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Effects of Bay K 8644 on contraction of the human isolated bronchus and guinea-pig isolated trachea

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1 The effects of Bay K 8644, a dihydropyridine which increases calcium flux through the potential-operated channels were studied on the contractions induced by histamine, acetylcholine, KCl and Ca^{2+} on human isolated bronchial strips and the results were compared to those obtained on guinea-pig isolated tracheal spirals. Subsequently the contractant effects of Bay K 8644 in K^+-enriched medium and in the presence of Ca^{2+} 0.03 mM were investigated.

2 In Krebs normal calcium medium, Bay K 8644 did not significantly modify the EC_{50} of acetylcholine or histamine on the human bronchus, but in concentrations of 10^{-7}–10^{-6} M it potentiated the effects of KCl on that preparation. It did not modify the EC_{50} of acetylcholine, histamine or KCl on the guinea-pig trachea.

3 In Ca^{2+}-free Krebs medium with additional K^+ (30 mM), Ca^{2+} concentration-response curves were displaced to the left by Bay K 8644 in the two preparations. Shifts were 0.52 ± 0.11 and 0.72 ± 0.16 log units respectively with Bay K 8644 10^{-8} and 10^{-7} M on human bronchus ($n = 4$) and 0.67 ± 0.16 and 1.06 ± 0.19 log units respectively with Bay K 8644 10^{-7} and 10^{-6} M on the guinea-pig trachea ($n = 5$).

4 In Krebs medium with Ca^{2+} 0.03 mM and K^+ 30 mM, Bay K 8644 (10^{-8} to 10^{-6} M) contracted both the human bronchus and the guinea-pig isolated trachea. This effect was competitively antagonized by nicardipine.

5 These results demonstrate the presence of dihydropyridine sites of action on human bronchus and confirm the minor role played by Ca^{2+} influx through potential-operated channels in the contractile effects of acetylcholine or histamine. They also demonstrate the similar reactivity of human bronchus and guinea-pig isolated trachea to Bay K 8644.

Introduction

Bay K 8644 is a dihydropyridine calcium agonist that increases cardiac muscle contractility and produces contraction of vascular smooth muscle (Schramm *et al.*, 1983a,b) and which has been postulated to act by accelerating the influx of Ca^{2+} through the potential-operated Ca^{2+} channels. Since different studies have shown that some vascular preparations and the guinea-pig isolated trachea react similarly to extracellular Ca^{2+} and to dihydropyridine calcium antagonists (Foster *et al.*, 1983a,b; Advenier *et al.*, 1984; Allen *et al.*, 1985), it seemed of interest to clarify the action of Bay K 8644 on the mechanical response of airway smooth muscle. Allen *et al.* (1985) have studied the effects of Bay K 8644 on the guinea-pig isolated trachea. They showed that in Krebs medium this substance had little or no effect of its own; it did not modify the effects of histamine or acetylcholine but potentiated the effects of tetraethylammonium (TEA) or KCl; it also potentiated the effects of Ca^{2+} in depolarizing medium; moreover Bay K 8644 was able to promote the cellular influx of Ca^{2+}, as evaluated by the lanthanum technique. They also showed that Bay K 8644 provided concentration-dependent protection against the inhibitory effects of verapamil, nifedipine and diltiazem vs KCl.

For further assessment of the action of Bay K 8644 on the bronchial smooth muscle, we studied its effects on human bronchus and compared them to those observed under similar experimental conditions on guinea-pig trachea. In a first series of experiments, we examined the effect of Bay K 8644 on contractions induced by histamine, acetylcholine (ACh) and KCl in a standard Krebs solution. We then experimented in a modified Krebs solution with additional K^+ (Godfraind *et al.*, 1968) to analyse the interaction between Ca^{2+} and Bay K 8644 and to determine the effects of

nicardipine and verapamil on Bay K 8644-induced contractions.

Methods

Guinea-pig tracheal spirals

Tracheal spirals were obtained from male guinea-pigs (250–350 g) anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and were equilibrated under an initial tension of 1.50 g in a Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2. After 1.25 h equilibration, the resting tension was between 0.4 and 0.6 g. Under these conditions, responses to agonists were reproducible. Tension was measured isometrically with a Gould strain gauge (UC 3) and was displayed on a Bryans BS 2H recorder.

The composition of the Krebs soltion was (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.7.

Human bronchus

Human bronchial tissue (usually with an inner diameter of 4–12 mm) obtained from patients undergoing surgery for lung cancer, but taken at a distance from the malignancy, was dissected free of parenchyma and transported to the laboratory in ice cold Krebs solution previously aerated with a mixture of 95% O_2 and 5% CO_2. The tissue was stored overnight at 4°C and the experiment was carried out on the next day. Published data have shown that overnight storage of tissue does not alter its reactivity (Brink *et al.*, 1980; Ghelani *et al.*, 1980; Guillot *et al.*, 1984; Vincenc *et al.*, 1984). Spirally cut strips from a segmental bronchus were suspended in Krebs solution under an initial tension of 2.5 g in the conditions described for guinea-pig isolated trachea.

Protocols

In all experiments, tracheal spirals or human bronchi were first contracted to maximal tension with histamine 2.2×10^{-4} M.

Cumulative concentration-response curves to histamine, acetylcholine or KCl were obtained by increasing concentration at 5–10 min intervals in logarithmic increments. Bay K 8644 was preincubated for 15 min.

In another series of experiments, Ca^{2+} dose-response curves were established according to Godfraind *et al.* (1968) and Advenier *et al.* (1984). Tracheal spirals or human bronchi were incubated for 1 h in Krebs solution but without $CaCl_2$, then for 15 min in $CaCl_2$-free Krebs solution in the presence of ethylen-

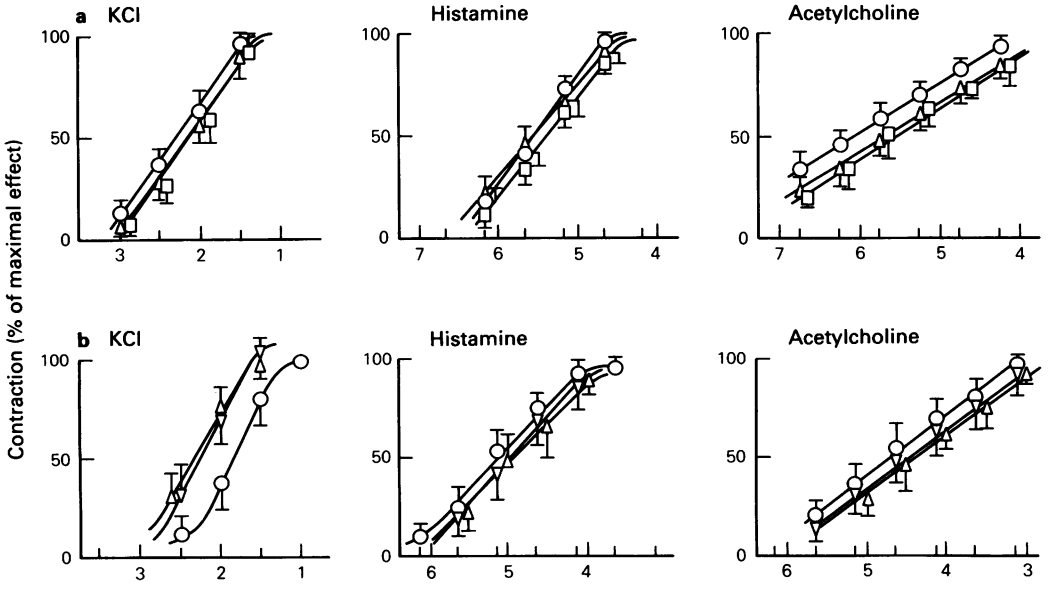

Figure 1 Effect of Bay K 8644 on the contraction of guinea-pig isolated tracheal spirals (a) and human bronchial strips (b) induced by potassium chloride, histamine or acetylcholine. Concentration-response relationship were observed in controls (○) or in the presence of Bay K 8644 10^{-8} (▽), 10^{-7} M (△) or 10^{-6} M (□). Experiments were performed on groups of 5 (guinea-pig trachea) or 4 (human bronchus) preparations. Vertical bars indicate s.e.mean.

ediaminetetraacetic acid 10^{-3} M. The preparations were washed at intervals of 15 min. In a second stage, the spirals were incubated in a calcium-free solution with additional K^+. The composition of the potassium-enriched solution was (mM): NaCl 90, KCl 29, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.7 (pH 7.46). After incubation the dose-response curves to Ca^{2+} 0.01 to 3 mM were determined by cumulative addition. Bay K 8644 was added to the bath 15 min before addition of Ca^{2+}; antagonists (nicardipine, and verapamil) were preincubated for 15 min, before Bay K 8644 was introduced.

The drug-induced contractions were expressed as percentage of the maximal effect. $-\log EC_{50}$ (pD_2) values were derived from the log concentration-effect curves, and were defined as the negative log of the drug concentration that caused 50% of maximal effect. These values were evaluated graphically from each experiment. pA_2 values were determined according to Arunlakshana & Schild (1959).

Statistical analysis of results

Statistical analysis of the results obtained was performed using Student's t test. All values in the text and table are expressed as mean ± s.e.mean.

Drugs

The drugs used were: Bay K 8644 (Bayer), nicardipine HCl (Sandoz, Basel), verapamil HCl (Biosédra, Paris), histamine HCl (Prolabo, Paris), acetylcholine di-HCl (Astra-Lematte et Boinot, Paris), KCl (Prolabo, Paris), calcium chloride (Prolabo, Paris).

Nicardipine and Bay K 8644 were dissolved daily in ethanol and the solution was further diluted with Krebs or Krebs modified solutions.

Results

In Krebs solution with normal Ca^{2+} and K^+ concentrations, Bay K 8644 did not significantly modify the concentration-response relationship of the guinea-pig isolated trachea to histamine, ACh or KCl, or the concentration-response relationship of human bronchus to histamine and ACh, although the curves were slightly displaced to the right with both preparations (Figure 1, Tables 1 and 2). In contrast, Bay K 8644 displaced the KCl curves to the left, thus indicating that the effect of KCl on human bronchus was potentiated; with Bay K 8644 concentrations of 10^{-8} M and 10^{-7} M, shifts corresponded to potentiations of 1.74 and 1.69 (Figure 1, Table 1). The maximal effect of KCl was slightly increased (Table 2).

In Ca^{2+}-free solution with additional K^+, Bay K 8644 displaced to the left the concentration-response relationships of both preparations to Ca^{2+} (Figures 2 and 3, Table 1). Potentiation of the effect of Ca^{2+} on the guinea-pig isolated trachea with Bay K 8644 10^{-7} and 10^{-6} M was 4.67 and 11.48 respectively; on the human bronchus and with Bay K 8644 10^{-7} M, potentiation was 5.24. The maximal effect of Ca^{2+} was slightly increased in both preparations (Table 2).

In Krebs solution with additional K^+ and in the presence of Ca^{2+} 0.03 mM, Bay K 8644 exerted a contractile effect on the guinea-pig isolated trachea and on the human bronchus preparations. This effect was proportionally to calcium more pronouced on the

Table 1 Negative log EC_{50} of histamine, acetylcholine, KCl and Ca^{2+} in the guinea-pig trachea ($n = 5$) and human bronchus ($n = 4$) and shifts induced by Bay K 8644.

	Histamine	*Acetylcholine*	*KCl*	*Ca^{2+}*
Guinea-pig trachea				
Control	5.56 ± 0.06	5.98 ± 0.12	2.27 ± 0.10	3.33 ± 0.17
Shift (log unit) in the presence of Bay K 8644 (M)				
10^{-8}	—	—	—	0.31 ± 0.14(L)
10^{-7}	0.03 ± 0.05(R)	0.31 ± 0.14(R)	0.15 ± 0.06(R)	0.67 ± 0.16(L)[a]
10^{-6}	0.13 ± 0.11(R)	0.33 ± 0.13(R)	0.14 ± 0.07(R)	1.06 ± 0.19(L)[b]
Human bronchus				
Control	5.15 ± 0.14	4.72 ± 0.21	1.85 ± 0.25	3.44 ± 0.09
Shift (log unit) in the presence of Bay K 8644 (M)				
10^{-8}	0.15 ± 0.06(R)	0.20 ± 0.08(R)	0.24 ± 0.06(L)[b]	0.52 ± 0.11(L)[b]
10^{-7}	0.17 ± 0.07(R)	0.24 ± 0.09(R)	0.23 ± 0.07(L)[a]	0.71 ± 0.16(L)[a]

(L) and (R) indicate respectively a shift to the left or to the right. Significant shift: [a] $P < 0.05$; [b] $P < 0.01$.

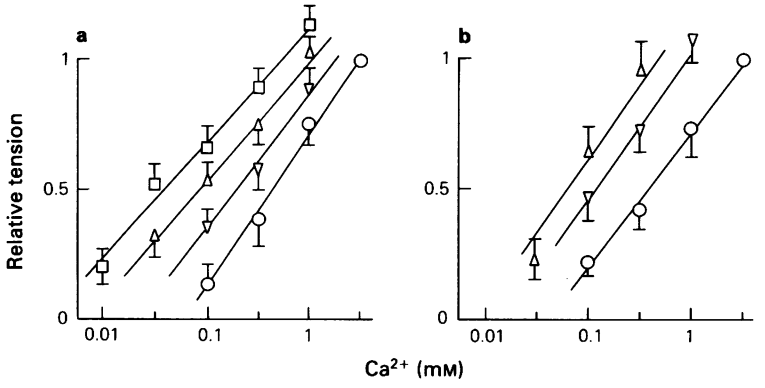

Figure 2 Ca^{2+} concentration-response relationship observed in guinea-pig isolated trachea (a) and human isolated bronchus (b) in the absence (○) or in the presence of Bay K 8644 10^{-8} M (▽), 10^{-7} (△) or 10^{-6} M (□). The contraction caused by Ca^{2+} 3 mM in the absence of Bay K 8644 is taken as 1.0. Experiments were performed on groups of 5 (guinea-pig trachea) or 4 (human bronchus) preparations. Vertical bars indicate s.e.mean.

guinea-pig trachea (Figure 4). The maximal effect of Bay K 8644 (10^{-7} M) was 665 ± 135 mg ($n = 5$) in guinea-pig trachea and 1161 ± 286 mg in human bronchus ($n = 4$); under the same experimental conditions, the maximal effect of Ca^{2+} (3 mM) was 723 ± 164 mg in guinea-pig trachea ($n = 5$) and 1787 ± 752 mg in human bronchus ($n = 4$). The contractile effect of Bay K 8644 was competitively antagonized in both guinea-pig trachea and human bronchus by nicardipine, with pA_2 values of 8.65 and 9.64 and regression slopes of 0.85 and 0.76 respectively. Finally, on the guinea-pig trachea concentration-response curves to Bay K 8644 observed in the presence of verapamil (10^{-7} to 10^{-6} M) would suggest a non-competitive antagonism.

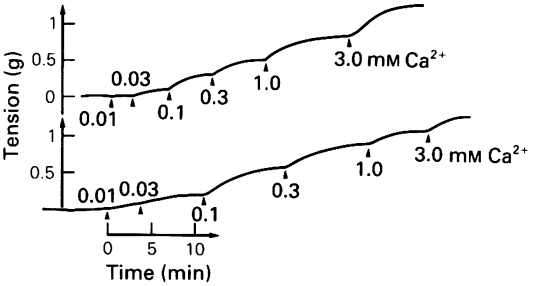

Figure 3 Example of cumulative concentration-response curve to Ca^{2+} in the absence (upper tracing) or in the presence (lower tracing) of Bay K 8644 (10^{-7} M) on the human isolated bronchus.

Table 2 Maximal tension (mg) induced by histamine, acetylcholine, KCl and Ca^{2+} in the guinea-pig trachea ($n = 5$) and human bronchus ($n = 4$) and variations (%) induced by Bay K 8644.

	Histamine	*Acetylcholine*	*KCl*	*Ca^{2+}*
Guinea-pig trachea				
Control (mg)	748 ± 120	1045 ± 84	1026 ± 105	688 ± 108
Variation (%) in the presence of Bay K 8644 (M)				
10^{-7}	− 2.7 ± 1.9	− 6.6 ± 4.2	− 7.3 ± 5.5	+ 31.2 ± 8.4*
10^{-6}	− 10.1 ± 4.7	− 3.1 ± 2.7	− 7.6 ± 5.1	+ 9.8 ± 4.1
Human bronchus				
Control (mg)	1625 ± 201	1949 ± 112	1323 ± 124	1146 ± 226
Variation (%) in the presence of Bay K 8644 (M)				
10^{-8}	− 6.1 ± 3.4	+ 6.4 ± 3.1	+ 21.0 ± 6.0*	+ 20.8 ± 6.1*
10^{-7}	− 12.6 ± 6.1	− 10.8 ± 4.2	+ 13.5 ± 4.6*	+ 32.4 ± 8.4*

Significant differences: $*P < 0.05$.

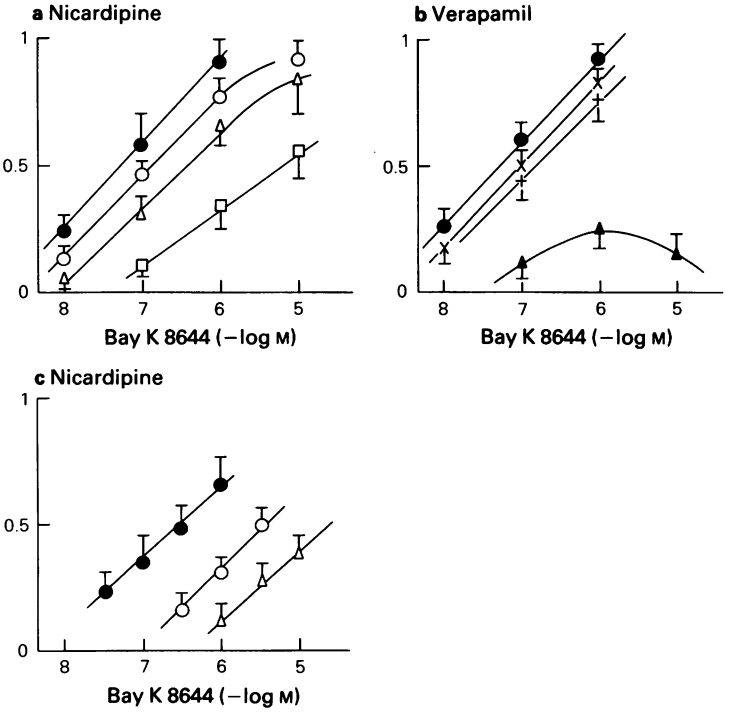

Figure 4 Bay K 8644 concentration-response relationship observed in guinea-pig isolated trachea (a,b) and human bronchus (c) in the absence (●) or in the presence of nicardipine (○: 3×10^{-9} M; ▽: 3×10^{-8} M; □: 3×10^{-7} M) and verapamil (×: 10^{-7} M; +: 3×10^{-7} M; ▲: 10^{-6} M). The contractions are expressed as tension relative to that induced by Ca^{2+} 3 mM which is taken as 1.0. Experiments were performed on groups of 4 or 5 preparations. Vertical bars indicate s.e.mean.

Discussion

Calcium movements through potential-operated channels (POC) have been demonstrated in guinea-pig airways muscle both directly, by measuring $^{45}Ca^{2+}$ uptake by the lanthanum method (Foster *et al.*, 1983b; Allen *et al.*, 1985), and indirectly, by using Ca^{2+} in a K^+-enriched solution as described by Godfraind *et al.* (1978) (Cerrina *et al.*, 1983; Advenier *et al.*, 1984); by investigating the effects of agents, such as potassium chloride, tetraethylammonium (TEA) or barium chloride, which tend to open POC (Cerrina *et al.*, 1983; Foster *et al.*, 1983a,b; Advenier *et al.*, 1984); or by studying the effects of dihydropyridines that stimulate (Bay K 8644) (Allen *et al.*, 1985) or antagonize (nifedipine, nicardipine or dazopidine) (Cerrina *et al.*, 1983; Advenier *et al.*, 1984) Ca^{2+} transport across the cell membrane.

These studies have shown that KCl, TEA and $BaCl_2$ contract the guinea-pig isolated trachea and that this effect is inhibited specifically by organic calcium antagonists, notably dihydropyridine derivatives. These derivatives also inhibit specifically the contractile effects of Ca^{2+} in K^+-enriched medium (Cerrina *et al.*, 1983; Advenier *et al.*, 1984). In this respect, the guinea-pig isolated trachea reacts to calcium antagonists very much like different types of vascular segments, such as rat aorta and mesenteric arteries, rabbit aorta and basilar artery, or dog coronary and mesenteric arteries (Advenier *et al.*, 1984).

The same studies with calcium antagonists have also shown that Ca^{2+} movements through POC play only a modest role in histamine- or ACh-induced contraction of the bronchial smooth muscle: these agents still induce a major contractile response after Ca^{2+} has been removed from the medium and very high concentrations of organic Ca^{2+} antagonists are needed to modify their effect (Cerrina *et al.*, 1983; Foster *et al.*, 1983a,b).

The studies of Allen *et al.* (1985) with Bay K 8644 on the guinea-pig trachea have given similar results concerning the effects of histamine and ACh.

However, these authors have shown that Bay K 8644 potentiates the effects of KCl, TEA and Ca^{2+} in depolarizing medium and that it increases the influx of Ca^{2+} as evaluated by the lanthanum method.

In our experiments Bay K 8644 did not significantly modify the effects of histamine and ACh on either human bronchus or guinea-pig trachea, which shows that in human bronchi the entry of Ca^{2+} into cells plays a modest role in the contractile effects of these mediators, as previously demonstrated on the guinea-pig trachea (Cerrina *et al.*, 1983; Foster *et al.*, 1983a,b; Allen *et al.*, 1985).

However, in our experiments Bay K 8644 in concentrations of 10^{-8} and 10^{-7} M potentiated the contractile effect of KCl on human bronchus but not on the guinea-pig trachea. These results conflict with those reported by Allen *et al.* (1985), who found that Bay K 8644 in concentrations of 10^{-6} M potentiated the effects of KCl on the guinea-pig trachea. This discrepancy is difficult to explain, since these authors experimented under conditions very similar to ours.

We also found that Bay K 8644 potentiated the contractile effects of Ca^{2+} in K^+-enriched Krebs solution on both preparations and that this potentiating effect was much more pronounced (ten fold) than that on KCl (two fold).

The results described here on human bronchus or guinea-pig trachea are comparable to those observed on other preparations, such as the rabbit aorta, where Bay K 8644 also potentiates the contractile effects of KCl but has no action on noradrenaline-induced contraction (Schramm *et al.*, 1983a,b), or on the rabbit mesenteric artery, where Bay K 8644 enhances the contractile effect of Ca^{2+} in a potassium-enriched solution (Kanmura *et al.*, 1984), or again on the taenia from the guinea-pig caecum, where Bay K 8644 also potentiates the contractile effect of calcium (Spedding & Berg, 1984).

Finally, in our experiments on the guinea-pig trachea and on the human bronchus, as in those performed on the rabbit aorta (Schramm *et al.*, 1983a,b; Yamamoto *et al.*, 1984), on the guinea-pig caecum (Spedding & Berg, 1984) and on the isolated heart of dog (Vaghy *et al.*, 1984), guinea-pig (Schramm *et al.*, 1983,b; Finet *et al.*, 1985) or rat (Finet *et al.*, 1985) Bay K 8644 exerted a contractile effect in a K^+-enriched medium. This effect was competitively inhibited by nicardipine, a dihydropyridine derivative. This finding is in agreement with the observation made by Vaghy *et al.* (1984) that Bay K 8644 and nitrendipine share the same binding sites and with the studies of Schramm *et al.* (1983b), Yamamoto *et al.* (1984), Spedding & Berg (1984), Kanmura *et al.* (1984), Spedding (1985) and Finet *et al.* (1985) which demonstrated a reversible antagonism between Bay K 8644 and other dihydropyridines (nifedipine, nisoldipine, dazopidine) on different preparations.

Furthermore, pA_2 values of nicardipine versus Bay K 8644 were similar in guinea-pig trachea and human bronchus and analogous with the values reported by Spedding (1985) with nifedipine when tested on a depolarized taenia preparation from the guinea-pig caecum.

Our experiments on the guinea-pig trachea would suggest that the antagonism between verapamil and Bay K 8644 is not of the competitive type. However, no definite conclusion can be drawn from the dose-response curve to Bay K 8644 in the presence of verapamil 10^{-6} M, since Allen *et al.* (1985), using a different protocol, have shown that Bay K 8644 (10^{-8} to 10^{-6} M) provided concentration-dependent protection against the depression induced by verapamil (10^{-6} M) vs KCl concentration-response curves.

In conclusion, these results show that the human bronchus responds to dihydropyridines, and notably to Bay K 8644, in a manner similar to that of the guinea-pig isolated trachea, and that in both preparations the potential-dependent calcium movements play a negligible part in the contractile effects of histamine and ACh.

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