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# Pressor responses induced by Bay K 8644 involve both release of adrenal catecholamines and calcium channel activation

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1 The dihydropyridine calcium channel activator, Bay K 8644, is believed to increase mean arterial blood pressure in several animal models, as a result of direct activation of vascular smooth muscle cells by increasing calcium influx through the voltage-dependent calcium channels. The purpose of the current study was to elucidate further the mechanism of action of Bay K 8644, by examining the possibility that the pressor response to Bay K 8644 may also be the result of indirect activation of the vascular smooth muscle cells by release of adrenal catecholamines.

2 Intravenous administration of Bay K 8644 increased mean arterial pressure in a dose-dependent manner in conscious, normotensive rats. This pressor response was blocked by calcium channel blockers (nifedipine, verapamil, and diltiazem) at doses lower than were necessary to decrease resting mean arterial pressure.

3 α-Adrenoceptor antagonists (phentolamine, yohimbine, and prazosin) completely blocked the Bay K 8644-induced pressor responses and converted them to depressor responses. Adrenalectomy did not alter the inhibitory effect of phentolamine on the pressor response to Bay K 8644. However, adrenalectomy or adrenal demedullectomy prevented the phentolamine-induced reversal of the Bay K 8644 pressor response to a depressor response. In addition, adrenalectomy did not affect the ability of phentolamine to reverse the pressor response to exogenous adrenaline administration to a depressor response.

4 These data suggest that the pressor response to Bay K 8644 may involve both direct activation of vascular smooth muscle cells and indirect activation of the muscle cells by release of adrenal catecholamines.

## Introduction

The importance of calcium in the regulation of cardiovascular function is well established. Calcium is necessary for contraction of both cardiac and vascular smooth muscle as well as for neural control of the heart and vasculature through its role in neurotransmitter release (for reviews see Kirpekar, 1975; Fleckenstein, 1977; Bolton, 1980). Although these calcium-dependent processes have been actively studied for several decades, the advent of organic compounds that specifically block the influx of calcium across cellular membranes has greatly enhanced these investigations (Fleckenstein, 1983). The dihydropyridine class of calcium channel blockers has been especially useful in the study of calcium and cardiovascular function (Kirpekar, 1975; Fleckenstein, 1983). In contrast to the dihydropyridine calcium channel blockers, Bay K 8644 is a dihydropyridine which activates calcium channels and thus results in an increase in calcium influx (Schramm *et al.*, 1983; Freedman & Miller, 1984).

Bay K 8644 has been shown to increase $^{45}Ca$ influx in rabbit aorta (Hwang & van Breemen, 1985; Dong & Wadsworth, 1986), guinea-pig trachealis (Allen *et al.*, 1985), and guinea-pig isolated ileum smooth muscle cells (Droogmans & Callewaert, 1986). Additionally, Bay K 8644 has been found to

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potentiate myocardial slow action potentials (Wahler & Sperelakis, 1984) and to increase the activity of cardiac sarcolemmal calcium channels incorporated into planar lipid bilayers (Ehrlich *et al.*, 1986). These facilitative actions of Bay K 8644 on calcium influx may be explained either by a Bay K 8644-induced increase in the probability that a single calcium channel will open more than once during a single membrane depolarization (Brown *et al.*, 1984), or that the channel will remain open for a longer period of time (Hess *et al.*, 1984).

Exposure of isolated strips of rabbit aorta (Schramm *et al.*, 1983), guinea-pig trachealis (Allen *et al.*, 1985), or swine carotid artery (Moreland & Moreland, 1987) to Bay K 8644 results in a concentration-dependent increase in force development. Furthermore, perfusion of guinea-pig isolated heart with Bay K 8644 increases left ventricular pressure (Schramm *et al.*, 1983). These effects of Bay K 8644 on isolated tissue preparations are presumably due to its calcium channel activating properties which increase the free intracellular calcium ion concentration. Intravenous injection of Bay K 8644 has been shown to increase mean arterial blood pressure and cardiac contractility (assessed by left ventricular $dP/dt$) in anaesthetized (Schramm *et al.*, 1983) and conscious (Gross *et al.*, 1985) dogs. Arterial pressure in anaesthetized rats has also been shown to increase following intravenous injection of Bay K 8644 (Lefer *et al.*, 1986).

The increase in arterial pressure *in vivo* following intravenous administration of Bay K 8644 is presumed to be the result of direct activation of either cardiac or vascular smooth muscle cells due to the calcium channel activating properties of this compound (Freedman & Miller, 1984). This premise is based on the evidence that exposure to Bay K 8644 results in an increase in cellular calcium and increased contractile activity in isolated cardiac and smooth muscle. The effects of Bay K 8644 on isolated tissues can be competitively antagonized by nifedipine by direct binding to the same receptor sites and can be non-competitively antagonized by verapamil and diltiazem via a generalized inhibition of calcium influx (Schramm *et al.*, 1983). However, the effects of Bay K 8644 are not limited to muscle cells. Bay K 8644 has also been shown to potentiate the release of catecholamines from adrenal glands (Montiel *et al.*, 1984) and nerve terminals (Cena *et al.*, 1985). The demonstration that catecholamine activation of vascular smooth muscle does not depend solely on the influx of extracellular calcium (Somlyo & Somlyo, 1968) may account for the incomplete inhibition of the pressor response to Bay K 8644 by calcium channel blockers demonstrated by Lefer *et al.* (1986). The purpose of the present study was to examine further the mechanism(s) by which administration of Bay K 8644 results in an increase in mean arterial blood pressure. Specifically, this study was designed to assess the relative contributions of direct (increased calcium influx) and indirect (catecholamine release) activation of the vasculature in the pressor response to Bay K 8644 in conscious, normotensive rats.

## Methods

### *Conscious rats*

Male Sprague-Dawley rats (150–250 g) were anaesthetized with sodium pentobarbitone and cannulated with indwelling aortic and vena caval catheters using a minor modification of the method described by Weeks & Jones (1960). The animals were allowed to recover from the surgery for at least two weeks before experimentation, during which time standard laboratory chow and water were provided *ad libitum*. The animals that were to be used for catecholamine depletion studies were adrenalectomized or adrenal demedullectomized one day before experimentation. On the day of the experiment, the animals were connected by their arterial catheters to Gould P23 ID pressure transducers and a Grass Model 7 polygraph for measurement of arterial pressure. Mean arterial blood pressure was determined by decreasing the high frequency response of the Grass driver amplifier used to monitor arterial pressure from 60 Hz to 0.1 Hz. Heart rates were determined by using a Grass model 7P4 tachograph interfaced to the driver amplifier of the Grass channel used to monitor arterial pressure. After the arterial pressure readings stabilized (approximately 1 h), control responses to Bay K 8644 (100 nmol $kg^{-1}$, i.v.) and adrenaline (3 nmol $kg^{-1}$, i.v.) were obtained. Test compounds (calcium channel blockers or adrenoceptor antagonists) were administered orally or intravenously to the appropriate groups of animals. Intravenous doses of Bay K 8644 and adrenaline were then administered at 5–20 min intervals until the pressor responses induced by Bay K 8644 or adrenaline returned to at least 80% of control before the administration of a higher dose of test compound. Data are presented as the mean ± s.e. mean for pressor responses from at least 4 rats.

The dihydropyridines, Bay K 8644 and nifedipine, were dissolved in polyethylene glycol (PEG) 400. Vehicle controls were performed at all doses of PEG 400 administered. The intravenous administration of these small amounts of PEG 400 had no effect on mean arterial blood pressure or heart rate. Adrenaline, diltiazem, verapamil, and all adrenoceptor antagonists were dissolved in water.

*Aortic strips*

For determination of the *in vitro* effect of test compounds on vascular smooth muscle, circumferential strips of rabbit thoracic aortae were used. New Zealand white rabbits were killed by an overdose of sodium pentobarbitone (50 mg kg$^{-1}$) injected into the marginal ear vein. The thoracic aorta was excised and cleaned of excess fat and connective tissue. Circumferential strips (2 mm) were cut from the cleaned aorta and suspended by gold clips between a stationary support and a Grass FT.03 force transducer mounted to a micrometer. The strips were bathed in physiological salt solution (PSS) of the following composition (in mM): NaCl 111.0, KCl 5.0, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25.0, $KH_2PO_4$ 1.0 and D-glucose, 11.5. The strips were maintained at 37°C and aerated with 95% $O_2$ and 5% $CO_2$ to produce a pH of 7.4. The strips were equilibrated in this solution for 1–2 h, during which time they were slowly stretched to 4 g resting force. Contractions of the smooth muscle cells were elicited by stimulation with 100 mM KCl (equimolar substitution for NaCl). When force reached a steady state value, the solution was changed to one containing 100 mM KCl plus an appropriate concentration of verapamil, diltiazem, or nifedipine and the strips were allowed to relax to a new steady-state force. The % relaxation was calculated by comparing the forces measured in the presence and absence of the calcium channel blocker. The concentration of calcium channel blocker necessary to produce a 50% inhibition of response ($IC_{50}$) was determined using logit analysis. $IC_{50}$ values for the calcium channel blockers in isolated aortic strips are presented as the mean ± 95% confidence interval for at least 4 aortic strips from different animals.

Nifedipine was dissolved in 95% ethanol to produce a stock solution such that the final concentration of ethanol in the muscle bath never exceeded 0.01%. The addition of ethanol alone to a final concentration of 0.01% had no effect on contractions elicited by 100 mM KCl. Verapamil and diltiazem were dissolved in water to produce a stock solution such that the change in muscle bath volume never exceeded 1%.

*Statistical analysis*

Data obtained from the conscious rats were normalized as a % of the maximal pressor response to either Bay K 8644 or adrenaline. Statistical analysis of these data was performed by subjecting the normalized data to an arcsine transformation before conducting a repeated measures ANOVA. Data obtained using isolated strips of rabbit thoracic aortae were normalized as a % relaxation of the response to 100 mM KCl. These data were arcsine transformed and submitted to an ANOVA. A $P$ value $<0.05$ was taken as significant. Any significant interactions identified by the ANOVA were analysed further by a Simple Effects Test. Alpha was adjusted by the number of tests conducted.

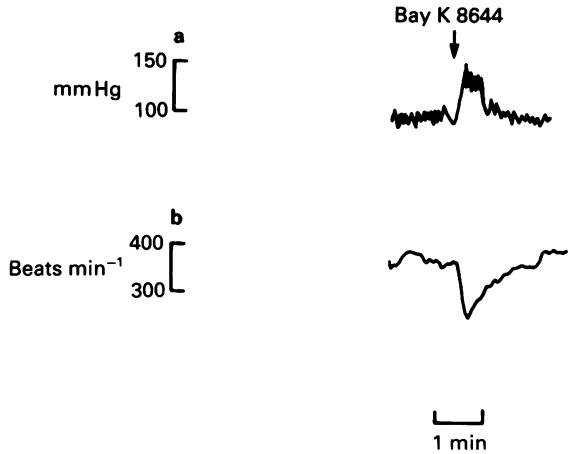


**Figure 1** Representative tracing of the effect of an intravenous injection of Bay K 8644 (100 nmol kg$^{-1}$) on (a) arterial blood pressure and (b) heart rate in a conscious, normotensive rat. Administration of Bay K 8644 resulted in a transient pressor response which lasted approximately 1 min. Arterial blood pressure and heart rate returned to control levels within 3 min after administration of Bay K 8644. Mean arterial blood pressure was derived by decreasing the high frequency response of the Grass driver amplifier used to monitor arterial pressure from 60 Hz to 0.1 Hz. This smoothed the signal giving a value representative of the mean.

## Results

Figure 1 shows a tracing of a typical experiment to test the effect of one concentration of Bay K 8644 (100 nmol kg$^{-1}$, i.v.) on the arterial blood pressure and heart rate of a conscious rat. The administration of Bay K 8644 resulted in a transient increase in arterial blood pressure and decrease in heart rate, both of which returned to baseline levels within 3 min after injection. This concentration of Bay K 8644 resulted in an increase in mean arterial blood pressure of 54 ± 5 mmHg above control (control = 104 ± 10 mmHg) and a decrease in heart rate to 81 ± 7% of control (control = 377 ± 26 beats min$^{-1}$).

Complete dose-response curves for the pressor effect of Bay K 8644 were generated in a manner similar to that shown in Figure 1. Conscious rats were injected with incremental doses of Bay K 8644,

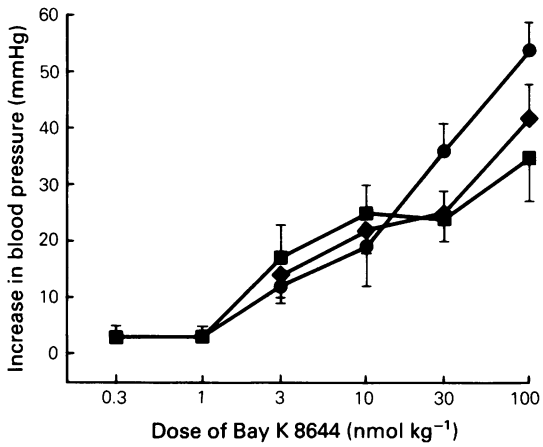


**Figure 2** Three cumulative dose-response curves to intravenous injections of Bay K 8644 (0.3–100 nmol $kg^{-1}$) in conscious rats were performed consecutively to ensure that the pressor response to Bay K 8644 was both reversible and reproducible. The pressor responses are plotted as the maximal increase in mean arterial blood pressure above control resting pressure levels at each dose of Bay K 8644. Administration of Bay K 8644 resulted in a dose-dependent increase in mean arterial blood pressure that was reproducible with respect to time and prior exposure to the drug. The responses to administration of Bay K 8644 are shown as first (●), second (■), and third (▲). There were no significant differences between the second and third responses at any dose of Bay K 8644 administered. Values shown are means of $n = 4$ rats; vertical lines indicate s.e. mean. Control mean arterial blood pressure was 106 ± 5 mmHg.

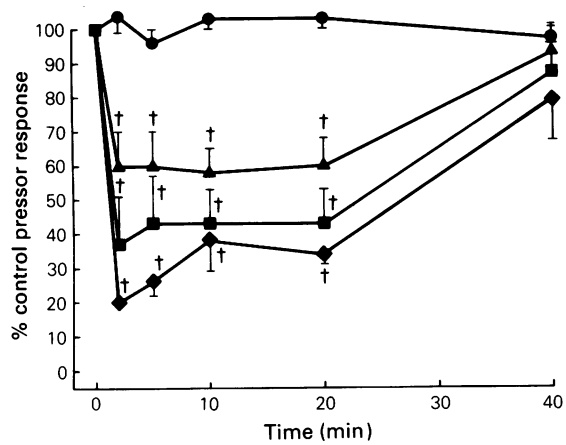


**Figure 3** Time course of the change in the pressor response to intravenous injections of Bay K 8644 (100 nmol $kg^{-1}$) in conscious rats before (●) and after intravenous injections of nifedipine at 0.1 (▲), 0.3 (■), and 1.0 (◆) μmol $kg^{-1}$. Administration of nifedipine resulted in a dose-dependent decrease in the pressor response to Bay K 8644. The maximal effect of the calcium channel blocker was measured 2 min after injection. Within 40 min the pressor responses to Bay K 8644 had returned to control levels. Values shown are means of $n = 4$ rats; vertical lines indicate s.e. mean. Normalized values were arcsine transformed before statistical analysis. † Significantly different as compared to the control response to 100 nmol $kg^{-1}$ Bay K 8644 in the absence of nifedipine.

from 0.3 to 100 nmol $kg^{-1}$ at 10 min intervals. The peak pressor response at each dose was determined. Figure 2 shows the results of these experiments. The threshold dose for an increase in mean blood pressure was greater than 1 nmol $kg^{-1}$. Increasing the dose between 1 and 100 nmol $kg^{-1}$ resulted in a dose-dependent increase in the pressor response to Bay K 8644. Doses of Bay K 8644 greater than 100 nmol $kg^{-1}$ were not administered, therefore the maximal effect of Bay K 8644 could not be determined. After administration of the highest dose, the rats were allowed a 15 min recovery period, then a second complete dose-response curve to Bay K 8644 was generated. A third dose-response curve was then generated in a similar manner. The effect of prior exposure to Bay K 8644 was observed only at the highest dose tested (100 nmol $kg^{-1}$) and only with the first administration of Bay K 8644 when compared to the second and third administration. There were no significant differences between any of the doses of Bay K 8644 administered during the second and third dose-response curves. This dose effect was alleviated in all subsequent studies by beginning each experiment with two equilibration doses of 100 nmol $kg^{-1}$ Bay K 8644 before the start of the experimental procedure. These curves are also shown in Figure 2 which demonstrates that the rats recovered quickly from the effects of Bay K 8644 and that the response to Bay K 8644 could be reproducibly tested with respect to time and prior exposure to Bay K 8644.

The time course of the effect of the dihydropyridine calcium channel blocker, nifedipine, on the pressor response to Bay K 8644 was examined and the results are shown in Figure 3. Nifedipine was administered intravenously in doses of 0.1, 0.3, or 1 μmol $kg^{-1}$. Two min after the injection of each dose of nifedipine, the pressor response to 100 nmol $kg^{-1}$ Bay K 8644 was significantly diminished. This depression of the pressor response remained relatively unchanged for 20 min after injection of nifedipine. By 40 min, however, the pressor response to Bay K 8644 had returned to control levels. Qualitatively similar results were obtained with two other calcium channel blockers, verapamil and diltiazem. Additionally, oral administration of the calcium channel blockers produced results quali-

**Table 1** Effects of intravenous doses of calcium channel blockers on pressor responses to Bay K 8644 (100 nmol $kg^{-1}$) and on resting mean arterial blood pressure

| *Drug* | *Dose* ($\mu$mol $kg^{-1}$) | *Pressor response to Bay K 8644* (% of control) | *Resting mean arterial blood pressure* (% of control) |
|---|---|---|---|
| Nifedipine | 0.03 | 71 ± 7* | 99 ± 1 |
| | 0.1 | 44 ± 7* | 86 ± 6 |
| | 0.3 | 38 ± 7* | 78 ± 8 |
| | 1.0 | 15 ± 15* | 88 ± 3 |
| | 3.0 | 0 ± 10* | 36 ± 7* |
| Verapamil | 0.3 | 57 ± 14* | 94 ± 3 |
| | 1.0 | 17 ± 10* | 71 ± 4* |
| | 3.0 | 0 ± 13* | 25 ± 8* |
| | 30.0 | 0 ± 0* | 13 ± 2* |
| Diltiazem | 0.3 | 65 ± 10* | 100 ± 2 |
| | 1.0 | 63 ± 8* | 95 ± 2 |
| | 3.0 | 8 ± 6* | 79 ± 4* |
| | 10.0 | 0 ± 0* | 20 ± 7* |

Control pressor responses to 100 nmol $kg^{-1}$ Bay K 8644 (in mmHg) were: 37 ± 2 (nifedipine), 30 ± 2 (verapamil), and 33 ± 1 (diltiazem). Resting mean arterial blood pressures (in mmHg) were: 103 ± 5 (nifedipine), 89 ± 6 (verapamil), and 101 ± 4 (diltiazem). Values shown are means ± s.e. mean for 4–12 rats. Normalized values were arcsine transformed before statistical analysis. * Significantly different as compared to control.

tatively similar to intravenous administration of the same channel blocker.

Experiments similar to the one shown in Figure 3 were conducted using intravenous injection of several different doses of each of the three calcium channel blockers (nifedipine, verapamil, and diltiazem). The maximal inhibition of the pressor response to 100 nmol $kg^{-1}$ Bay K 8644 at each concentration of the channel blockers was recorded and complete dose-response inhibition curves were generated (Figure 4). The results of these studies are shown in Table 1. Intravenous administration of the calcium channel blockers was associated with a dose-dependent depression of the maximal pressor effect of 100 nmol $kg^{-1}$ Bay K 8644. The doses of calcium channel blocker necessary to produce a 50% depression of the pressor effect ($IC_{50}$) were: 0.075 $\mu$mol $kg^{-1}$ nifedipine, 0.37 $\mu$mol $kg^{-1}$ verapamil, and 1.3 $\mu$mol $kg^{-1}$ diltiazem. Interestingly, Table 1 shows that inhibition of the pressor response to Bay K 8644 by the calcium channel blockers was evident at doses that did not significantly depress resting mean blood pressure.

Table 2 shows data from experiments identical to those described above except that the calcium channel blockers were administered orally rather than intravenously. Bay K 8644 was injected at 15 min intervals after oral administration of the channel blockers. All three calcium channel blockers produced a dose-dependent inhibition of the pressor response to 100 nmol $kg^{-1}$ Bay K 8644. The $IC_{50}$ values for oral administration of the calcium channel blockers were: 1.3 $\mu$mol $kg^{-1}$ nifedipine, 5.3 $\mu$mol $kg^{-1}$ verapamil, and 10.9 $\mu$mol $kg^{-1}$ diltiazem. In addition, as was the case with intravenous administration, oral administration of the calcium channel blockers inhibited the pressor response to

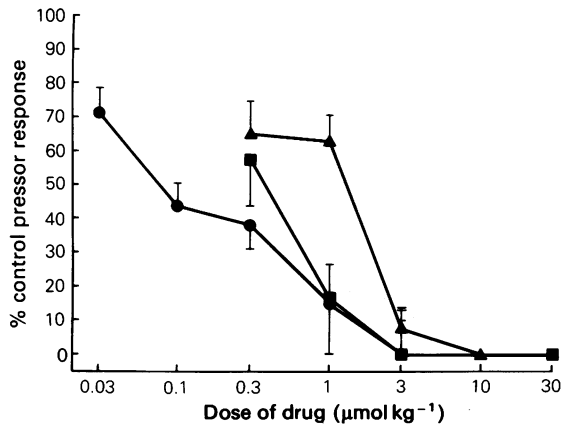


**Figure 4** Maximum change in the pressor response to 100 nmol $kg^{-1}$ Bay K 8644 in conscious rats following intravenous injections of nifedipine (●), verapamil (■) and diltiazem (▲). Pressor responses are plotted as a % of the peak pressor response in the absence of calcium channel blocker. Administration of each calcium channel blocker resulted in a dose-dependent decrease in the pressor response to Bay K 8644. Control responses to 100 nmol $kg^{-1}$ Bay K 8644 and resting mean arterial blood pressures are listed in Table 1. Values shown are means of $n$ = 4–12 rats; vertical lines indicate s.e. mean.

Bay K 8644 at doses lower than those which significantly lowered resting mean blood pressure. As would be expected, the dose of each calcium channel blocker required to produce a given level of inhibition of the pressor response to Bay K 8644 was greater with oral than with intravenous administration.

In an attempt to equate the effects of the calcium channel blockers in the conscious rat with effects on arterial muscle, a series of experiments was performed using circumferential strips of rabbit thoracic aortae. The three calcium channel blockers, nifedipine, verapamil, and diltiazem, were tested for their ability to relax KCl-stimulated strips as an indication of calcium channel blockade (Brittain & Moreland, 1985). The responses of rabbit thoracic aortic strips to 100 mM KCl are primarily the result of membrane depolarization and the influx of extracellular calcium. Release of noradrenaline from nerve terminals during KCl stimulation does not represent a major portion of these contractions as 1 $\mu$M phentolamine or yohimbine produces only a 10% depression of the KCl responses and 1 $\mu$M prazosin has no effect (Brittain & Moreland, 1985). Non-cumulative concentration-response curves for relaxation of KCl-stimulated aortic strips were generated and are shown in Figure 5. Each of the calcium channel blockers relaxed the KCl stimulated aortic strips in a concentration-dependent fashion. The concentrations of the calcium channel blockers necessary to relax a KCl-stimulated aortic strips by 50% ($IC_{50}$) were: 1.8 $\pm$ 0.2 nM nifedipine, 0.19 $\pm$ 0.02 $\mu$M verapamil, and 0.69 $\pm$ 0.07 $\mu$M diltiazem.

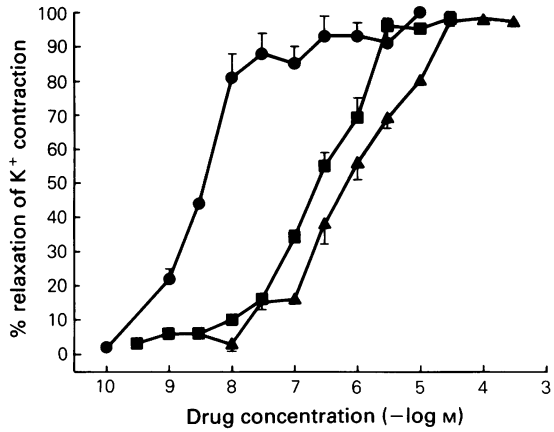


**Figure 5** Non-cumulative concentration-response curves for relaxation of KCl-stimulated contractions by calcium channel blockers in rabbit thoracic aortic strips. The calcium channel blockers studied were: nifedipine (●), verapamil (■) and diltiazem (▲). The % relaxation of a contraction elicited by 100 mM KCl is plotted for each concentration of calcium channel blocker tested. Each calcium channel blocker resulted in a dose-dependent decrease in the force. Values shown are means of $n = 4$–11 strips, each from different rabbits; vertical lines indicate s.e. mean.

To determine if a mechanism of action other than the direct activation of vascular smooth muscle is involved in the pressor response to Bay K 8644, a series of experiments was performed investigating the possible role of the adrenergic system in this

**Table 2** Effects of oral doses of calcium channel blockers on pressor responses to Bay K 8644 (100 nmol kg$^{-1}$) and on resting mean arterial blood pressure

| *Drug* | *Dose* ($\mu$mol kg$^{-1}$) | *Pressor response to Bay K 8644* (% of control) | *Resting mean arterial blood pressure* (% of control) |
|---|---|---|---|
| Nifedipine | 1 | 57 ± 9* | 100 ± 3 |
| | 5 | 10 ± 6* | 74 ± 5 |
| | 15 | 0 ± 0* | 73 ± 4 |
| | 45 | 0 ± 0* | 60 ± 6* |
| Verapamil | 5 | 51 ± 10* | 97 ± 1 |
| | 15 | 34 ± 10* | 84 ± 4 |
| | 45 | 19 ± 5* | 64 ± 2* |
| Diltiazem | 1 | 73 ± 5 | 80 ± 7 |
| | 5 | 56 ± 10* | 94 ± 2 |
| | 15 | 48 ± 4* | 89 ± 1 |
| | 45 | 51 ± 12* | 79 ± 4* |
| | 135 | 7 ± 7* | 61 ± 12* |
| | 405 | 8 ± 8* | 60 ± 4* |

Control pressor responses to 100 nmol kg$^{-1}$ Bay K 8644 (in mmHg) were: 36 $\pm$ 2 (nifedipine), 33 $\pm$ 5 (verapamil), and 31 $\pm$ 2 (diltiazem). Resting mean arterial blood pressures (in mmHg) were: 100 $\pm$ 5 (nifedipine), 102 $\pm$ 5 (verapamil), and 98 $\pm$ 3 (diltiazem). Values shown are means $\pm$ s.e. mean for 3–8 rats. Normalized values were arcsine transformed before statistical analysis. * Significantly different as compared to control.

pressor response. One group of rats was given an oral dose of the non-selective α-adrenoceptor antagonist phentolamine (160 μmol kg$^{-1}$), then challenged with 100 nmol kg$^{-1}$ Bay K 8644 or 3 nmol kg$^{-1}$ adrenaline at various times after administration of phentolamine. This dose of adrenaline resulted in a pressor response with a magnitude similar to that caused by 100 nmol kg$^{-1}$ Bay K 8644. The results of these experiments are shown in Figure 6. Phentolamine, given orally, produced a time-dependent inhibition of the pressor responses to both Bay K 8644 and adrenaline. This inhibition was evident by 10 min in response to Bay K 8644 and by 30 min for adrenaline (earliest time measured). The slow onset of the inhibition is presumably due to the time required for absorption of phentolamine following oral administration. However, within 40–60 min after the oral administration of phentolamine, intravenous administration of either Bay K 8644 (100 nmol kg$^{-1}$) or adrenaline (3 nmol kg$^{-1}$) resulted in a depressor response in contrast to the pressor response elicited in the absence of phentolamine.

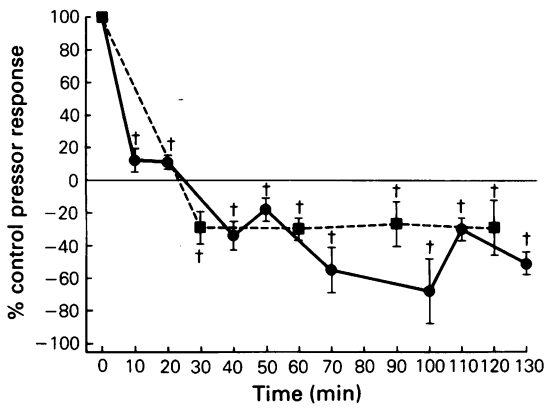


**Figure 6** Time course of the change in the maximal pressor response to Bay K 8644 (100 nmol kg$^{-1}$, ●——●) or adrenaline (3 nmol kg$^{-1}$, ■---■) following oral dosing of conscious rats with phentolamine (160 μmol kg$^{-1}$). Pressor responses to the vasoactive compounds are plotted as a % of the control response of the appropriate compound in the absence of phentolamine. Pressor responses to both Bay K 8644 and adrenaline were time-dependently depressed and then converted to depressor responses during the first hour after phentolamine dosing. The control response to 100 nmol kg$^{-1}$ Bay K 8644 was 51 ± 4 mmHg, whereas the control response to 3 nmol kg$^{-1}$ adrenaline was 62 ± 4 mmHg. The two pressor responses were not significantly different. Resting mean arterial blood pressure was 79 ± 8 mmHg and did not significantly change after the administration of phentolamine. Values shown are means of $n = 4$ rats; vertical lines indicate s.e. mean. Normalized values were scored from 0 to 1, such that −100% control pressor response was 0 and 100% control pressor response was 1, and arcsine transformed before statistical analysis. † Significantly different as compared to control responses in the absence of phentolamine.

The inhibitory effect of adrenoceptor antagonists on the pressor response to Bay K 8644 in the conscious rat was further examined with selective $\alpha_1$ (prazosin)- and selective $\alpha_2$ (yohimbine)-adrenoceptor antagonists and with a non-selective β-adrenoceptor antagonist (propranolol). The results of these experiments are shown in Figure 7. Conscious rats were given two different doses of either yohimbine (1 and 3 μmol kg$^{-1}$, i.v.), prazosin (40 and

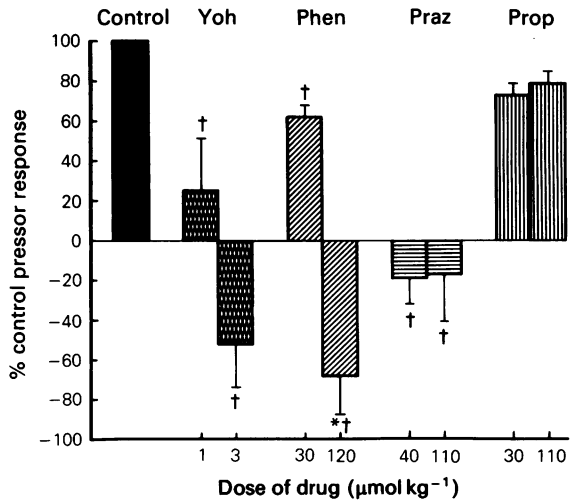


**Figure 7** Maximum change in the pressor response to 100 nmol kg$^{-1}$ Bay K 8644 following the intravenous administration of yohimbine (Yoh) or the oral administration of propranolol (Prop), prazosin (Praz), or phentolamine (Phen) in conscious rats. Pressor responses are plotted as a % of the control response to Bay K 8644 in the absence of the appropriate adrenergic intervention. Administration of α-adrenoceptor antagonists inhibited the pressor response to Bay K 8644 and converted it to a depressor response. The β-adrenoceptor antagonist did not significantly affect the pressor response to Bay K 8644. Values shown are means of $n = 3$–6 rats; vertical lines indicate s.e. mean. Control responses to 100 nmol kg$^{-1}$ Bay K 8644 ranged from 32 ± 8 to 51 ± 4 mmHg and control mean arterial blood pressures ranged from 79 ± 8 to 113 ± 2 mmHg in the different experimental groups of rats. Mean arterial blood pressure was not significantly affected by the addition of any of the adrenoceptor antagonists used in this study. Normalized values were scored from 0 to 1, such that −100% control pressor response was 0 and 100% control pressor response was 1, and arcsine transformed before statistical analysis. † Significantly different as compared to control responses to Bay K 8644. * Significantly different as compared to the lower dose of adrenoceptor antagonist.

110 $\mu$mol kg$^{-1}$, p.o.), phentolamine (30 and 120 $\mu$mol kg$^{-1}$, p.o.), or propranolol (30 and 110 $\mu$mol kg$^{-1}$, p.o.) and then challenged with 100 nmol kg$^{-1}$ Bay K 8644. All three $\alpha$-adrenoceptor antagonists inhibited the pressor response to Bay K 8644 and, in fact, reversed it to a depressor response. Time-dependent changes in the response to Bay K 8644 in the presence of prazosin or yohimbine were similar to that with phentolamine shown in Figure 6 (data not shown). The non-selective $\beta$-adrenoceptor antagonist, propranolol, had no effect on the pressor response to Bay K 8644 at the doses tested.

The possible role of endogenous catecholamine release in the pressor and depressor responses to Bay K 8644 in the conscious rat was examined by adrenal catecholamine depletion studies. These studies were performed with rats that were either adrenalectomized or adrenal demedullectomized as described in the methods. Adrenalectomy or adrenal demedullectomy did not significantly reduce resting mean arterial blood pressure, although a non-significant decrease from control was noted. Mean arterial blood pressures for the experimental groups of animals ranged from 73 ± 3 mmHg to 85 ± 5 mmHg, whereas the mean arterial blood pressures for control groups of animals ranged from 79 ± 8 mmHg to 113 ± 2 mmHg. The catecholamine depleted rats were administered an oral dose of phentolamine (160 $\mu$mol kg$^{-1}$) and then challenged with either Bay K 8644 (100 nmol kg$^{-1}$ i.v.) or adrenaline (3 nmol kg$^{-1}$, i.v.). the time-dependent changes in mean arterial blood pressure were monitored. The results of such experiments using adrenalectomized rats are shown in Figure 8. Similar to the results shown in Figure 6 (intact rats), phentolamine inhibited the pressor responses to both Bay K 8644 and adrenaline within approximately 20–30 min. However, in contrast to the experiments utilizing intact rats, adrenalectomy prevented the phentolamine-induced reversal of the pressor response to Bay K 8644 to a depressor response. Adrenalectomy did not affect the phentolamine-induced reversal of the pressor response to exogenous adrenaline. Qualitatively similar results were obtained with adrenal demedullectomized rats (data not shown).

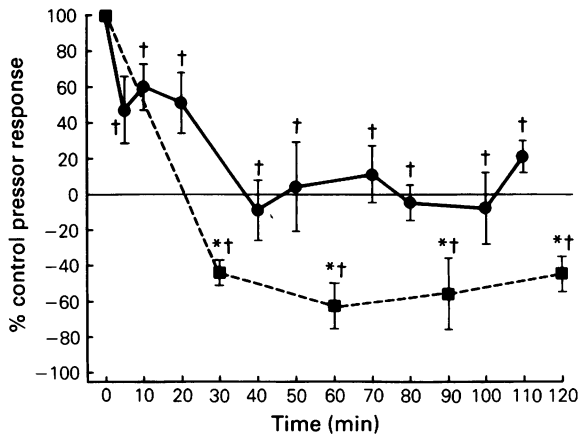


**Figure 8** Time course of the change in the pressor response to Bay K 8644 (100 nmol kg$^{-1}$, ●——●) or adrenaline (3 nmol kg$^{-1}$, ■---■) following oral dosing with phentolamine (160 $\mu$mol kg$^{-1}$) in conscious adrenalectomized rats. Pressor responses are plotted as a % of the control response to the appropriate vasoactive compound in the absence of phentolamine. Adrenalectomy prevented the phentolamine-induced reversal of the pressor response to Bay K 8644 to a depressor response, but did not affect the ability of phentolamine to inhibit the pressor responses to Bay K 8644 or adrenaline. Adrenalectomy did not prevent the phentolamine-induced reversal of the pressor response of adrenaline to a depressor response. The control response to 100 nmol kg$^{-1}$ Bay K 8644 was 37 ± 3 mmHg and the control response to 3 nmol kg$^{-1}$ adrenaline was 40 ± 1 mmHg. Control mean arterial blood pressure in the adrenalectomized rats was 85 ± 5 mmHg. Phentolamine did not significantly affect the resting mean arterial blood pressure. Values shown are means of $n = 4$ rats; vertical lines indicate s.e. mean. Normalized values were scored from 0 to 1, such that −100% control pressor response was 0 and 100% control pressor response was 1, and arcsine transformed before statistical analysis. Differences in pressor response to Bay K 8644 and adrenaline were analysed within blocks of time: 30–40 min, 60–70 min, and 90–100 min. † Significantly different as compared to control response. * Significantly different as compared to the response elicited by 100 nmol kg$^{-1}$ Bay K 8644 within the same 10 min interval after phentolamine administration.

## Discussion

The dihydropyridine calcium channel activator, Bay K 8644, has been shown to enhance calcium influx through voltage-dependent calcium channels in several tissues, both muscle and non-muscle (Schramm *et al.*, 1983; Freedman & Miller, 1984; Hwang & van Breemen, 1985; Dong & Wadsworth, 1986; Droogmans & Callewaert, 1986; Ehrlich *et al.*, 1986). This action of Bay K 8644 has been demonstrated by its ability to activate an inward calcium current (Brown *et al.*, 1984; Hess *et al.*, 1984; Wahler & Sperelakis, 1984; Droogmans & Callewaert, 1986) and increase the influx of $^{45}$Ca (Hwang & van Breemen, 1985; Dong & Wadsworth, 1986). The cardiovascular ramifications of exposure to this compound are an increase in cardiac contractility and vascular constriction with a resultant increase in arterial blood pressure (Schramm *et al.*, 1983; Gross

et al., 1985; Preuss et al., 1985). The results of the present study also demonstrate that administration of Bay K 8644 to a conscious normotensive rat increases mean arterial blood pressure. The precise mechanism(s) by which this increase in mean arterial blood pressure occurs, however, have not been delineated. Several studies have suggested that the pressor response is strictly the result of activation of calcium channels in either cardiac or vascular smooth muscle and, therefore, due to a direct increase in calcium-mediated contractile force development (Schramm et al., 1983; Preuss et al., 1985; Lefer et al., 1986). Conversely, other investigators have presented evidence that Bay K 8644 potentiates the release of adrenal catecholamines (Montiel et al., 1984) and stimulates the release of noradrenaline from nerve terminals (Cena et al., 1985). These latter mechanisms would increase contractile activity indirectly by release of vasoactive compounds rather than by a direct activation of the muscle cells. The present study was designed to investigate the relative contributions of these mechanisms (both indirect and direct activation of the muscle cells) in the pressor response to Bay K 8644 in the conscious rat.

The results of this study demonstrate that administration of Bay K 8644 produces a pressor response in the conscious rat and that this response is dose-dependently depressed by calcium channel blockers. Nifedipine was the most potent blocker of the pressor response to Bay K 8644. Verapamil and diltiazem were less potent than nifedipine but, nonetheless, completely inhibited the response to Bay K 8644. This difference in potency between dihydropyridine (nifedipine) and non-dihydropyridine (verapamil and diltiazem) calcium channel blockers is in accord with their potency in other studies of calcium channel blocker activity and probably a result of the different sites of action of the different classes of blockers. The inhibition of the pressor response to Bay K 8644 by the calcium channel blockers is not an indirect effect through a decrease in resting mean arterial blood pressure, as shown in Tables 1 and 2. The calcium channel blockers resulted in a 50% or greater reduction in the pressor response to Bay K 8644 at doses that did not affect resting mean arterial blood pressure.

The inhibition of the pressor response to Bay K 8644 by addition of the calcium channel blockers is consistent with the hypothesis that Bay K 8644 increases mean arterial blood pressure by a direct activation of the vascular smooth muscle cells (Gross et al., 1985; Preuss et al., 1985; Lefer et al., 1986). However, further examination of the response to Bay K 8644 suggests a role of the adrenal catecholamines. The non-specific α-adrenoceptor antagonist phentolamine depressed the pressor response to Bay K 8644 as did both the specific $\alpha_1$- and $\alpha_2$-adrenoceptor antagonists. This suggests that Bay K 8644 may produce vasoconstriction by an α-adrenoceptor-mediated event. At least two possible mechanisms could be responsible for these results. The first is a direct activation of α-adrenoceptors by Bay K 8644. We know of no evidence to suggest this and, because Bay K 8644 does not fulfil any of the structural criteria for α-adrenoceptor agonists, this mechanism does not seem likely. The second possibility is that Bay K 8644 induced release of catecholamines from the adrenal glands and/or other stores in the rat. This suggestion is supported by work of other investigators demonstrating that Bay K 8644 potentiates the release of catecholamines from nerve terminals (Cena et al., 1985) and adrenal glands (Montiel et al., 1984).

In the present study, addition of phentolamine not only completely blocked the pressor responses to both Bay K 8644 and adrenaline, but also converted them to depressor responses. After adrenalectomy or adrenal demedullectomy, phentolamine blocked the pressor response to Bay K 8644 but did not reverse it to a depressor response. In the presence of phentolamine, administration of exogenous adrenaline continued to induce a depressor response after adrenalectomy, demonstrating that the release of adrenal catecholamines by Bay K 8644 is at least partially responsible for the depressor response. Bay K 8644 may also release noradrenaline from nerve terminals, but the present study did not address this question. An additional factor that may be important in these studies is adrenal cortical function. We have no information concerning the functional status of the adrenal cortex in the adrenal demedullectomized animals. However, because similar effects of phentolamine administration on the pressor responses to adrenaline were observed with intact and experimental groups of animals, we assume the adrenal cortex does not play an important role in these experiments. We believe that adrenaline released by administration of Bay K 8644 is the most likely mechanism for the depressor response and probably plays a significant role in the pressor response.

Contraction of vascular smooth muscle by adrenoceptor agonists has been shown to be due to pharmacomechanical coupling and is primarily independent of extracellular calcium (Somlyo & Somlyo, 1968). If the release of catecholamines from adrenals and other stores is involved in the pressor response to Bay K 8644, the calcium channel blockers should not be able to inhibit completely the pressor response to Bay K 8644. This is because the calcium channel blockers should not significantly inhibit contractions brought about by pharmacomechanical coupling. However, the release of catecholamines is calcium-dependent (Kirpekar, 1975). Therefore, Bay K 8644 may induce catechol-

amine release by enhancing calcium influx into the adrenal glands. In the presence of calcium channel blockers, this influx, and therefore the catecholamine release, would be inhibited. Thus, the role of adrenal catecholamines in the pressor response to Bay K 8644 can only be determined by indirect methods such as were used in this study.

In a similar study, Lefer *et al.* (1986) found no effect of phentolamine on the pressor response to Bay K 8644. We know of no obvious reason for the discrepancy in these results. Possibly differences in the doses of phentolamine used by Lefer *et al.* (1986) ($100\,\mu g\,kg^{-1}$, i.v.) as compared to this study ($50\,mg\,kg^{-1}$, p.o.) may explain these findings. However, in both studies the dose of phentolamine was adequate to inhibit the effects of exogenously administered catecholamines. Another interesting difference between these studies is that Lefer *et al.* (1986) demonstrated only a 62–84% inhibition of the pressor response to Bay K 8644 by calcium channel blockers, whereas we were able to show complete blockade of the pressor response to Bay K 8644 in the present study. Additionally, Gross *et al.* (1985) demonstrated that phenoxybenzamine did not affect the pressor response to Bay K 8644 in the conscious dog. Again, we have no simple explanation for this difference in results, except possibly the kinetics of catecholamine release from the adrenals of rats and dogs may differ.

The pressor response to Bay K 8644 is not mediated by $\beta$-adrenoceptor activation, as shown by the complete lack of effect of $\beta$-adrenoceptor antagonists. The inability of $\beta$-adrenoceptor antagonists to inhibit the cardiovascular responses to Bay K 8644 has been previously demonstrated in anaesthetized (Schramm *et al.*, 1983) and conscious (Preuss *et al.*, 1985) dogs. However, $\beta$-adrenoceptor activation most likely plays a role in the depressor response to Bay K 8644.

In summary, this study has shown that the pressor response to Bay K 8644 may be the result of two separate mechanisms. Firstly, Bay K 8644 may directly activate cardiac and vascular smooth muscle cells by activation of calcium influx through dihydropyridine-sensitive, voltage-dependent calcium channels, as demonstrated by several laboratories (Schramm *et al.*, 1983; Hwang & van Breemen, 1985; Dong & Wadsworth, 1986; Droogmans & Callewaert, 1986). Secondly, Bay K 8644 releases adrenal catecholamines and thereby indirectly results in vasoconstriction and an increase in cardiac contractility. Both mechanisms would result in increases in cardiac output, total peripheral resistance and hence mean arterial blood pressure. Moreover, both direct activation of the muscle cells by Bay K 8644 itself and indirect activation of the muscle cells by Bay K 8644-induced release of adrenal catecholamines are inhibited by calcium channel blockers.

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## References

ALLEN, S.L., FOSTER, R.W., SMALL, R.C. & TOWART, R. (1985). The effects of the dihydropyridine Bay K 8644 in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **86**, 171–180.

BRITTAIN, R.J. & MORELAND, S. (1985). Relaxation of $K^+$ contracted rabbit aortic strips implies calcium channel blockade. *The Physiologist*, **28**, 325.

BOLTON, T.B. (1980). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BROWN, A.M., KUNZE, D.L. & YATANI, A. (1984). The agonist effect of dihydropyridines on Ca channels. *Nature*, **311**, 570–572.

CENA, V., GARCIA, A.G., KHOYI, M.A., SALAICES, M. & SANCHEZ-GARCIA, P. (1985). Effect of the dihydropyridine Bay K 8644 on the release of [$^3$H]-noraderenaline from the rat isolated vas deferens. *Br. J. Pharmacol.*, **85**, 691–696.

DONG, Y.J. & WADSWORTH, R.M. (1986). Effects of Bay K 8644 on $^{45}$Ca uptake and efflux and on contraction in the rabbit aorta. *Br. J. Pharmacol.*, **87**, 139–145.

DROOGMANS, G. & CALLEWAERT, G. (1986). $Ca^{2+}$-channel current and its modification by the dihydropyridine agonist Bay K 8644 in isolated smooth muscle cells. *Pfluegers Arch.*, **406**, 259–65.

EHRLICH, B.E., SCHEN, C.R., GARCIA, M.L. & KACZOROWSKI, G.J. (1986). Incorporation of calcium channels from cardiac sarcolemmal membrane vesicles into planar lipid bilayers. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 193–197.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Ann. Rev. Pharmacol. Toxicol.*, **17**, 149–166.

FLECKENSTEIN, A. (1983). *Calcium antagonism in heart and smooth muscle. Experimental facts and therapeutic prospects.* New York: John Wiley and Sons.

FREEDMAN, S.B. & MILLER, R.J. (1984). Calcium channel activation: A different type of drug action. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 5580–5583.

GROSS, R., KAYSER, M., SCHRAMM, M., TANIEL, R. & THOMAS, G. (1985). Cardiovascular effects of the calcium-agonistic dihydropyridine BAY K 8644 in conscious dogs. *Arch. Int. Pharmacodyn.*, **277**, 203–216.

HESS, P., LANSMAN, J.B. & TSIEN, R.W. (1984). Different modes of Ca channel gating behaviour favoured by

dihydropyridine Ca agonists and antagonists. *Nature*, **311**, 538–544.

HWANG, K.S. & VAN BREEMEN, C. (1985). Effects of the Ca agonist Bay K8644 and net Ca uptake into rabbit aortic smooth muscle. *Eur. J. Pharmacol.*, **116**, 299–305.

KIRPEKAR, S.M. (1975). Factors influencing transmission at adrenergic synapses. *Prog. Neurobiol.*, **4**, 63–98.

LEFER, A.M., WHITNEY, C.C. & HOCK, C.E. (1986). Mechanism of the pressor effect of the calcium agonist, BAY k 8644, in the intact rat. *Pharmacology*, **32**, 181–189.

MONTIEL, C., ARTALEJO, A.R. & GARCIA, A.G. (1984). Effects of the novel dihydropyridine Bay k 8644 on adrenomedullary catecholamine release evoked by calcium reintroduction. *Biochem. Biophys. Res. Commun.*, **120**, 851–857.

MORELAND, S. & MORELAND, R.S. (1987). Effects of dihydropyridines on stress, myosin phosphorylation, and $V_0$ in smooth muscle. *Am. J. Physiol.*, **252**, H1049–H1058.

PREUSS, K.C., BROOKS, H.L., GROSS, G.L. & WARLTIER, D.C. (1985). Positive inotropic actions of the calcium channel stimulator, Bay k 8644, in awake, unsedated dogs. *Basic Res. Cardiol.*, **80**, 326–332.

SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK, G. (1983). Novel dihydropyridines with positive inotropic action through activation of $Ca^{2+}$ channels. *Nature*, **303**, 535–537.

SOMLYO, A.V. & SOMLYO, A.P. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **159**, 129–145.

WAHLER, G.M. & SPERELAKIS, N. (1984). New Ca agonist (Bay K 8644) enhances and induces cardiac slow action potentials. *Am. J. Physiol.*, **247**, H337–H340.

WEEKS, J.R. & JONES, J.A. (1960). Routine direct measurement of arterial pressure in unanesthetized rats. *Proc. Soc. Exp. Biol. Med.*, **104**, 646–648.

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