared of adhering tissue.

Three ring segments, approximately 5 mm wide, were obtained from each vein. Each ring was suspended between two wire hooks, one attached to a Grass FT03C force displacement transducer and the other to a stationary support in a 20 ml organ bath containing Krebs solution pH 7.4 of the following composition (mM): NaCl 118.41, $NaHCO_3$ 25.00, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, glucose 11.10, $CaCl_2$ 2.50 and indomethacin 1 $\mu g\,ml^{-1}$. This was maintained at 37°C and continuously gassed with 95% O_2:5% CO_2. Tissue responses were measured as changes in isometric force and recorded on Gould BS 272 pen recorders.

Guinea-pig trachea

Male Dunkin Hartley guinea-pigs (400 g) were killed by a blow to the head and the trachea was removed and cleared of fat and connective tissue. The trachea was cut open longitudinally to expose the trachealis muscle. Transverse strips containing 3 cartilage rings were cut and suspended by cotton thread in the organ bath, as described above. Responses were measured as changes in isometric force.

Experimental protocols

Human washed platelets: In most experiments prostaglandin receptor agonists were incubated in the platelet suspension for 6 min before addition of ADP (50 μM). The reproducibility of the ADP-induced aggregation response was checked at intervals throughout the experiment. When an antagonist was used it was added 2 min before the agonist. Responses to agonists were expressed as percentage inhibitions of the standard ADP-induced aggregation. The total volume of solutions added to the suspension was always less than 5% of the platelet suspension volume.

In experiments with PGD_2 the order of addition of agents was reversed. A steady level of aggregation was induced by ADP (50 μM) before addition of PGD_2. Antagonist was incubated in the suspension for 5 min before ADP was added.

Rabbit jugular vein: These experiments followed a paired curve design. This was necessary due to substantial inter-tissue variation in the position of prostaglandin receptor agonist concentration-effect curves. Also the thromboxane antagonist BM 13.177 (Patscheke *et al.*, 1984) was routinely used in order to inhibit thromboxane receptor-mediated effects which occurred at higher concentrations of the agonists. Data illustrating the effect of BM 13.177 on responses to PGD_2 are shown in Figure 2, together with the results of an experiment indicating that BM 13.177 acts as a competitive antagonist of U46619, the stable thromboxane-mimetic (Coleman *et al.*, 1980a), in the jugular vein assay, demonstrating the same affinity as described previously for its effects in rabbit aorta and human platelets (Patscheke *et al.*, 1984).

Initially, jugular vein ring segments were subjected to a force of 0.75 g then allowed to stabilize for a period of 60 min during which time preparations were washed three times. BM 13.177 (30 μM) was incubated for 60 min, then histamine (1 μM) was added in order to produce tone. After establishing a stable contraction, relaxation concentration-effect curves were obtained using prostaglandin receptor agonists. Responses were expressed as percentage relaxations of the histamine-induced force. Preparations were then washed thoroughly and allowed to re-stabilise. After re-introduction of BM 13.177, antagonists, when used, were incubated for 60 min. The preparations were contracted again with histamine (1 μM) and a second prostaglandin agonist concentration-effect curve was obtained.

A separate experiment established that repeated histamine concentration-effect curves were superimposable (data not shown).
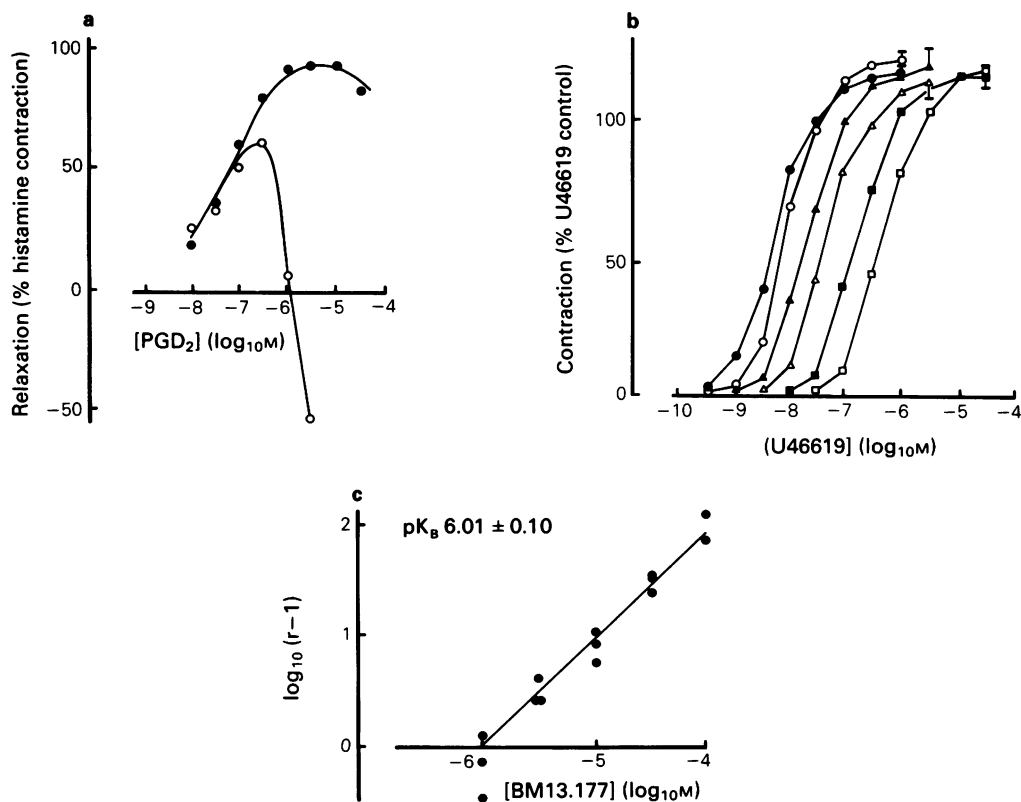

Figure 2   (a) Representative prostaglandin D_2 (PGD_2) concentration-effect curves in rabbit jugular vein in the absence ($\bigcirc$) and presence ($\bullet$) of 30 μM, BM 13.177. (b) Antagonism of U46619 effects in rabbit jugular vein by BM 13.177 at the following concentrations (number of replicate curves in parentheses): zero ($\bullet$), (3); 1 μM ($\bigcirc$), (3); 3 μM ($\blacktriangle$), (3); 10 μM ($\triangle$), (3); 30 μM ($\blacksquare$), (3); 100 μM ($\square$), (2). Error bars denote standard errors. (c) Schild plot for the antagonism of U46619 by BM 13.177. Analysis of [A_{50}] values by Equation (2) showed that n, the slope parameter was not significantly different from unity, and the line drawn through the data set was obtained by fitting the data to Equation 2 with n constrained to unity.

The experiment which demonstrated the effects of BM 13.177 on U46619 contractions was conducted by a one curve per preparation design. U46619 was used to provide a reference contraction then, following thorough washing BM 13.177 or vehicle was added to the organ baths for 60 min before obtaining cumulative U46619 concentration-effect curves.

Guinea-pig trachea: An initial force of 1 g was applied to the tissues. After stabilization the tissues were challenged with 10 μM histamaine to establish viability and to provide a reference contraction that was used to normalise subsequent agonist responses. After thorough washout of histamine, BW A868C or vehicle was added to the organ bath 60 min before cumulative addition of PGD$_2$. A single concentration-effect curve was obtained from each preparation. Contractile responses were expressed as a percentage of the histamine control response.

Rat fundic strip and guinea-pig longitudinal ileum muscle These tissues were used for exclusion assays and were set up in a similar manner to that already described for rabbit jugular vein. In both cases atropine sulphate 0.4 μM was added to the bathing medium. Single cumulative concentration-effect curves were performed on each tissue.

Analysis of data

Curve fitting Each concentration-effect curve data set was fitted to a logistic function of the form:

$$E = \frac{\alpha[A]^m}{[A_{50}]^m + [A]^m} \qquad (1)$$

in which α, $[A_{50}]$ and m are the asymptote, location and slope parameters respectively. Location parameters were actually estimated as $-\log_{10}[A_{50}]$.

Analysis of antagonism: single curves designs: A one-way analysis of variance tested for treatment effects on the computed estimates of α and m. In the platelet study inhibition of aggregation greater than 100% of the ADP aggregation cannot be obtained therefore analysis of variance on the asymptote (α) was inappropriate. Tests for parallelism relied on slope analysis in this case. If the antagonist did not significantly modify these parameters then computed $\log_{10}[A_{50}]$ values were fitted to the linear form of the Schild equation used previously (Black *et al.*, 1985a,b):

$$\log_{10}[A_{50}] = \log_{10}[A_{50}^c] + \log_{10}(1 + [B]^n/K_B) \qquad (2)$$

where $[A_{50}^c]$ is a control A_{50} value, $[B]$ is the concentration of antagonist, K_B is its equilibrium disso-

ciation constant and n is its order of reaction with the receptor. If n was not significantly different from unity, indicating simple competition, it was constrained to this value in order to obtain an estimate of K_B. The dissociation constant was actually estimated as pK_B ($-\log_{10} K_B$). In this experimental design a complete study of antagonism was conducted using each suspension or tissue and so equation (2) was used to provide an estimate of pK_B in each case. The average of these values is quoted in the text together with the standard error.

Paired curve design: One-way analyses of variance were performed on the differences between computed α and m estimates for each pair of concentration-effect curves. Further analysis of curve displacements was inappropriate in this case as will be seen, although a concentration-ratio was calculated for each pair and used for display in Schild plot form.

Computer-simulation of two-receptor systems: In order to interpret the data from one of the experiments it was necessary to consider the action of a selective antagonist on a one agonist: two-receptor system. The model of Furchgott (1981) was used to simulate the expectations of such an interaction. This model is essentially an extension of the traditional model for a one-receptor system (Stephenson, 1956; Furchgott, 1966) in which pharmacological effect is assumed to be a function of stimulus. In the two-receptor case the combined stimulus is imparted by the interaction of the agonist at each receptor, thus:

$$S = S_1 + S_2 = e_1 p_1 + e_2 p_2 \qquad (3)$$

where e_1 and e_2 are the efficacies of the agonist at each receptor and p_1 and p_2 are the respective fractional occupancies.

Furchgott assumed that the relation between S and pharmacological effect can be described by:

$$\frac{E}{E_m} = \frac{S^n}{1 + S^n} = \frac{(e_1 p_1 + e_2 p_2)^n}{1 + (e_1 p_1 + e_2 p_2)^n} \qquad (4)$$

When a competitive antagonist able to interact with each receptor is present the occupancies p_1 and p_2 are defined by:

$$p_1 = \frac{[A]}{K_{A1}(1 + [B]/K_{B1}) + [A]} \qquad (5)$$

$$p_2 = \frac{[A]}{K_{A2}(1 + [B]/K_{B2}) + [A]} \qquad (6)$$

Equations (4), (5) and (6) were used to generate theoretical agonist concentration-effect curves in the presence and absence of competitive antagonists. Concentration-ratios were measured between the curves in order to produce theoretical Schild plot profiles.

Drugs

PGD$_2$, PGE$_1$, PGE$_2$ and U46619 (9,11-dideoxy-9α,11α-methanoepoxy-PGF$_{2\alpha}$) were purchased from Cayman Chemical, Denver, Colorado, U.S.A. Carbacyclin ((5E)-6a-carbaprostaglandin I$_2$), epoprostenol sodium, Grade 1 (PGI$_2$), BW245C (5-(6-carboxyhexyl) - 1 - (3 - cyclohexyl - 3 - hydroxypropyl-hydantoin), BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl) - 1 - (2 - cyclohexyl - 2 - hydroxyethylamino)-hydantoin) were all obtained from Wellcome Research Laboratories. Fluprostenol sodium was a gift from Coopers Animals Health Ltd., Berkhamsted, Herts. AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) was a gift from Glaxo Group Research Ltd., Ware, Herts. BM13.177 (4-[2-(benzenesulphonamido)-ethyl]-phenoxyacetic acid, Boehringer Mannheim GmbH) was prepared at the Medicinal Chemical Laboratories, Wellcome Research Laboratories. Indomethacin, adenosine-5-diphosphate, histamine-HCl and atropine sulphate were all purchased from Sigma Chemical Co. Ltd. Phenoxybenzamine hydrochloride was obtained from Smith, Kline and French, Welwyn Garden City, Herts, and fibrinogen was obtained from Kabi Vitrum, Stockholm, Sweden.

Results

Antagonism of PGD$_2$ by BW A868C in human washed platelets

Figure 3 shows the effects of BW A868C on PGD$_2$-mediated responses in human washed platelets. This compound had no intrinsic effect on the aggregations. The results illustrated are from one platelet suspension; they typify those of two other replicate experiments. Although BW A868C evidently antagonized the deaggregatory effects of PGD$_2$ in a concentration-dependent way, the 'bell-shaped' nature of the PGD$_2$ concentration-effects curve precluded quantitative analysis of the antagonism.

Antagonism of BW245C by BW A868C in human washed platelets

Unlike PGD$_2$, BW245C was shown in preliminary experiments to be devoid of pro-aggregatory effects. Therefore this putative DP-receptor agonist (Town *et al.*, 1983; Whittle *et al.*, 1983) was investigated in the presence of BW A868C. Figure 4 shows the results of this study. BW A868C displaced BW245C concentration-effect curves in a manner consistent with simple competitive antagonism. No significant differences between curve gradients were detected by analyses of variance. Analysis of com-
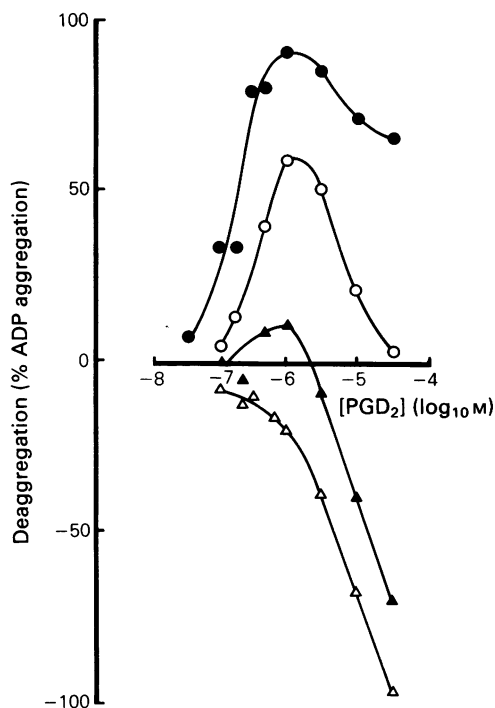

Figure 3 Representative effects of prostaglandin D$_2$ (PGD$_2$) in the presence of increasing concentrations of BW A868C; control (●); 10 nM (○); 30 nM (▲); 100 nM (△) PGD$_2$ was added to human washed platelet suspensions just after maximum aggregation to ADP was obtained. On the ordinate scale, positive values indicate deaggregation, negative values indicate further aggregation.

puted [A$_{50}$] values by equation (2) indicated that n, the Schild slope parameter, was 1.25 ± 0.04 (s.e. 3 d.f.) which was significantly greater than unity. The broken line in Figure 4b corresponds to this fit.

We considered the possibility that this departure from simple competitive behaviour was the consequence of inadequate antagonist equilibration (Kenakin, 1980). In order to examine this in more detail the inhibition of aggregation achieved after incubating BW A868C and BW245C for differing periods of time (1–10 min) before the addition of ADP was measured. Time-course profiles were constructed in the absence of antagonist or in the presence of 10 nM or 300 nM BW A868C. A steady state response to BW245C in the absence of antagonist, or in the presence of 300 nM BW A868C was achieved within 6 min. However, in the presence of 10 nM BW A868C there was, with increasing incubation time, a gradual decrease in the degree of agonism. A steady state response appeared to have been achieved after 10 min incubation.
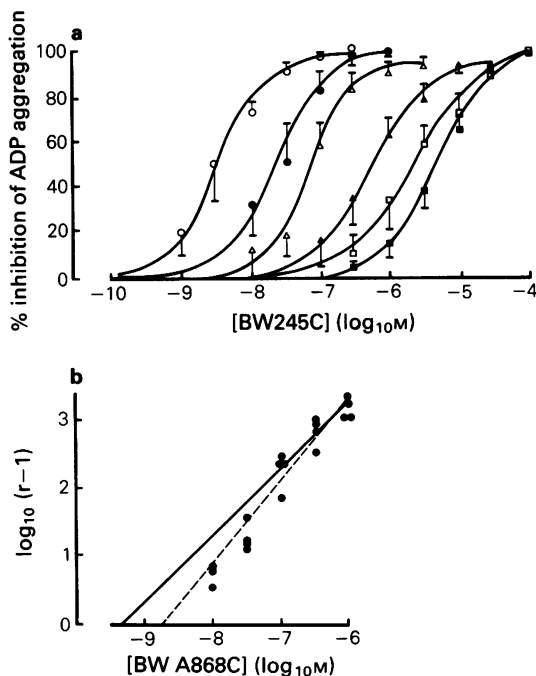
Figure 4 (a) Antagonistic effects of BW A868C on BW245C concentration-effect curves in human washed platelet suspension at the following concentrations (replicate number of curves in parentheses): zero (O), (4); 10 nM (●), (3); 30 nM (△), (4); 100 nM (▲), (4); 300 nM (□), (4); 1 μM (■), (4). Error bars represent standard errors. The lines drawn through the data are the results of logistic curve fitting using Equation (1). (b) Schild plot for the antagonism of BW245C by BW A868C. [A_{50}] values were analysed using Equation (2). The broken line drawn through the data was calculated using all the data, as described in the Methods section, and has a slope of 1.25 ± 0.04 (s.e. 3 d.f.). The solid line has a slope of unity and was calculated, omitting data points at 10 nM and 30 nM BW A868C, as explained in the results section.

The antagonist study was, therefore, repeated (in triplicate) using an extended period of agonist and antagonist co-incubation. This period was limited (in total) to 10 min for reasons of experimental feasibility. Under these conditions the estimate of n was still greater than unity, 1.14 ± 0.05, although the departure was less than previously, a result which would be expected if an equilibration problem existed.

However, under both conditions analysis of the higher concentration-ratio data (obtained at 0.1, 0.3 and 1 μM BW A868C, see Figure 4b) provided estimates of n of 0.89 ± 0.10 (6 min incubation) and 1.09 ± 0.08 (10 min incubation), neither of which is significantly different from unity. Again, these results

are consistent with the above explanation which, if accepted, means that the pK_B estimates obtained from the higher concentration-ratios with n constrained to unity would be the most reliable (Kenakin, 1980). When analysed in this way, the data shown in Figure 4 provided a pK_B estimate of 9.26 ± 0.10 (s.e., 3 d.f.), corresponding to the continuous line in Figure 4b.

An equivalent experiment was carried out with platelet-rich plasma (obtained as described in the Methods section). Although broadly parallel rightward shift was obtained, the curve displacements accorded with a much lower affinity for BW A868C, and the resulting Schild plot was curvilinear. It is likely that these results are a result of antagonist loss due to binding to plasma proteins.

Antagonism of BW245C by BW A868C in rabbit jugular vein

The effects of BW A868C on BW245C concentration-effect curves are shown in Figure 5a. The antagonist had no intrinsic effects on the tone of preparations. The control curve shown is the average of all the first curves obtained and the antagonist treatment curves are average second curves. Paired control curves were also obtained during the course of the experiment (8 replicates) and found to be superimposable. Although low concentrations (10 nM and 30 nM) of the antagonist caused displacement of the curves, no further rightward shift could be achieved at higher concentrations. As the curves shown in Figure 5a consist of average data they do not accurately represent paired information. The average paired concentration-ratios, which reflect the antagonistic effects of BW A868C more accurately, are displayed in Figure 5b.

The profile of the Schild plot accords with expectations of a one-agonist: two-receptor system (Furchgott, 1981) in which (i) the agonist can elicit full effect through both receptors, (ii) the two agonist-receptor processes are separated in potency terms by approximately an order of magnitude, (iii) the antagonist acts exclusively on the more potent process. Equations (4), (5) and (6) (after Furchgott, 1981) were used to simulate the present data assuming that BW245C acts non-selectively and BW A868C acts exclusively at the DP-receptor. Figure 5b shows that the data were fitted adequately assessing a pK_B for BW A868C of 8.7 (the other parameters used are given in the figure legend).

The presence of a second phase of agonism, resistant to antagonism by BW A868C was also detected in the case of PGD_2 in the jugular vein. The nature of this secondary action was investigated further by examining BW245C and PGD_2 concentration-effect curves in the presence of AH6809 (Coleman *et al.*,
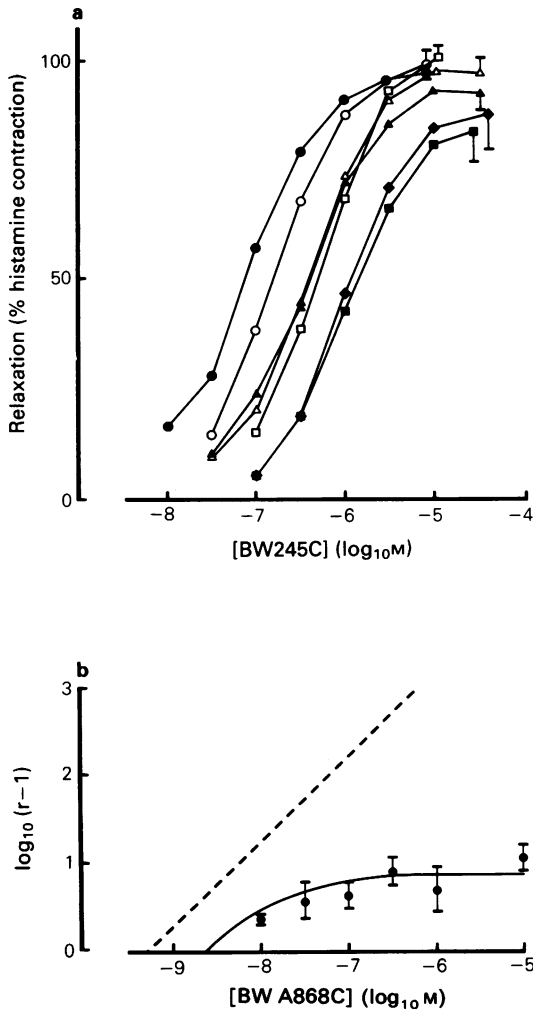
Figure 5 (a) Antagonism by BW A868C of BW245C effects in rabbit isolated jugular vein rings precon-stricted with $1\,\mu M$ histamine. Concentration-effect curves were obtained in a paired design using the fol-lowing concentrations of BW A868C: zero (●), 10 nM (○); 30 nM (▲); 100 nM (△); 300 nM (■); $1\,\mu M$ (□); $10\,\mu M$ (◆). The diagram shows the mean of the 24 repli-cate control curves obtained by this design and the average of four curves obtained at each BW A868C concentration. (b) Schild plot for the antagonism by BW A868C of BW245C in rabbit jugular vein. The paired curve experimental design allowed a concentra-tion ratio (r) to be calculated for each pair of curves. The data points are the mean for four experiments; s.e. shown by vertical bars. The line through the data was generated from a computer-simulation of a one agonist: two-receptor system, as described in the Methods section. The dashed line indicates the position of the Schild plot obtained from the equivalent platelet experi-ment (Figure 4b).

1985b). This putative EP_1-receptor antagonist, at 10^{-5} M, had no effect on the BW A868C resistant phase, in each case, the latter being applied at $1\,\mu M$ in order to expose the secondary action. This experi-ment was performed in triplicate using BW245C, and on one occasion using PGD_2 (data not shown).

The effect of BW A868C on responses to PGD_2 in guinea-pig trachea

PGD_2 caused a dose-dependent contraction of iso-lated segments of guinea-pig trachea, although at high concentrations ($10\,\mu M$ or greater) a dose-dependent relaxation occurred, which resulted in a bell-shaped concentration-effect curve. This curve was unaffected by the presence of $10\,\mu M$ BW A868C (Figure 6) and BW A868C alone had no effect on basal tone.

Selectivity of BW A868C

Concentration-effect curves for PGI_2, carbacyclin (a stable PGI_2 analogue), PGE_1 and U46619 were unaffected by BW A868C up to $1\,\mu M$ (in platelets) and $10\,\mu M$ (in jugular veins). Also, basal tone and PGE_2 concentration-effect curves in guinea-pig
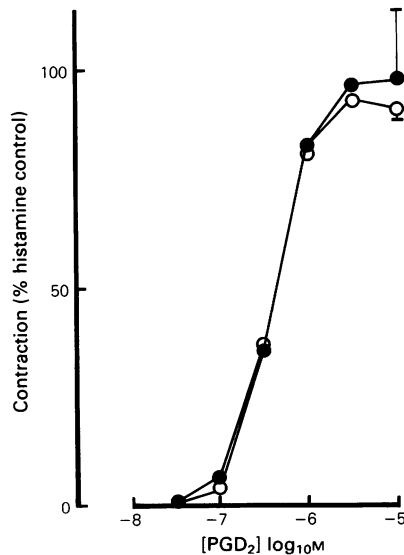

Figure 6 Prostaglandin D_2 (PGD_2) concentration-effect curves in isolated segments of guinea-pig trachea in the absence (○) and presence of BW A868C $10\,\mu M$ (●). Each point is the mean of three experiments and bars represent standard errors.

ileum, and fluprostenol concentration-effect curves in rat stomach fundic strips were unaffected by $10 \mu M$ BW A868C, as were PGE_2-mediated relaxations of cat trachea pre-contracted with $3 \mu M$ bethanechol. These experiments were performed in triplicate.

Discussion

In this paper we have shown that BW A868C acts as a highly potent antagonist at the DP-receptor in human washed platelets. The DP-designation relies partly on the prior designation of BW245C as an agonist at this receptor. Binding studies (Town *et al.*, 1983) as well as agonist potency order information (Whittle *et al.*, 1983) indicate that BW245C and PGD_2 interact with the same receptor on platelets. Also, in the present experiments, BW A868C was shown to antagonize PGD_2-induced deaggregatory responses, albeit in a complex way. Together these results support the classification of BW A868C.

Evidently, this antagonist is extremely selective for DP-receptors. In the platelet assay neither IP-receptor-mediated inhibition of aggregation (by PGI_2, PGE_1 and carbacyclin) nor TP-receptor-mediated aggregation (by U46619) was affected by concentrations of BW A868C 10^3 times higher than those at which DP-receptor antagonism is demonstrable. Vehicle (ethanol) effects prevented use of BW A868C at higher concentrations. In the jugular vein, IP-receptor-mediated relaxations and TP-receptor-mediated constrictions were unaffected by yet higher concentrations, in this case 10^4 times greater than the DP-receptor K_B. The same concentration of BW A868C failed to alter the contractile effects of PGE_2 in the guniea-pig ileum, or relaxant effects in the cat trachea, which are claimed to be mediated by the EP_1- and EP_2-receptors respectively (Kennedy *et al.*, 1982; Coleman *et al.*, 1984; 1987a). The exposure of a BW A868C resistant relaxation response in the rabbit jugular vein assay, which we presume to be mediated by the putative EP_2-receptor (this is discussed below), in itself provides evidence for selectivity of the compound over this receptor type. As shown by Figure 5, concentrations of BW A868C 10^4 times higher than the apparent DP-receptor K_B again failed to displace this relaxation effect. The contractile effects of the putative selective FP-receptor agonist fluprostenol (Dukes *et al.*, 1974; Coleman *et al.*, 1984) in the rat fundus preparation (Dong *et al.*, 1986) were similarly unchanged by this concentration of BW A868C.

Although BW A868C is evidently highly potent and selective for DP-receptors it did not satisfy all the criteria for simple competitive antagonism under the conditions employed in the platelet assay. While the antagonist produced apparently parallel displacement of BW245C concentration-effect curves, the Schild plot slope parameter, n, exceeded unity. This deviation was significant according to the method of data analysis used here, that is, on the basis of the average of four independent estimates of n made in different platelet suspensions. In fact, the same estimate of n, namely 1.25, was obtained when all the curve $[A_{50}]$ data from the four experiments were pooled and analysed (analysis not shown), although, in this case, the estimated standard error (0.16) made the deviation appear insignificant. However, in the interests of rigour it seemed appropriate to accept the implications of the more stringent statistical evaluation. As it was found that increasing the equilibrium period for the antagonist lowered the value of n, albeit not to unity, we conclude that the estimate of 1.25 was meaningful as well as significant. On this basis, as explained in the results section, a reliable estimate of antagonist affinity could only be derived from the concentration-ratio data obtained at higher concentrations of BW A868C. This estimate 9.26 is, in fact, very similar to that obtained in a human adenylate cyclase assay as described in the following paper (Trist *et al.*, 1989).

It was not possible to confirm this estimate of affinity with the natural agonist, PGD_2, in the platelet assay due to expression of pro-aggregatory effects by PGD_2. In the jugular vein assay also, PGD_2 demonstrated additional properties producing contractile effects, which due to their BM 13.177 susceptibility, appeared to be TP-receptor-mediated. These results are consistant with similar reports of PGD_2 actions in other smooth muscle preparations (Jones *et al.*, 1982; Toda *et al.*, 1986). Although the pro-aggregatory effects of PGD_2 in the human platelet assay were not examined in the presence of BM 13.177 it is likely that they are TP-receptor-mediated in view of previous evidence of PGD_2 action at this receptor in guinea-pig platelet (Hamid-Bloomfield & Whittle, 1986).

Unfortunately, the BW A868C-resistant relaxation responses of BW245C meant that a reliable affinity estimate for the antagonist could not be made in the jugular vein assay with this agonist either. However, assuming that a one agonist: two receptor system was operating here, it was possible to show that antagonistic effects were consistent with the antagonism of BW245C-mediated effects by BW A868C with a pK_B at the DP-receptor of 8.7. This value is clearly lower than that obtained in the platelet assay(s) but under the circumstances we feel it is unwise to invoke receptor heterogeneity.

Further evidence, using other agonist and antagonist probes, is necessary for the similarity or otherwise of the platelet and vascular DP-receptors to be established. We are currently undertaking such studies.

Regarding the BW A868C-resistant relaxation responses of BW245C and also PGD_2, the receptor mediating these effects is unlikely to be of the EP_1-type as AH6809 produced no antagonism at concentrations approaching two orders of magnitude higher than the reported EP_1-receptor affinity of this compound (Coleman *et al.*, 1985). It is also unlikely that these responses are IP-receptor-mediated because the potency of PGI_2 is higher in the platelet than in the jugular vein assay (data not shown) implying that any BW245C-mediated IP-receptor effect should have been more marked in the platelet assay rather than absent as was actually the case. Also, binding studies have shown the BW245C has negligible affinity for PGI_2 binding sites in platelets (Town *et al.*, 1983). In the absence of evidence to the contrary and for the sake of parsimony it seems reasonable to conclude that these effects are mediated by the EP_2-receptor class. Previous reports indicate that this receptor subserves relaxation responses in dog vasculature (Lumley *et al.*, 1982; Dong *et al.*, 1986) although firm classification of this receptor as a distinct type awaits the discovery of further selective agonist and antagonist probes.

In addition to its effects in platelets and vasculature, PGD_2 has also been shown to mediate bronchoconstriction in the dog *in vivo* (Wasserman *et al.*,

1977) and contraction of guinea-pig isolated tracheal preparations (Coleman & Kennedy, 1980). Here we have shown in the guinea-pig trachea that the receptor(s) mediating contraction to PGD_2 is not of the DP-class; $10 \mu M$ BW A868C had no effect on these responses. Indeed it has previously been suggested that PGD_2 produces these effects through TP- and EP_1-receptors (Jones *et al.*, 1982; Coleman & Kennedy, 1985).

In summary, BW A868C appears to be a very potent competitive antagonist of DP-receptors in human washed platelets. It demonstrates negligible affinity for TP- IP-, EP_1-, EP_2- and FP-receptors at concentrations three to four orders of magnitude higher than its expressed DP-receptor dissociation constant. Antagonists of this potency and selectivity have not been previously reported for DP-receptors or for most of the other putative prostaglandin receptor classes. The exception is the TP-class for which antagonists of similar profile exist: EP045 (Jones *et al.*, 1982), BM 13,177 (Patscheke *et al.*, 1984), AH23848 (Brittain *et al.*, 1984). As in the case of the available TP-receptor antagonists, BW A868C should prove to be an important tool for the reliable identification and quantitative classification of prostaglandin receptors.

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