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Electrical and mechanical effects of BRL34915 in guinea-pig isolated trachealis

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1 BRL34915 (0.1–10 μM) suppressed the spontaneous tone of guinea-pig isolated trachealis in a concentration-dependent manner. BRL34915 was not antagonized by propranolol (1 μM).

2 In trachea where spontaneous tone was suppressed by indomethacin (2.8 μM) but subsequently restored to the same level with acetylcholine or histamine, the relaxant potency of BRL34915 was reduced.

3 In Krebs solution containing K^+ (120 mM), isolated trachealis muscle developed near-maximal tension. The relaxant effects of BRL34915 were virtually abolished in this medium.

4 Concentration-effect curves for KCl, acetylcholine and histamine were constructed in tissues treated with indomethacin (2.8 μM). BRL34915 (10 μM) depressed the foot of the concentration-effect curve for KCl and caused minor rightward shifts in the concentration-effect curves of acetylcholine and histamine.

5 Four K^+-channel inhibitors were tested. Apamin (0.1 μM) did not modify the action of BRL34915. Tetraethylammonium (8 mM) had little effect but procaine (5 mM) and 4-aminopyridine (5 mM) each significantly inhibited the relaxant action of BRL34915.

6 Intracellular electrophysiological recording showed that BRL34915 (0.1 μM) caused very minor relaxation and little, if any, electrical change. Higher concentrations (1–10 μM) evoked relaxation, suppression of spontaneous electrical slow waves and marked hyperpolarization of the trachealis cells. In the presence of TEA (8 mM) or procaine (5 mM) the hyperpolarization induced by BRL34915 was significantly reduced.

7 In trachealis skinned of its plasma membranes, tension development induced by Ca^{2+} (20 μM) was unaffected either by BRL34915 (10 μM) or by nicorandil (1 mM).

8 In studies of the efflux of $^{86}Rb^+$ from muscle-rich strips of trachea, BRL34915 (1 and 10 μM) increased the effux rate constant.

9 It is concluded that BRL34915 evokes relaxation of the trachealis by a mechanism that involves neither β-adrenoceptor activation nor direct reduction of the sensitivity of the intracellular contractile machinery to cytosolic free Ca^{2+}. The action of BRL34915 may depend on the opening of K^+ channels in the plasma membrane which are permeable to $^{86}Rb^+$. The opening of these channels, or the effects of their opening, may be reduced by K^+-channel inhibitors such as 4-aminopyridine, procaine and TEA but not by apamin.

Introduction

BRL34915, (±)6-cyano-3,4-dihydro-2, 2-dimethyl-*trans*-4- (2-oxo-1-pyrrolidyl)-2H-benzo[b] pyran-3-ol, is a novel antihypertensive agent (Ashwood *et al.*, 1984) which has inhibitory effects on vascular and gastrointestinal smooth muscle. In rat portal vein these inhibitory effects are manifest as suppression of spontaneous tension waves, suppression of spontaneous spike potentials and hyperpolarization of the cell membrane to a value close to the K^+ equilibrium potential. In rat portal vein and aorta, BRL34915 suppresses spasm induced by K^+ but only for those concentrations ($<$20 mM) of K^+ where the K^+-equilibrium potential is more negative than the transmembrane potential. In rat portal vein and guinea-pig

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taenia caeci previously loaded with $^{86}Rb^+$, BRL34915 stimulates the efflux of $^{86}Rb^+$. Evidence of this kind has led to the suggestion that the smooth muscle inhibitory effects of BRL34915 depend on its ability to open K^+ channels in the plasma membrane (Hamilton *et al.*, 1986; Weir & Weston, 1986a,b).

In the present study we have examined the effects of BRL34915 on guinea-pig isolated trachealis with a view to gaining more insight into the smooth muscle relaxant effects of this compound and into the nature of the K^+-channels of the trachealis cell membrane.

Methods

Guinea-pigs (300–750 g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

Tissue bath studies of mechanical activity of the trachealis

Small segments of trachea were set up for the isometric recording of tension changes as described by Foster *et al.* (1983). At the outset of each experiment tissues were subjected to imposed tension of 1 g. Approximately 20 min later aminophylline (1 mM) was added in order to determine the recorder pen position at zero tone. The aminophylline was washed from the tissues and when tone subsequently became maximal, study of relaxant drugs started.

Effects of BRL34915 on spontaneous tracheal tone

The relaxant effects of isoprenaline (0.001 to 1 μM) and BRL34915 (0.1 to 10 μM) were studied by constructing cumulative concentration-effect curves. Ten fold concentration increments were used at intervals of 4 (isoprenaline) or 8 min (BRL34915). Following construction of initial log concentration-effect curves for the relaxant drugs, tissues were allocated randomly to test or time-matched control groups. Test tissues were treated with Krebs solution containing propranolol (1 μM) and allowed to equilibrate with this antagonist for at least 10 min before the log concentration-effect curves of isoprenaline and BRL34915 were reconstructed. Time-matched control tissues were treated similarly but were not exposed to propranolol.

Effects of BRL34915 on tracheal tone supported by acetylcholine or histamine

Following construction of an initial log concentration-effect curve for BRL34915, tissues were exposed to Krebs solution containing indomethacin (2.8 μM). When all spontaneous tone had been suppressed (30–40 min) it was restored to the pre-indomethacin level with either acetylcholine (2–20 μM) or histamine (10–30 μM). The log concentration-effect curve for BRL34915 was then reconstructed.

Effects of K^+-rich Krebs solution or K^+-channel inhibitors on the relaxant action of BRL34915

Following construction of an initial log concentration-effect curve for BRL34915, tissues were allocated randomly to test or time-matched control groups. Test tissues were exposed to K^+-rich Krebs solution (see below) or Krebs solution containing a K^+-channel inhibitor (apamin 0.1 μM; TEA, 8 mM; procaine, 5 mM; 4-aminopyridine, 5 mM). These modifying agents were allowed at least 20 min equilibration with the test tissues before the log concentration-effect curve of BRL34915 was reconstructed. Control tissues were treated similarly but were not exposed to the modifying agents.

Effects of BRL34915 on log concentration-effect curves of KCl, acetylcholine or histamine

These experiments were carried out in Krebs solution containing indomethacin (2.8 μM). Cumulative concentration-effect curves were constructed for KCl (5–40 mM), acetylcholine (1–1000 μM) or histamine (2–200 μM). Two fold concentration increments were used for KCl (12 min intervals) and ten fold increments for acetylcholine (3 min intervals) and histamine (6 min intervals). Following the construction of an initial concentration-effect curve for one of these spasmogens, tissues were allocated randomly to test or time-matched control groups. Test tissues were exposed to Krebs solution containing BRL34915 (10 μM). The BRL34915 was allowed at least 10 min equilibration with test tissues before the spasmogen concentration-effect curve was reconstructed. Control tissues were treated similarly but were not exposed to BRL34915.

Intracellular electrophysiological recording from trachealis

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed by use of the technique of Dixon & Small (1983).

The effects of BRL34915 on spontaneous electrical and mechanical activity of the trachealis were studied as follows. After impalement of a trachealis cell, 3 min were allowed to elapse to check that the record of electrical activity had stabilised. BRL34915 (0.1, 1 or 10 μM) was then added to the Krebs solution. The effects of BRL34915 were monitored for 8 min. The

drug was then washed from the tissue and recovery of electrical and mechanical activity was monitored until the pre-BRL34915 activity was regained or the microelectrode became dislodged from the cell.

Similar procedures were adopted when assessing the electrical responses to BRL34915 in tissue pretreated (<20 min) with TEA (8 mM) or procaine (5 mM).

Estimation of effects of BRL34915 on $^{86}Rb^+$ efflux

Muscle-rich strips of trachea were prepared as described by Foster *et al*, (1983). The efflux of $^{86}Rb^+$ from the tissues was then studied essentially as described by Allen *et al.* (1986a,b). After pre-incubation at 37.5°C in 5 ml of Krebs solution bubbled with 95% O_2:5% CO_2, all tissues were loaded with $^{86}Rb^+$ by 150 min incubation with 74 MBq l^{-1} and 17 μM $^{86}RbCl$ in Krebs solution.

Each tissue was then transferred to the first of a series of 19 washing samples of 5 ml of Krebs solution at 37.5°C. Tissues remained in each washing sample for 4 min. In the case of test tissues the 10th, 11th and 12th washing samples contained BRL34915 (1 or 10 μM).

At the end of the efflux period, tissue digests and media were assayed for radioactivity and values of efflux rate constant were calculated as previously described (Allen *et al.*, 1986a).

Experiments with trachealis skinned of its plasma membranes

Segments of trachea were prepared as described above. The trachealis was then skinned of its plasma membranes with Triton X-100 and was arranged for tension recording as previously described (Allen *et al.*, 1986b). Tension development was induced by addition of $CaCl_2$ in amount calculated to yield a free Ca^{2+} concentration of 20 μM.

In test tissues three such Ca^{2+} challenges were performed. BRL34915 (10 μM) or nicorandil (1 mM) was present for 8 or 6 min respectively before and throughout the 2nd Ca^{2+} challenge. Acetylcholine (100 μM) was added to the tissues once full relaxation was achieved after the third Ca^{2+} challenge. Time-matched control tissues were treated similarly except that they were not exposed to BRL34915 or nicorandil.

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The following substances were used: acetylcholine chloride (Sigma), aminophylline (BDH), 4-aminopyridine (Sigma), apamin (Sigma), BRL34915 (Beechams Research Laboratories), histamine acid phosphate (Sigma), indomethacin (Sigma), (−)-isoprenaline hydrochloride (Sigma), nicorandil (Chugai, Japan), procaine hydrochloride (Sigma), propranolol hydrochloride (ICI), tetraethylammonium bromide (Sigma).

Stock solutions of BRL34915 and isoprenaline were prepared in 70% v/v ethanol and 0.1 M HCl respectively, those of other agents in twice-distilled water. Dilutions of isoprenaline were prepared in distilled water containing 0.57 mM ascorbic acid as an antioxidant.

The Krebs solution used in the majority of experiments had the following composition (mM): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 125, HCO^-_3 25, SO_4^{2-} 1.2, $H_2PO^-_4$ 1.2 and glucose 11.1. The K^+-rich Krebs solution was of identical osmolality to Krebs solution and had the following composition (mM): Na^+ 26, K^+ 120, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 125, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1.

The solutions used in experiments with skinned trachea were as previously described (Allen *et al.*, 1986b).

The significance of differences between two means was assessed by a one-tailed or a two-tailed unpaired *t* test. Larger groups were assessed by analysis of variance and the Studentized range test. A difference between means was assumed to be significant when $P<0.05$.

Results

Tissue bath studies of mechanical activity

Effects of BRL34915 on spontaneous tracheal tone Isoprenaline (0.001–1 μM) and BRL34915 (0.1 to 10 μM) each caused concentration-dependent suppression of spontaneous tracheal tone. Relaxation evoked by isoprenaline reached equilibrium within 4 min whilst that evoked by BRL34915 required 8 min to equilibrate. BRL34915 (10 μM) yielded relaxation which closely approached the isoprenaline maximum.

In time-matched control tissues the log concentration-effect curves for both isoprenaline and BRL34915 moved slightly to the left after tissue incubation in Krebs solution. In the corresponding test tissues, propranolol (1 μM) evoked a rightward shift of the isoprenaline curve which was greater than 100 fold. However, the log concentration-effect curve for BRL34915 was shifted only 2 fold to the right (Figure 1). Following correction for shifts seen in control tissues, the mean of values of the $\log_{10}$ dose-ratio for BRL34915 was not significantly ($P>0.05$) different from zero, indicating that propranolol had not antagonized BRL34915.

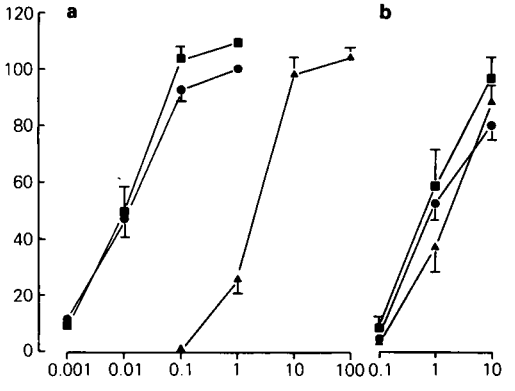

Figure 1 The effects of propranolol (1 μM) on the relaxant actions of isoprenaline and BRL34915 in guinea-pig isolated trachealis. The abscissa scale indicates the concentration (μM) of isoprenaline (a) or BRL34915 (b) on a log scale. The ordinate scale represents relaxation as a % of the inital maximal response to isoprenaline. (●) = pooled initial log concentration-effect curve for test and control tissues. (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation with Krebs solution. (▲) = log concentration-effect curve constructed in test tissues after at least 10 min exposure to propranolol (1 μM). Data indicate the means of values from at least 6 tissues; s.e. mean shown by vertical bars.

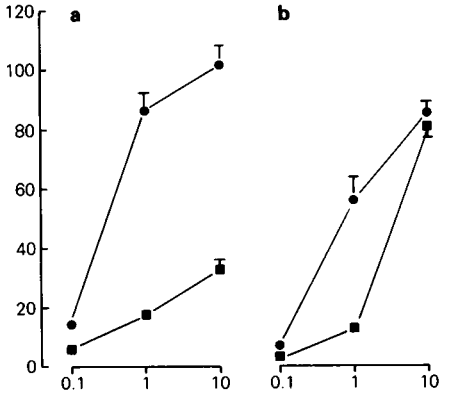

Figure 2 Acetylcholine and histamine as tone-inducing agents: their effects on the relaxant action of BRL34915 in guinea-pig trachealis. The abscissa scale indicates the concentration (μM) of BRL34915 on a log scale. The ordinate scale represents relaxation as a % of the maximal relaxation induced by aminophylline. (●) = initial log concentration-effect curve constructed in normal Krebs solution. (■) = log concentration-effect curve constructed following abolition of tissue tone by indomethacin (2.8 μM) and its subsequent restoration to control levels using either acetylcholine (a) or histamine (b). Data indicate the means of values from at least 6 tissues; s.e. mean shown by vertical bars.

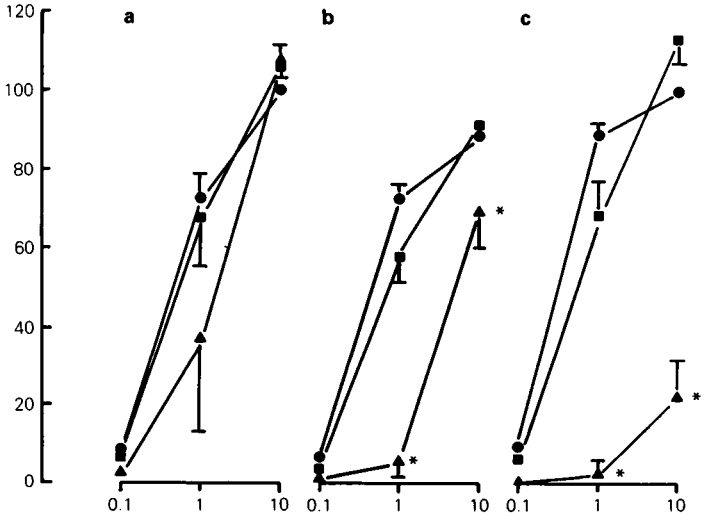

Figure 3 The effects of some K^+-channel inhibitors on the relaxant action of BRL34915 in guinea-pig isolated trachealis. The abscissae indicate the concentration (μM) of BRL34915 on a log scale. The ordinates represent relaxation as a % of the initial maximum. (●) = pooled initial log concentration-effect curve for test and control tissues. (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution. (▲) = log concentration-effect curve constructed in test tissues after at least 10 min exposure to TEA (8 mM, a), to procaine (5 mM, b) or to 4-aminopyridine (5 mM, c). Data indicate means of values from at least 6 tissues; s.e. mean shown by vertical bars. * indicates a significant difference from the corresponding point for control tissues.

Effects of BRL34915 on tracheal tone supported by acetylcholine or histamine

When acetylcholine was used to restore tone in indomethacin-treated tissues, the log concentration-effect curve for BRL34915 was markedly depressed (Figure 2). In the presence of histamine as a tone-restoring agent, the log concentration-effect for BRL34915 was moved to the right but there was no evidence to suggest that the maximal effect of BRL34915 had been reduced.

Effects of K^+-channel inhibitors on the relaxant action of BRL34915

Apamin (0.1 μM) itself caused no change in tracheal tone and did not modify the relaxant action of BRL34915. TEA (8 mM), procaine (5 mM) and 4-aminopyridine (5 mM) each evoked tracheal spasm. There was a tendency (particularly in the case of TEA and procaine) for this spasm to be tonic initially but to become phasic subsequently.

Procaine and 4-aminopyridine each antagonized BRL34915 (Figure 3), the antagonism being more marked in the case of 4-aminopyridine. TEA caused some depression of the central part of the log concentration-effect curve of BRL34915 but this did not reach the level of significance ($P = 0.1$).

Effects of K^+-rich Krebs solution on BRL34915-induced relaxation

As reported previously (Allen *et al.*, 1986a,b) preparations of trachealis exposed to Krebs solution containing K^+ (120 mM) developed tension which was tonic, near-maximal and well-maintained. In this medium the relaxant effects of BRL34915 were all but abolished (Figure 4).

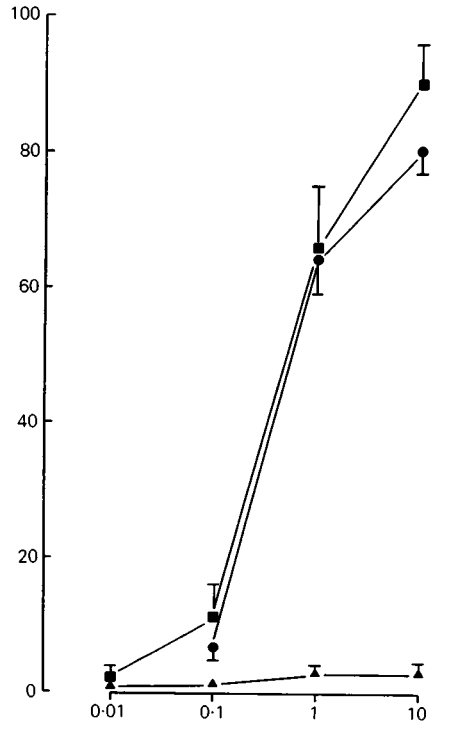

Figure 4 The effects of K^+-rich Krebs solution on the relaxant action of BRL34915 in guinea-pig isolated trachealis. The abscissa scale indicates the concentration (μM) of BRL34915 on a log scale. The ordinate scale represents relaxation as a % of the maximal relaxation induced by aminophylline. (●) = pooled initial log concentration-effect curve for test and control tissues. (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution. (▲) = log concentration-effect curve constructed in test tissues after 40 min exposure to K^+-rich (120 mM) Krebs solution. Data indicate means of values from at least 9 tissues; s.e. mean shown by vertical bars.

Table 1 The effects of BRL34915 on the membrane potential of smooth muscle cells of the guinea-pig isolated trachealis bathed either by normal Krebs solution or Krebs solution containing TEA (8 mM) or procaine (5 mM)

BRL34915 concentration (μM)	*Change in membrane potential (mV) after 8 min*		
	Normal Krebs	*TEA* (8 mM)	*Procaine* (5 mM)
0.1	−2.3 ± 2.0	------	------
1	+11.9 ± 2.8	+5.9 ± 4.3	−0.4 ± 0.7*
10	+26.3 ± 2.9	+15.7 ± 2.7*	------

Data represent the means of observations from at least 6 cells ± s.e. mean. A positive value indicates hyperpolarization. * indicates a significant ($P < 0.05$) change in the hyperpolarization compared with that seen in normal Krebs solution (two-tailed unpaired *t* test).

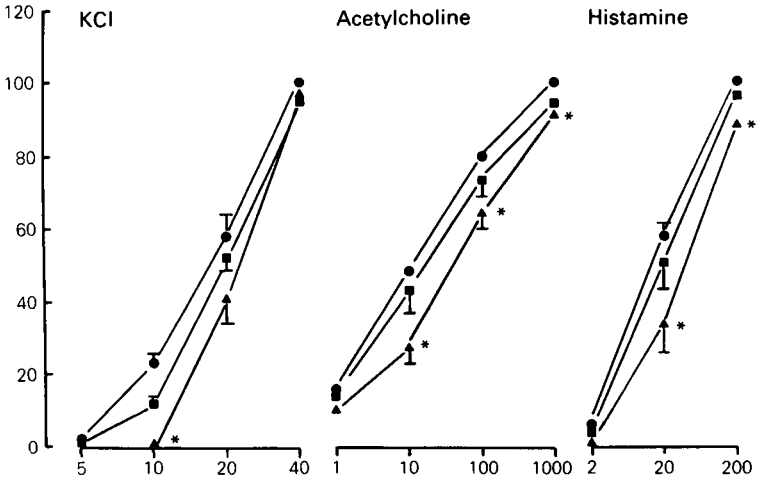

Figure 5 The effect of BRL34915 on the log concentration-effect curves of KCl, acetylcholine and histamine in guinea-pig isolated trachealis. The abscissa scales represent the concentrations of KCl (mM), acetylcholine (μM) or histamine (μM) on a log scale. The ordinate scale represents tension as a % of the greatest tension achieved in the initial log concentration-effect curve of each spasmogen. (●) = pooled initial log concentration-effect curve for the test and control tissues. (■) = subsequent log concentration-effect curve constructed in control tissues after further incubation in Krebs solution. (▲) = log concentration-effect curve constructed in test tissues treated with 10 μM BRL34915. Indomethacin (2.8 μM) was present throughout these experiments. Data indicate the means of values from at least 6 tissues; s.e. mean shown by vertical bars. * indicates a significant difference from the corresponding point for control tissues.

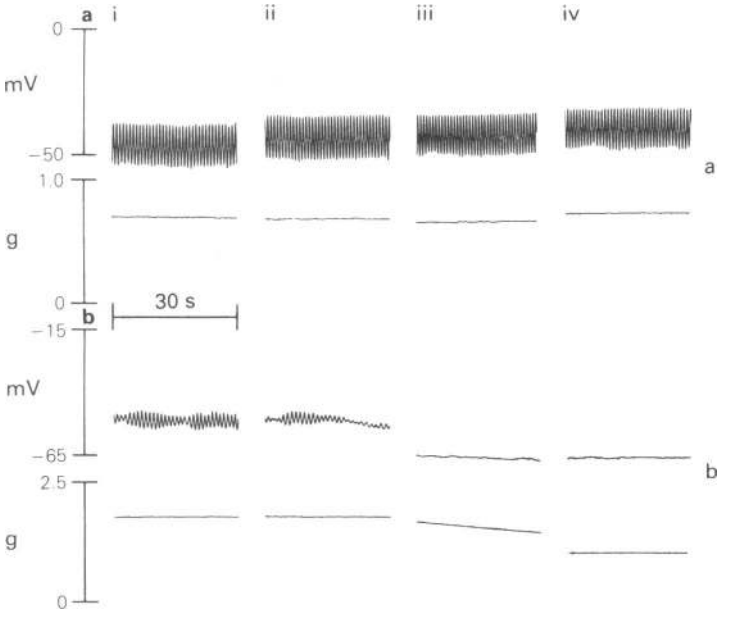

Figure 6 Effects of BRL34915 (0.1 and 1 μM) on the electrical and mechanical properties of guinea-pig isolated trachealis. The upper (a) and lower (b) rows of records indicate results obtained from two different cells. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. Activity was recorded (i) before, (ii) 1.5, (iii) 4 and (iv) 8 min after the addition of BRL34915 0.1 μM (a) or 1 μM (b).

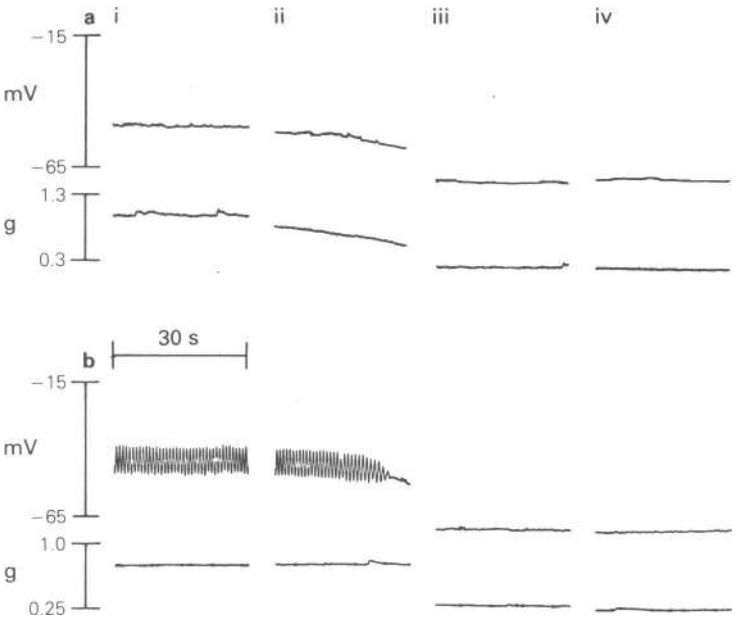

Figure 7 Effects of BRL34915 (10 μM) on the electrical and mechanical properties of guinea-pig isolated trachealis. The upper (a) and lower (b) rows of records indicate results obtained from a quiescent and a spontaneously active cell respectively. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In (a) activity was recorded (i) before, (ii) 2, (iii) 4 and (iv) 8 min after addition of BRL34915 10 μM. In (b) activity was recorded (i) before, (ii) 1, (iii) 4 and (iv) 8 min after addition of BRL34915 10 μM.

The influence of BRL34915 on log concentration-effect curves of KCl, acetylcholine and histamine

These experiments were conducted in Krebs solution containing indomethacin (2.8 μM) to suppress spontaneous tone. Under these conditions KCl (5–40 mM), acetylcholine (1–1000 μM) and histamine (2–200 μM) each caused concentration-dependent tension development. The use of time-matched control tissues showed that the log concentration-effect curves of the three spasmogens moved slightly to the right when reconstructed following further tissue incubation in

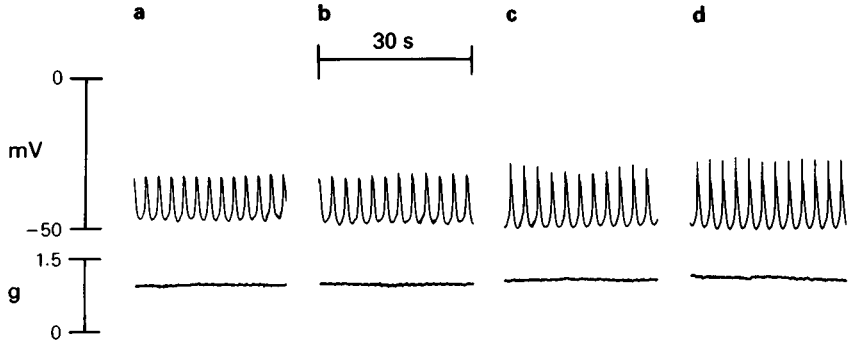

Figure 8 The effects of BRL34915 (1 μM) on the electrical and mechanical activity of guinea-pig isolated trachealis pretreated (> 20 min) with procaine (5 mM). In each case the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. All electrical recordings are from the same cell. Activity was recorded (a) before, (b) 1.5, (c) 4 and (d) 8 min after the addition of BRL34915 1 μM. Note the very minor changes in resting membrane potential and mechanical tone evoked by BRL34915 in the procaine-treated tissue.

indomethacin-containing Krebs solution (Figure 5).

Treatment of test tissues with 10 μM BRL34915 caused very minor rightward shifts in the log concentration-effect curves of acetylcholine and histamine. The concentration-effect curve of KCl was differently affected by BRL34915 in that the foot of the curve (below 20 mM KCl) was selectively depressed (Figure 5).

Intracellular electrophysiological recording

In these experiments BRL34915 (0.1 μM) caused very slight relaxation. The simultaneously-recorded membrane potential changes were also small and were variable in direction (Figure 6 and Table 1). The spontaneous discharge of electrical slow waves (if present) was not affected by this concentration of BRL34915. Higher concentrations (1 and 10 μM) evoked concentration-dependent hyperpolarization (Figures 6 and 7, Table 1) which required more than 6 min to develop fully. Spontaneous slow waves were abolished within 4 (1 μM) or 3 (10 μM) min drug contact and marked relaxation occurred.

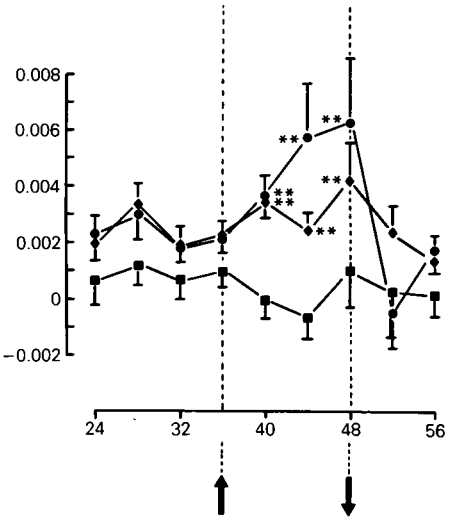

Figure 9 The effects of BRL34915 on the efflux of $^{86}Rb^+$ from muscle-rich strips of guinea-pig isolated trachea. The abscissa scale indicates time (min) and the ordinate scale represents the relative efflux rate constant (min^{-1}). Drug (test tissues only) was present during the period between the arrows. Time-matched control tissues (■); Test tissues treated with BRL34915 1 μM (◆) or 10 μM (●). Data indicate means of values from 8 tissues:vertical bars indicate s.e. mean. ** indicates a significant ($P < 0.01$) difference from the corresponding point in the time-matched control tissues.

In tissue treated with TEA (8 mM), trachealis muscle cells generated slow waves with superimposed spike potentials. Sometimes spikes were discharged continuously and sometimes periodically. The associated tension development could also be tonic or phasic. In the presence of TEA the ability of BRL34915 to hyperpolarize the tissue was reduced (Table 1). Nevertheless, BRL34915 could suppress TEA-induced oscillations of membrane potential and evoke relaxation.

Trachealis cells treated with procaine (5 mM) often generated large slow waves. Sometimes these were surmounted by spike potentials. In the presence of procaine the hyperpolarization and relaxation induced by BRL34915 (1 μM) were markedly reduced (Table 1 and Figure 8).

$^{86}Rb^+$ efflux studies

After an initial very high value, the efflux rate constant of the muscle-containing tracheal strips quickly settled to a low and consistent value as described previously (Allen *et al.*, 1986a,b). Test tissues were exposed to BRL34915 for the period between 36 and 48 min after the start of efflux.

That BRL34915 had affected the $^{86}Rb^+$ efflux rate constant was suggested by the results of within-group (the 8 tissues similarly treated) comparisons over the time period 24–76 min. No sample in the control group differed ($P > 0.05$) from any other. The third sample collected in the presence of 1 μM BRL34915 was greater than 3 subsequent samples. The second and third samples collected in the presence of 10 μM BRL34915 were greater than 5 subsequent samples. The effect of BRL34915 was more clearly revealed by between-group comparisons. Such comparisons (made over the same time period) revealed highly significant differences between test (1 or 10 μM BRL34915) tissues and controls. These differences occurred only during the period of drug contact with the test tissues (Figure 9). Concentration-dependency of the effect is indicated by the greater enhancement of efflux produced by 10 μM BRL34915.

Experiments with skinned trachea

As reported previously (Allen *et al.*, 1986b), skinned trachea responded to an initial Ca^{2+} (20 μM) challenge by generating tension which reached a peak value within 25 min. Responses to subsequent Ca^{2+} challenges became progressively smaller (Figure 10). Acetylcholine (100 μM) never evoked spasm of the skinned preparation.

When test tissues were compared with their appropriate time-matched controls it was evident that neither BRL34915 (10 μM) nor nicorandil (1 mM) reduced the spasm evoked by Ca^{2+} in skinned trachea.

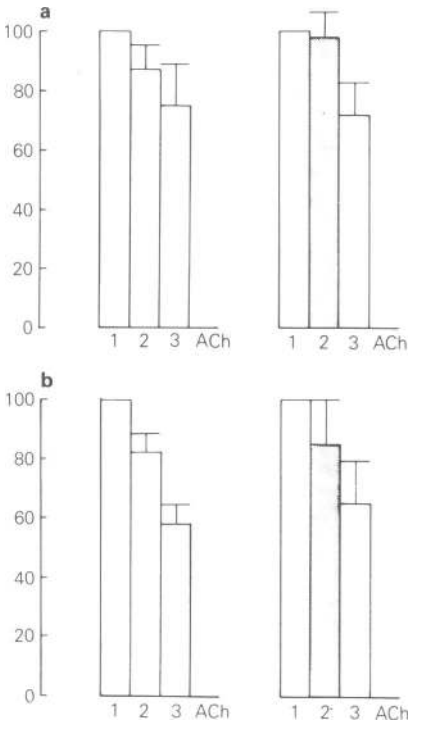

Figure 10 The effects of BRL34915 and nicorandil on responses of skinned trachealis to Ca^{2+}. The abscissa scales indicate the first, second and third (1,2,3) challenge with 20 μM Ca^{2+} or with 100 μM acetylcholine (ACh). Left hand panels: time-matched control tissues. Right hand panels: test tissues. Shaded columns indicate the presence of BRL34915 10 μM (Row a) or nicorandil 1 mM (Row b). Column heights represent means of experimental values from at least 7 tissues; s.e.mean shown by vertical bars. There was no significant difference ($P > 0.05$) between column 2 means either in Row a or b (two-tailed unpaired t test).

Discussion

BRL34915-induced relaxation: an action on the intracellular contractile machinery or the plasma membrane?

The failure of acetylcholine to evoke spasm has been used as a test of the functional completeness of the process of skinning trachealis cells of their plasma membranes (Ito & Itoh, 1984; Allen *et al.*, 1986b). Such a test was satisfied in the present study. The failure of either BRL34915 or nicorandil to reduce the Ca^{2+}-induced spasm of skinned trachea indicates that neither of these agents acts directly to reduce the sensitivity of the intracellular contractile machinery to Ca^{2+}.

If BRL34915 instead acts on the trachealis plasma membrane then such an action does not involve the activation of β-adrenoceptors, for propranolol (1 μM) profoundly antagonized isoprenaline yet failed to modify the action of BRL34915.

Hamilton *et al.* (1986) observed that BRL34915 caused hyperpolarization of the smooth muscle cells of rat portal vein. A similar membrane potential change was observed in trachealis cells of the guinea-pig (present study) and supports the suggestion (Hamilton *et al.*, 1986; Weir & Weston, 1986a,b) that BRL34915 may open membrane K^+-channels.

In the trachealis this suggestion is strengthened by the fact that BRL34915 was able to raise the membrane potential to a value approaching the calculated (− 77 mV; Kirkpatrick, 1981) K^+ equilibrium potential. The ability of the K^+-channel inhibitors procaine and TEA to reduce BRL34915-induced hyperpolarization adds additional weight to the argument.

Nature of the K^+-channels opened by BRL34915

The failure of apamin to modify the mechanical tone of guinea-pig trachealis or its spontaneous electrical activity (Allen *et al.*, 1985; 1986a; present study) suggest that apamin-sensitive K^+-channels do not play a role in tone maintenance in this tissue. The present failure of apamin to antagonize BRL34915 acting on the trachea is consistent with the apamin-resistant action of BRL34915 in the taenia caeci (Weir & Weston, 1986a) and argues against the involvement of apamin-sensitive K^+ channels in the smooth muscle relaxant action of BRL34915.

Nicorandil and BRL34915 each causes apamin-resistant relaxation of the trachea. Each promotes $^{86}Rb^+$ efflux from the tissue and each causes hyperpolarization which can be reduced by procaine and, to a lesser extent, by TEA (Allen *et al.*, 1986a; present study). It is possible, therefore, that BRL34915 and nicorandil open the same K^+ channel, a channel which might be similar to the apamin-insensitive, Ca^{2+}-dependent K^+ channel opened by nicorandil (Yamanaka *et al.*, 1985) in guinea-pig small intestine. However, the present results cannot be regarded as proof of this hypothesis. The present results with K^+-channel inhibitors do not permit more detailed characterization of the K^+ channels opened by BRL34915 in trachealis, for procaine is a non-selective inhibitor of smooth muscle K^+-channels (Yamanaka *et al.*, 1985) and the selectivity of TEA and 4-aminopyridine among such channels remains to be determined.

Role of hyperpolarization in BRL34915-induced relaxation

Previous electrophysiological studies (Inoue *et al.*, 1983; Allen *et al.*, 1985; 1986a,b) have shown that

hyperpolarization is not crucial for the tracheal relaxant actions of isoprenaline, nicorandil and aminophylline. For each of these agents some separation was evident between their log concentration-relaxation and log concentration-hyperpolarization curves as observed in normal Krebs solution. Each of these agents was able to evoke relaxation in a K^+-rich (120 mM) medium in which the K^+-equilibrium potential and the transmembrane potential were virtually coincident. Furthermore, K^+-channel inhibitors such as procaine and TEA could reduce the hyperpolarization induced by these agents with little or no antagonism of their relaxant actions.

BRL34915 therefore clearly differs from isoprenaline, nicorandil or aminophylline, for there was little evidence of separation between its log concentration-relaxation and log concentration-hyperpolarization curves. The relaxant effect of BRL34915 was abolished in the K^+-rich (120 mM) medium and procaine reduced both the hyperpolarization and the relaxant activity of BRL34915. These observations suggest that the relaxant action of BRL34915 is very dependent upon its ability to hyperpolarize the trachealis cells. This conclusion is supported by the observation (Figure 5) that BRL34915 was able to depress the foot but not the central or upper parts of the KCl log concentration-effect curve. Similar effects have been observed in rat portal vein and aorta (Hamilton *et al.*, 1986; Weir & Weston, 1986b). The critical point on the KCl concentration axis below which BRL34915 inhibits spasm is where the K^+ equilibrium potential is sufficiently more negative than the transmembrane potential to allow K^+-channel opening to cause hyperpolarization great enough to close voltage-operated Ca^{2+} channels and therefore mediate inhibition.

Possible roles of K^+-channels and BRL34915 in asthma

We have recently (Small & Foster, 1986) reviewed evidence which suggests that airways smooth muscle cells are normally of low excitability due to the presence of potential-dependent K^+-channels in the plasma membrane. By opening as resting membrane potential falls, such channels exert a powerful rectifying effect against the depolarization evoked by excitatory stimuli. One consequence of this is that airways smooth muscle cells are normally incapable of supporting spike-like action potentials. Instead these cells may exhibit slow waves which represent action potentials aborted by the opening of the potential-dependent K^+-channels.

In the presence of K^+-channel inhibitors such as TEA, rectification is reduced. The smooth muscle cells exhibit spike-like action potentials and, in consequence, can develop tension more rapidly and powerfully. Inhibition of K^+-channel opening might therefore account for the hyper-reactivity of airway smooth muscle seen in asthma. One observation in support of this hypothesis is provided by the extracellular recording of larger and more frequent action potentials from human airways muscle during asthmatic episodes (Akasaka *et al.*, 1975).

If the hyper-reactivity of airways smooth muscle which is characteristic of asthma indeed represents altered gating properties of membrane K^+ channels, then a drug like BRL34915 might prove useful as a therapeutic agent acting to return the membrane K^+-channels towards their normal state of opening. However, a finding which might suggest only limited efficacy in asthma is the relatively poor antagonism provided by BRL34915 against acetylcholine and histamine. In any event BRL34915 will surely prove to be a useful scientific tool for investigating the nature and function of K^+-channels in the smooth muscle cell membrane.

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