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Partial agonist effects of BW A868C, a selective DP receptor antagonist, on Cl^- secretion in dog tracheal epithelium

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Abstract

We examined the interactions of prostaglandin D_2, BW245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin) a selective DP receptor agonist and BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin) a selective DP receptor antagonist on Cl^- secretion using dog isolated tracheal epithelial preparations in Ussing chambers. Both prostaglandin D_2 and BW245C stimulated Cl^- secretion as reflected by increased short-circuit current (I_{sc}) in the epithelial cells where the latter was more potent than the former. BW A868C produced, consistently, weak but significant partial agonism on Cl^- secretion in these preparations in addition to its expected antagonism at the DP receptors. A pK_B estimate of 8.16 ± 0.06 ($n = 11$) for BW A868C from its antagonism to BW245C was found to be comparable with its estimates of both $p[A]_{50}$ (8.19 ± 0.14, $n = 5$) and pK_A (8.00 ± 0.20, $n = 5$). In addition, no significant effect by BW A868C up to 1 μM on Cl^- secretory responses to other prostanoids, such as prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 9α,11β-prostaglandin $F_{2\alpha}$, was detected in the system. These results are consistent with previous findings that BW A868C is a selective antagonist at the DP receptors mediating Cl^- secretion by epithelial cells. To our knowledge, this is a (the first) confirmation of partial agonist properties of BW A868C in an isolated tissue system.

Keywords: BW A868C; DP receptor; Partial agonist; Cl^- secretion

1. Introduction

Prostaglandin D_2 is involved in the modulation of ion transport by epithelial cells where the effects are considered to be predominantly mediated by DP receptors. Both secretory and antisecretory effects of prostaglandin D_2 have been reported recently. Tamaoki et al. (1992) demonstrated that, in dog cultured tracheal epithelium, prostaglandin D_2 increased Cl^- secretion which was not altered by pre-incubation of cells with the autonomic antagonists phentolamine, propranolol, atropine, the lipoxygenase inhibitor AA-861, the protein kinase C inhibitor H-7 or the Na^+ channel blocker amiloride, but was inhibited by indomethacin, piroxicam, the Cl^- channel blocker diphenylamine-2-carboxylate, the Cl^- transport inhibitor furosemide or Cl^--free medium. In the epithelial preparations of rabbit ileum, prostaglandin D_2 was shown to stimulate Cl^- secretion (Musch et al., 1987). Exposure of submucosal preparations of guinea-pig colon to prostaglandin D_2 resulted in stimulation of electrogenic Cl^- secretion (Frieling et al., 1994). In the epithelial preparations of rat and dog colons, however, prostaglandin D_2 exhibited antisecretory effects which was shown to be mediated by DP receptors and probably associated with inhibition of acetylcholine release (Keenan and Rangachari, 1991; Diener et al., 1992).

Earlier, we have observed in anaesthetized dogs that increased airway fluid production in response to inhaled prostaglandin D_2 appeared to be dose-dependent. In order to characterise the receptors involved we examined the interactions of prostaglandin D_2, BW 245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin) a selective DP receptor agonist and BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin) a selective DP receptor antagonist, in the present study, on Cl^- secretion using dog isolated tracheal epithelial preparations in Ussing chambers. A brief account of this study was communicated at a recent American Thoracic Society Conference (Seattle, May 1995) and an abstract has been published (Liu et al., 1995).

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2. Materials and methods

2.1. Dog isolated tracheal epithelial preparation

The method employed to prepare dog isolated tracheal epithelial sheets was essentially the same as that described by Boucher and Larsen (1988). Briefly, Beagle dogs (10–16 kg) of either gender were killed by an intravenous injection of Euthatal (200 mg/ml pentobarbitone sodium) at 1.0 ml per 1.4 kg body weight. The thorax was cut open and a segment of the trachea close to the carina was dissected out and cleared of connective and adipose tissues. The tracheal segment was cut open along the dorsal midline and pinned flat and placed in a dissection dish 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 at room temperature. A sheet of the epithelium was dissected off the underlying cartilaginous tissues and mounted in a 10 ml Ussing chamber which was filled with the Krebs buffer aerated with 5% CO_2 in O_2 and maintained at 37 ± 0.5°C. Both sides of the preparation were bathed with indomethacin (2.8 μM) to prevent possible interference by cyclo-oxygenase products. In addition, amiloride (0.1 mM) a Na^+ channel blocker was included in the buffer bathing the apical side of the preparation to abolish Na^+ absorption. The Ussing chamber system was linked to a voltage clamp amplifier via 4.0% agar and 3.0 M potassium chloride salt bridges connected to silver-silver chloride pellet electrodes. Under these conditions, changes in the short-circuit current (I_{sc}) required to clamp the trans-epithelial potential difference to zero would reflect changes in Cl^- secretion by the epithelial cells.

2.2. Experimental protocol

Tracheal epithelial preparations from each dog were used in random pairs simultaneously to provide a time control. From preliminary investigations, it was observed that the second cumulative concentration-effect (E/[A]) curve to prostaglandin D_2 or BW245C appeared to be rightward shifted when compared with the first in a preparation and, the difference between the two consecutive curves was sometimes significant. A single curve regime, that is one E/[A] curve per preparation, was therefore adopted in the present study. Although dosing on the basal side of a preparation could also allow a drug to reach its active sites in the epithelium it usually requires much longer time to reach equilibrium. Therefore, in the present study, drug dosing was performed on the apical side. After a stable basal I_{sc} had been established, the preparation was challenged with a maximal dose of ATP (30 μM) and afterwards washed with fresh buffer preheated to 37 ± 0.5°C. Once the preparation had recovered as indicated by restoration of the basal I_{sc} level, the maximal dose ATP challenge was repeated until a reproducible response had been obtained. When examined for its antagonist activity, BW A868C was pre-incubated for 30 min before the start of the E/[A] curves to BW245C or prostaglandin D_2. Drug-induced responses were expressed as percentages of a stable response to the ATP challenge, referred to as sighter. At the end of an experiment, bumetanide (0.1 mM) a Cl^--cation co-transport inhibitor was added to reverse the induced response as a confirmation of Cl^- secretion.

2.3. Data analysis

2.3.1. Hill equation

Agonist E/[A] curve data from individual preparations were computer-fitted to the Hill equation of the form (Jenkinson et al., 1995),

$$E = \frac{\alpha[A]^{n_H}}{[A]_{50}^{n_H} + [A]^{n_H}} \quad \text{(I)}$$

to provide parameter estimates of α, the upper asymptote of the curve in an $E/\log_{10}$[A] space; $[A]_{50}$, the midpoint location; and n_H, the Hill coefficient, respectively. All curve-fittings were performed by 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. Operational model of agonism

In order to obtain an estimate of the dissociation equilibrium constant for BW A868C as a partial agonist, we assumed that BW245C is a full agonist in the system and employed the operational model of agonism of the form (Black and Leff, 1983),

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \quad \text{(II)}$$

where K_A is the agonist dissociation equilibrium constant which is an agonist-dependent quantity for a receptor type, τ is the agonist efficacy in a tissue which depends on both the agonist and the tissue system, E_m is the maximal effect that can be generated in a tissue system which, in practice, is the maximal effect by a full agonist, and n is the transducer slope index which measures the sensitivity of the system transducing the agonist-receptor complex into an effect. In the present study, a comparative method (Leff et al., 1990) was adopted where individual E/[A] curve data for BW245C were computer-fitted to equation (I) from which estimates of the assumed common parameters, between equations (I) and (II), α equivalent to E_m and n_H equivalent to n were obtained. Individual E/[A] curve data for BW A868C were then computer-fitted to equation (II) using the above E_m and n estimates to produce a common K_A estimate, as well as individual estimates for τ.

2.3.3. Schild equation

In order to obtain an estimate of the dissociation equilibrium constant for BW A868C as an antagonist, we computer-fitted individual midpoint location parameter estimates for BW245C in the absence ($[A]^c_{50}$) and presence ($[A]^t_{50}$) of the antagonist to a modified form of the Schild equation (Waud and Parker, 1971; Stone and Angus, 1978; Black et al., 1985):

$$\log_{10}[A]^t_{50} = \log_{10}[A]^c_{50} + \log_{10}\left(1 + [B]^b/K_B\right) \qquad \text{(III)}$$

If the Schild plot slope b was found not to be significantly different from unity, an estimate of the antagonist dissociation equilibrium constant K_B was therefore obtained with b constrained to unity.

2.3.4. 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.

2.4. Drugs

Prostaglandin D_2 was purchased from Cascade Biochemicals, Reading, Berkshire, UK. Indomethacin, amiloride, bumetanide, ATP, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were purchased from Sigma Chemical Co., Poole, Dorset, UK. 9α,11β-Prostaglandin $F_{2\alpha}$ was purchased from ICN Pharmaceuticals, Costa Mesa, CA 92626, USA. BW A868C ((±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin) and BW 245C ((±)-5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin) were gifts from Glaxo-Wellcome, London, UK.

Ethanolic stock solutions of the prostanoids, from which fresh drug solutions were made by dilution with 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 Ussing chamber did not exceed 5% of the buffer volume.

3. Results

3.1. Agonist effects of prostanoids on Cl^- secretion

In the dog isolated tracheal epithelial preparations, both BW245C and prostaglandin D_2, stimulated Cl^- secretion in a concentration-dependent fashion (Fig. 1). BW245C appeared to be 10 times more potent than prostaglandin D_2

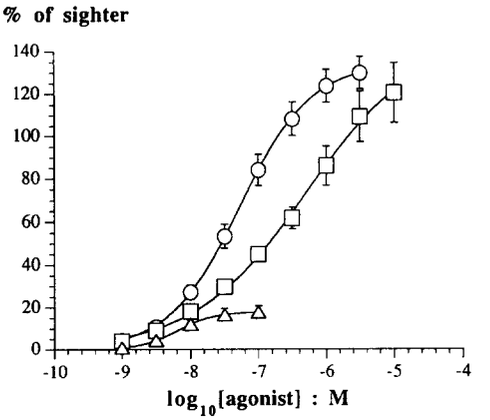

Fig. 1. Cl^- secretory concentration-effect curves of BW245C (○, $n = 7$), PGD_2 (□, $n = 5$) and BW A868C (△, $n = 5$) in dog isolated tracheal epithelial preparations in Ussing chambers. The curves super-imposed upon the mean experimental data points were simulated by using the parameter estimates from computer-fitting the data to the Hill equation (refer to 2.3.1.). Error bars represent S.E.M.

where estimates of the $p[A]_{50}$ were 7.27 ± 0.09 ($n = 7$) and 6.27 ± 0.10 ($n = 5$), respectively. BW A868C the selective DP receptor antagonist also produced, consistently, a weak but definite Cl^- secretory response (Fig. 1) with a $p[A]_{50}$ estimate of 8.19 ± 0.14 ($n = 5$). The partial agonist activity of BW A868C was further analysed by using a comparative method (refer to 2.3.2.), assuming BW245C to be a full agonist in the system, and a pK_A estimate of 8.00 ± 0.20 ($n = 5$) was obtained. In addition, it was also observed that prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 9α,11β-prostaglandin $F_{2\alpha}$ all induced Cl^- secretory responses in the system (Fig. 2) where the responses to prostaglandin E_2 appeared to be sensitive to selective EP receptor antagonism (data not shown).

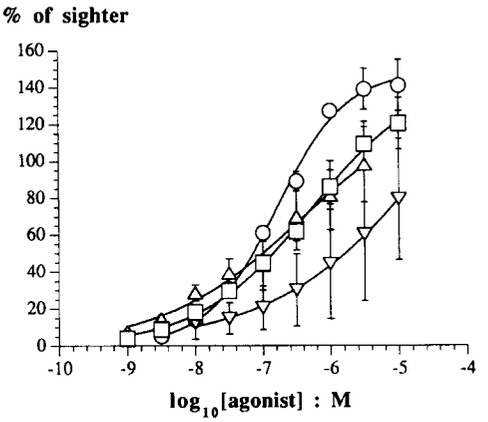

Fig. 2. Cl^- secretory concentration-effect curves of PGE_2 (○, $n = 4$), PGD_2 (□, $n = 5$), $PGF_{2\alpha}$ (△, $n = 4$) and 9α,11β-$PGF_{2\alpha}$ (▽, $n = 2$) in dog isolated tracheal epithelial preparations in Ussing chambers. The curves super-imposed upon the mean experimental data points were simulated by using the parameter estimates from computer-fitting the data to the Hill equation (refer to 2.3.1.). Error bars represent S.E.M.

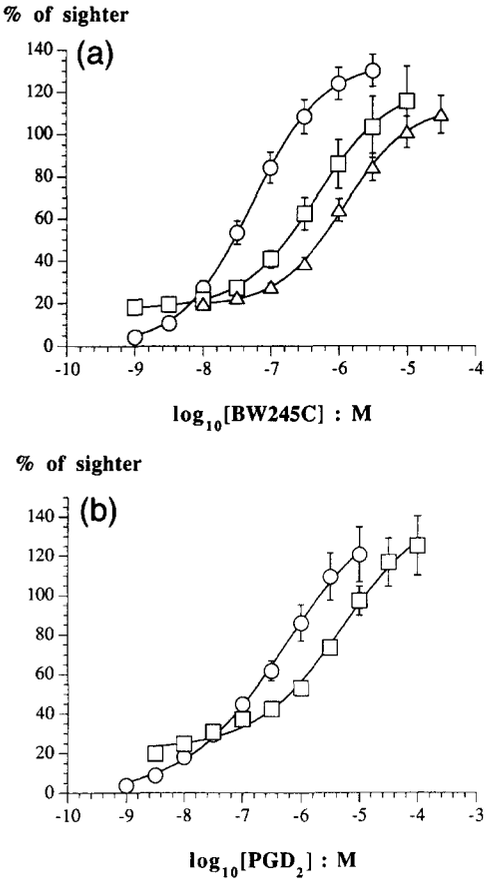

Fig. 3. (a) Cl^- secretory concentration-effect curves of BW245C in the absence (○, $n = 7$) and presence of 30 nM (□, $n = 4$) and 100 nM (△, $n = 7$) of BW A868C in dog isolated tracheal epithelial preparations in Ussing chambers. (b) Cl^- secretory concentration-effect curves of PGD_2 in the absence (○, $n = 5$) and presence of 30 nM (□, $n = 5$) of BW A868C in dog isolated tracheal epithelial preparations in Ussing chambers. The curves super-imposed upon the mean experimental data points were simulated by using the parameter estimates from computer-fitting the data to the Hill equation (refer to 2.3.1.). Error bars represent S.E.M.

3.2. Antagonist effects of BW A868C on prostanoid-induced Cl^- secretion

The Cl^- secretory responses to BW245C and prostaglandin D_2 were antagonised by BW A868C in a concentration-dependent fashion (Fig. 3). The antagonist profile of BW A868C on BW245C-induced responses appeared to be one that is reversible competitive at one receptor population (Fig. 3a) which was reflected by parallel rightward shifts with no significant change in the upper asymptotes and by a Schild plot slope not significantly different from unity. The acquired pK_B estimate of 8.16 ± 0.06 ($n = 11$) for BW A868C from its antagonism to BW245C was comparable with its estimates of $p[A]_{50}$ and pK_A (Table 1). In addition, no significant effect by BW A868C up to 1 μM on the Cl^- secretory responses to the other prostanoids such as prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 9α,11β-prostaglandin $F_{2\alpha}$ was detected in the system (data not shown).

Table 1
Pharmacological analyses of the agonist and antagonist activities of BW A868C in dog tracheal epithelium (refer to 2.3.)

Potency	Affinity	
$p[A]_{50}$	Antagonist pK_B [a]	Agonist pK_A [b]
8.19 ± 0.14	8.16 ± 0.06	8.00 ± 0.20
$n = 5$	$n = 11$	$n = 5$

[a] The Schild plot slope b was constrained to unity. [b] The mean efficacy τ estimate was 0.18 ± 0.03.

4. Discussion

Active absorption and/or secretion of ions across airway epithelia are important processes in the regulation of airway mucociliary clearance, in that they modulate the volume and composition of airway surface fluid. Such ion transports across epithelial cells are the driving forces which generate trans-epithelial potential difference and short-circuit current (I_{sc}), and passive diffusion of water (Widdicombe, 1991).

Here, we have demonstrated that activation of prostanoid DP receptors in the dog isolated tracheal epithelial preparations, using prostaglandin D_2 and BW245C a selective DP receptor agonist, stimulates Cl^- secretion as indicated by the increased I_{sc}. Furthermore, BW A868C a selective DP receptor antagonist has been demonstrated to be a weak but definite partial agonist in the system. The present observations that the experimentally acquired pK_A estimate for BW A868C as a partial agonist, while assuming BW245C is a full agonist in the system, was comparable with its pK_B estimate obtained from its antagonism to BW245C and together with that BW A868C up to 1 μM did not significantly affect the Cl^- secretory responses to the other prostanoids such as prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 9α,11β-prostaglandin $F_{2\alpha}$ confirmed that the partial agonist activity of BW A868C was probably mediated by DP receptors. Recently, Hirata et al. (1994) reported partial agonist activity by BW A868C using a cAMP assay in cultured Chinese hamster ovary (CHO) cells expressing a cloned mouse DP receptor gene. In their assay, however, BW A868C appeared to be approximately 100-fold less potent than in the present system whereas BW245C and prostaglandin D_2 appeared to be approximately 100-fold more potent than in the present system. In addition, BW A868C appeared to be more efficacious in their assay than in the present system, while using BW245C as a reference agonist in both systems. It appeared difficult to reconcile these different findings on the ground of possible difference in the receptor expression levels in their CHO cells as compared with the present system, assuming the same DP receptors were involved. To our

knowledge, this is a (the first) confirmation of partial agonist properties of BW A868C in an isolated tissue system as in the present study.

In earlier investigations, it was observed that when two cumulative $E/[A]$ curves to prostaglandin D_2 or BW245C were performed in one preparation with washings (3 replacements of the buffer for 4 times at 10 min intervals) and recovery (restoration of the basal I_{sc} level) in between, the second curve was in some experiments displaced significantly to the right of the first as indicated by their $p[A]_{50}$ estimates. It appeared unlikely that this was due to inadequate washing because this phenomenon occurred only in some of the experiments performed under the same conditions and further washings had not made a difference. Furthermore, in the present system, a progressive fall in the basal I_{sc} level over time had also been observed which was similar to the observations made by Jarnigan et al. (1983). This gradual decline in the basal I_{sc} level over time could not be prevented by modifying the experimental conditions and was, in most experiments, steady and gentle. It could be speculated that a gradual reduction in the activity of the basolateral Cl^--cation co-transport and/or Na^+-K^+-ATPase under the experimental conditions might be involved. Only one cumulative $E/[A]$ curve was, therefore, performed in each preparation and the preparations from each dog were used in random pairs simultaneously to provide a time control.

A range of values of the dissociation equilibrium constant for BW A868C from its antagonism to BW245C has been reported: 9.3 from human washed platelet and 8.7 from rabbit jugular vein (Giles et al., 1989); 8.6 and 8.3 from term pregnancy and non-pregnant human myometrium, respectively (Senior et al., 1992, 1993); 8.5 from rabbit saphenous vein (Lydford et al., 1994) and 7.4 from dog dorsal nasal vein (Liu and Jackson, 1995). In the present study, from dog tracheal epithelium a pK_B estimate of 8.2 for BW A868C was obtained from its antagonism to BW245C by using a modified Schild equation (refer to 2.3.3.). A comparable pK_A estimate of 8.0 for BW A868C was also obtained from its partial agonism by using a comparative method for the operational model of agonism (refer to 2.3.2) while assuming BW245C to be a full agonist in the system. In addition, data from the present study were also applied to an extended operational model of agonism, describing the interaction between a partial agonist and a full agonist at the same receptors (Leff et al., 1993), and the result was in accordance with the above findings (data not shown). In the light of such a wide range of values of the dissociation equilibrium constant for BW A868C over a variety of species and tissue systems and in the absence of further evidence suggesting possible DP receptor heterogeneity, it is prudent to suggest that these different affinity estimates for BW A868C could be due to species or tissue system-related variations in the functional expression of the DP receptors.

In summary, prostaglandin D_2 and BW245C have been shown to stimulate Cl^- secretion in the dog isolated tracheal epithelial preparations where BW245C was more potent than prostaglandin D_2. This potency order is consistent with the previous findings from the dog vascular smooth muscle preparations (Liu et al., 1996). Furthermore, in the present system, BW A868C a selective DP receptor antagonist has been shown to be a weak partial agonist. The consistent affinity estimates for BW A868C obtained from several analyses appeared to indicate that this partial agonist activity was mediated by the known DP receptors.

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