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Tracheal relaxation induced by potassium channel opening drugs: its antagonism by adrenergic neurone blocking agents

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1 We have studied the ability of some adrenergic neurone blocking agents to inhibit the tracheal relaxant actions of isoprenaline, theophylline and the potassium channel openers (KCOs) BRL 38227, pinacidil and RP 52891.

2 BRL 38227, isoprenaline, pinacidil, RP 52891 and theophylline each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. The maximal relaxant effects of isoprenaline and pinacidil were equal to that of theophylline. In contrast, the maximal effects of BRL 38227 and RP 52891 were approximately 85–95% of that of theophylline.

3 Guanethidine (5–500 μM) did not itself modify the spontaneous tone of the trachealis muscle but antagonized BRL 38227 in a concentration-dependent manner. Guanethidine (50 μM) also antagonized pinacidil and RP 52891. However, guanethidine did not antagonize either isoprenaline or theophylline.

4 Bretylium (50 μM) did not itself modify the spontaneous tone of the trachealis muscle but antagonized BRL 38227, pinacidil and RP 52891. Bretylium did not antagonize either isoprenaline or theophylline.

5 Guanidine (50 and 500 μM) did not itself modify the spontaneous tone of the trachea and failed to modify the tracheal relaxant activity both of BRL 38227 and theophylline.

6 BRL 38227 (1 and 10 μM) stimulated, in a concentration-dependent manner, the efflux of $^{86}Rb^+$ from strips of bovine trachealis muscle that had been pre-loaded with the radiotracer. Guanethidine (50 μM), bretylium (50 μM) and debrisoquine (50 μM) did not themselves modify the efflux of $^{86}Rb^+$ from bovine trachealis but each of these agents markedly inhibited the stimulant effect of BRL 38227 (10 μM) on $^{86}Rb^+$ efflux.

7 It is concluded that the adrenergic neurone blocking agents guanethidine and bretylium can inhibit the tracheal relaxant actions of KCOs such as BRL 38227, pinacidil and RP 52891 without antagonizing isoprenaline or theophylline. The ability of the adrenergic neurone blocking agents to antagonize BRL 38227 in promoting $^{86}Rb^+$ efflux from trachealis muscle may suggest that the adrenergic neurone blocking agents act to prevent the opening of the plasmalemmal K^+-channel that is involved in the tracheal relaxant actions of the KCOs.

Keywords: Trachealis muscle; BRL 38227; RP 52891; theophylline; isoprenaline; guanethidine; bretylium; debrisoquine; guanidine; $^{86}Rb^+$ efflux

Introduction

In order to study the effects of cromakalim (a potassium channel opener) on excitatory, non-adrenergic, non-cholinergic (NANC) neuroeffector transmission in guinea-pig isolated trachealis, Burka *et al.* (1991) set up tracheal segments for the isometric recording of tension changes in Krebs solution containing atropine (1 μM), indomethacin (2.8 μM) and propranolol (1 μM). The tracheal segments were subjected to transmural electrical stimulation and cromakalim was observed to cause a concentration-dependent inhibition of the NANC contractile responses. Whilst performing further, similar experiments, we decided to compare the effects of isoprenaline with those of cromakalim against excitatory NANC neuroeffector transmission in the trachea. The use of isoprenaline in these experiments dictated that an alternative sympatholytic agent be substituted for propranolol as a component of the antagonist cocktail in the Krebs solution. Accordingly, guanethidine (50 μM) was substituted for propranolol. Under these conditions we noticed that the inhibitory potency of cromakalim against excitatory NANC neuro-effector transmission was reduced. This raised the possibility that guanethidine might antagonize cromakalim in suppressing the spontaneous tone of guinea-pig trachealis muscle.

Our preliminary observation that guanethidine, indeed, antagonized cromakalim in suppressing the spontaneous tone of the trachea prompted the experiments of the present study. In these experiments we have attempted to assess the selectivity of guanethidine as an antagonist of BRL 38227 (a benzopyran derivative; the active enantioner of cromakalim), whether guanethidine antagonizes other KCOs (e.g. the cyanoguanidine derivative, pinacidil and the tetrahydrothiopyran derivative, RP 52891) and whether the antagonism of KCOs provided by guanethidine is a property shared by other adrenergic neurone blocking agents (e.g. debrisoquine and bretylium).

Methods

Tissue preparation

Guinea-pigs (300–500 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. The open trachea was cut into small segments each containing 3–4 cartilage rings. Bovine tracheae were collected from the local abbatoir and transported to the laboratory immersed in cold Krebs solution. The bovine tracheae were opened in a fashion similar to that described for guinea-pig

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tissue. However, a segment of bovine tracheal wall containing the trachealis muscle was then dissected from the opened organ and pinned out under Krebs solution with the adventitial surface uppermost. Adventitial tissue was carefully dissected from the preparation to expose the dorsal surface of the trachealis muscle. The preparation was then pinned out mucosal surface uppermost and the mucosal and submucosal tissue was carefully removed in order to expose the ventral surface of the trachealis muscle. Strips (approximately 10 mm in length) of the cleaned trachealis muscle were prepared by cutting in the longitudinal axis of the muscle bundles.

Tissue bath studies of mechanical activity of guinea-pig trachealis

Small segments of guinea-pig trachea were set up for the isometric recording of tension changes essentially as described by Foster *et al.* (1983). At the outset of each experiment, tissues were subjected to an imposed tension of 1.5 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 (initial wash followed by two further washes at 10 min intervals) and when tone subsequently became maximal (40 min after initial wash), study of bronchodilator drugs started.

Cumulative log concentration-effect curves were constructed for all relaxant drugs studied. Half-$\log_{10}$ unit concentration increments were used. Each concentration increment of BRL 38227, pinacidil or RP 52891 was allowed 8 min tissue contact. For isoprenaline and theophylline the contact times were 4 and 5 min respectively. In order to minimize desensitization to the KCOs, a concentration-effect curve for theophylline was always interposed between successive concentration-effect curves of each KCO. The effects of the various relaxants were measured in terms of the maximal relaxation induced by aminophylline.

Following the construction of initial log concentration-relaxation curves for the bronchodilator drugs, test tissues were exposed to Krebs solution containing one of the following modifying agents: bretylium (50 μM), debrisoquine (16 or 50 μM), guanethidine (5, 16, 50 or 500 μM), or guanidine (50 or 500 μM). Following 40 min tissue equilibration with the modifying agent, the log concentration-relaxation curves of the bronchodilator drugs were reconstructed in the presence of the modifying agent. Time-matched control tissues were treated identically to the test tissues but were not exposed to the modifying agent.

Studies of $^{86}Rb^+$ efflux from bovine trachealis

The efflux of $^{86}Rb^+$ from bovine trachealis was studied essentially as described by Longmore *et al.* (1991). In brief, strips of bovine trachealis (wet weight approximately 150 mg) were impaled on hypodermic needles and loaded with $^{86}Rb^+$ by incubation for 90 min in Krebs solution maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. This loading medium contained 185 kBq ml^{-1} $^{86}Rb^+$ and the Rb^+ concentration was less than 50 μM. Following loading with the radiotracer, each tissue strip was transferred to the first of a series of efflux tubes containing 3 ml of Krebs solution gassed with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. Each tissue strip was transferred to the next tube in the series at 3 min intervals. At the end of the efflux period the contents of each of the efflux tubes was assayed for radioactivity in a gamma counter. Each tissue strip was blotted and similarly assayed for radioactivity.

In the case of test tissues, BRL 38227 (1 or 10 μM) was present in the efflux medium for the period 30 to 60 min from the start of the efflux. In the case of time-matched control tissues, the efflux medium contained the appropriate vehicle for BRL 38227 over the same time period. In experiments where the effects of guanethidine (50 μM), bretylium (50 μM) or debrisoquine (50 μM) alone, or in combination with BRL 38227 (10 μM) were examined, the adrenergic neurone blocking agent (or appropriate vehicle) was added to the efflux medium for the whole of the efflux period.

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: aminophylline (BDH), bretylium tosylate (Wellcome Foundation), BRL 38227 ((−)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol, SmithKline Beecham Pharmaceuticals), debrisoquine sulphate (Roche Products Ltd), guanethidine sulphate (Ciba), guanidine sulphate (Sigma), (−)-isoprenaline hydrochloride (Sigma), (±)-pinacidil (Leo), RP 52891 ((−)-N-methyl-2-(3-pyridinyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide, RhonePoulenc-Rorer), theophylline (Sigma).

A stock solution of isoprenaline was prepared in 0.1 M HCl. Dilutions from this stock were prepared with distilled water containing 0.57 mM ascorbic acid as an antioxidant. Stock solutions of BRL 38227, pinacidil and RP 52891 were prepared in 70% ethanol. Stock solutions of other drugs were prepared using twice-distilled water.

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1. This solution (pH 7.4) was maintained at 37°C and was gassed with a mixture of 95% O_2 and 5% CO_2.

The significance of differences between means was assessed by use of a two-tailed, unpaired t test. The null hypothesis was rejected when $P<0.05$.

Results

Tissue bath studies of mechanical activity of guinea-pig trachealis

Isoprenaline (1–300 nM), theophylline (3 μM–1 mM), BRL 38227 (0.03–100 μM), pinacidil (0.1–100 μM) and RP 52891 (0.03–100 μM) each evoked concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. The maximal relaxant effects of BRL 38227 and RP 52891 were only 85–95% of that of theophylline, but the maximal relaxant effects of pinacidil and isoprenaline were identical to that of theophylline (Figures 1–3).

Guanethidine (5, 16, 50 or 500 μM) did not itself modify the spontaneous tone of the tracheal segments (Table 1). However, guanethidine antagonized BRL 38227 in a concentration-dependent manner. The antagonism was characterized by a rightwards shift in the log concentration-relaxation curve for BRL 38227 (Figure 1). The greatest rightward shift (approximately 10 fold) was obtained with guanethidine (50 μM). Increasing the concentration of guanethidine to 500 μM produced no greater antagonism. Guanethidine (50 μM) also antagonized RP 52891 and pinacidil. In the presence of guanethidine, the log concentration-relaxation curve for RP 52891 was shifted to the right and there was some reduction in the slope of the curve and in the maximal response. In contrast, the presence of guanethidine caused a rightward shift of the log concentration-relaxation curve of pinacidil without reducing the maximal response (Figure 2). Guanethidine (50 μM) did not affect the shape or position of the log concentration-relaxation curve for theophylline but caused approximately 4 fold potentiation of isoprenaline (Table 2, Figure 2).

Bretylium (50 μM) did not itself modify the spontaneous tone of the tracheal segments (Table 1). However, bretylium antagonized BRL 38227, pinacidil and RP 52891. In the case of pinacidil the antagonism was characterized by a rightward shift in the log concentration-relaxation curve of the KCO without depression of the maximal response. In the cases of

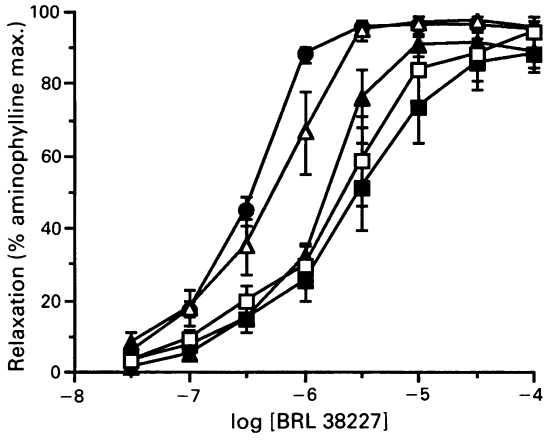

Figure 1 Effect of guanethidine on the action of BRL 38227 in suppressing the spontaneous tone of guinea-pig isolated trachealis. Abscissa scale: molar concentration of BRL 38227 on a $\log_{10}$ scale. Ordinate scale: relaxation as a percentage of the maximum relaxation induced by aminophylline. Symbols: (●) indicates the log concentration-effect curve for BRL 38227 obtained in time-matched control tissues; (△), (▲), (■) and (□) indicate subsequent log concentration-effect curves for BRL 38227 obtained in test tissues equilibrated with guanethidine 5, 16, 50 and 500 μM respectively. Data points indicate means of values from at least 8 tissues. Vertical bars indicate s.e.mean.

Table 1 Effects of some adrenergic neurone blocking agents and guanidine on the spontaneous tone of guinea-pig isolated trachealis

Agents tested	*Vehicle-treated, time-matched control tissues*	*Test tissues*
Bretylium (50 μM)	95.1 ± 1.5	94.3 ± 2.2
Debrisoquine (50 μM)	98.7 ± 2.4	44.8 ± 6.0*
Guanethidine (5 μM)	104.5 ± 3.4	101.4 ± 3.4
Guanethidine (16 μM)	103.5 ± 3.2	105.2 ± 5.9
Guanethidine (50 μM)	99.4 ± 1.8	99.7 ± 2.3
Guanethidine (500 μM)	102.5 ± 2.3	98.4 ± 4.0
Guanidine (50 μM)	94.0 ± 2.2	94.8 ± 4.5

Data indicate the spontaneous tone observed after 40 min tissue equilibration with vehicle (time-matched control tissues) or with the indicated adrenergic neurone blocking agent (test tissues) and expressed as a percentage of the spontaneous tone observed at the beginning of the experiment. Data indicate the mean (± s.e.mean) of values from at least 12 tissues.

* indicates a significant ($P<0.05$) difference from the corresponding time-matched control tissues (two-tailed, unpaired t test).

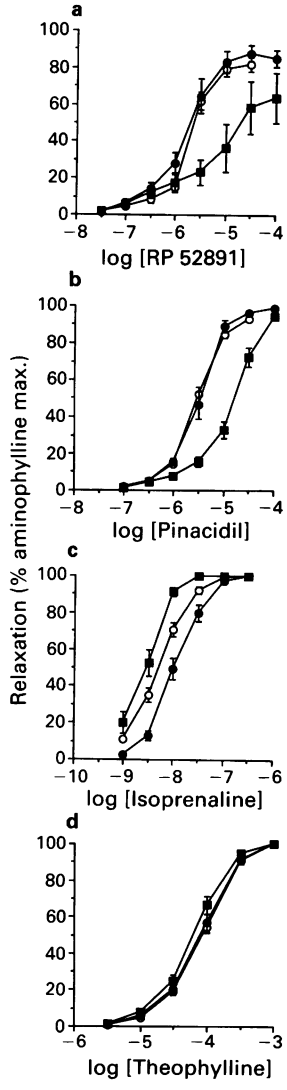

Figure 2 Effects of guanethidine (50 μM) on the actions of RP 52891 (a), pinacidil (b), isoprenaline (c) and theophylline (d) in suppressing the spontaneous tone of guinea-pig isolated trachealis. Abscissa scales: molar concentration of RP 52891, pinacidil, isoprenaline or theophylline on a $\log_{10}$ scale. Ordinate scale: relaxation as a percentage of the maximal relaxation evoked by aminophylline. Symbols: (○) indicates the pooled initial log concentration-relaxation curve for RP 52891, pinacidil, isoprenaline or theophylline; (●) indicates the subsequent log concentration-relaxation curve obtained in time-matched control tissues; (■) indicates the subsequent log concentration-relaxation curve for RP 52891, pinacidil, isoprenaline or theophylline obtained in test tissues equilibrated with guanethidine (50 μM). Data points indicate means of values obtained from at least 6 tissues. Vertical bars indicate s.e.mean.

BRL 38227 and RP 52891 the log concentration-relaxation curves were shifted rightward with some reduction in slope and maximal response (Figure 3). Bretylium (50 μM) failed to affect the shape and position (Table 2) of the log concentration-relaxation curves of theophylline or isoprenaline.

In contrast to guanethidine and bretylium, debrisoquine (16 and 50 μM) tended to suppress the spontaneous tone of the tracheal segments and to induce phasic tension changes (Table 1). These effects made it extremely difficult to test debrisoquine for inhibitory activity against the tracheal relaxant activity of the KCOs. However, in the 4 out of 12 tissues where debrisoquine failed to suppress spontaneous tone, it was clear that debrisoquine antagonized BRL 38227 and that such antagonism was characterized by a reduction in the maximal response to BRL 38277 (data not shown). Since the guanidine moiety forms part of the structure of guanethidine, it seemed worthwhile to examine whether guanidine might be the active species rendering guanethidine able to antagonize the KCOs. Guanidine (50 and 500 μM) did not modify the spontaneous tone of the tracheal segments (Table 1), nor did it antagonize BRL 38227 or theophylline (Table 2).

Studies of $^{86}Rb^+$ efflux from bovine trachealis

Fifteen minutes after the start of the efflux period, the $^{86}Rb^+$ efflux rate coefficient had declined to a relatively low (approximately 0.3% min^{-1}) value, suggesting that the rapidly-exchanging compartment for $^{86}Rb^+$ had cleared. This was true for both test and control tissues. In the case of the control tissues, the $^{86}Rb^+$ efflux rate coefficient thereafter fell very slowly throughout the remainder of the efflux period (Figures 4 and 5). Addition of the ethanolic vehicle for BRL

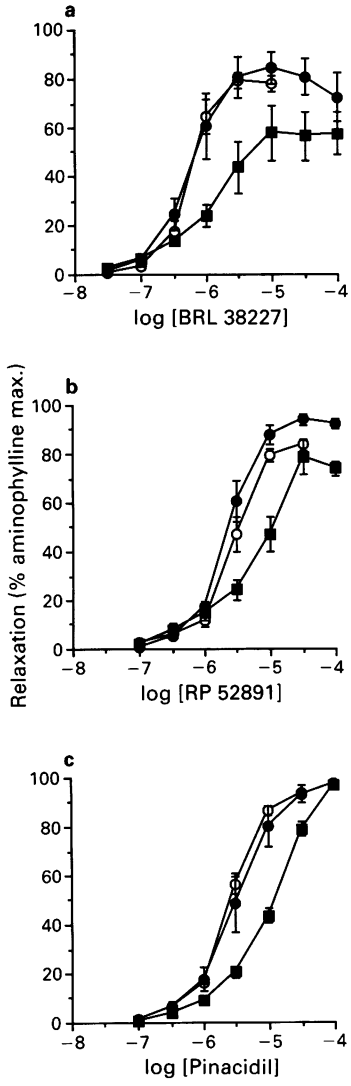

Figure 3 Effect of bretylium (50 μM) on the actions of BRL 38227 (a), RP 52891 (b) and pinacidil (c) in suppressing the spontaneous tone of guinea-pig isolated trachealis. Abscissa scale: molar concentration of BRL 38227, RP 52891 or pinacidil on a $\log_{10}$ scale. Ordinate scales: relaxation as a percentage of the maximal relaxation evoked by aminophylline. Symbols: (○) indicates the pooled initial log concentration-relaxation curve for BRL 38227, RP52891 or pinacidil; (●) indicates the subsequent log concentration-relaxation curve obtained in time-matched control tissues; (■) indicates the subsequent log concentration-relaxation curve for BRL 38227, RP 52891 or pinacidil obtained in test tissues equilibrated with bretylium (50 μM). Data points indicate means of values from at least 6 tissues. Vertical bars indicate s.e.mean.

Table 2 The effects of some adrenergic neurone blocking agents and guanidine on the potencies of some tracheal relaxant drugs

	−log EC_{50} for isoprenaline	
	Control tissues	*Test tissues*
Guanethidine (50 μM)	7.96 ± 0.07	8.55 ± 0.09*
Bretylium (50 μM)	7.92 ± 0.09	7.99 ± 0.07
	−log EC_{50} for theophylline	
	Control tissues	*Test tissues*
Guanethidine (50 μM)	4.42 ± 0.04	4.62 ± 0.10
Bretylium (50 μM)	4.09 ± 0.05	4.14 ± 0.08
Guanidine (50 μM)	4.28 ± 0.04	4.24 ± 0.04
Guanidine (500 μM)	4.24 ± 0.03	4.23 ± 0.04
	−log EC_{50} for BRL 38227	
	Control tissues	*Test tissues*
Guanidine (50 μM)	6.35 ± 0.02	6.32 ± 0.05
Guanidine (500 μM)	6.30 ± 0.05	6.22 ± 0.05

Data indicate the mean (± s.e.mean) of values from at least 6 tissues.
* indicates a significant ($P < 0.05$) difference from the corresponding value for the vehicle-treated, time-matched control tissues.

38227 to the efflux medium (30 min into the efflux period) caused no detectable change in the efflux rate coefficient. In contrast, the addition of BRL 38227 (1 or 10 μM) to the efflux medium at this time caused a concentration-dependent increase in the efflux rate coefficient (Figure 4). The concentration-dependency of the effect was manifest principally by the rate at which the peak effect was attained.

Addition of guanethidine (50 μM) to the efflux medium caused no change in the efflux rate coefficient at any time point as evident from comparisons with vehicle-treated control tissues. However, the presence of guanethidine markedly inhibited the ability of BRL 38227 to increase the efflux rate coefficient (Figure 4). Similar results were obtained with bretylium (50 μM) and debrisoquine (50 μM) (Figure 5).

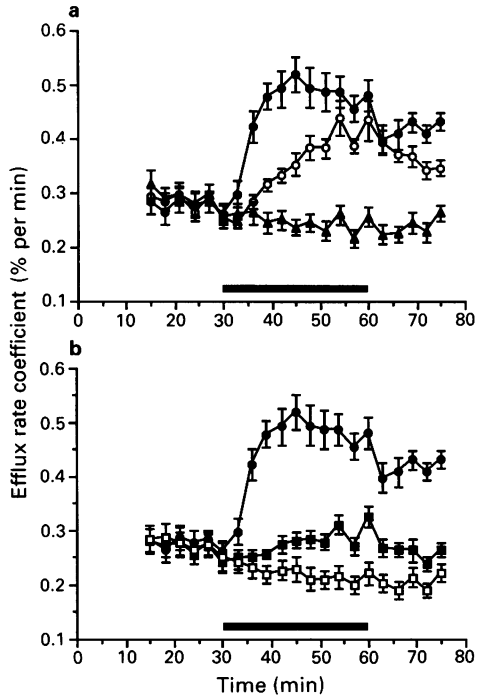

Figure 4 The effect of guanethidine (50 μM) on the action of BRL 38227 in promoting the efflux of $^{86}Rb^{+}$ from strips of bovine trachealis muscle preloaded with the radiotracer. In each panel the abscissa scale indicates the time (min) measured from the start of the efflux period. The ordinate scale indicates the efflux rate coefficient (% per min). In (a) the effects of ethanolic vehicle (▲), 1 μM BRL 38227 (○) and 10 μM BRL 38227 (●) are shown; (b) the effects of 50 μM guanethidine alone (□) of 10 μM BRL 38227 alone (●) [same data as in (a)] and 10 μM BRL 38227 in combination with 50 μM guanethidine (■). Data points indicate the mean of values from tissue strips from at least 6 tracheae. Vertical bars indicate s.e.mean. Where used, the adrenergic neurone blocking agent was present throughout. The horizontal bar indicates the period for which BRL 38227 or its ethanolic vehicle was present.

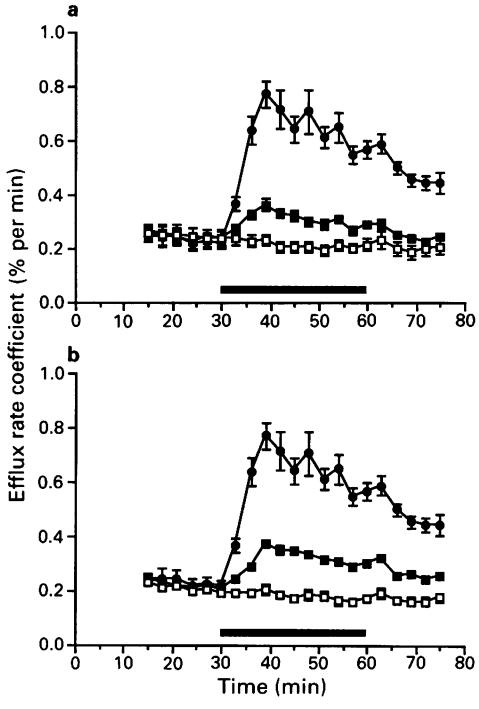

Figure 5 The effect of bretylium and debrisoquine (each 50 μM) on the action of BRL 38227 in promoting the efflux of $^{86}Rb^+$ from strips of bovine trachealis muscle preloaded with the radiotracer. In each panel the abscissa scale indicates the time (min) measured from the start of the efflux period. The ordinate scale indicates the efflux rate coefficient (% per min). In (a) are shown the effects of bretylium (50 μM) alone (□), BRL 38227 (10 μM) alone (●) and bretylium (50 μM) in combination with BRL 38227 (10 μM) (■); (b) shows the effects of debrisoquine (50 μM) alone (□), BRL 38227 (10 μM) alone (●) [same data as in (a)] and debrisoquine (50 μM) in combination with BRL 38227 (10 μM) (■). Data points indicate the mean of values from tissue strips from at least 6 tracheae. Vertical bars indicate s.e.mean. Where used, the adrenergic neurone blocking agent was present throughout. The horizontal bar indicates the period for which BRL 38227 or its ethanolic vehicle was present.

Discussion

Interactions between adrenergic neurone blocking agents and drugs causing tracheal relaxation

The ability of guanethidine to potentiate isoprenaline in suppressing the spontaneous tone of the guinea-pig isolated trachea (Figure 2) confirms results obtained in our earlier studies (Boyle *et al.*, 1987) and may be explained in terms of guanethidine inhibiting the extraneuronal uptake of isoprenaline (Foster, 1969). The present observation that bretylium failed to potentiate isoprenaline (Table 2) may suggest that, in contrast to guanethidine, bretylium does not inhibit the sequestration of isoprenaline into extraneuronal sites.

Among the group of KCOs chosen for study, BRL 38227 is a benzopyran derivative, pinacidil is a cyanoguanidine derivative and RP 52891 is a tetrahydrothiopyran derivative (Small *et al.*, 1992). The experiments of the present study have shown that guanethidine can antagonize each of these agents in suppressing the spontaneous tone of the trachea without antagonizing the effect of isoprenaline or theophylline (Figures 1 and 2). Clearly, the antagonism provided by guanethidine exhibits selectivity for the KCOs as opposed to agonists at β-adrenoceptors or alkylxanthines. Furthermore, the antagonism of KCOs provided by guanethidine is not restricted to one particular chemical class (e.g. benzopyrans) of KCO. The present experiments have also shown that bretylium can antagonize BRL 38227, pinacidil and RP 52891 in suppressing spontaneous tracheal tone (Figure 3) without antagonizing isoprenaline or theophylline (Table 2). In addition, guanethidine, bretylium and debrisoquine were each able to antagonize BRL 38227 in promoting the efflux of $^{86}Rb^+$ from strips of bovine trachealis muscle preloaded with the radiotracer (Figures 4 and 5). These observations suggest that antagonism of the tracheal relaxant actions of KCOs is a property shared by several agents classically described (Louis & Howes, 1990) as adrenergic neurone blocking agents. The ability of the adrenergic neurone blocking agents selectively to inhibit the airways smooth muscle relaxant actions of the KCOs without antagonizing agonists at β-adrenoceptors or alkylxanthines is shared both by sulphonylureas such as glibenclamide (Murray *et al.*, 1989; Black *et al.*, 1990; Nielsen-Kudsk *et al.*, 1990; Berry *et al.*, 1991; Raeburn & Brown, 1991) and by phentoamine (Murray *et al.*, 1989; McPherson & Angus, 1990). That such a structurally-diverse group of agents can provide selective antagonism of the KCOs may have important implications concerning the site or mechanism of action of the KCOs.

Mechanisms by which adrenergic neurone blocking agents antagonize KCOs in suppressing the spontaneous tone of the trachea

The scientific literature currently contains very few reports linking the cellular pharmacology of adrenergic neurone blocking agents to the activity of plasmalemmal K^+-channels. Kirpekar *et al.* (1978) observed that the K^+-channel inhibitors tetraethylammonium (TEA) and 4-aminopyridine (4-AP) could reverse the ability of guanethidine to inhibit the neural release of noradrenaline in the *in situ* perfused spleen of the cat. Stutzin *et al.* (1983) observed that the K^+-channel inhibitor, apamin, could antagonize guanethidine in inhibiting contractile responses to the electrical stimulation of nerves in the guinea-pig isolated vas deferens. Such observations led Stutzin *et al.* (1983) to suggest that, by promoting an increase in the cytosolic concentration of free Ca^{2+}, guanethidine might cause the opening of Ca^{2+}-dependent K^+-channels in the neuronal plasmalemma, hyperpolarize the neuronal terminals and thereby inhibit transmitter release. However, the present finding that adrenergic neurone blocking agents antagonize KCOs acting on airways smooth muscle is difficult to reconcile with the idea that the adrenergic neurone blocking agents promote K^+-channel opening.

Tested on cardiac myocytes from the chick embryo, bretylium (applied either extracellularly or intracellularly) was observed to inhibit the outward K^+-current induced by a depolarizing voltage step (Bkaily *et al.*, 1988) and the antidysrhythmic activity of this agent has been attributed to K^+-channel blockade (Bacaner *et al.*, 1986). The present observation that adrenergic neurone blocking agents, including bretylium, antagonize KCOs in suppressing the spontaneous tone of the trachea is entirely consistent with the idea that the former group of compounds have K^+-channel inhibitory activity.

If guanethidine, indeed, is an inhibitor of the K^+-channels opened by BRL 38227, pinacidil and RP 52891, then the failure of guanethidine to convert the spontaneous electrical slow waves of guinea-pig trachealis into regenerative action potentials (Boyle *et al.*, 1987) suggests that the K^+-channel opened by BRL 38227, pinacidil and RP 52891 does not play an important role in determining the strong outward rectifying behaviour of the trachealis cells. Electrophysiological studies of the effect of glibenclamide and phentolamine (Murray *et al.*, 1989) have led to a similar conclusion.

The present observation that guanethidine, bretylium and debrisoquine each inhibited the ability of BRL 38227 to promote $^{86}Rb^+$ efflux from strips of bovine trachealis (Figures 4 and 5) is consistent with the idea that these adrenergic neurone blocking agents inhibit the K^+-channel that is opened by the KCOs. However, at present, it has not been established whether the KCOs act directly on the K^+-

channel protein or whether they act at a site distant from the channel and, there, initiate a series of biochemical changes that culminate in the opening of the channel. In view of this, the mechanism of action of any agent having antagonist activity against the KCOs must remain somewhat speculative.

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