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Differential antagonism by Bay k 8644, a dihydropyridine calcium agonist, of the negative inotropic effects of nifedipine, verapamil, diltiazem and manganese ions in canine ventricular muscle

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1 Antagonism between either the dihydropyridine calcium agonist, Bay k 8644, or high external Ca^{2+} and the calcium antagonists, nifedipine, verapamil and diltiazem, and Mn^{2+} was investigated in canine isolated ventricular trabeculae.

2 Bay k 8644 (10^{-7}–10^{-5} M) produced a slowly developing increase in developed tension which reached a maximum at 10^{-6} M. A small decrease in the positive inotropic effect of Bay k 8644 at 10^{-5} M was probably due to the negative inotropic effect of the solvent, 0.5% ethanol.

3 Bay k 8644 (10^{-7}–10^{-5} M) produced a rightward parallel shift of the concentration-response curves for the negative inotropic effects of nifedipine (10^{-8}–10^{-5} M) and verapamil (10^{-7}–3×10^{-5} M). The slopes of the Schild plots were -0.92 for nifedipine (pA_2 value = 6.58) and -0.48 for verapamil.

4 Bay k 8644 (10^{-6} and 10^{-5} M) produced only a slight rightward shift of the concentration-response curves for the negative inotropic effect of diltiazem (10^{-7}–3×10^{-5} M) and did not affect the negative inotropic effect of Mn^{2+} (10^{-4}–10^{-2} M).

5 Addition of 2.5×10^{-3} M Ca^{2+} (5.05×10^{-3} M Ca^{2+}) to the medium produced a greater maximum positive inotropic effect than Bay k 8644. The concentration-response curves for the negative inotropic effects of nifedipine, verapamil and diltiazem obtained under these conditions were not essentially different from those under control conditions (2.55×10^{-3} M Ca^{2+}).

6 These results indicate that Bay k 8644, while producing a positive inotropic effect, antagonizes the negative inotropic effect of nifedipine by competing with the latter for the same site closely associated with the calcium channel. In contrast, Bay k 8644 antagonizes the negative inotropic effects of verapamil and diltiazem by interfering allosterically with the binding of these calcium antagonists to their sites of action. Bay k 8644 does not antagonize the negative inotropic effect of Mn^{2+}. No pharmacological antagonism was observed between the three organic calcium antagonists and high external Ca^{2+}.

Introduction

Bay k 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), unlike its parent dihydropyridine calcium antagonists such as SK&F 24260 and nifedipine, produces positive inotropic, chronotropic, and vasoconstrictor effects (Schramm *et al.*, 1983a,b; Satoh *et al.*, 1984). The activation of the calcium channel has been proposed as the mechanism underlying these actions of Bay k 8644 (Schramm *et al.*, 1983a,b). Thus, the compound has been designated a calcium agonist (Schramm *et al.*, 1983a,b). Moreover, it has been shown that the contractile effect of Bay k 8644 on partially depolarized rabbit aortic strips is competitively antagonized by nifedipine but only functionally inhibited by the non-dihydropyridine calcium antagonists, verapamil and diltiazem (Schramm *et al.*, 1983a,b). It has also been shown that the positive inotropic effect of Bay k 8644 is competitively antagonized by nifedipine (Schramm *et al.*, 1983a,b). However, no information is available regarding the interaction between Bay k 8644 and non-dihydropyridine calcium antagonists or inorganic calcium antagonists on cardiac muscle. The present experiments attempted to settle this question. It has been suggested that Bay k 8644 binds to dihydropyridine receptors closely associated with the calcium channel and thereby

activates the latter (Schramm *et al.*,1983a,b). More recently, the specific binding of Bay k 8644 to dihydropyridine receptors has been shown by a receptor binding study using [^{3}H]-Bay k 8644 and [^{3}H]-nimodipine, a dihydropyridine calcium antagonist, on cultured myocardial cells (Bellemann, 1984).

Methods

Mongrel dogs of either sex weighing 5 to 14.5 kg were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.v.) and the hearts were excised. Right ventricular trabeculae (smaller than 1.2 mm in width) were dissected from the hearts in cold Tyrode solution, and mounted in 20 ml organ baths containing Krebs-Henseleit or HEPES buffered solution. Krebs-Henseleit solution was gassed with 95% O_2 and 5% CO_2 and HEPES buffered solution with 100% O_2 at a temperature of 37°C. The composition (mmol l^{-1}) of the solutions was as follows: (1) Krebs-Henseleit solution: NaCl 118, KCl 4.7, $CaCl_2$ 2.55, $MgSO_4$ 1.18, KH_2PO_4 1.18, $NaHCO_3$ 24.9 and glucose 11.1, (2) HEPES buffered solution: HEPES 3, NaCl 140, KCl 4.7 $CaCl_2$ 2.55, $MgCl_2$ 2.5 and glucose 11.1 (pH 7.4). HEPES buffered solution was used to avoid the precipitation of insoluble salts in the experiments investigating the effects of Mn^{2+}.

Muscle preparations were stretched to produce a resting tension of about 500 mg and electrically stimulated with square-wave pulses of a voltage of about 20% above threshold and 5 ms duration, at a frequency of 0.5 Hz. In experiments using Mn^{2+}, the electrical threshold was sometimes raised and the voltage was therefore increased to produce contractions. Tissues were allowed to equilibrate for at least 1 h and washed once or twice during the equilibration period. Tension of the muscles was recorded on a thermal pen writing oscillograph (NEC San-ei Instrument, 8K-23) by means of force displacement transducers (Grass FT03B; Shinkoh UL). In all experiments 4 muscles were run in parallel and one of them served as control, that is. a cumulative concentration-response curve for one of the calcium antagonists, diltiazem, nifedipine and verapamil, or divalent cation Mn^{2+} was determined in the absence of Bay k 8644. In the remaining muscles similar curves were determined in the presence of various concentrations of Bay k 8644. In the latter experiments calcium antagonists or $MnCl_2$ were administered after the developed tension had reached a plateau. It took 60–120 min for an increase in developed tension to reach a plateau. In a previous study on canine ventricular muscles (Endoh *et al.*, 1980) the negative inotropic effects of the calcium antagonists, nicardipine (YC-93) and D-600 (methoxyverapamil), developed gradually over the course of 60 min to reach a steady level and the negative inotropic effects of these agents at 30 min were more than 75% of those at steady state. Therefore, we allotted 30 min intervals to increasing concentrations of the calcium antagonists. As Bay k 8644 produced a positive inotropic effect, possible modification by the organic calcium antagonists and Mn^{2+} of the positive inotropic effect of high external Ca^{2+} (5.05×10^{-3} M) was also examined for comparison. Some preparations were not used for determining drug effects because of unstable resting tension. Only data with controls were used to construct concentration-response curves in the presence of Bay k 8644. In some preparations the effect of ethanol (the solvent for Bay k 8644) was examined, and in some preparations the effect of nadolol on Bay k 8644 was examined.

The drugs used were methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay k 8644, Bayer), diltiazem hydrochloride (Tanabe Seiyaku), nifedipine (100 μg ml^{-1} in ampoules, Bayer), verapamil hydrochloride (Knoll) and nadolol base (Squibb). Bay k 8644 was dissolved in 99.5% ethanol at a concentration of 2×10^{-3} M. Nadolol base was dissolved in 0.5 N HCl to give a concentration of 3.2×10^{-2} M. The stock solutions of Bay k 8644, nadolol and nifedipine were diluted with distilled water to the desired concentrations. Other

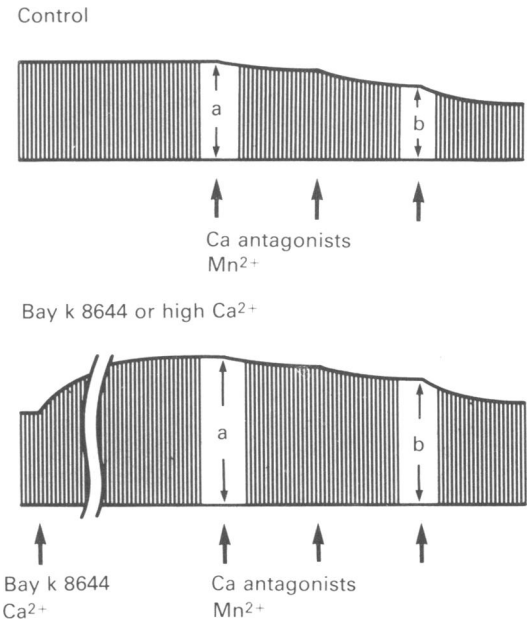

Figure 1 Schematic representation of calculation of suppression of developed tension of canine ventricular muscles caused by calcium antagonists or Mn^{2+} in the absence (control) (upper panel) and presence of Bay k 8644 or high external Ca^{2+} (lower panel). Suppression (%) = ((a − b)/a) × 100.

drugs were dissolved in distilled water in the desired concentrations.

Cumulative concentration-response curves for the negative inotropic effects of the calcium antagonists and Mn^{2+}, i.e., suppression of developed tension, were expressed as percentage changes from the developed tension just before administration of these negative inotropic agents in the absence (control) or presence of each concentration of Bay k 8644 or high external Ca^{2+} (5.05×10^{-3} M), as shown in Figure 1. Each preparation was subject to only one concentration of Bay k 8644. Therefore, the concentration-response curve for Bay k 8644 was constructed from pooled data.

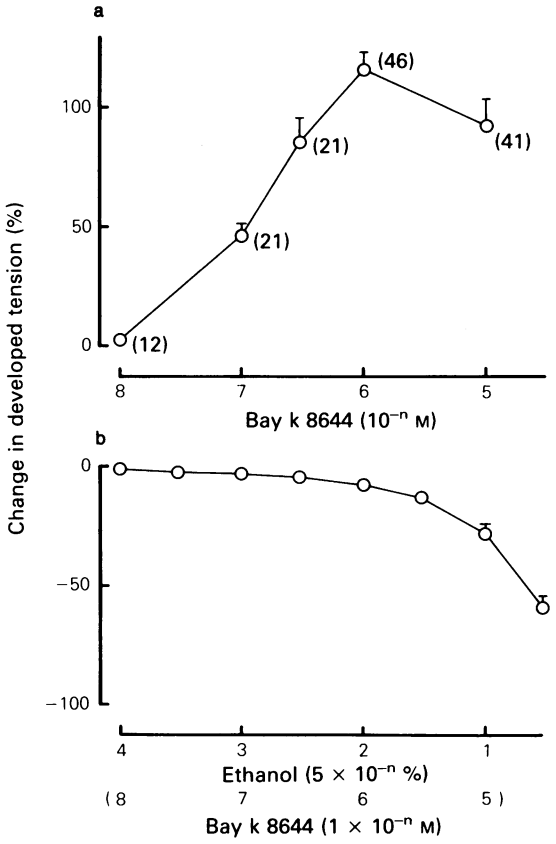

Figure 2 (a) Concentration-response curve to Bay k 8644 for increase in developed tension of canine ventricular muscles. The increase in developed tension is expressed as percentage of the basal developed tension. The number of muscles is given in parentheses. (b) Concentration-response curve to ethanol (the solvent for Bay k 8644) for decrease in developed tension of canine ventricular muscles. The decrease in developed tension is expressed as percentage of the basal developed tension ($n = 5$). The corresponding concentrations of Bay k 8644 are given under its solvent concentrations. Vertical lines show s.e.mean.

Experimental values were expressed in terms of means ± s.e.mean. Differences between mean values were analysed using Student's t test. Parallelism of concentration-response curves was analysed by the use of analysis of covariance techniques described by Snedecor & Cochran (1967). The concentration-response curves were treated as linear regressions and analysed for similarities in slope. The criterion for significance was P values less than 0.05.

Results

Effects of Bay k 8644 on the basal developed tension

In canine ventricular muscle Bay k 8644 (10^{-8}–10^{-6} M) produced a concentration-dependent increase in developed tension up to 120% of the basal value at 10^{-6} M, but the increase at 10^{-5} M was slightly less (about 25%) than that at 10^{-6} M (Figure 2a). The positive inotropic effect of Bay k 8644 developed very slowly to reach a plateau over a period of 60–120 min. Ethanol (the solvent for Bay k 8644) (0.0005–1.5%) decreased the developed tension in a concentration-dependent manner, and 0.5% ethanol (the amount required to dissolve 10^{-5} M Bay k 8644) decreased it by about 27% of the basal value (Figure 2b). Nadolol (10^{-6} M) failed to modify the positive inotropic effect of Bay k 8644 (10^{-6} or 10^{-5} M) (data not shown) indicating no involvement of β-

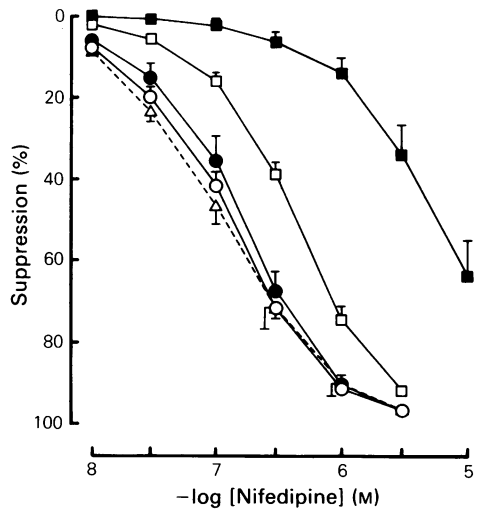

Figure 3 Concentration-response curves for the negative inotropic effect of nifedipine on canine ventricular muscles in the absence (○) ($n = 12$) and presence of Bay k 8644 10^{-7} M (●) ($n = 6$), 10^{-6} M (□) ($n = 5$), 10^{-5} M (■) ($n = 7$) or high external Ca^{2+} (5.05×10^{-3} M) (△) ($n = 6$). Responses are expressed as shown in Figure 1. Vertical lines show s.e.mean.

adrenoceptor mechanisms in the positive inotropic effect of Bay k 8644.

Effects of Bay k 8644 on the negative inotropic actions of organic calcium antagonists

In control preparations nifedipine (10^{-8}–3×10^{-6} M) exerted a concentration-dependent negative inotropic effect, and at 3×10^{-6} M the developed tension decreased to less than 5% of the basal value. The effects of various concentrations of Bay k 8644 on the concentration-response curves for the negative inotropic action of nifedipine are shown in Figure 3. The concentration-response curves for nifedipine were shifted to the right by Bay k 8644 in a concentration-dependent and parallel manner; the pD_2 values for nifedipine were reduced from 6.89 ± 0.05 in the absence, down to 5.35 ± 0.14 in the presence, of 10^{-5} M Bay k 8644 (Table 1). The Schild plot was constructed from all the data except those obtained from the following 3 preparations: in 1 of 6 preparations in which 10^{-7} M Bay k 8644 was examined, the ED_{50} value for nifedipine was not larger than that of control, and in 2 of 7 preparations in which 10^{-5} M Bay k 8644 was examined, even 10^{-5} M nifedipine failed to decrease the developed tension to less than 50%. The pA_2 value obtained from the Schild plot was 6.58 and the slope of the regression line -0.92 (the correlation coefficient $r = -0.94$) (Figure 4).

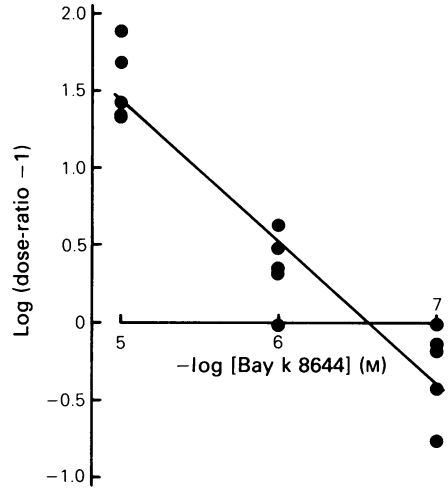

Figure 4 Schild plot showing the competitive antagonism of the negative inotropic effect of nifedipine by Bay k 8644 in canine ventricular muscles. The pA_2 value is 6.58 with the slope of the regression line being -0.92. The correlation coefficient is -0.94.

Verapamil was about 10 times less potent in producing a negative inotropic effect than nifedipine, and the concentration range examined was 10^{-7} to 3×10^{-5} M. In control preparations verapamil in these

Table 1 Effects of Bay k 8644 and high external Ca^{2+} on the developed tension and the pD_2 values for three calcium antagonists

		n	pD_2 value	Developed tension: Basal (mg)	Developed tension: Increase (%)
Nifedipine					
Control		12	6.89 ± 0.05	999 ± 129	
Bay k 8644	10^{-7} M	6	6.80 ± 0.09	634 ± 168	164.7 ± 11.0
	10^{-6} M	5	6.36 ± 0.05*	327 ± 103	224.3 ± 8.4
	10^{-5} M	7	5.35 ± 0.14* ($n = 5$)	801 ± 161	159.5 ± 16.8
High Ca^{2+}	(5.05×10^{-3} M)	6	6.92 ± 0.10	252 ± 42	294.5 ± 43.0
Verapamil					
Control		13	5.77 ± 0.09	855 ± 155	
Bay k 8644	10^{-7} M	5	5.66 ± 0.07	1052 ± 314	139.5 ± 8.6
	10^{-6} M	9	5.12 ± 0.04*	556 ± 81	210.5 ± 18.0
	10^{-5} M	6	4.95 ± 0.06*	744 ± 75	180.7 ± 21.0
High Ca^{2+}	(5.05×10^{-3} M)	8	5.67 ± 0.04	306 ± 49	268.4 ± 45.7
Diltiazem					
Control		11	5.46 ± 0.06	979 ± 181	
Bay k 8644	10^{-7} M	5	5.50 ± 0.09	335 ± 138	153.8 ± 10.0
	10^{-6} M	6	5.17 ± 0.05*	1018 ± 223	169.6 ± 15.3
	10^{-5} M	6	5.11 ± 0.06*	1041 ± 290	190.7 ± 30.7
High Ca^{2+}	(5.05×10^{-3} M)	5	5.38 ± 0.08	509 ± 70	243.0 ± 35.1

Results are given as mean ± s.e.mean; n = number of muscles.
*Significantly different from control values ($P < 0.01$).

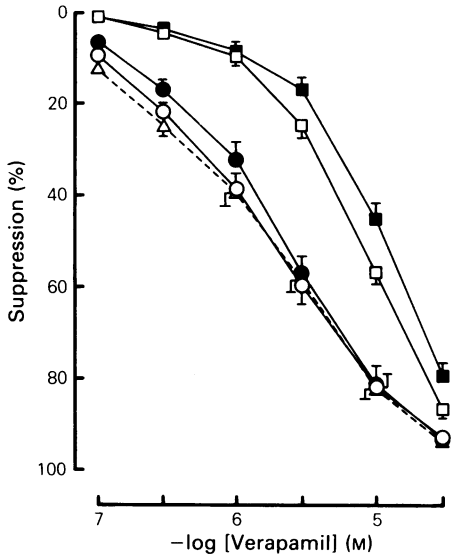

Figure 5 Concentration-response curves for the negative inotropic effect of verapamil on canine ventricular muscles in the absence (○) ($n = 13$) and presence of Bay k 8644 10^{-7} M (●) ($n = 5$), 10^{-6} M (□) ($n = 9$), 10^{-5} M (■) ($n = 6$) or high external Ca^{2+} (5.05×10^{-3} M) (△) ($n = 8$). Responses are expressed as shown in Figure 1. Vertical lines show s.e.mean.

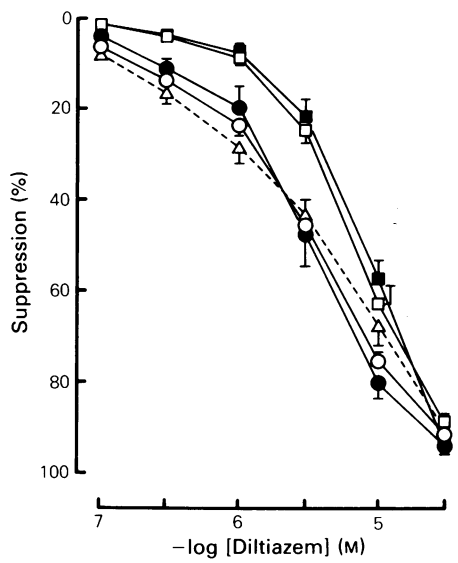

Figure 7 Concentration-response curves for the negative inotropic effect of diltiazem on canine ventricular muscles in the absence (○) ($n = 11$) and presence of Bay k 8644 10^{-7} M (●) ($n = 5$), 10^{-6} M (□) ($n = 6$), 10^{-5} M (■) ($n = 6$) or high external Ca^{2+} (5.05×10^{-3} M) (△) ($n = 5$). Responses are expressed as shown in Figure 1. Vertical lines show s.e.mean.

concentrations decreased the developed tension in a concentration-dependent manner, down to less than 10% of the basal value at 3×10^{-5} M. The effect of Bay k 8644 on the concentration-response curves for the negative inotropic action of verapamil is shown in Figure 5. Bay k 8644 (10^{-7}–10^{-5} M) shifted the concentration-response curves for verapamil to the right in a parallel way, but 10^{-6} and 10^{-5} M Bay k 8644 were nearly equi-effective in shifting the curves for verapamil. The pD_2 values for verapamil were reduced from 5.77 ± 0.09 in the absence, down to 4.95 ± 0.06 in the presence, of 10^{-5} M Bay k 8644 (Table 1). The slope of the Schild plot was -0.48 and $r = -0.52$ (Figure 6).

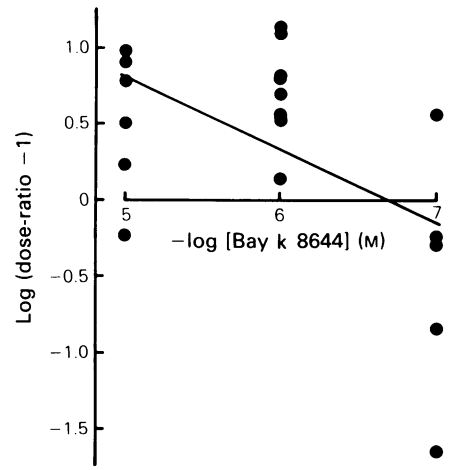

Figure 6 Schild plot showing the antagonism of the negative inotropic effect of verapamil by Bay k 8644 in canine ventricular muscles. The slope of the regression line is -0.48 with a correlation coefficient of -0.52.

Diltiazem (10^{-7}–3×10^{-5} M) exerted a concentration-dependent negative inotropic effect on control preparations, and at 3×10^{-5} M the developed tension decreased to less than 10% of the basal value (Figure 7). In this respect diltiazem resembled verapamil. The concentration-response curves for diltiazem were shifted to the right to a small extent by Bay k 8644 (10^{-6} and 10^{-5} M) (Figure 7); the pD_2 value was 5.11 ± 0.06 in the presence of 10^{-5} M Bay k 8644 as against 5.46 ± 0.06 in control (Table 1). In 4 of 5 preparations which were exposed to 10^{-7} M Bay k 8644 and in 1 of 6 preparations subjected to 10^{-6} M Bay k 8644, ED_{50} values for diltiazem were not larger than those in each control (i.e. the (dose-ratio − 1) was negative in those 5 paired preparations). Therefore, a Schild plot could not be calculated in the case of diltiazem.

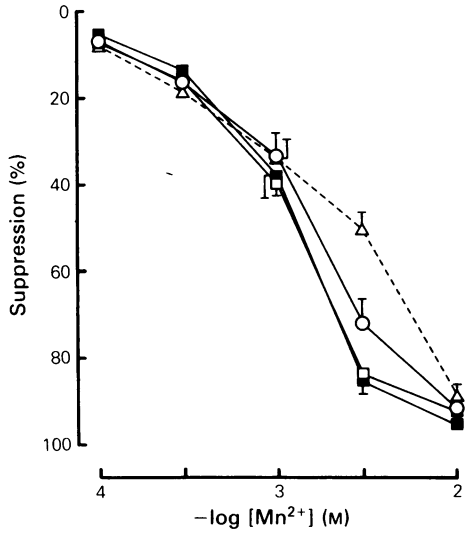

Figure 8 Concentration-response curves for the negative inotropic effect of Mn^{2+} on canine ventricular muscles in the absence (○) ($n = 6$) and presence of Bay k 8644 10^{-6} M (□) ($n = 6$), 10^{-5} M (■) ($n = 6$ or 5) or high external Ca^{2+} (5.05×10^{-3} M) (△) ($n = 6$ or 5). Responses are expressed as shown in Figure 1. Vertical lines show s.e.mean.

Effects of high external Ca^{2+} on the negative inotropic actions of organic calcium antagonists

Developed tension was increased to a greater extent by adding 2.5×10^{-3} M Ca^{2+} than by the most effective concentration of Bay k 8644 (10^{-6} M) (Table 1). However, the concentration-response curves for the negative inotropic effects of all three calcium antagonists examined (diltiazem, nifedipine and verapamil) in the presence of high external Ca^{2+} (5.05×10^{-3} M) were not significantly different from those in control conditions (2.55×10^{-3} M Ca^{2+}) (Figures 3, 5 and 7).

Effects of Bay k 8644 and high external Ca^{2+} on the negative inotropic action of Mn^{2+}

The developed tension was decreased by Mn^{2+} (10^{-4}–10^{-2} M) in a concentration-dependent manner. Bay k 8644 (10^{-6}–10^{-5} M) scarcely affected the negative inotropic effect of Mn^{2+}, whereas high external Ca^{2+} (adding 2.5×10^{-3} M to the bath) significantly reduced the negative inotropic effect of 3×10^{-3} M Mn^{2+} (Figure 8).

Discussion

Bay k 8644 produced a positive inotropic effect in canine isolated ventricular muscle as previously observed in anaesthetized dogs and Langendorff preparations of guinea-pigs (Schramm *et al.*, 1983a,b), and in isolated, blood-perfused papillary muscle preparations of the dog (Satoh *et al.*, 1984). This positive inotropic effect was not modified by the β-adrenoceptor antagonist, nadolol. Therefore, the positive inotropic effect of Bay k 8644 does not seem to involve the release of catecholamines, although Bay k 8644 has been found to potentiate catecholamine release evoked by K^+ in adrenomedullary chromaffin cells (García *et al.*, 1984). In the previous studies (Schramm *et al.*, 1983a,b) the positive inotropic effect of Bay k 8644 (measured as an increase in left ventricular pressure in the guinea-pig isolated perfused heart) reached a maximum at 10^{-6} M, and it was less at 10^{-5} M than at 10^{-6} M. In keeping with these observations, in the present experiments the maximum positive inotropic effect of Bay k 8644 was attained at 10^{-6} M and the effect became less at 10^{-5} M. Thus, it is tempting to speculate that this phenomenon reflects the action of Bay k 8644 as a partial calcium agonist. However, it is likely that this phenomenon can be attributed to the solvent, 0.5% ethanol, required to dissolve 10^{-5} M Bay k 8644, since it produced a negative inotropic effect similar in extent to the reduction of the positive inotropic effect at 10^{-5} M Bay k 8644 from that at 10^{-6} M (Figure 2).

While producing a positive inotropic effect Bay k 8644 has been shown to antagonize competitively the negative inotropic effect of nifedipine (Schramm *et al.*, 1983a,b). The antagonism between the negative inotropic effects of the calcium antagonists and the increased developed tension induced by high external Ca^{2+} (5.05×10^{-3} M) was also examined, for comparison. The concentration-response curves for the negative inotropic effects of all of the three organic calcium antagonists were not essentially different under control conditions and in the presence of the positive inotropic effect of high external Ca^{2+}, whereas they were modified by Bay k 8644, even though the developed tension was increased to a greater extent by high external Ca^{2+} than by 10^{-6} M Bay k 8644. Here it should be recalled that in the present study the concentraton-response curves for the negative inotropic effects of the three calcium antagonists and Mn^{2+} in the absence (control), and presence, of Bay k 8644 or high external Ca^{2+} were all normalized, i.e., expressed as percentage suppression from the respective developed tension measured before administration of these negative inotropic agents. The virtual absence of an effect of high external Ca^{2+} on the concentration-response curves for the negative inotropic effects of the three calcium antagonists means two things. (1) The sensitivity of canine ventricular muscle to the calcium antagonists was not modified by high external Ca^{2+}. (2) Each dose of the calcium antagonists produces a greater decrease

in developed tension in the presence of the positive inotropic effect of high external Ca^{2+} than under control conditions. These properties of calcium antagonists do not seem to be reconciled with their definition. If the mechanism of action of calcium antagonists is opposite to that of Bay k 8644 which has been elucidated at the level of single calcium channels (Hess *et al.*, 1984), the mechanism of action of calcium antagonists would be (1) to decrease the probability of opening (2), to decrease open times or (3) to increase closed times of single calcium channels. Elevation of external Ca^{2+} increases the driving force of Ca^{2+}. If the mechanism of action of calcium antagonists is as postulated above, even in the presence of high external Ca^{2+} a percentage decrease in the increased developed tension, produced by a single concentration of calcium antagonist, should be the same as the percentage decrease under control conditions. Only the negative inotropic effect of Mn^{2+} was antagonized by high external Ca^{2+}. This is understandable if these two ions compete for the binding sites at the calcium channel (Hagiwara & Byerly, 1981).

The concentration-response curves for the negative inotropic effect of nifedipine, constructed as described above, were shifted to the right by increasing concentrations of Bay k 8644. The Schild plot for Bay k 8644 against nifedipine gave a regression line with a slope of -0.92 and a pA_2 value of 6.58. Thus, it is reasonable to conclude that specific dihydropyridine receptors exist in canine ventricular muscle and that Bay k 8644 and nifedipine compete for the same site. The present conclusion is consistent with that obtained from receptor binding studies (García *et al.*, 1983; Bellemann, 1984).

The concentration-response curves for the negative inotropic effect of verapamil were also shifted by 10^{-6} and 10^{-5} M Bay k 8644, but the rightward shifts caused by these two concentrations of Bay k 8644 were similar. The Schild plot of the data gave a regression line with a slope of -0.48 and the correlation coefficient was -0.52. This indicates that Bay k 8644 and verapamil do not compete for the same binding site. Verapamil has been reported to reduce allosterically the binding of dihydropyridines (Ehlert *et al.*, 1982; Glossmann *et al.*, 1982; Murphy *et al.*, 1983). The non-competitive inhibition by verapamil of the Bay k 8644-induced contraction of rabbit aortic strips observed by Schramm *et al.* (1983a,b) might be ascribed to this allosteric interaction, because in their experiments Bay k 8644 was administered in the presence of verapamil. In the present experiments the negative inotropic effect of verapamil was examined in the presence of Bay k 8644. If verapamil displaces bound Bay k 8644, the negative inotropic effects of verapamil may be greater in the presence of Bay k 8644 than in its absence. In the present experiments, however, the results were opposite to what was expected; Bay k 8644 inhibited the negative inotropic effects of verapamil. This suggests that Bay k 8644 does not compete with verapamil for the same site of action but instead reduces the binding of verapamil.

As to the interaction between Bay k 8644 and diltiazem, a Schild plot could not be calculated since the concentration-response curves for the negative inotropic effect of diltiazem were shifted to a much smaller extent than those for the two other calcium antagonists. This can be explained if Bay k 8644 reduces binding of diltiazem, or diltiazem increases binding of Bay k 8644 by slowing dissociation as has been demonstrated by receptor binding studies (Ferry & Glossmann, 1982).

In contrast to the results obtained with the organic calcium antagonists, the negative inotropic effect of the inorganic divalent cation, Mn^{2+}, was not affected by Bay k 8644, but was inhibited by high external Ca^{2+} (5.05×10^{-3} M) although only at a concentration of 3×10^{-3} M. Inorganic divalent cations possessing calcium antagonistic properties, such as Mn^{2+}, Co^{2+} and Ni^{2+}, have been thought to compete with Ca^{2+} for the same binding site in the calcium channel (Hagiwara & Byerly, 1981). The inhibition by Ca^{2+} of the negative inotropic effect of Mn^{2+} may be attributed to such competition.

In conclusion, the present study suggests that specific dihydropyridine receptors may exist in canine ventricular muscle whose occupation by Bay k 8644 causes some interaction with organic calcium antagonists but not with inorganic calcium antagonists. Competitive antagonism occurs between Bay k 8644 and nifedipine but the antagonistic effects of Bay k 8644 on responses to verapamil or diltiazem are clearly of a non-competitive nature.

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