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The effects of the dihydropyridine Bay K 8644 in guinea-pig isolated trachealis

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1 In trachea bathed by Krebs solution containing indomethacin 0.8 μmol l^{-1}, Bay K 8644 (0.01–1 μmol l^{-1}) evoked mild spasm. Peak tension was achieved after 10 min and was generally less than 20% of an acetylcholine (ACh) maximum. The effect of Bay K 8644 was not potentiated by addition of 2.5 mmol l^{-1} potassium chloride (KCl) to the Krebs solution.

2 Bay K 8644 (1 μmol l^{-1}) caused a small potentiation of KCl and tetraethylammonium (TEA). In contrast it did not modify the actions of ACh or histamine.

3 Bay K 8644 (1 μmol l^{-1}) caused a small potentiation of the effect of calcium chloride ($CaCl_2$) tested in trachea bathed by a K^+-rich, Ca^{2+}-free, MOPS-buffered physiological salt solution.

4 Organic inhibitors of calcium influx such as nifedipine (0.1 μmol l^{-1}), verapamil (1 μmol l^{-1}) or diltiazem (10 μmol l^{-1}) each caused marked depression of concentration-effect curves to KCl. Bay K 8644 (0.01–1 μmol l^{-1}) provided concentration-dependent protection against this effect in all three cases.

5 Estimation of calcium influx by the lanthanum technique revealed that Bay K 8644 (1 μmol l^{-1}) was able to promote the cellular influx of Ca^{2+}.

6 Intracellular electrophysiological recording showed that Bay K 8644 (1 μmol l^{-1}) caused no change in the resting membrane potential of trachealis cells and no change in the properties of the spontaneous electrical slow waves. However, Bay K 8644 was able to delay the slow wave suppression evoked by 1 μmol l^{-1} nifedipine.

7 The ability of Bay K 8644 to promote Ca^{2+} influx and its ability to protect against the effects of several structurally-unrelated inhibitors of Ca^{2+} influx are consistent with Bay K 8644 acting as an agonist at the dihydropyridine receptor associated with the voltage-operated Ca^{2+} channel (VOC) of trachealis muscle. By this action it potentiates those spasmogens (KCl, TEA) which act by permitting Ca^{2+} influx through VOCs. In contrast it has no effect on those spasmogens (ACh, histamine) which principally act to liberate Ca^{2+} from intracellular sites of sequestration.

Introduction

Schramm *et al.* (1983a,b) reported that the dihydropyridine derivative Bay K 8644 increases the force of cardiac contraction and causes spasm of partially depolarized vascular smooth muscle. These authors suggested that the effects of Bay K 8644 were mediated by the promotion of Ca^{2+} influx through voltage-operated channels (VOCs). This suggestion receives direct support from the observation (Freedman & Miller, 1984; Schramm & Towart, 1984; Yamamoto *et al.*, 1984) that Bay K 8644 promotes the uptake of $^{45}Ca^{2+}$ into partially depolarized neuroblastoma and vascular smooth muscle cells.

Patch clamping experiments on ventricular muscle cells of the guinea-pig and frog (Hess *et al.*, 1984) have revealed that Bay K 8644 increases the peak inward Ca^{2+} current evoked by a depolarizing voltage step. Furthermore, patch clamp recording of the activity of single Ca^{2+} channels showed that Bay K 8644 and other dihydropyridines produce distinct changes in the pattern of Ca^{2+} channel opening. Hess and co-workers postulated that the VOC of cardiac muscle has three modes of gating. Mode 0 represents a state where the channel is unavailable for opening and this state is stabilized by dihydropyridine inhibitors of Ca^{2+} influx

such as nimodipine. Mode 1 is a state characterized by brief channel openings occurring in rapid bursts. Mode 2 is a state characterized by prolonged channel openings and abbreviated closings. Bay K 8644 is suggested to stabilize the channel in Mode 2.

Small & Foster (1985) have recently reviewed evidence which suggests that VOCs exist in airways smooth muscle. These authors argued that while some bronchoconstrictor agents (e.g. KCl, TEA) act principally to open VOCs and thereby permit the cellular influx of Ca^{2+}, those more likely to have roles as mediators in bronchial asthma (e.g. acetylcholine, histamine) act principally to release Ca^{2+} from intracellular sites of sequestration. If this suggestion is correct and if Bay K 8644 promotes Ca^{2+} influx through the VOCs of airways smooth muscle, then Bay K 8644 should discriminate between KCl and TEA on the one hand and acetylcholine (ACh) and histamine on the other.

The present experiments were designed to determine whether Bay K 8644 facilitates Ca^{2+} influx through the VOCs of guinea-pig trachealis and whether Bay K 8644 can selectively potentiate bronchoconstrictors which open VOCs. A preliminary account of this work has been communicated the British Pharmacological Society (Foster *et al.*, 1985).

Methods

Guinea-pigs (350–700 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. Subsequent experiments were carried out under sodium lamp illumination (to minimize the photolysis of dihydropyridines) and in physiological salt solutions (see below) containing indomethacin, 0.8 μmol l^{-1} (to inhibit prostaglandin production).

Assessment of the spasmogenic effects of Bay K 8644

In these and all other tissue bath experiments small segments of trachea were set up for the isometric recording of tension changes as described by Foster *et al.* (1983). Tissues were bathed by Krebs solution (see below) or Krebs solution to which 2.5 mmol l^{-1} KCl had been added. Cumulative concentration-effect curves for Bay K 8644 (0.01–1 μmol l^{-1}) were constructed with a 10 min contact time for each concentration. One hour later the Bay K 8644 concentration-effect curve was redetermined. Finally ACh (1–10 mmol l^{-1}) was added to determine the maximal tension attainable by each tissue.

Assessment of the interactions between Bay K 8644 and ACh, histamine, KCl or TEA

Spasmogen (ACh, histamine, KCl or TEA) action was studied by the construction of cumulative concentration-effect curves as previously described (Foster *et al.*, 1984). In most experiments a curve for ACh was constructed initially, followed by a curve for one of the other spasmogens (histamine, KCl or TEA). Tissues were then incubated for 10 min either with vehicle (controls) or with 1 μmol l^{-1} Bay K 8644 (test tissues). In the continued presence of vehicle or Bay K 8644 the two spasmogen concentration-effect curves were reconstructed, though in the reverse spasmogen order.

In a separate group of experiments a concentration-effect curve for ACh alone was constructed before and after incubation with vehicle or Bay K 8644.

Assessment of the interaction between Bay K 8644 and $CaCl_2$

Tissues were initially set up in MOPS-PSS (see below) but this was then changed to K^+-rich, Ca^{2+}-free MOPS-PSS. Spasm evoked by the depolarizing medium was dissipated by changing the bath fluid every 15 min. Full relaxation was achieved after 105 min.

A cumulative concentration-effect curve for $CaCl_2$ (0.01–10 μmol l^{-1}) was then constructed using tenfold concentration increments and a 15 min contact time for each concentration. $CaCl_2$-induced spasm was then dissipated by washing for 105 min. Test tissues and control tissues were incubated with Bay K 8644 (1 μmol l^{-1}) or vehicle, respectively, for 30 min. Concentration-effect curves for $CaCl_2$ were then reconstructed in the continued presence of Bay K 8644 or vehicle.

Assessment of the interaction between Bay K 8644 and organic inhibitors of Ca^{2+} influx

These experiments were designed to determine whether Bay K 8644 could protect KCl-induced contractions against the depressant effect of inhibitors of calcium influx. Accordingly a cumulative log concentration-effect curve for KCl was constructed. Tissues were then incubated in Krebs solution for 1 h (controls) or Krebs solution to which a calcium influx inhibitor (nifedipine 0.1, verapamil 1, or diltiazem 10 μmol l^{-1}) had been added alone or with Bay K 8644 (0.01, 0.1 or 1 μmol l^{-1}).

During the hour of incubation the effects of dihydropyridine photolysis were minimized by changing the bath fluid at 15 min intervals. At each change of bath fluid the calcium influx inhibitor and Bay K 8644 were replaced by fresh material. Finally the

concentration-effect curve for KCl was reconstructed either in the absence (control tissues) or presence of the appropriate modifying agent(s).

Estimation of calcium influx by the lanthanum technique

Tracheae were opened as described above and pinned out, mucosal surface uppermost, on a paraffin wax block. That segment of tracheal wall containing the trachealis muscle (and minimal cartilage) was dissected from the organ by making two cuts running longitudinally at the tips of the cartilage arches. The strip of tissue thus prepared was divided into three equal segments (laryngeal, central and carinal). Tissue segments were subsequently allocated to control or test experimental groups in such a way that laryngeal, central and carinal tissue was present in equal proportion in each group.

Following at least 75 min tissue equilibration at 37.5°C with MOPS-PSS and 5 min exposure to $^{45}Ca^{2+}$ (0.5 mCi l^{-1}), test tissue strips were incubated (in the continued presence of $^{45}Ca^{2+}$) either with Bay K 8644 (1 μmol l^{-1}) or with a concentration (3.85 mmol l^{-1}) of KCl calculated to be equispasmogenic with Bay K 8644 (1 μmol l^{-1}). Control tissues were treated with vehicle.

Twelve minutes later tissues were removed from these media and placed in 5 ml ice cold, oxygenated Ca^{2+}-free MOPS-PSS containing $LaCl_3$ (10 mmol l^{-1}). Tissues were transferred to fresh 5 ml samples of this washing medium at times of 1, 3, 5, 9, 19, 29, 59, 89 and 119 min. One ml aliquots of washing media from control tissues were added to 4 ml Rialuma (Lumac) liquid scintillation mixture and counted for radioactivity in order to follow the efflux of $^{45}Ca^{2+}$ from the tissues. All initial incubation media were radioassayed in the same way.

At the end of the efflux period the tissues were blotted, weighed and solubilized in Soluene 350 (Packard) 0.5 ml, in the warm, overnight. To this 0.5 ml of HCl 0.5 mmol l^{-1} and 4 ml of Lumagel (Lumac) were added for radioassay. Quench correction was by the automatic external standard channels ratio method: mean counting efficiency was 85%. The lanthanum-resistant calcium fraction was estimated as the tissue: medium ratio (apparent volume of distribution of $^{45}Ca^{2+}$) at 119 min.

Intracellular electrophysiological recording

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 Bay K 8644 were studied in single cells in each of six tissues. Following impalement 5 min were allowed to elapse to check that the record of electrical activity had stabilized. Bay K 8644 (1 μmol l^{-1}) was then added to the Krebs solution. The effects of Bay K 8644 were monitored for a minimum of 10 min before the microelectrode was deliberately withdrawn from the cell to obtain an estimate of the resting membrane potential. The change in mechanical tone occurring during this period was also measured.

The effects of nifedipine (1 μmol l^{-1}) alone or preceded by Bay K 8644 (1 μmol l^{-1}) were studied in a similar manner, though microelectrode withdrawal was performed when spontaneous electrical activity had ceased.

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. Where KCl was used as a spasmogen the stated concentration excludes the KCl provided by the formulation of the physiological salt solution. The following substances were used: acetylcholine chloride (BDH), Bay K 8644 (methyl 1, 4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, Bayer), calcium chloride (BDH), diltiazem hydrochloride (Synthelabo), histamine acid phosphate (BDH), indomethacin (Sigma), nifedipine (Bayer), potassium chloride (Hopkin & Williams), tetraethylammonium bromide (Sigma) and verapamil hydrochloride (Knoll). Stock solutions of acetylcholine, Bay K 8644, indomethacin and nifedipine were prepared in absolute ethanol, those of other drugs in twice-distilled water.

The Krebs solution used in the majority of experiments had the following composition (mmol l^{-1}): 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.

In the radioisotope experiments and in experiments where $CaCl_2$ was used as a spasmogen, the tissue was bathed in MOPS-buffered physiological salt solution (MOPS-PSS, Jetley & Weston, 1980): in the latter case it was K^+-rich (40 mmol l^{-1}), Ca^{2+}-free MOPS-PPS.

The significance of differences between means was assessed using a one or two-tailed paired or unpaired *t* test or by analysis of variance.

Results

Spasmogenic effects of Bay K 8644

In 5 of the 12 tissues examined, Bay K 8644 (0.01–1 μmol l^{-1}) was devoid of spasmogenic effect. Where Bay K 8644-induced spasm was observed it was

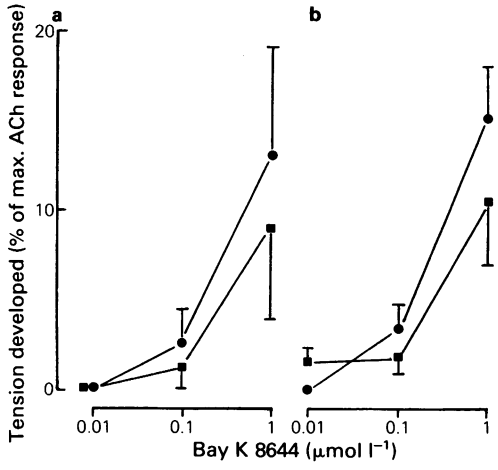

Figure 1 The spasmogenic effects of Bay K 8644 in guinea-pig isolated trachealis bathed by Krebs solution (a) or by Krebs solution to which 2.5 mmol l^{-1} KCl had been added (b). The abscissa scale represents the concentration of Bay K 8644 (μmol l^{-1}) on a log scale. The ordinate scale represents the tension developed as a % of the maximal response to acetylcholine. (●) = Initial log concentration-effect curve; (■) = log concentration-effect curve obtained 1 h later. Data represent the means of values from 12 tissues; s.e.mean shown by vertical bars.

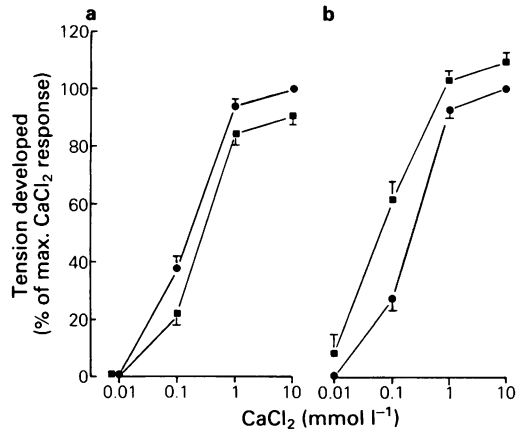

Figure 2 Effects of Bay K 8644 (1 μmol l^{-1}) on responses of guinea-pig isolated trachealis to $CaCl_2$ in K^+-rich, Ca^{2+}-free MOPS-PSS. The abscissa scale represents the concentration of $CaCl_2$ (mmol l^{-1}) on a log scale. The ordinate represents tension developed as a % of the initial maximal response to $CaCl_2$. (●) Initial log concentration-effect curve for $CaCl_2$; (■) log concentration-effect curve for $CaCl_2$ obtained after tissue equilibration for 135 min in K^+-rich Ca^{2+}-free MOPS-PSS in controls (a) or with 1 μmol l^{-1} Bay K 8644 in test tissues (b). Data represent the means of values from six tissues and vertical lines the s.e.mean.

slowly-developing and, during the 10 min contact time, rarely exceeded 20% of the ACh maximum. Bay K 8644-induced spasm could be dissipated by washing and some depression of the log concentration-effect curve for Bay K 8644 was evident when the curve was reconstructed an hour later (Figure 1a).

Concentrations for KCl just subthreshold for tension development render Bay K 8644 spasmogenic in vascular smooth muscle (Schramm *et al.*, 1983a,b). This observation prompted our testing the effects of Bay K 8644 in tissue bathed by Krebs solution to which 2.5 mmol l^{-1} KCl had been added (Figure 1b). The spasmogenic effects of Bay K 8644 were not significantly increased in this medium. Higher concentrations of KCl were themselves spasmogenic.

Interactions of Bay K 8644 with ACh, histamine, KCl or TEA

The results of these experiments are indicated by the data presented in Table 1. Control experiments revealed that log concentration-effect curves for all the spasmogens moved slightly to the right when reconstructed following incubation in Krebs solution con-

Table 1 Interactions of Bay K 8644 with some spasmogens in guinea-pig isolated trachealis.

Spasmogen	*Bay K 8644* 1 μmol l^{-1}	*Time-matched vehicle control*	*Net shift due to Bay K 8644*
KCl	− 0.17 ± 0.05*	0.14 ± 0.06	− 0.31 ± 0.07*
TEA	− 0.08 ± 0.05	0.42 ± 0.06*	− 0.50 ± 0.08*
ACh	0.31 ± 0.08*	0.20 ± 0.13	0.10 ± 0.15
Histamine	0.44 ± 0.12*	0.60 ± 0.15*	− 0.16 ± 0.18

Data represent mean $\log_{10}$ dose-ratio for the indicated spasmogen ± s.e.mean ($n > 6$). A negative sign signifies a leftward shift.

* Indicates a mean value of $\log_{10}$ dose-ratio which is significantly different from zero ($P < 0.05$ two-tailed, unpaired t test).

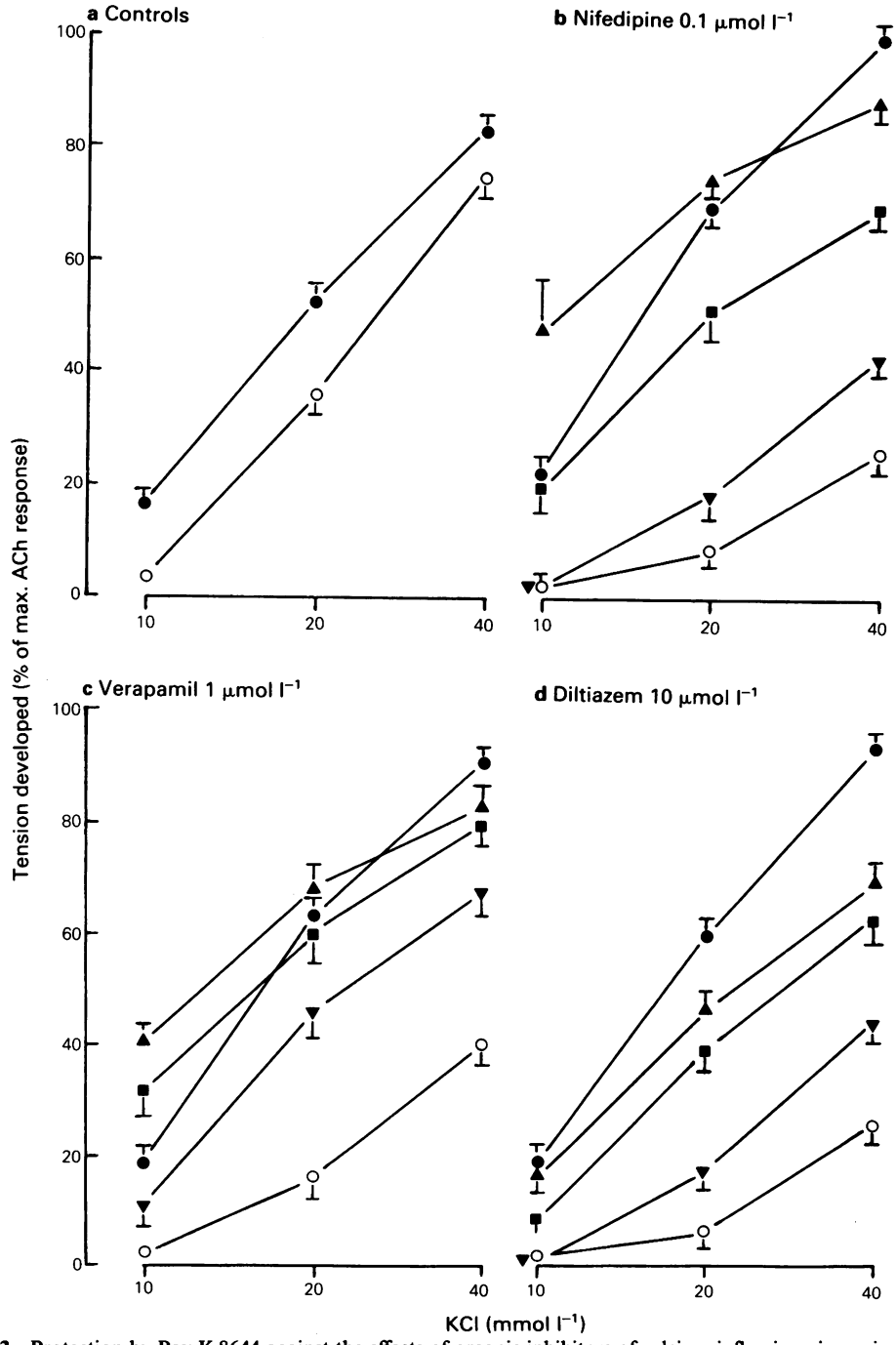

Figure 3 Protection by Bay K 8644 against the effects of organic inhibitors of calcium influx in guinea-pig isolated trachealis. The abscissa scale represents concentration of KCl (mmol l^{-1}) on a log scale. The ordinate scale represents tension developed as a % of the maximal response to acetylcholine. (●) Initial log concentration-effect curve for KCl. Other curves for KCl were obtained after 1 h of incubation with (○) Krebs solution (controls) or the indicated influx inhibitor alone, (▼) Bay K 8644 0.01 μmol l^{-1} + influx inhibitor, (■) Bay K 8644 0.1 μmol l^{-1} + influx inhibitor, (▲) Bay K 8644 1 μmol l^{-1} + influx inhibitor. Data represent the means of values from at least seven tissues; s.e.mean shown by vertical bars.

taining vehicle. Rightward shifts of the concentration-effect curves of ACh and histamine commonly occurred in test tissues treated with 1 μmol l^{-1} Bay K 8644. When the test data were corrected for shifts seen in concurrent matched control tissues, it was evident that Bay K 8644 did not influence the position of concentration-effect curves for ACh and histamine.

In contrast, the log concentration-effect curves of KCl and TEA generally moved to the left in tissues treated with Bay K 8644. Significant ($P < 0.05$) potentiation of KCl (two fold) and of TEA (three fold) was evident, following correction of test data for shifts seen in time-matched controls (Table 1).

The failure of Bay K 8644 to potentiate the effects of ACh was not a consequence of the order of spasmogen administration for, in experiments where ACh was the only spasmogen used, Bay K 8644 again failed to cause potentiation.

Interaction of Bay K 8644 with $CaCl_2$

In trachea bathed by K^+-rich, Ca^{2+}-free MOPS-PSS, $CaCl_2$ 0.01–10 mmol l^{-1} caused concentration-dependent spasm. Control experiments (Figure 2a) showed that the log concentration-effect curve of $CaCl_2$, when reconstructed after a 135 min period of tissue incubation, always underwent a small rightward shift (mean $\log_{10}$ dose-ratio ± s.e.mean = + 0.147 ± 0.021). In every tissue the maximal response to $CaCl_2$ was also slightly depressed.

When tissues were treated with 1 μmol l^{-1} Bay K 8644 (Figure 2b), the maximal response to $CaCl_2$ always increased slightly. The log concentration-effect curve for $CaCl_2$ always underwent a small leftward shift (mean $\log_{10}$ dose-ratio ± s.e.mean = − 0.488 ± 0.121). This was significantly ($P = 0.005$) different from that in the control tissues indicating that Bay K 8644 had potentiated $CaCl_2$.

Interactions of Bay K 8644 with organic inhibitors of Ca^{2+} influx

In control experiments (Figure 3a) the log concentration-effect curve of KCl was observed to move slightly to the right when reconstructed after 1 h of tissue incubation in Krebs solution. There was little or no change in the slope of the curve.

Incubation of tissues for 1 h with the inhibitors of Ca^{2+} influx nifedipine (0.1 μmol l^{-1}), verapamil (1 μmol l^{-1}), or diltiazem (10 μmol l^{-1}) caused marked depression of the log concentration-effect curve for KCl. When tissues were incubated with mixtures of one of these inhibitors of Ca^{2+} influx and Bay K 8644 (0.01, 0.1 or 1 μmol l^{-1}) it was evident that Bay K 8644 provided concentration-dependent protection against depression of the KCl curve evoked by nifedipine, verapamil or diltiazem (Figure 3b,c,d).

Table 2 Effects of KCl and Bay K 8644 on the lanthanum resistant calcium fraction of guinea-pig isolated trachealis muscle.

Control	*KCl* 3.85 mmol l^{-1}	*Bay K 8644* 1 μmol l^{-1}
0.087 ± 0.009	0.09 ± 0.011	0.110 ± 0.015*

Data represent mean tissue: medium ratio (ml g^{-1}) for $^{45}Ca^{2+}$ at 119 min ± s.e.mean ($n = 9$).
*Indicates a significant increase over the control value ($P = 0.036$, single-tailed paired t test).

Indeed Bay K 8644 1 μmol l^{-1} could move the KCl curve to the left of the control position despite the presence of the organic inhibitor of Ca^{2+} influx.

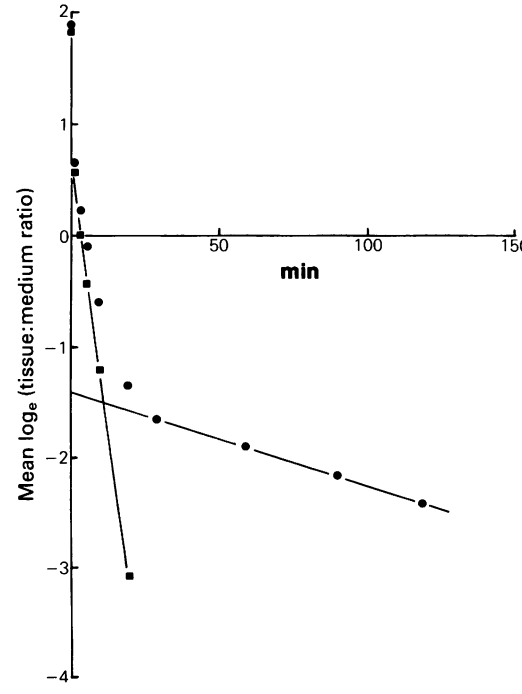

Figure 4 Efflux of $^{45}Ca^{2+}$ from guinea-pig isolated trachealis into ice-cold Ca^{2+}-free MOPS-PSS containing $LaCl_3$ (10 mmol l^{-1}). The abscissa scale represents time (min). The ordinate scale represents $\log_e$ (tissue: medium ratio) (ml g^{-1}). (●) Experimental data points; (■) calculated linear regression of mean $\log_e$ (tissue: medium ratio) against time for the intermediate, moderately fast phase of efflux (exposed by stripping the late, very slow phase of efflux indicated by the straight line of shallower slope). Data represent the means of values from 9 tissues. Curve stripping (Riggs, 1963) reveals that tissue:medium ratio at time, t, = $4.62e^{-2.88t} + 1.74e^{-0.191t} + 0.24e^{-0.0087t}$.

Estimation of calcium influx by the lanthanum technique

Figure 4 illustrates the efflux of $^{45}Ca^{2+}$ from the tracheal strips into ice-cold Ca^{2+}-free MOPS-PSS containing $LaCl_3$ and how the efflux may be separated into an initial very rapid phase, an intermediate, less rapid phase and a late, very slow phase.

Measurements of the lanthanum-resistant calcium fraction were made at the end of the efflux period. Bay K 8644 (1 μmol l^{-1}) caused a significant increase in

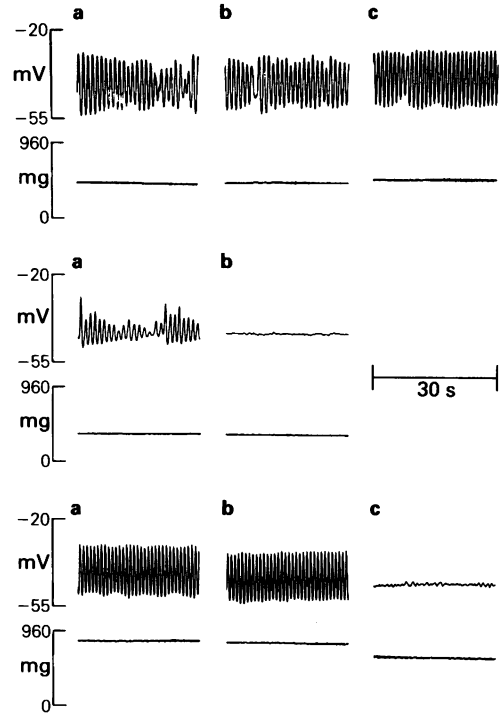

Figure 5 Effects of Bay K 8644 and nifedipine on the electrical and mechanical properties of guinea-pig isolated trachealis. In each row of records the upper trace represents membrane potential and the lower trace the mechanical activity of a contiguous segment of trachea. In each row all electrical records were taken from the same cell. Upper row: activity recorded before (a) and 1.5 (b) or 9 min (c) after Bay K 8644 1 μmol l^{-1}. Middle row: activity recorded before (a) and 1.5 min (b) after nifedipine 1 μmol l^{-1}. Lower row: activity recorded in a tissue pretreated with Bay K 8644 1 μmol l^{-1} (a) and 1.5 min (b) or 9 min (c) following addition of nifedipine 1 μmol l^{-1}. Note in (c) that the slow waves were at the point of being abolished 9 min after addition of nifedipine.

Table 3 Effects of dihydropyridines on the electrical and mechanical properties of guinea-pig isolated trachealis.

	Properties of cells before experimental period			*Measurements made at end of experimental period*			
Treatment	*Apparent resting membrane potential* (mV)	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)	*Change in membrane potential* (mV)	*Change in mechanical tone* (mg)
Bay K 8644 1 μmol l^{-1}	− 54.2 ± 4.0	13.8 ± 2.1	1.12 ± 0.1	13.8 ± 2.3	1.1 ± 0.07	− 0.6 ± 1.0	+60.0 ± 30.6
Nifedipine 1 μmol l^{-1}	− 47.7 ± 1.8	8.6 ± 1.1	0.97 ± 0.08	0	0	+ 0.3 ± 0.9	− 2.9 ± 2.9
Bay K 8644 1 μmol l^{-1} + nifedipine 1 μmol l^{-1}	− 51.8 ± 3.6	15.5 ± 2.9	1.12 ± 0.1	0	0	− 4.6* ± 1.6	− 63.3 ± 32.4

Data represent mean ± s.e.mean of values from at least 6 tissues. Negative changes in membrane potential or tension signify depolarization and relaxation respectively. For Bay K 8644 alone the experimental period was 10 min, otherwise it corresponded to the time required for slow wave abolition.

*Indicates a significant ($P < 0.05$, two-tailed t test) change in membrane potential or mechanical tone. The small depolarization seen in the presence of Bay K 8644 + nifedipine may be a consequence of the duration of the impalements rather than the action of either drug.

the lanthanum-resistant calcium fraction compared with that of control tissues (Table 2). KCl (3.58 mmol l^{-1}) appeared to cause a lesser increase in the lanthanum-resistant calcium fraction which did not reach the level of significance.

Electrophysiological effects of dihydropyridines

The effects of Bay K 8644 (1 μmol l^{-1}) were assessed 10 min after its first application to the tissue. Bay K 8644 did not significantly alter the resting membrane potential of trachealis cells and did not modify the maximal amplitude or frequency of spontaneous slow waves (Figure 5 and Table 3). However, it caused a small increase in mechanical tone.

In contrast nifedipine (1 μmol l^{-1}) abolished spontaneous slow wave discharge after 1.7 ± 0.3 min (mean ± s.e.mean, $n = 7$), confirming the observations of Ahmed *et al.* (1985). This action of nifedipine was associated with very minor changes in membrane potential and minor reduction in mechanical tone.

When tissues were pretreated with Bay K 8644 (1 μmol l^{-1}) there was a significant ($P = 0.03$) increase in the time (7.2 ± 1.8 min) required for nifedipine (1 μmol l^{-1}) to abolish spontaneous slow waves.

Discussion

The desaturation curve of trachealis loaded with $^{45}Ca^{2+}$ and washed with ice-cold, Ca^{2+}-free MOPS-PSS containing La^{3+} (Figure 4) behaves as though it were composed, by summation, of three exponential components. The occurrence of the late, very slow phase (rate coefficient less than 1%) is an expression of the failure of $LaCl_3$ 10 mmol l^{-1} completely to prevent the leakage of $^{45}Ca^{2+}$ from the intracellular compartment. The late, very slow phase of efflux is uncontaminated by the more rapidly eluting material at 29 min and therefore the lanthanum-resistant calcium fraction could in future be assessed after washing for only 29 min rather than the 119 min used here.

The intermediate, moderately fast phase of efflux shows a $t_{\frac{1}{2}}$ very similar to that found when efflux of the extracellular fluid marker [^{14}C]-sorbitol was studied in trachea (Foster, 1968). In view of its large coefficient it probably represents not only the clearance of dissolved $^{45}Ca^{2+}$ but also the clearance of readily-desorbed, surface-bound material. The initial, very fast phase of efflux is interpreted as the carry over of adhering incubation medium into the first aliquot of washing medium.

Examination of concentration-response data suggested that KCl (3.85 mmol l^{-1}) should be equi-spasmogenic with Bay K 8644 (1 μmol l^{-1}). Accordingly, this concentration of KCl was compared with Bay K 8644 in the $^{45}Ca^{2+}$ influx experiments. Table 2 shows that Bay K 8644 but not KCl increased the lanthanum-resistant calcium fraction over control values. We conclude that Bay K 8644 promotes Ca^{2+} influx into the trachealis muscle. This conclusion seems to be substantiated by the results of the tissue bath studies.

When $CaCl_2$ is added to smooth muscle bathed by a K^+-rich, Ca^{2+}-free medium, the resultant contraction is due to the influx of Ca^{2+} through VOCs (Spedding, 1982). In the present study the ability of Bay K 8644 to potentiate $CaCl_2$ (Figure 2) indicates that Bay K 8644 may facilitate Ca^{2+} entry into trachealis cells. Alternatively it could act to sensitize the contractile machinery to cytosolic Ca^{2+}.

The latter mechanism seems unlikely for Kanmura *et al.* (1984) have reported that Bay K 8644 does not modify the sensitivity of rabbit skinned mesenteric artery to Ca^{2+}. Furthermore, the present failure of Bay K 8644 to potentiate either ACh or histamine (Table 1) is not consistent with its sensitizing the contractile machinery to Ca^{2+}. In contrast several groups of workers (Freedman & Miller, 1984; Schramm & Towart, 1984; Yamamoto *et al.*, 1984) have observed that Bay K 8644 promotes the cellular influx of Ca^{2+}. Since we have now extended this finding to guinea-pig trachealis, it seems highly probable that Bay K 8644 potentiates $CaCl_2$ acting on trachealis by facilitating the transmembrane passage of Ca^{2+} through VOCs.

Bay K 8644 may act at a receptor site for dihydropyridines (Towart & Schramm, 1984) which modulates (up or down) the Ca^{2+} conductance of the VOC. If this is true for trachealis muscle, then several pieces of evidence suggest that the dihydropyridine receptor is activated rather than simply occupied by Bay K 8644. Firstly, Bay K 8644 increases Ca^{2+} influx over control values (Table 2). Secondly, the effects of nifedipine (a dihydropyridine inhibitor of Ca^{2+} influx) can be reversed rather than simply nullified by Bay K 8644 (Figure 3b). Thirdly, Bay K 8644 can protect against the effects of verapamil and diltiazem. These latter two inhibitors of Ca^{2+} influx act at membrane sites which are discrete from the dihydropyridine receptor (Towart & Schramm, 1984) and Bay K 8644 is thus likely to antagonize them not by occupancy of their receptor but by activating the dihydropyridine receptor and thereby evoking the opposite physiological response.

Nifedipine and other organic inhibitors of Ca^{2+} influx readily suppress the spontaneous electrical slow waves of guinea-pig trachealis (Small, 1982; Ahmed *et al.*, 1985; Small & Foster, 1985; present study). Slow waves are also abolished in a Ca^{2+}-free bathing medium (Foster *et al.*, 1983). These observations strongly suggest that the depolarizing phase of the slow waves may be associated with VOC opening.

Bay K 8644 initiates electrical activity in guinea-pig

taenia caeci and augments its electrical responses to depolarizing current (Godfraind *et al.*, 1984). In view of this observation and the probable involvement of VOC opening in the depolarizing phase of trachealis slow waves we were surprised to find that Bay K 8644 did not modify the spontaneous electrical activity of the trachea (Table 3). However, the concentration of Bay K 8644 used (1 μmol l^{-1}) had only very minor spasmogenic activity and may simply have been too low to evoke detectable membrane potential changes. Nevertheless a protective effect of Bay K 8644 was apparent as judged by its ability to delay the slow wave suppression induced by nifedipine.

Evidence that KCl and TEA cause contraction of trachealis by a mechanism which differs from that used by ACh or histamine has been reviewed by Small & Foster (1985). The action of KCl, for example, is potential-dependent, susceptible to organic inhibitors of Ca^{2+} influx, susceptible to Ca^{2+}-free media and associated with the cellular influx of Ca^{2+}. In contrast, the action of ACh is potential-independent, relatively resistant to organic inhibitors of Ca^{2+} influx, relatively resistant to Ca^{2+}-free media and is not associated with measurable Ca^{2+} influx. On the basis of such evidence it has been proposed that KCl and TEA act principally to open VOCs and thereby permit Ca^{2+} influx. In contrast ACh and histamine act principally to liberate Ca^{2+} from intracellular sites of sequestration.

If it can be assumed that Bay K 8644 acts to promote Ca^{2+} influx through trachealis VOCs, then the results of the present study add weight to these proposed differences in mechanism of spasmogen action, for Bay K 8644 caused significant potentiation of KCl and TEA but failed to modify the potency of ACh or histamine (Table 1). An ability of Bay K 8644 to discriminate between spasmogens acting by different mechanisms has also been reported for vascular smooth muscle (Yamamoto *et al.*, 1984).

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