A Dihydropyridine (Bay k 8644) That Enhances Calcium Currents in Guinea Pig and Calf Myocardial Cells

A New Type of Positive Inotropic Agent

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SUMMARY. Bay k 8644 is a structural analog of nifedipine with positive inotropic activity. The mechanism of drug action was evaluated by measuring the effects of Bay k 8644 on twitch tension, action potential configuration, and calcium channel currents in myocardial cells. Bay k 8644 increases twitch tension in guinea pig atria without changing the time course of tension development. The drug does not occlude the effect of isoproterenol on twitch tension. The effects of Bay k 8644 on atrial twitch tension are highly dependent on the frequency of stimulation. Maximal inotropic effects are observed at ~0.5 Hz, but no inotropic effect occurs at 0.003 Hz (a rested-state contraction). Since positive inotropic effects only occur with frequent electrical stimulation, they are not due to an intracellular action or to mechanisms that elevate cell calcium in quiescent muscle, such as inhibition of the Na,K-ATPase. Bay k 8644 increases the action potential duration of calf ventricular muscle and Purkinje fibers. Effects on action potential duration are occluded by 1 μM nisoldipine, which specifically blocks calcium channels. The interaction of Bay k 8644 with calcium channels in calf Purkinje fibers was studied using the two-microelectrode voltage clamp technique. Strontium was used as a charge carrier to minimize current through calcium-activated channels and to avoid changes in calcium conductance due to changes in intracellular calcium. Bay k 8644 increases strontium currents and alters the time- and voltage-dependence of channel opening. The greatest percent increase in strontium current occurs for weak depolarizations. For strong depolarizations, strontium current is increased most at the beginning of a test pulse. The drug-induced changes in calcium channel gating are inconsistent with a calcium- or cyclic adenosine monophosphate-mediated effect, and indicate a novel mechanism of action on calcium channels. Thus, Bay k 8644 is the first positive inotropic agent shown to act specifically and directly on calcium channels. (Circ Res 56: 87-96, 1985)

CALCIUM channels play an important role in excitation-contraction coupling in cardiac and vascular smooth muscle. Agents that block these channels produce vasodilation and depress cardiac contractility. The most potent and specific of the Ca++ channel blockers are the 1,4-dihydropyridines, typified by nifedipine (Vater et al., 1972). Recently, this laboratory reported on a nifedipine-derivative that acts as a positive inotropic agent (Schramm et al., 1983a, 1983b). The drug, Bay k 8644 (shown in Fig. 1), acts as a competitive antagonist of nifedipine in contractility experiments, suggesting that it acts by increasing Ca++ influx through voltage-gated Ca++ channels (Schramm et al., 1983a, 1983b). Other agents are known to exert positive inotropic effects by increasing Ca++ currents, but some of them, such as the cardiac glycosides, increase Ca++ currents indirectly by increasing intracellular Ca++ (Marban and Tsien, 1982; Lederer and Eisner, 1982). Consequently, tension measurements alone cannot define the primary site of drug action. For this reason, we have investigated the mechanism of drug action in greater detail by correlating the mechanical and electrical effects of Bay k 8644 on myocardial cells.

Four different categories of drug action were tested for by measuring the effects of Bay k 8644 on twitch tension, action potential configuration, and Ca++ channel currents: (1) an intracellular effect, such as increasing the Ca++ sensitivity of the myofibrils or altering Ca++ uptake and release by the sarcoplasmic reticulum; (2) elevation of cell Na+, which results in increased cell Ca++ due to Na-Ca exchange; (3) a direct effect on Ca++ channels, resulting in increased Ca++ influx; and (4) prolongation of the action potential duration by an effect on Na+ or K+ channels, which indirectly increases current through Ca++ channels or increases Ca++ release from the sarcoplasmic reticulum. We found that Bay k 8644 directly interacts with Ca++ channels to promote Ca++ entry. No other site of action was detected. The drug represents a new type of positive inotropic agent because it affects Ca++ channels in a unique way.

Preliminary reports of parts of this work have
been published previously (Thomas et al., 1983a, 1983b; Cohen and Chung, 1984).

Methods
Preparations
Mechanical studies were performed on guinea pig left atria. Pirbright-white guinea pigs (250-300 g) of either sex were killed by a blow to the head. Electrophysiological experiments were performed on calf ventricular trabeculae or Purkinje fibers. Calf hearts were obtained from a local slaughterhouse and transported to the laboratory in ice-cold physiological saline. Voltage clamp experiments were performed on short segments of Purkinje fibers (~200 μm in diameter and 0.5–1.5 mm long) obtained from either ventricle.

Twitch Tension Measurements
Left atria were separated from the ventricle, suspended in an organ bath, and maintained under a constant resting tension of 0.5–1.0 g. Square pulse field stimuli were 2 msec and 16V (~2.5x threshold). Contractions were measured isometrically with Stratham UC2 strain gauges and recorded with a chart recorder (Hellige) and digital storage oscilloscope (Vuko VKS 22-16).

Voltage Clamp Measurements
Two microelectrode voltage clamp experiments were performed as previously described (Kass et al., 1979). Command pulses were rounded with a time constant of 100 μsec to reduce cell damage. Membrane current and voltage were filtered with an 8-pole low pass Bessel filter (Frequency Devices) and recorded on an LSI 11/23 computer. Cut-off frequencies and sampling rates are indicated in the appropriate figure legends. In order to equate membrane current with Ca ++ channel current, all current pathways other than Ca ++ channels must be blocked or inactivated. In particular, the transient outward and delayed rectifier currents must be blocked. Previous studies have shown that these currents can be minimized by loading the fibers with Cs + by using nystatin (Marban, 1981) or by injecting quaternary ammonium salts, such as tetrabutylammonium (Kass et al., 1982). We used a modification of these techniques: Cs + was injected into the fibers under voltage clamp using current-passing microelectrodes filled with 2.5 M Cs acetate plus 1.0 M CsCl. Cs + salts were used instead of tetraethylammonium salts because they have a higher solubility and ionic mobility, so that outward currents could be passed more readily with unbeveled micropipettes. Cs + was iontophoresed into the fibers until block of outward currents was maximal (~15 minutes). In addition, extracellular Ca ++ and K + were replaced by Sr ++ and Cs + (see composition of solutions below). The Ca ++-dependent transient outward is only weakly activated by Sr ++ (Siegelbaum and Tsien, 1980; Siegelbaum et al., 1981; Cohen and Carmeliet, 1982). The use of Sr ++ as a charge carrier also allowed us to determine whether drugs directly affect Ca ++ channels (see Results).

Solutions and Drugs
Tension measurements were conducted in Krebs-Henseleit solution containing (mM): NaCl, 118; KCl, 4.8; CaCl2, 1.8; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25; glucose, 10; pH, 7.4. Solutions were gassed with 95% O2, 5% CO2 and maintained at 35 ± 1°C. Voltage clamp experiments were conducted in (mM): NaCl, 134; CsCl, 20; SrCl2, 5.4; MgCl2, 0.5; HEPES, 10; glucose, 5; pH, 7.4. Temperature was maintained at 30 ± 1°C. Solutions for electrophysiological experiments were gassed with 100% O2. Changes in divalent cation composition were made without compensating for the change in tonicity.

Bay k 8644 is methyl 1,4-dihydropyridine 2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, and its structure is given in Figure 1. The structural formula for nifedipine is also shown to illustrate the close structural analogy of these compounds. Bay k 8644, nifedipine (Bay e 5009), and nisoldipine (Bay k 5552) are all products of Bayer AG. They were dissolved in 40% dimethyl sulfoxide (DMSO) or polyethylene glycol 400 (Baker). Final solvent concentrations in these experiments were <0.03%.

The solvents have no effect on Ca ++ channel currents (Kass, 1982) or twitch tension at those low concentrations. Sodium vapor lights were used during experiments with dihydropyridines because the compounds are light sensitive. Isoproterenol sulfate was obtained from Boehringer Ingelheim.

Results
Positive Inotropic Effects
The effect of Bay k 8644 on twitch tension in guinea pig atria is shown in the top panel of Figure 2. Twitch tension is approximately doubled after a 5-minute exposure to 280 nM Bay k 8644, with little change in the time course of tension development. Bay k 8644 and elevated external Ca ++ have similar effects on twitch tension (middle panel). In contrast, the β-adrenoceptor agonist, isoproterenol, shortens the time-to-peak tension and speeds the rate of relaxation (bottom panel). These results suggest that Bay k 8644 is not a sympathomimetic agent. Likewise, the positive inotropic effect of Bay k 8644 is unaffected by propranolol, a β-adrenoceptor antagonist (Thomas et al., 1983b).

Figure 3 further contrasts the inotropic effects of Bay k 8644 and β-adrenoceptor agonists. This figure shows that maximally effective doses of Bay k 8644 do not occlude the effect of isoproterenol on twitch tension. 1 μM Bay k 8644 was a maximally effective dose because increasing the concentration to 3 μM did not have an additional effect on tension. However, 10 nM isoproterenol further increased twitch tension. This result suggests that the two drugs do not.

![Structure of nifedipine (Bay a 1040) and Bay k 8644.](image-url)
positive inotropic agents that alter intracellular Ca++ release or load the cell with Ca++ during rest (see the discussion section for references). This behavior is illustrated in Figures 5 and 6.

The upper panels of Figure 5 show twitch tension when an atrium was rested for 10 minutes and then stimulated at 1.0 Hz. In each panel, a large rested-state contraction was followed by smaller contractions that progressively increased with further stimulation. The left panel shows the control response. The middle panel shows that 830 nM nitrendipine depresses steady state twitch tension for 1.0 Hz stimulation, but does not alter the rested-state contraction. In contrast, elevating external Ca++ in the

not share a common mechanism of action. It also indicates that the response to Bay k 8644 had a maximum that was not due to a mechanical limitation.

The positive inotropic effect of Bay k 8644 was studied under a wide range of concentrations and stimulation rates. Figure 4 shows that Bay k 8644 is a very potent positive inotropic agent during 1 Hz stimulation. Increases in cardiac contractility are consistently produced by 10 nM drug, and maximal effects are produced at 1 μM.

The inotropic effects of Bay k 8644 were evaluated in mammalian atrial cells, because insight into the mechanism of drug action is readily obtained from the dependence of inotropy on stimulation rate in this tissue. In these cells, the first contraction after a long rest period (a rested-state contraction) should be insensitive to agents that act primarily on Ca++ channels, but these contractions are enhanced by
Figure 5. Effect of nitrendipine and increased Ca++ on rested-state contractions and twitch tension during steady stimulation in a guinea pig atrium. Each of the upper panels is a tension recording from an atrium during 1 Hz stimulation under control conditions (a), after the addition of 830 nM nitrendipine (b), and in the presence of nitrendipine after increasing Ca++ from 1.8 to 3.6 mM (c). Each train was preceded by a rest period of >10 minutes. The train shown in panel b was the second one in the presence of nitrendipine. The rested-state contractions are reproduced on a fast time scale in the lower panel. The arrow marks the time of stimulation.

The continued presence of nitrendipine does alter the rested-state contraction (right panel). The rested-state contractions for all three panels are superimposed on an expanded time scale in the lower panel. Nitrendipine was studied because its specificity for blocking Ca++ channels in myocardial cells is well documented (Lee and Tsien, 1983; Shibata et al., 1984), but it alters Ca++ transport by the sarcoplasmic reticulum only at very high doses (Colvin et al., 1982; Chamberlain et al., 1984). In contrast, changes in external Ca++ result in changes in intracellular Ca++, even in the absence of electrical stimulation (Sheu and Fozzard, 1982; Lakatta and Lappé, 1981).

Figure 6 shows the result when the same stimulation pattern is applied in the presence of 280 nM Bay k 8644. This dose increases the steady state twitch tension for 1.0 Hz stimulation (as shown in Fig. 2) and also increases the second twitch in a train by 20.4%, but does not affect the rested-state contraction (see the lower panel for an expanded time scale). This experiment suggests that the inotropic effect of Bay k 8644 is not due to an intracellular action, or to any mechanism that alters cell Ca++ at rest, such as inhibition of the Na,K-ATPase.

The effect of Bay k 8644 on the steady state force-frequency relationship is shown in Figure 7. Increasing the stimulation rate above 0.2 Hz normally produces a positive force staircase in mammalian atria (Koch-Weser and Blinks, 1963). Bay k 8644 shifts the positive staircase effect to lower frequencies of stimulation. Consequently, the maximal percent increase in twitch tension occurs for stimulation at about 0.5 Hz.

Electrophysiological Effects

The dependence of the mechanical effects of Bay k 8644 on continual electrical stimulation suggests that the drug alters a sarcolemmal ionic conductance. The nature of the conductance change was explored in electrophysiological studies on calf ventricular muscle and Purkinje fibers. Figure 8 shows...
the effect of Bay k 8644 on the cardiac action potential. The upper left panel shows that the action potential duration of ventricular muscle is progressively increased as the concentration of Bay k 8644 increases from 3 to 300 nM. The effects on action potential duration are similar to those of elevated external Ca$^{++}$ (upper right panel), but the drug increases the plateau height less than elevated Ca$^{++}$. The lower left panel shows that Bay k 8644 also increases the action potential duration in Purkinje fibers. This action contrasts with the effect of increased external Ca$^{++}$ (Dudel et al., 1966; Tente and Davis, 1967; Kass and Tsien, 1976; Colatsky and Hogan, 1980; but see Reuter, 1967) or $\beta$-adrenergic receptor agonists (Tsien et al., 1972), which usually decrease the action potential duration. Similar effects of Bay k 8644 on the action potential were seen in one other calf Purkinje fiber and in one rabbit Purkinje fiber.

The effect of Bay k 8644 on action potential duration indicates a decrease in net outward current during repolarization. If the drug affects only inward Ca$^{++}$ currents (and, indirectly, Ca$^{++}$-activated outward currents), then a high dose of the specific Ca$^{++}$ channel blocker nisoldipine should eliminate the effect of Bay k 8644 on action potential configuration (Kazda et al., 1980; Kass, 1982). Figure 9 shows a test of this prediction. A Purkinje fiber was exposed to 1 $\mu$M nisoldipine, which shortened the action potential and depressed the plateau height (Kass, 1982). However, this dose does not alter the delayed rectifier current, $I_n$ (Kass, 1982). 30 nM Bay k 8644 was then added in the continued presence of nisoldipine (a low dose was chosen so as not to reverse the nisoldipine block of Ca$^{++}$ channels). No additional change in action potential was observed, in contrast to the result of Figure 8. A similar result was obtained in another calf Purkinje fiber.

**Figure 8.** Effect of Bay k 8644 and external Ca$^{++}$ on the cardiac action potential. Upper panels: action potentials recorded in calf ventricular muscle when Bay k 8644 was progressively increased to 3, 30, and 300 nM. Larger increases in action potential duration were sometimes observed when Bay k 8644 was increased from 30 to 300 nM. At right, the effect of increasing external Ca$^{++}$ from 1.8 to 5.4 mM in the same preparation. Stimulation at 0.5 Hz (50 V stimulus for 1.0 msec). $T = 35.6 \pm 1.0^\circ$C. Lower panels: effect of 30 nM Bay k 8644 on the action potential recorded in calf Purkinje fiber. At right, the effect of increasing external Ca$^{++}$ from 1.8 to 5.4 mM. Stimulation at 1.0 Hz (20 V stimulus for 1.4 msec). $T = 34.0 \pm 0.4^\circ$C. All voltage measurements were sampled at 250 Hz. Please note the change in time scale between the upper and lower panels.

**Figure 9.** Effect of 30 nM Bay k 8644 on the Purkinje fiber action potential in the presence of 1 $\mu$M nisoldipine. Action potentials recorded in 1 $\mu$M nisoldipine (labeled 0) and in 1 $\mu$M nisoldipine + 30 nM Bay k 8644 are superimposed. Steady state response during 1.0 Hz stimulation (28 V stimulus for 1.0 msec). $T = 36.7 \pm 0.1^\circ$C. The voltage measurements were sampled at 250 Hz.
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The results presented thus far indicate that Bay k 8644 enhances $\text{Ca}^{++}$ currents in myocardial cells. Recent voltage clamp studies of $I_{Ca}$ suggest that all positive inotropic agents may have this effect, because the $\text{Ca}^{++}$ channel conductance often increases in parallel with intracellular $\text{Ca}^{++}$ (Isenberg, 1977; Weingart et al., 1978; Noble and Shimoni, 1981; Marban and Tsien, 1982; Noble and Shimoni, 1981). This indirect drug effect on $\text{Ca}^{++}$ conductance can be eliminated by using Sr$^{++}$ as the charge carrier (Marban and Tsien, 1982). To determine whether Bay k 8644 acts directly on voltage-gated $\text{Ca}^{++}$ channels to enhance $\text{Ca}^{++}$ entry, we measured Sr$^{++}$ currents with a two-microelectrode voltage clamp in calf Purkinje fibers. Purkinje fibers were used because the effects of other inotropic agents on $\text{Ca}^{++}$ channels are well characterized in this tissue, and because run-down of $\text{Ca}^{++}$ currents is negligible.

Figure 10 shows the effect of Bay k 8644 on Sr$^{++}$ currents ($I_{Sr}$). $I_{Sr}$ was elicited by stepping the membrane potential from $-45$ to $+10$ mV. The current recordings show (from top to bottom) the effect of 0, 30, 100, or 300 nM Bay k 8644. Bay k 8644 increases $I_{Sr}$, and changes the time course of channel gating. $I_{Sr}$ is most enhanced immediately after the voltage step, so that large increases in peak $I_{Sr}$ are observed. The rate of current decay is speeded, so that only a small increase in $I_{Sr}$ is observed at the end of the depolarization.

Bay k 8644 could increase peak $I_{Sr}$ and speed the rate of channel inactivation by uniformly shifting the voltage-dependence of channel gating to more negative voltages. Figure 11 indicates that this is not the case. Comparison of the control record at $-15$ mV with the record at $-30$ mV in Bay k 8644 suggests a voltage shift of $\sim 15$ mV, but other pairwise comparisons indicate much larger effects on the voltage-dependence of channel gating. In general, the time course of $I_{Sr}$ in the presence of 300 nM Bay k 8644 was unlike the current elicited by stronger depolarizations without drug. Figure 12 shows a plot of the peak current measured at each voltage. The maximal inward current was seen at more negative potentials in the presence of Bay k 8644, indicating that the largest percent increase in $I_{Sr}$ was seen for weak depolarizations. This type of change in the current-voltage relationship can occur as an artifact of inadequate voltage control during the current measurement (Jack et al., 1975; chapter 12). However, the same change in the I-V relationship occurred when the effect of Bay k 8644 on peak current was offset by reducing external Sr$^{++}$ from 5.4 to 1.8 mM. Similar effects of Bay k 8644 on the I-V relationship were observed in four other experiments.

Deactivation of $\text{Ca}^{++}$ channels after repolarization is normally so fast that it occurs while the membrane capacitance is being discharged in this preparation. However, a slow component of this "tail current" can be seen in the presence of Bay k 8644 (this is most easily seen by comparing the two current recordings at $+15$ mV after repolarization in Fig. 11). These tail currents are blocked by nisoldipine with the same potency as the inward current during the test pulse, indicating that they are through $\text{Ca}^{++}$ channels (unpublished results).

**Mechanism of Drug Action**

Earlier contractility studies from this laboratory suggested that Bay k 8644 acts on the same $\text{Ca}^{++}$ channels as nifedipine, but that the two dihydropyridines have a diametrically opposite effect on these channels: nifedipine blocks the channels, while Bay k 8644 enhances $\text{Ca}^{++}$ entry (Schramm et al., 1983a, 1983b). The voltage clamp experiments presented in this paper provide direct experimental support for this idea. Bay k 8644 directly interacts with $\text{Ca}^{++}$ channels to increase $\text{Ca}^{++}$ entry into myocardial cells. Our findings are consistent with ligand binding studies with $[^{3}H]$-Bay k 8644, which indicate that the drug acts at the same site as nitrendipine and nifedipine (Bellemann, 1984; Janis et al., 1984). Similar electrophysiological findings have recently been reported by others (Hess et al., 1984; Sanguinetti and Kass, 1984; Ochi et al., 1984).
The lack of effect of Bay k 8644 on atrial rested state contractions indicates that the drug does not alter cell Ca\(^{++}\) in the absence of electrical activity. This distinguishes Bay k 8644 from cardiac glycosides, which increase these contractions (Koch-Weser and Blinks, 1962; Tuttle and Farah, 1962; Vincenzi, 1967), presumably because inhibition of the sodium pump increases intracellular Ca\(^{++}\) even at rest (Bers and Ellis, 1982; Sheu and Fozzard, 1982). It also distinguishes Bay k 8644 from a number of cardiotonic agents that alter the intracellular transport or binding of Ca\(^{++}\), such as caffeine (Lewartowski et al., 1978), ryanodine (Frank and Sleator, 1975), metabolic inhibitors (Katzung et al., 1957), isoproterenol (G. Thomas, unpublished observations), and phosphodiesterase inhibitors (Reinhardt et al., 1977). Hence, the positive inotropic effect of Bay k 8644 is not due to inhibition of the Na,K-ATPase or to increased sensitivity of the myofibrils to Ca\(^{++}\).

The effects of nitrendipine and Bay k 8644 on twitch tension further clarify the role of Ca\(^{++}\) channels in atrial muscle. Although tension development at physiological stimulation rates is sensitive to changes in I\(_{Ca}\), this source of Ca\(^{++}\) seems to play a minor role in atrial rested-state contractions. For example, Ni\(^{++}\) has a small effect on rested-state contractions at concentrations that block a substantial fraction of I\(_{Ca}\) (Lewartowski et al., 1978). Nitrendipine is a more specific blocker of Ca\(^{++}\) channels than drugs previously used to characterize atrial rested state contractions. Nitrendipine, 830 nm, should block a significant fraction of I\(_{Ca}\) at all stimulation rates, although more block is likely at faster rates of stimulation (Lee and Tsien, 1983; Sanguinetti and Kass, 1984). This dose had no effect on...
Comparison with Other Cardiotonic Agents

The best characterized and most often used cardiotonic agents are the cardiac glycosides and sympathomimetic amines. Both classes of drugs, like Bay k 8644, increase cardiac contractility by altering sarcoplasmal Ca++ transport. Serious questions have been raised about their usefulness in chronic therapy to increase cardiac output. The increase in intracellular Ca++ produced by these drugs can aggravate the effects of ischemia and cause arrhythmias (Smith, 1975; Braunwald et al., 1976, chapter 12; Hamer, 1979; Fleckenstein, 1983). Most of the currently available cardiotonic agents are plagued by the difficulties associated with Ca++ overload and, consequently, have a low therapeutic index. Katz has even suggested that this problem is inherent to positive inotropic agents and questions the usefulness of new drugs of this type (Katz, 1978).

Bay k 8644 is a particularly interesting drug because it increases ICa in a unique way that should allow the extra Ca++ influx to be used very efficiently. Consequently, less Ca++ entry should be needed to achieve a given inotropic effect. As discussed above, Bay k 8644 increases Ca++ entry primarily during the first 50 m sec of systole, with smaller increases taking place during the action potential plateau, and probably no increment in influx during diastole. Recent studies suggest that Ca++ entry at the beginning of an action potential has a greater effect on tension development than entry at other times.

Simultaneous measurements of tension and ICa under voltage clamp indicate that Ca++ entry throughout the action potential is important for tension development (Beeler and Reuter, 1970; Gibbons and Fozzard, 1975; Wier and Isenberg, 1982). However, two studies indicate that Ca++ entry during the action potential plateau is used for loading intracellular stores of Ca++ and affects tension development only on subsequent beats, whereas Ca++ release from these stores is determined only by the first few milliseconds of depolarization: (1) twitch tension is not increased when test pulses are made longer than 100 m sec if the effects of prior electrical stimulation are eliminated by a long rest period (Gibbons and Fozzard, 1971); (2) a 50-m sec depolarization elicits as much tension as a 500-m sec depolarization immediately after a change in pulse duration (Wier and Isenberg, 1982; compare Fig. 5, a and d).

The Ca++ influx occurring during the first few milliseconds of a cardiac action potential is only a small fraction of the Ca++ that activates myofibrils (Wier and Isenberg, 1982; Fabiato, 1983; Malloy and Morad, 1984), but is likely to serve as a trigger for the intracellular release of Ca++ (Fabiato and Fabiato, 1975a). Ca++-induced Ca++ release from the sarcoplasmic reticulum is a graded function of the activating Ca++ concentration, rather than an all-or-nothing process (Fabiato and Fabiato, 1975a; Fabiato and Fabiato, 1975b; Tada and Katz, 1982) and increase the delayed rectifier K+ channel current (Ix) (Tsien et al., 1972). Ca++ does not speed the rate of relaxation of tension (Fig. 2), suggesting a lack of effect on Ca++ uptake by the sarcoplasmic reticulum. Furthermore, Bay k 8644 increases the action potential duration over a broad concentration range, but /3-adrenoceptor agonists can decrease the action potential duration due to enhanced I x (Tsien et al., 1972). Bay k 8644 does not speed the rate of activation is slowed by isoproterenol, and isoproterenol increases [Noma et al., 1980; Isenberg and Klockner, 1982; Fabiato and Fabiato, 1975a] agents that increase cyclic AMP also speed the rate of ICa is unimportant in supply-
biation, 1983). Hence, the increase in Ca++ entry produced by Bay k 8644 should be amplified by increased release of Ca++ from the sarcoplasmic reticulum.

Agents that alter ICa via cyclic AMP are prone to produce Ca++ overload for three reasons: (1) Ca++ entry is not preferentially increased at the beginning of systole, so that Ca++ release from the sarcoplasmic reticulum is not optimized; (2) the sensitivity of myofibrils to Ca++ is reduced, so that a greater increment in intracellular Ca++ must occur for a given inotropic effect [Ray and England, 1976; McClellan and Winegrad, 1978; Allen and Kurihara, 1980: Marban et al., 1980; but Fabiato and Fabiato (1975b) did not find this effect]; and (3) the positive inotropic effect of increased cyclic AMP is always accompanied by an increase in heart rate. Bay k 8644 has none of these effects, and, thus, offers a promising new way to increase cardiac output. Unfortunately, Bay k 8644 is not clinically useful because it increases coronary resistance, apparently by a direct effect on vascular smooth muscle (Schramm et al., 1983a, 1983b). Although it is uncertain whether the vasoconstrictor properties of these dihydropyridines can be eliminated, it is clear that the discovery of superior positive inotropic agents is, in principle, possible.

Bay k 8644 is the most specific positive inotropic agent for increasing Ca++ channel currents. As such, it should be a useful pharmacological tool for defining the role of Ca++ in physiological processes, complementing the use of the Ca++ channel blockers.

Note added in proof:

After this manuscript was submitted for publication, S. Kokubun and H. Reuter [Proc. Natl. Acad. Sci. (Wash.) 81: 4824-4827 (1984)] and A. M. Brown et al. [Nature 311: 570-572 (1984)] reported voltage clamp studies that demonstrated direct effects of Bay k 8644 on cardiac Ca++ channels.

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