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Effects of BW A868C, a selective prostaglandin DP receptor antagonist, in dog isolated vascular preparations

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Abstract

The effects of the selective prostaglandin DP receptor antagonist BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin), 3.0 nM to 0.3 μM) were examined against prostaglandin D_2 and BW245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin)-induced smooth muscle relaxation on dog isolated vascular preparations pre-contracted with a sub-maximal concentration of KCl (50 mM). In dog dorsal nasal vein BW245C was found to be more potent than prostaglandin D_2 with $p[A]_{50}$ estimates of 7.6 ± 0.1 (S.E.M., $n = 8$) and 5.8 ± 0.1 ($n = 5$), respectively. BW A868C, up to 0.3 μM, displaced the relaxant concentration-effect curves to BW245C in dog dorsal nasal vein in an apparently competitive manner with parallel rightward shifts and no significant changes in the upper asymptotes. The data were analysed by using a modified Schild equation which not only gives equal weight to all agonist concentration-effect data but also allows a direct plot in Clark plot space. A pK_B estimate of 7.3 ± 0.8 ($n = 20$) was obtained with a unity Schild plot slope ($b = 1.0 \pm 0.1$). This affinity estimate, however, is lower than the values previously reported in other studies. The affinity estimates of BW A868C against BW245C and prostaglandin D_2 obtained from dog dorsal nasal vein, major palatine artery and saphenous vein were found to be consistent. The relatively low affinity estimates of BW A868C at DP receptors as observed in the present study may be due to species- or tissue-related variations or may be indicative of the possible existence of DP receptor subtypes.

Keywords: BW A868C; Prostaglandin D_2; BW245C; DP receptor; Antagonist affinity

1. Introduction

The demonstration that BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin) is a selective prostaglandin DP receptor antagonist (Giles et al., 1989) supported the original classification of DP receptors (Kennedy et al., 1982; Coleman et al., 1982) which was based largely on potency orders of the naturally occurring prostanoids. Since then BW A868C has become a very useful research tool in studies involving various prostanoid receptors. The nature of the antagonism produced by BW A868C at DP receptors has been examined in a variety of isolated tissue preparations from different species. A range of equilibrium dissociation constant estimates has been reported, using BW245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin), a hydantoin prostaglandin analogue, as the agonist, for example, by Giles et al. (1989), 9.3 in human washed platelet and 8.7 in rabbit jugular vein; by Senior et al. (1992, 1993), 8.3 and 8.6 in non-pregnant and term pregnancy human myometrium, respectively, and by Lydford et al. (1994), 8.5 in rabbit saphenous vein. These different values reflected possible effects of species- or tissue-related variations on the affinity estimates of an antagonist. The possibility that DP receptor subtypes may exist, on the other hand, has been suggested primarily based on the different profiles of prostaglandin D_2 and its analogues in relation to BW A868C (Woodward et al., 1993).

In preliminary studies, we found that prostaglandin D_2 and its analogues induced smooth muscle relaxation on pre-contracted dog major palatine artery, dorsal nasal and saphenous veins in vitro and the responses were sensitive to BW A868C. The aim of the present study was to examine the antagonism of BW A868C using prosta-

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glandin D_2 and BW245C in an attempt to characterize its antagonist profile at DP receptors in these vascular preparations obtained from the same species. A brief account of the present study was communicated at a recent British Pharmacological Society Meeting at Canterbury (April, 1995) and the abstract has been published (Liu and Jackson, 1995).

2. Materials and methods

2.1. Tissue preparations

Beagle dogs (10–16 kg) of either gender were killed by an intravenous injection of Euthatal (200 mg ml^{-1} pentobarbitone sodium) at 1.0 ml per 1.4 kg body weight. Lengths of the major palatine artery, dorsal nasal vein and saphenous vein were then dissected out and cleared of adherent tissues and endothelium. The vessels were subsequently cut into equal ring segments of approximately 3.0 mm in length. Each of these ring preparations was mounted on to two parallel stainless steel hooks suspended in a 10 ml organ bath. One hook was fixed to a racking mechanism and the other was connected to a force-displacement transducer for data acquisition (Ormed Beam). The organ baths were filled with modified Krebs buffer of the following composition (mM): NaCl 117.56, KCl 5.36, $CaCl_2$ 2.55, $MgSO_4$ 1.18, NaH_2PO_4 1.15, $NaHCO_3$ 25.00, glucose 11.10, aerated with 5% CO_2 in O_2 and maintained at 37 ± 0.5°C. A pre-load of 1.0 g was applied to the preparations and the organ bath fluid was replaced with prewarmed buffer 3 times at 15 min intervals before antagonist pretreatments. Isometric forces were continuously recorded on chart recorders (Sekonic SS-250F).

2.2. Experimental protocol

In all experiments, indomethacin (2.8 μM) and GR32191B ([1α(Z),2β,3β,5α]-(+)-7-[5-[1,1'-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)-cyclopentyl]-4-heptenoic acid, 5.0 μM) were included in the buffer to prevent possible interference by cyclo-oxygenase products and TP receptor activation, respectively. In preliminary investigations to determine an experimental protocol, the second cumulative concentration-effect (E/[A]) curves to prostaglandin D_2 or BW245C were found to be rightward shifted from the first to various degrees which ruled out a paired-curve protocol and, no significant difference in the pA_2 estimates was detected from pretreatments with BW A868C at 30 nM for 60, 120 and 180 min which indicated that 60 min would be sufficient. Antagonist pretreatments with BW A868C (3.0 nM to 0.3 μM), 60 min prior to the start of the concentration-effect (E/[A]) curves to prostaglandin D_2 or BW245C, were allocated according to a randomised block-design to minimise possible external errors. The preparations were pre-contracted with a submaximal dose of KCl (50 mM) and stable plateaus were obtained. Relaxation curves to prostaglandin D_2 or BW245C, one curve per preparation, in the absence and presence of BW A868C were performed simultaneously.

2.3. Theory

2.3.1. Logistic function

Agonist $E/\log_{10}[A]$ data from individual preparations were fitted to a logistic function of the form:

$$E = \frac{\alpha[A]^p}{[A]_{50}^p + [A]^p} \quad (1)$$

to provide parameter estimates of α (the upper asymptote), $\log_{10}[A]_{50}$ (the midpoint location) and p (the slope index), respectively. All curve-fittings were performed using a data analysis package KaleidaGraph on a Macintosh Centris 650 computer. These parameter estimates, expressed as means ± S.E.M., were used for subsequent analysis and display of data.

2.3.2. Schild analysis

Where agonist $E/\log_{10}[A]$ curves were rightward shifted in parallel with no significant changes in the upper asymptotes by an antagonist, the midpoint location parameter estimates in the absence ($\log_{10}[A]_{50}^c$) and presence ($\log_{10}[A]_{50}^t$) of the antagonist ([B]) were fitted to a modified Schild equation of the form:

$$\log_{10}[A]_{50}^t = \log_{10}\left([A]_{50}^c / K_B\right) + \log_{10}\left(K_B + [B]^b\right) \quad (2)$$

Like the previously modified Schild equation (Waud and Parker, 1971; Stone and Angus, 1978; Black et al., 1985) the present equation gives equal weight to all agonist concentration-effect data. In addition it also provides a direct plot in Clark plot space ($\log_{10}[A]_{50}^t$ vs. $\log_{10}(K_B + [B])$, $b = 1$). If the Schild plot slope (b) was found not to be significantly different from unity, an estimate of the antagonist equilibrium dissociation constant (pK_B) was obtained. If a significant difference was found, however, in the upper asymptotes or in the slope indexes of the agonist $E/\log_{10}[A]$ curves in the absence and presence of the antagonist or, if the Schild plot slope was found to be significantly different from unity, the estimate would be expressed as an apparent pK_B (pK_{Bapp}) without prejudice to mechanism of action.

2.3.3. Statistical analysis

Parameter estimates from individual treatments were compared by a one-way analysis of variance or a Student's t-test, where the sample frequency (n) refers to the number of individual cases used to generate the parameter(s), and the standard error of mean (S.E.M.) were derived from the variance. A probability of $P < 0.05$ was considered to be significant.

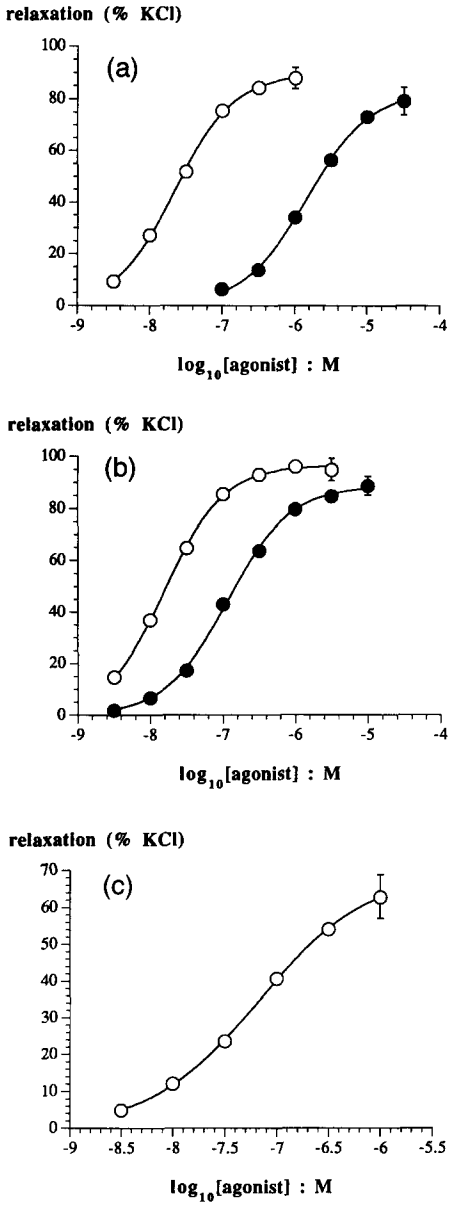

Fig. 1. Relaxant concentration-effect curves of BW245C (○) and PGD_2 (●) in dog dorsal nasal vein (a), major palatine artery (b) and saphenous vein (c) pre-contracted with 50 mM KCl. The curves super-imposed upon the mean experimental data points were simulated by using the parameter estimates from the logistic curve-fitting (equation 1 in Methods). Error bars represent S.E.M.

2.4. Drugs

Prostaglandin D_2 was purchased from Cascade Biochemicals, Reading, Berkshire, UK. Indomethacin was purchased from Sigma Chemical Co., Poole, Dorset, UK. BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin), BW245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin) and GR32191B ([1α(Z),2β,3β,5α]-(+)-7-[5-[1,1'-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)-cyclopentyl]-4-heptenoic acid) were gifts from Glaxo-Wellcome, UK.

Ethanolic stock solutions of the prostanoids, from which fresh drug solutions were made by dilution in the buffer before use, were stored at −20°C and used within 4 weeks. The total volume of drug solutions added in a 10 ml organ bath did not exceed 5% of the buffer volume.

3. Results

3.1. BW245C and prostaglandin D_2 relaxant $E / log_{10}[A]$ curves

In dog dorsal nasal vein, BW245C and prostaglandin D_2 induced concentration-dependent relaxations with mean $p[A]_{50}$ estimates of 7.6 ± 0.1 ($n = 8$) and 5.8 ± 0.1 ($n = 5$), respectively (Fig. 1a). Similar effects were observed in dog major palatine artery with $p[A]_{50}$ estimates of 7.8 ± 0.1 ($n = 3$) and 6.9 ± 0.1 ($n = 4$) for BW245C and prostaglandin D_2, respectively (Fig. 1b). In dog saphenous vein, a $p[A]_{50}$ estimate of 7.2 ± 0.2 ($n = 5$) for BW245C was obtained (Fig. 1c).

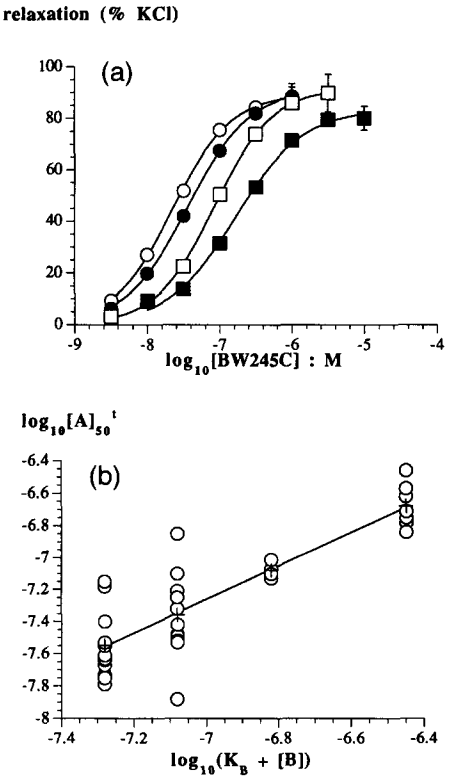

Fig. 2. (a) Relaxant concentration-effect curves of BW245C in the absence (○, $n = 8$) and presence of 30 nM (●, $n = 10$), 100 nM (□, $n = 4$) and 300 nM (■, $n = 8$) of BW A868C in dog dorsal nasal vein pre-contracted with 50 mM KCl. (b) A direct plot of the data from (a) derived from the modified Schild equation (equation 2 in Methods).

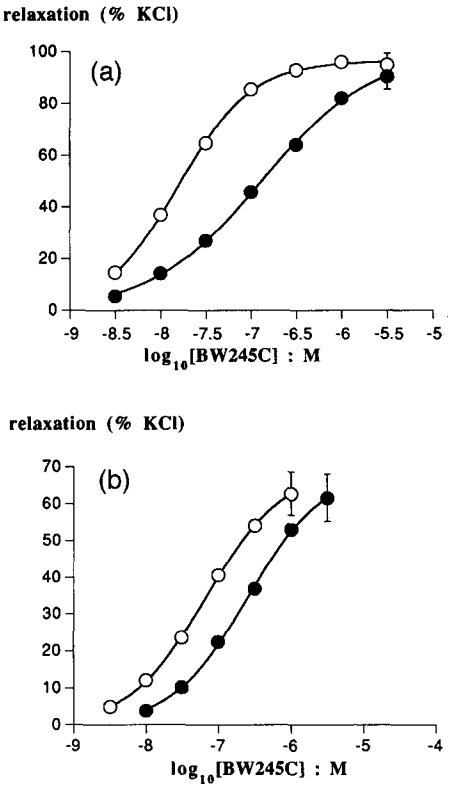

Fig. 3. Relaxant concentration-effect curves of BW245C in the absence (○) and presence of 300 nM (●) of BW A868C in dog major palatine artery (a) and saphenous vein (b) pre-contracted with 50 mM KCl.

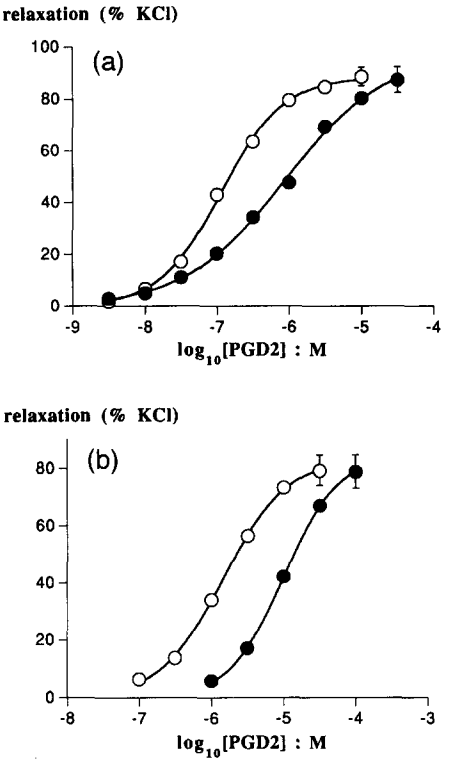

Fig. 4. Relaxant concentration-effect curves of PGD_2 in the absence (○) and presence of 300 nM (●) of BW A868C in dog major palatine artery (a) and dorsal nasal vein (b) pre-contracted with 50 mM KCl.

3.2. Antagonism of BW A868C (3.0 nM to 0.3 μM)

In dog dorsal nasal vein BW A868C, up to 0.3 μM in concentration, displaced the $E/\log_{10}[A]$ curves to BW245C in a competitive manner, yielding a pK_B estimate of 7.3 ± 0.8 ($n = 20$) with a unity Schild plot slope ($b = 1.0 \pm 0.1$, Fig. 2a,b). This affinity estimate, however, is clearly lower than the values previously reported in other studies (Giles et al., 1989; Senior et al., 1992, 1993). In dog major palatine artery and saphenous vein, similar antagonism was observed with pK_{Bapp} estimates of 7.6 ± 0.2 ($n = 8$) and 7.1 ± 0.1 ($n = 12$), respectively (Fig. 3a,b). The $E/\log_{10}[A]$ curves to prostaglandin D_2, in dog major palatine artery and dorsal nasal vein, were similarly rightward shifted in the presence of BW A868C in parallel with no significant changes in the upper asymptotes, producing comparable pK_{Bapp} estimates of 7.3 ± 0.2 ($n = 4$) and 7.3 ± 0.2 ($n = 9$) (Fig. 4a,b and Table 1).

Table 1
Affinity estimates (pK_B or pK_{Bapp}, means ± S.E.M.) of antagonist BW A868C interacting with BW245C and prostaglandin D_2 in dog isolated major palatine artery (MPA), dorsal nasal vein (DNV) and saphenous vein (SV)

	MPA	DNV	SV
BW245C	7.6 ± 0.2 (8)	7.3 ± 0.8 (20)	7.1 ± 0.1 (12)
PGD_2	7.3 ± 0.2 (4)	7.3 ± 0.2 (9)	

4. Discussion

The data presented in this paper demonstrated the antagonist profiles of BW A868C against BW245C and prostaglandin D_2 in dog dorsal nasal vein, major palatine artery and saphenous vein. In these dog vascular preparations comparable estimates of the antagonist equilibrium dissociation constant for BW A868C at prostaglandin DP receptors was obtained (Table 1) which are lower than the values previously reported in other studies by Giles et al. (1989), 9.3 in human washed platelet and 8.7 in rabbit jugular vein; by Senior et al. (1992, 1993), 8.3 and 8.6 in non-pregnant and term pregnancy human myometrium, respectively, and by Lydford et al. (1994), 8.5 in rabbit saphenous vein. The differences could be attributed to species- or tissue-related variations or may be indicative of the existence of DP receptor subtypes. The possible existence of DP receptor subtypes has previously been suggested by Woodward et al. (1993), where prostaglandin D_2, but not the selective DP receptor agonists BW245C and SQ 27986, was found to increase conjunctival microvascular permeability in guinea pigs and this action, in particular, was sensitive to BW A868C.

In examining the pharmacological properties of BW A868C in the present study, we started with a concentration of 3.0 nM of the antagonist with half a log unit increments. This was based on the high affinity value of $pK_B = 9.3$ in human washed platelet as previously reported (Giles et al., 1989). No significant rightward shift of the $E/\log_{10}[A]$ curves to BW245C and prostaglandin D_2 was observed until concentrations of BW A868C higher than 30 nM were used which provided an early indication that BW A868C may be less potent in the dog vascular preparations than in human washed platelet. In dog saphenous vein, BW A868C up to 1 μM did not affect the relaxant $E/\log_{10}[A]$ curves to PGE_2 (data not shown), which confirmed earlier findings that BW A868C, up to 1 μM or 1000 times of its dissociation constant at DP receptors, was inactive at other prostanoid receptors (Giles et al., 1989). In addition, no significant partial agonist activity was detected with BW A868C using concentrations up to 0.1 mM in the present study. The fact that prostaglandin D_2 appeared to be approximately 10 times more potent in dog major palatine artery than in dog dorsal nasal vein (Results, Fig. 1a,b) where the potencies of BW245C were comparable could be indicative of that the mechanism of the prostaglandin D_2 responses may be more complex than being mediated by DP receptors. In the absence of GR32191B a TP receptor antagonist, prostaglandin D_2 at concentrations higher than 0.1 mM was found to induce significant contractions in the dog vascular preparations which were sensitive to the antagonist and necessitated the inclusion of the antagonist in the buffer to prevent possible interference by TP receptor activation in the present study. It has become a routine practice in our studies to include indomethacin wherever cyclo-oxygenase may be present because its products may cause interference.

It has long been known that species- or tissue-related variations or coexistence of heterogeneous receptor populations may be a contributing, if not a causal, factor for different pharmacological parameters obtained by use of agonists and antagonists at prostanoid receptors. Having discussed the possibility that inadequate antagonist equilibration, obvious at low antagonist concentrations, was responsible for the steep Schild plot slope which could result in underestimation of antagonist affinity, Giles et al. (1989) recognized that a pK_B value of 8.7 for BW A868C in the rabbit jugular vein is 'clearly lower' than that of 9.3 in human washed platelet. The presence of a BW A868C-resistant component of the BW245C-induced relaxation responses in the rabbit jugular vein could be responsible for the difference. BW245C is now known to be a selective and potent DP receptor agonist (Town et al., 1983; Whittle et al., 1983), also showing weak agonism at EP receptors (Smith et al., 1994). The two pA_2 values of 8.3 and 8.6 for BW A868C with flat Schild plot slopes, as reported by Senior et al. (1992, 1993), were not significantly different statistically from each other. In these studies, the important changes in the uterus from non-pregnant state to term pregnancy did not result in a different profile of the DP receptor activity. The difference in the antagonist affinity values for BW A868C between human washed platelet (Giles et al., 1989) and human myometrium (Senior et al., 1992, 1993) could be due to tissue-related variations. These together with our own data presented in the present study highlighted once again the problems associated with pharmacological classifications of prostanoid receptors using animal tissues, in the drive to develop selective agonists or antagonists for human disorders. In order to achieve a higher probability of being clinically efficacious it would be commendable to use human tissues or cells from the target systems where it is necessary.

In conclusion, we have presented evidence obtained from dog dorsal nasal vein, major palatine artery and saphenous vein demonstrating that the consistent estimates of the antagonist equilibrium dissociation constant for BW A868C at prostaglandin DP receptors are clearly lower than the values previously reported in other studies. This difference could be due to species- or tissue-related variations or may be indicative of the possible existence of DP receptor subtypes. Further studies using a variety of antagonists and agonists in several species and tissues would be required to resolve this issue.

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