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Inhibitory action of the potassium channel opener BRL 38227 on agonist-stimulated phosphoinositide metabolism in bovine tracheal smooth muscle

Agents which increase the probability of potassium channel opening have been shown to cause hyperpolarization and relaxation of a variety of smooth muscles [1, 2]. The prototypic potassium channel opener cromakalim has been shown to be an effective inhibitor of agonist-induced bronchospasm in guinea-pigs *in vivo* [3], and *in vitro* [4] and *in vivo* [5] studies suggest that this class of agent also exhibits similar anti-spasmogenic properties in human subjects.

Spasmogenic agonists, such as acetylcholine and histamine, initiate airway smooth muscle contraction by activation of phosphoinositidase C to increase the rate of inositol phospholipid hydrolysis [6, 7]. The initial transient increase in inositol 1,4,5-trisphosphate has been causally related to initiation of contraction, whilst the sustained increase in phosphoinositide turnover, and consequent production of 1,2-diacylglycerol, may play an important role in the maintenance of the contractile response [6, 7]. Despite the wealth of functional data on the relaxant properties of potassium channel openers, little is known about the molecular mechanisms by which they bring about this action. In the present study, the consequences of potassium channel opening on spasmogen-stimulated phosphoinositide turnover has been investigated in bovine tracheal smooth muscle.

* Abbreviations: BTSM, bovine tracheal smooth muscle; [^{3}H]InsP$_x$, [^{3}H]inositol phosphates.

Materials and Methods

Cromakalim was synthesized in SmithKline Beecham Laboratories and was resolved into (−)- and (+)-enantiomers (BRL 38227 and 38226, respectively), as described previously [8]. All reagents were obtained from the same suppliers as previously [9, 10]. In addition, [2,8-^{3}H]adenosine 3′,5′-cyclic monophosphate was purchased from Amersham International (Amersham, U.K.) and glibenclamide was purchased from the Sigma Chemical Co. (Poole, U.K.)

Incubation techniques. Bovine tracheal smooth muscle (BTSM*) slices (300 μm × 300 μm) were prepared and incubated, and tissue inositol phospholipids were labelled with 1 μCi/mL [^{3}H]inositol, as described previously [9]. Aliquots of gravity-packed BTSM slices (75 μL) were incubated in a final volume of 500 μL of Krebs–Henseleit buffer containing 1 μCi/mL [^{3}H]inositol and 5 mM LiCl for 30 min, with regular gassing of vials with O_2/CO_2 (19:1). Additions of BRL 38227, BRL 38226 and/or glibenclamide were made 15 min before those of carbachol or histamine, and unless otherwise stated incubations were then continued for 30 min. All incubations were terminated by addition of 500 μL ice-cold 1 M trichloroacetic acid.

Measurement of [^{3}H]*InsP$_x$ and* [^{3}H]*inositol phospholipids.* After 20 min on ice, samples were centrifuged, the supernatant neutralized by repeated extraction with water-saturated diethylether and total [^{3}H]InsP$_x$ separated on Dowex 1-×8 (100–200 mesh, Cl^- form) columns [9, 11].

Incorporation of [^{3}H]inositol into inositol phospholipids was also assessed, as described previously [9, 11].

Measurement of cyclic nucleotides. Cyclic AMP and cyclic GMP concentrations were determined in neutralized BTSM tissue extracts using the methods described previously [10, 12]: for all samples the protein concentration in the tissue pellet was determined to allow cyclic nucleotide content to be expressed as pmol/mg of protein.

Results and Discussion

The effects of pre-incubation of BTSM slices in the presence of 5 μM BRL 38227 on the subsequent stimulation of [^{3}H]InsP$_x$ accumulation by carbachol and histamine are shown in Fig. 1. Maximally effective concentrations of carbachol (100 μM) caused an approximate 40-fold increase in [^{3}H]InsP$_x$ accumulation both in the absence and presence of BRL 38227. However, pre-addition of BRL 38227 significantly inhibited [^{3}H]InsP$_x$ accumulation evoked by low concentrations of carbachol (inhibition at 1 μM carbachol: 59 ± 4%, $P < 0.001$), causing a rightward-shift in the carbachol concentration–response curve (Fig. 1a). In contrast, 5 μM BRL 38227 caused a marked inhibition of [^{3}H]InsP$_x$ accumulation at all concentrations of histamine studied. Thus, the response to 1 mM histamine was inhibited by 68 ± 6% in the presence of BRL 38227 (Fig. 1b).

The differential effects of BRL 38227 on carbachol- and histamine-stimulated [^{3}H]InsP$_x$ accumulations in BTSM are similar to those reported for the inhibitory actions of β-adrenoceptor agonists, cyclic nucleotide phosphodiesterase inhibitors and other agents which elevate cyclic AMP concentrations in this tissue [13–15]. Therefore, the effects of BRL 38227 on cyclic nucleotide concentrations in BTSM slices was assessed under identical conditions to those used in the phosphoinositide turnover studies, except that [^{3}H]-inositol was omitted from the pre-incubation and incubation media. The presence of 5 μM BRL 38227 had no effect on tissue cyclic AMP or cyclic GMP concentrations (Table 1), whereas 10 μM isoprenaline and 100 μM nitroprusside caused highly significant increases in cyclic AMP and cyclic GMP levels, respectively. Both carbachol (1 μM) and histamine (100 μM) caused significant increases in the tissue concentrations of both cyclic nucleotides measured 10 min

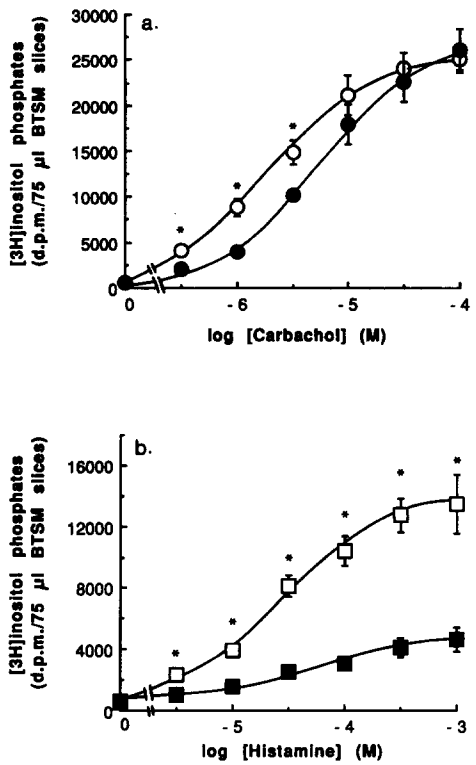

Fig. 1. Effects of BRL 38227 on (a) carbachol- and (b) histamine-stimulated [^{3}H]InsP$_x$ accumulations in BTSM. BRL 38227 (5 μM) was added 15 min prior to challenge with the indicated concentrations of carbachol or histamine. All incubations were continued for 30 min. Values are expressed as means ± SEM for at least three experiments performed in triplicate. Statistically significant differences (Student's *t*-test for unpaired observations) between incubations in the absence (○, □) and presence (●, ■) of BRL 38227 are indicated as *$P < 0.05$.

Table 1. Effects of BRL 38227 on cyclic nucleotide concentrations in BTSM slices challenged with carbachol or histamine

Addition	Time (min)	±BRL 38227	Cyclic nucleotide concn (pmol/mg of protein) cAMP	cGMP
—	0	−	2.17 ± 0.18	0.30 ± 0.05
		+	2.40 ± 0.16	0.40 ± 0.05
Isoprenaline	10	−	4.27 ± 0.20‡	0.35 ± 0.03
Nitroprusside	10	−	2.35 ± 0.08	10.8 ± 2.0‡
Carbachol	10	−	2.70 ± 0.07*	1.14 ± 0.10‡
		+	2.68 ± 0.08*	1.20 ± 0.18†
	30	−	2.52 ± 0.10	0.87 ± 0.07‡
		+	2.54 ± 0.11	0.84 ± 0.08†
Histamine	10	−	2.82 ± 0.19*	1.00 ± 0.15†
		+	2.81 ± 0.14	0.96 ± 0.20*
	30	−	2.34 ± 0.15	0.40 ± 0.08
		+	2.36 ± 0.12	0.46 ± 0.03

Where indicated BRL 38227 (5 μM) was added 15 min prior to carbachol (1 μM) or histamine (100 μM) additions for the stated times. Isoprenaline (10 μM) or sodium nitroprusside (100 μM) were used as positive controls for increasing the rates of cyclic AMP and cyclic GMP synthesis. Values are means ± SEM for three experiments performed in duplicate: statistically significant differences (Student's *t*-test for unpaired observations) from respective ± BRL 38227 control values are indicated as *$P < 0.05$; †$P < 0.01$ or ‡$P < 0.001$.

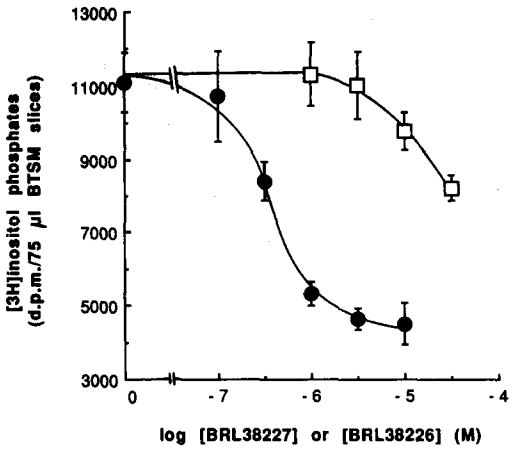

Fig. 2. Effects of increasing concentrations of BRL 38227 (●) or BRL 38226 (□) on histamine-stimulated [^{3}H]InsP$_x$ accumulations in BTSM. The indicated concentrations of BRL 38227 or BRL 38226 were added 15 min prior to challenge with 100 μM histamine. Values are expressed as means ± SEM for three experiments performed in triplicate. Basal [^{3}H]InsP$_x$ accumulations in the absence of histamine were: control, 645 ± 66; +10 μM BRL 38227, 599 ± 72; +30 μM BRL 38226, 641 ± 80 dpm/75 μL BTSM slices.

after agonist addition; however, pre-addition of BRL 38227 had no effect on the magnitude or duration of these responses (Table 1). We conclude that BRL 38227 is very unlikely to exert the observed inhibitory effect on spasmogen-stimulated [^{3}H]InsP$_x$ accumulations through an action on cyclic nucleotide metabolism. A similar conclusion was reached by other workers in studies on the effects of cromakalim on cyclic nucleotide concentration in guinea-pig trachealis muscle [16].

The effects of pre-incubation of BTSM slices with different concentrations of BRL 38227 or BRL 38226 on subsequent [^{3}H]InsP$_x$ accumulations stimulated by 100 μM histamine are shown in Fig. 2. BRL 38227 potently inhibited histamine-stimulated [^{3}H]InsP$_x$ accumulation with a maximal 65 ± 5% inhibition of this response observed at 10 μM BRL 38227. The concentration of BRL 38227 necessary to cause a half-maximal inhibition of the histamine-stimulated [^{3}H]InsP$_x$ response (IC_{50}) was determined to be 357 ± 54 nM using computer-assisted curve-fitting. The IC_{50} value for this effect on spasmogen-stimulated phosphoinositide metabolism is in good agreement with the IC_{50} values obtained for inhibition of spontaneous and prostaglandin E_2-induced tone in guinea-pig tracheal spirals by cromakalin [3]. Furthermore, the stereo-selectivity of the inhibitory response for the (−)-enantiomer is clearly illustrated in Fig. 2 with BRL 38226 being at least 100-fold less potent in causing this inhibitory effect. The action of BRL 38227 on histamine-stimulated [^{3}H]InsP$_x$ accumulation could be completely blocked (at least up to 30 μM BRL 38227) by the sulphonylurea glibenclamide (10 μM).

These data clearly demonstrate that the potassium channel opener BRL 38227 can potently inhibit [^{3}H]InsP$_x$ accumulations stimulated by histamine and sub-maximally effective concentrations of carbachol. Although these effects are qualitatively similar to those evoked by β-adrenoceptor agonists [13–15] they do not appear to be mediated by changes in tissue cyclic nucleotide concentrations. A more likely explanation, supported by preliminary results which suggest that glibenclamide can block the inhibitory effects of BRL 38227, is that opening of BRL 38227- and sulphonylurea-sensitive plasmalemmal potassium channels can directly or indirectly affect spasmogen-stimulated phosphoinositidase C activity. The target protein for BRL 38227 in tracheal smooth muscle is unlikely to be the large, Ca^{2+}-dependent K^+-channels with which this tissue is richly endowed [17, 18], but may be similar to the ATP-regulated K^+-channels found in other tissues [19].

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Department of Pharmacology and Therapeutics
University of Leicester
University Road
Leicester LE1 9HN; and
†*SmithKline Beecham Pharmaceuticals*
Biosciences Research Centre
Great Burgh, Epsom
Surrey KT18 5XQ, U.K.

R. A. JOHN CHALLISS*
NEELA PATEL
DAVID ADAMS
JONATHAN R. S. ARCH†

* Corresponding author: R. A. John Challiss, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, U.K.

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