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MODULATION OF CALCIUM CHANNEL CURRENTS IN GUINEA-PIG SINGLE VENTRICULAR HEART CELLS BY THE DIHYDROPYRIDINE BAY K 8644

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SUMMARY

1. A single glass micropipette voltage clamp technique with intracellular dialysis was used to study Ba^{2+} currents in isolated ventricular cells from guinea-pig hearts. Effects of the 1,4-dihydropyridine Bay K 8644 on whole-cell currents were evaluated at 37 °C.

2. Bay K 8644 increased the Ba^{2+} peak currents at test potentials between -50 and $+20$ mV and shifted the current–voltage relationships towards hyperpolarizing potentials (leftward shift for Ca^{2+} channel activation, $13{\cdot}8 \pm 4{\cdot}1$ mV; $n = 9$; Bay K 8644, 5 μmol/l).

3. The peak times of the Ba^{2+} currents were diminished over the voltage range tested between -40 and $+20$ mV after Bay K 8644 in parallel with a shortening of the time constant of activation that was estimated from fits of the recorded currents with a d^2f model.

4. The decay of the Ba^{2+} currents was fitted with two exponentials including a pedestal. The compound Bay K 8644 accelerated the fast decay over the whole voltage range. The amplitude of the rapidly inactivated component of the Ba^{2+} currents was strikingly increased after application of Bay K 8644.

5. The steady-state inactivation using a 0·5 or 5 s pre-pulse was shifted towards hyperpolarizing potentials (leftward shift $10{\cdot}3 \pm 5{\cdot}2$ mV; $n = 4$; Bay K 8644, 5 μmol/l).

6. The change in the time course of Bay K 8644-modified Ba^{2+} currents cannot be described solely by a decrease of the backward rate coefficient from an open to a closed state of the Ca^{2+} channel (Sanguinetti, Krafte & Kass, 1986). The described effects of Bay K 8644 on the inactivation can be both qualitatively and quantitatively described by a model of current-dependent inactivation (Standen & Stanfield, 1982), assuming a lower affinity of an internal binding site for Ba^{2+} than for Ca^{2+}.

INTRODUCTION

Modulators of Ca^{2+} channels are among the most potent tools which have been used to affect many important cellular functions such as muscle contraction, neurosecretion and metabolic processes controlled by Ca^{2+}-dependent proteins.

One of these tools, the 1,4-dihydropyridine Ca^{2+} agonist Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), also seems to be a particularly helpful compound for studying the fundamental properties of Ca^{2+} channels (Hess, Lansman & Tsien, 1984; Reuter, Porzig, Kokubun & Prod'hom, 1985; Tsien, Bean, Hess, Lansman, Nilius & Nowycky, 1986). Although single-channel studies have given a comprehensive insight into modulatory effects of Bay K 8644 at the molecular level (Hess *et al.* 1984; Kokubun & Reuter, 1984; Ochi, Hino & Niimi, 1984) there are still unresolved problems concerning the interpretation of the macroscopic effects of Bay K 8644 on whole-cell currents. We report here on an acceleration of the activation of Ba^{2+} currents through Ca^{2+} channels, besides the well-described shift in the voltage range of activation towards hyperpolarizing potentials (Hess *et al.* 1984; Sanguinetti & Kass, 1984; Thomas, Chung & Cohen, 1985; Sanguinetti, Krafte & Kass, 1986). Furthermore, Bay K 8644 has been found to induce alterations in the process of inactivation which have not been described hitherto. We find that inactivation can be explained by a current-dependent process.

METHODS

Experiments were carried out in single ventricular cells isolated from guinea-pig hearts by use of an enzymatic dissociation procedure modified from that described by Kao, Christman, Chu, Krauhs, Tyers & Williams (1980). The dissociated cells were stored in an Eagle's minimum essential medium. After settling of the cells in a glass-bottomed 0·1 ml chamber we superfused a HEPES-buffered solution containing (mmol/l): NaCl, 150; KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 0·5; glucose, 11; HEPES, 5; adjusted to pH 7·4 with NaOH and HCl. The temperature of the solution in the chamber was controlled at 37 ± 1 °C.

In order to measure ionic currents we used an internal dialysis technique as described in detail by Benndorf, Boldt & Nilius (1985). In short, pipettes pulled from Pyrex glass tubes were fire-polished to a final tip diameter of 4–6 μm. A polyethylene tube inlet was positioned as near as possible to the tip (about 150 μm behind the tip). Cells were sucked onto the pipettes and their cell membrane was disrupted by suction after formation of a high-resistance seal. Intracellular perfusion was performed using a negative pressure of 50 cmH_2O applied to the tip of the inlet. The intracellular solution contained (mmol/l): Tris-Cl, 140; EGTA, 2; $MgCl_2$, 2; ATP, 5; pH 7·1 titrated with Tris and H_3PO_4. The cells used were of medium size of about 160 μF (see also Isenberg & Klöckner, 1982; Cavalie, Pelzer & Trautwein, 1986). Each measurement was started with a recording of the fast Na^+ current. Thereafter, to measure Ca^{2+} channel currents with a minimal interdependence with other ionic currents, we rapidly switched to the following external solution (mmol/l): caesium aspartate, 85; glucose, 5; Tris-Cl, 5; $BaCl_2$, 10; titrated to pH 7·4 with aspartate and Tris. During washing of the sucked cell with Na^+-free solution Na^+ currents disappeared leaving tetrodotoxin (TTX)-resistant slowly inactivating Ba^{2+} currents.

The voltage clamp arrangement, the compensation of the series resistance, the effectiveness of the intracellular dialysis, and problems arising from inhomogeneities of the voltage control have been described in detail elsewhere (Benndorf *et al.* 1985). The linear leakage resistance as well as linear capacitive currents were compensated in the conventional way.

In order to describe Ca^{2+} channel activation and inactivation we fitted theoretical curves to the experimental data using the Levenberg–Marquard algorithm as described by Brown & Dennis (1972). Averaged values are given as means ± standard error of means.

The sample of Bay K 8644 was kindly provided by Dr M. Schramm, Bayer AG, Wuppertal, F.R.G. We used it from a stock solution diluted to only one final concentration of 5 μmol/l in all experiments.

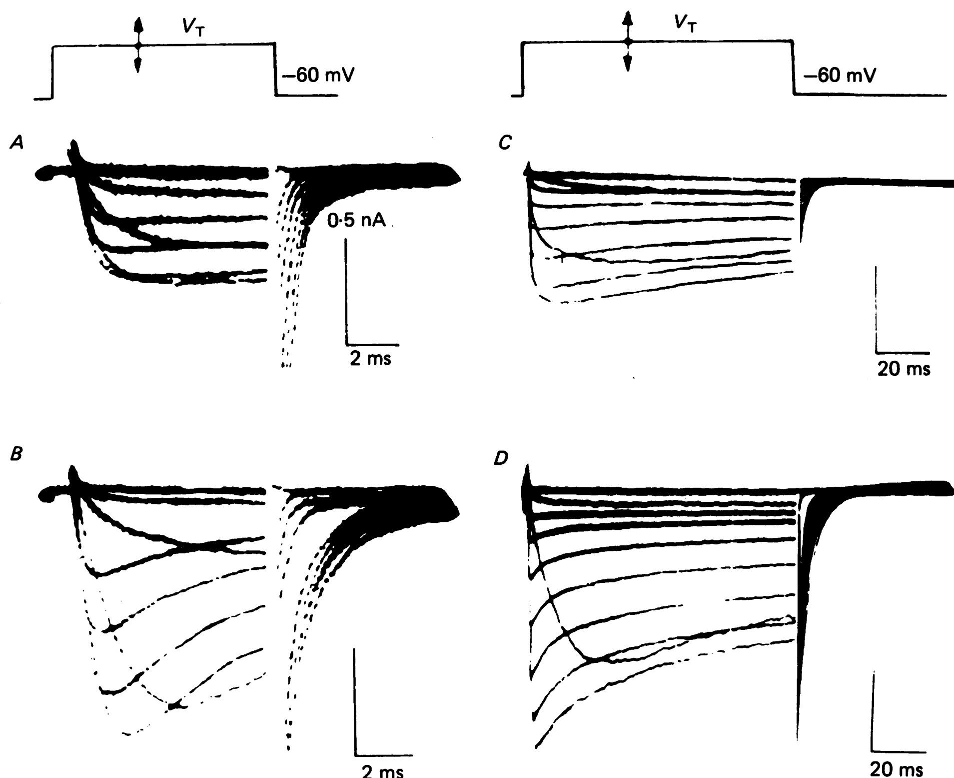


Fig. 1. Ba^{2+} currents through voltage-activated Ca^{2+} channels at different test potentials (V_T). The holding potential was −60 mV in each case. The spacing of the voltage steps is 10 mV. *A*: control currents, the first clearly visible inward current being activated by a step to −30 mV. *B*: Ba^{2+} currents about 3 min after application of Bay K 8644 (5 μmol/l), the first but very small inward current appearing at −50 mV (same cell as in *A*). *C*: control currents recorded with a lower time resolution (note time calibration; current calibration for all records is 0·5 nA). The first but small inward current appeared at −40 mV. *D*: Bay K 8644-modulated currents 9 min after application, the first current again appearing at −50 mV (same cell as in *C*, but different from that in *A* and *B*; this was also the same cell as that used in Fig. 6*C* and *D*).

RESULTS

Effects of Bay K 8644 on the activation of calcium channel currents

The compound Bay K 8644 causes multiple effects on Ba^{2+} currents through Ca^{2+} channels. Figure 1 shows families of Ba^{2+} currents from two different cells clamped at −60 mV; the currents in *B* and *D* were measured 3 min after addition of Bay K 8644 to the bath. We measured an increase in the magnitude of the peak inward current over the whole tested voltage range between −50 and +20 mV. Obviously, Bay K 8644 accelerated the inactivation and enabled us to measure Ba^{2+} currents at test potentials more negative than those under control conditions. Figure 2 shows a typical example of the effects of Bay K 8644 on the current–voltage relationship. The peak currents were plotted at different test potentials, unveiling a distinct leftward shift towards hyperpolarizing potentials of both the current maximum and

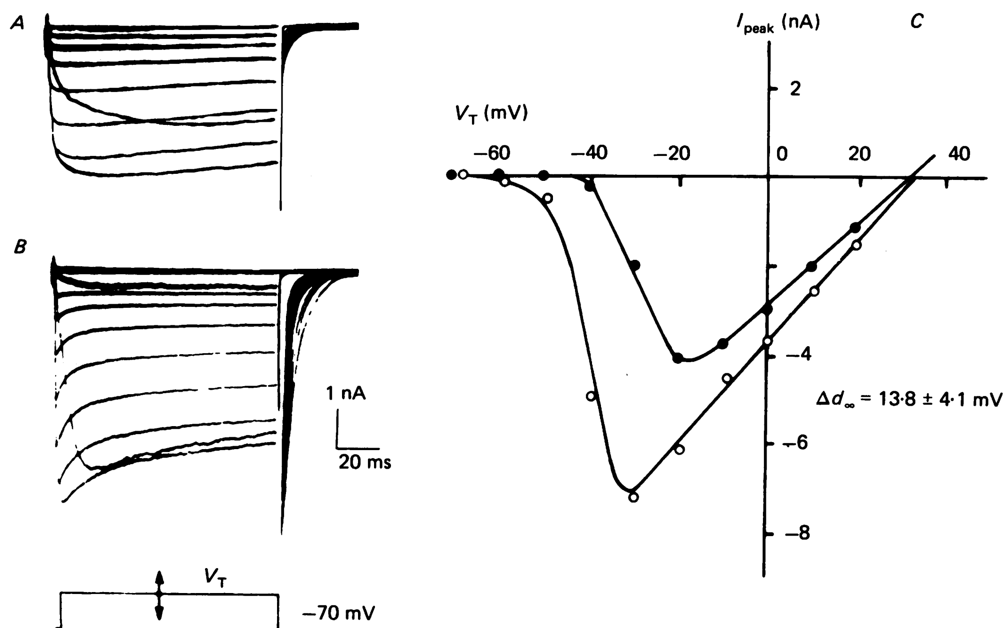


Fig. 2. Effects of Bay K 8644 on current–voltage relationships of the Ba^{2+} channel currents. *A*: voltage-activated inward currents in control solution; the holding potential is −70 mV, voltage steps are spaced 10 mV. *B*: Ba^{2+} currents of the same cells as in *A* activated from the same holding potential. Note the slowly deactivated inward tails in comparison to the controls and the shift of the current peaks towards shorter times than in control. *C*: current–voltage relationships obtained from the currents shown at the left-hand side. The theoretical curve to which the experimental data were fitted was

$$I_{peak} = (1+\exp((V_T-a_3)/a_4))^{-1}a_1(V_T-a_2) \quad \text{(eqn (1) in text)}$$

The following parameters were obtained:

	Control	Bay K 8644
a_1 (μS)	0·09	0·14
a_2 (mV)	28·8	24·7
a_3 (mV)	−27·8	−40·7
a_4 (mV)	3·7	3·1

the activation range of the currents. At negative membrane potentials the Ba^{2+} currents were strikingly increased by Bay K 8644. This increase is even more accentuated because it overlapped with the run-down of Ca^{2+} channel currents in our cells (run-down was quantified by a mean time up to 50% decrease of the peak currents of 19±5 min from four cells). We described the current–voltage relationships by a tentative four-parameter model to quantity the effects of Bay K 8644:

$$I_{peak} = (1+\exp((V_T-a_3)/a_4))^{-1}a_1(V_T-a_2), \quad (1)$$

where I_{peak} means the maximum inward current at the test potential V_T in nA, a_1 is the positive slope conductance, a_2 the extrapolated reversal potential of the Ba^{2+} current, a_3 the potential of half-maximal activation, and a_4 the slope parameter of

the Ca^{2+} channel activation in mV. From nine cells the following results were obtained: (i) the slope conductance increased from $0{\cdot}11 \pm 0{\cdot}03$ μS in the controls to $0{\cdot}18 \pm 0{\cdot}03$ μS under Bay K 8644, (ii) the extrapolated reversal potential a_2 was not significantly changed (control: $29{\cdot}7 \pm 4{\cdot}3$ mV; Bay K 8644: $27{\cdot}2 \pm 4{\cdot}8$ mV) and (iii) the potential of half-maximal activation of Ca^{2+} channel currents was shifted from

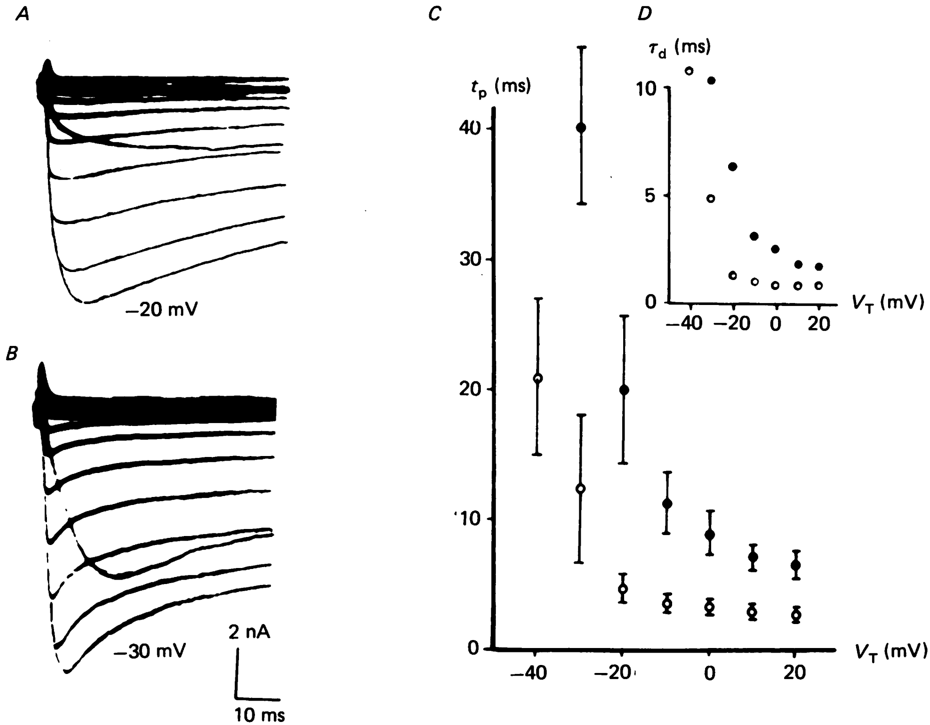


Fig. 3. Effects of Bay K 8644 on the time course of the activation of Ba^{2+} currents. *A*: control currents elicited by depolarizing test potentials 10 mV spaced, starting from holding potential of -60 mV. *B*: Ba^{2+} currents 3 min after application of Bay K 8644; same voltage protocol as in *A*. Note the shorter time to peak current in comparison to *A*. *C*: mean values of time to peak currents (t_p) determined from eight cells (●: control, ○: Bay K 8644). *D*: voltage dependence of the activation time constant τ_d, calculated from the mean values in *C* by $t_p = \tau_d \ln(2\tau_f/\tau_d + 1)$ assuming a d^2-activation scheme. τ_f is the fast time constant of inactivation taken from Fig. 5*A*, and τ_d is the time constant of activation.

$-25{\cdot}6 \pm 2{\cdot}3$ to $-39{\cdot}3 \pm 5{\cdot}5$ mV; Bay K 8644 induced a mean leftward shift of the activation range of Ca^{2+} channels of $13{\cdot}8 \pm 4{\cdot}1$ mV, (iv) the slope of the activation was steepened by Bay K 8644 from $3{\cdot}4 \pm 0{\cdot}8$ to $2{\cdot}6 \pm 0{\cdot}6$ mV. The main finding of a shift of the current–voltage relationship towards hyperpolarizing potentials nicely matches the results of other authors with different preparations (Sanguinetti & Kass, 1984; Thomas, Chung & Cohen, 1985; Sanguinetti *et al.* 1986) and also with guinea-pig ventricular cells (Hess *et al.* 1984). A further effect of Bay K 8644 on the Ca^{2+} channel activation is seen in Fig. 3. After application of Bay K 8644 Ba^{2+} currents peaked in shorter times than in the controls (Fig. 3*A* and *B*). This effect cannot be

interpreted as a loss of voltage control during the onset of the currents because in this case longer delays should be expected. As measured in eight cells with no sign of inhomogeneities of the clamp, the time to peak current was shifted towards smaller values after Bay K 8644 (Fig. 3*C*). If the activation was described with a d^2 model we revealed highly reliable fits between -40 and 0 mV. At stronger depolarizations potency functions with higher powers than 2 were necessary. From both the fits and the recalculation of the time constant of activation (τ_d) from the peak times we obtained shorter time constants of activation after administration of Bay K 8644 than in the controls (Fig. 3*D*).

Changes in barium current inactivation induced by Bay K 8644

To describe the time course of inactivation we used long pulses. It turned out that the best fits could be obtained with the following five-parameter model:

$$I = I_1 \exp(-t/\tau_1) + I_2 \exp(-t/\tau_2) + I_\infty, \quad (2)$$

where I_1 is the amplitude of a fast-inactivating current component with the time constant τ_1. The terms I_2 and τ_2 describe a slowly decaying current component, and I_∞ is a pedestal of a non-inactivating current. Figure 4*A* and *B* shows a control current activated by a step from -50 to -10 mV and the semilog plot of the inactivating part of the current, respectively. Two time constants of inactivation can be detected. Figure 4*C* shows the same current–voltage relationship for the peak current and the current I_∞ measured at the end of the 5 s pulse. Equation (2) was fitted to the experimental data in all experiments with the highest reliability.

The compound Bay K 8644 strikingly influenced the fast component of the current inactivation. As shown in Fig. 5*A* the time constant of the fast inactivation was decreased over the whole voltage range studied after Bay K 8644 application in comparison with that of the control currents. Even at potentials between -40 and -30 mV a clear fast-inactivating current component could be observed after Bay K 8644 but not in the controls. As shown in Fig. 5*B*, a drug-induced increase of the contribution of the fast-inactivating current component could be observed. For reliable measurements of the slow inactivation, pulses of several seconds were found to be necessary. It turned out that stimulation programmes with long-lasting pulses accelerated the run-down of the cells and therefore we only measured the slow time constant of inactivation sporadically. From the fits using eqn (2) for 100 ms pulses we could not find any significant changes in the time constant of the slow inactivation between controls and Bay K 8644. The time constants were scattered between 0·8 and 3·7 s in the voltage range studied.

The compound Bay K 8644 was also found to shift the steady-state inactivation curve towards hyperpolarizing potentials. Figure 6 shows Ba^{2+} currents elicited by voltage steps to 0 mV after pre-pulses of 0·5 s (Fig. 6*A* and *B*), and 5 s (Fig. 6*C* and *D*) duration. At both pre-pulse durations Bay K 8644 shifted the inactivation curve (Fig. 7) towards hyperpolarizing potentials but strikingly unveiled a long-lasting onset of inactivation: with short pre-pulses the inactivation was only partial whereas with a 5 s pre-pulse the inactivation was nearly complete.

The conventionally recorded inactivation curves were fitted by:

$$f_\infty = (1-b_3)/(1+\exp((V_p-b_1)/b_2))+b_3, \quad (3)$$

where f_∞ is the degree of inactivation measured from the normalized peak currents, b_1 describes the half-maximal steady-state inactivation, b_2 is the slope parameter of the inactivation–voltage relationship, and b_3 fits the non-inactivated current (pedestal). Figure 7 shows two examples of inactivation curves with different duration of the pre-pulses. From four cells the following results were obtained: (i) the

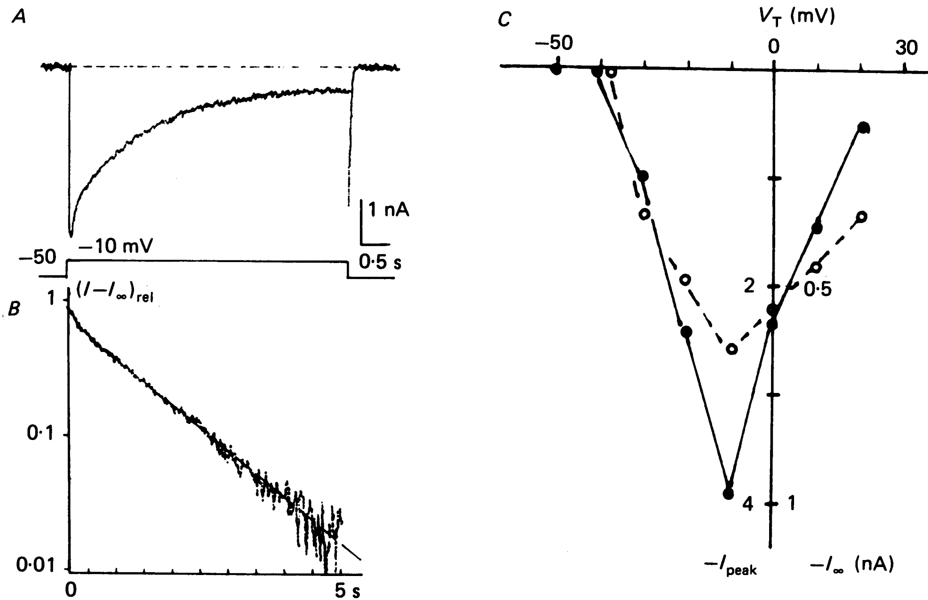


Fig. 4. Evaluation of a suitable model to describe the inactivation of Ba^{2+} currents. *A*: Ba^{2+} inward current following voltage steps of 5 s duration from −50 to −10 mV with no Bay K 8644. The decay of the current was fitted by

$$I = I_1 \exp(-t/\tau_1) + I_2 \exp(-t/\tau_2) + I_\infty \qquad \text{(eqn (2))}$$

with $I_1 = 1{\cdot}4$ nA, $\tau_1 = 52$ ms, $I_2 = 2{\cdot}5$ nA, $\tau_2 = 1289$ ms, $I_\infty = 0{\cdot}4$ nA. All decays were described by the above model. *B*: the semilog plot of the decay of the Ba^{2+} current unveils the two time constants. Only 32 % of the total current is carried by the fast-inactivating component. This component, however, determines the decay seen in a 100 ms pulse as usually applied. *C*: peak Ba^{2+} current and pedestal show the same voltage dependence (●: peak current (I_{peak}); ○: current at the end of a 5 s pulse (I_∞); mean values from two cells).

potential of half-maximal inactivation (b_1) was shifted from $-37{\cdot}9 \pm 2{\cdot}3$ (control) to $-47{\cdot}9 \pm 5{\cdot}8$ mV (with Bay K 8644), an average shift of $-10{\cdot}1 \pm 5{\cdot}6$ mV towards hyperpolarizing potentials being measured, (ii) the slope b_2 was not significantly changed ($5{\cdot}4 \pm 0{\cdot}3$ mV for the control and $5{\cdot}9 \pm 1{\cdot}2$ mV with Bay K 8644) and (iii) the fraction of the non-inactivated current (b_3) was $0{\cdot}36 \pm 0{\cdot}06$ (control) and $0{\cdot}17 \pm 0{\cdot}13$ (Bay K 8644) for the 0·5 s pre-pulse and $0{\cdot}04 \pm 0{\cdot}01$ (control); $0{\cdot}07 \pm 0{\cdot}02$ (Bay K 8644) for the 5 s pre-pulse.

Alterations of inactivation induced by Bay K 8644 are due to current dependent inactivation

Striking evidence has suggested that the inactivation of Ca^{2+} channels depends on Ca^{2+} entry in a number of tissues (see Eckert & Chad, 1984, for a review) including cardiac cells (Nilius & Hencek, 1983; Kass & Sanguinetti, 1984; Mentrard, Vassort & Fischmeister, 1984; Bechem & Pott, 1985; Lee, Marban & Tsien, 1985; Nilius & Benndorf, 1986). Even if Ba^{2+} is the charge carrier we observed the following

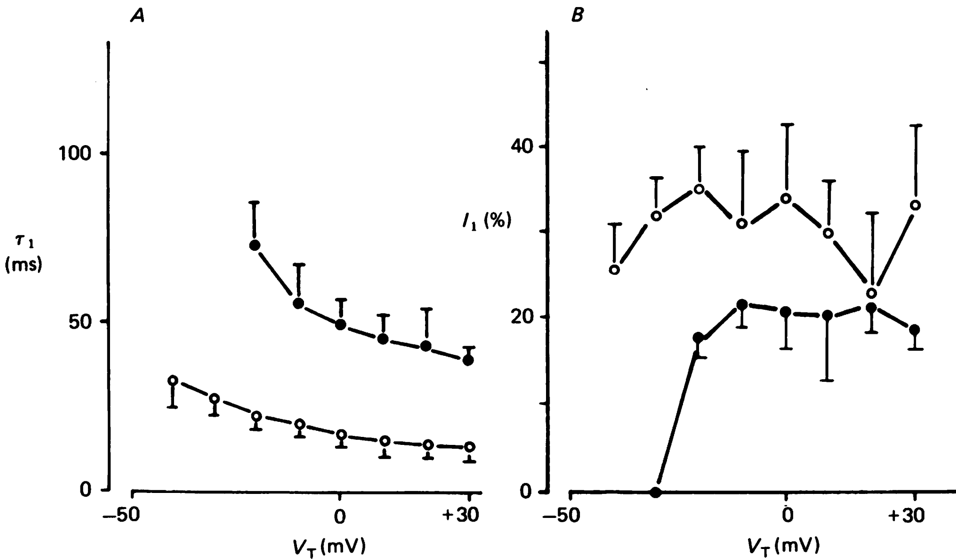


Fig. 5. Voltage dependence of the fast time constant of inactivation and the contribution of the fast-decaying current to the total current. *A*: Bay K 8644 shifts the time constant of the fast inactivation towards smaller values over the whole voltage range tested. *B*: Bay K 8644 increases the contribution of the fast-inactivated current to the total current. The effect is pronounced at negative membrane potentials (●: control; ○: Bay K 8644, 5 μmol/l, means ± S.E. of means from eight cells).

properties of Ca^{2+} channel currents that encouraged us to explain our experimental findings by the mechanism of current-dependent inactivation: (i) the inactivation was dependent on both the magnitude of the current and the speed of activation, (ii) using a double-pulse programme we found an increase in the test currents following successively stronger depolarizing pre-pulses beyond +30 mV (Fig. 8) and (iii) the inactivation of the Ba^{2+} currents became decelerated at constant test steps if the current amplitude was diminished due to run-down of the Ca^{2+} channels. In three cells we observed a linear correlation between the peak current amplitude and the reciprocal time constant of fast inactivation with a correlation coefficient of $r = 0.92$. We described our results with a simple model for Ca^{2+} channel inactivation based on the assumption that Ba^{2+} can also be bound at a Ca^{2+} binding site. We used the same model as previously proposed by Standen & Stanfield (1982). This model predicts an acceleration of the fast inactivation of Ca^{2+} channel currents if (i) the activation is

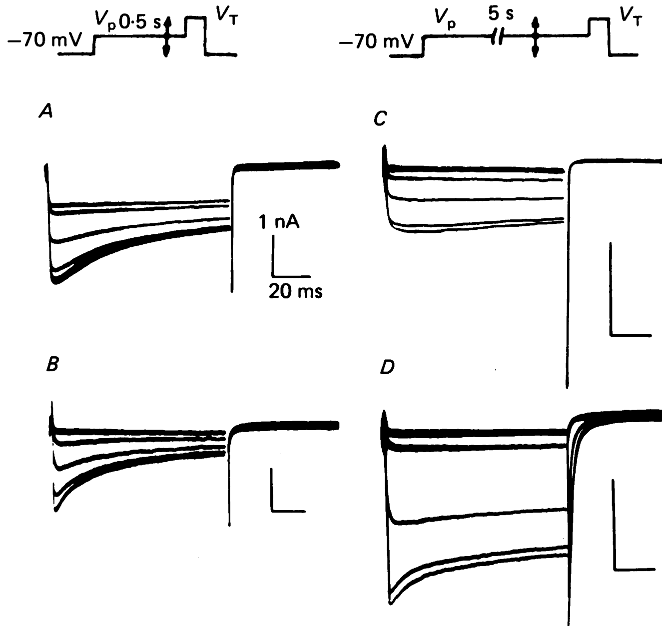


Fig. 6. Barium inward currents before and after the application of Bay K 8644. Pre-pulses of 0·5 or 5 s were followed by a test step to 0 mV. *A*: Ba^{2+} currents after stepping from 500 ms pre-pulse to 0 mV. The holding potential is −70 mV. The decrease of the test current is due to more positive pre-pulses. Pre-pulses are 10 mV spaced. Note the incomplete inactivation and the slowing of the decay of the test currents with depolarizing potentials. *B*: same cell as in *A* but 6 min after application of Bay K 8644 (same calibration as in *A*). *C*: long-lasting pre-pulse (5 s) decreased the non-inactivated part, unveiling a slow onset of inactivation. Same pulse protocol as in *A*. *D*: same cell as in *C* (but different from that in *A* and *B*), 6 min after Bay K 8644 was applied to the bath. The inactivation is nearly complete. Note again the delayed tail currents. This was the same cell as that used in Fig. 1*C* and *D*).

speeded up and (ii) the currents are increased. Both conditions nicely fit our findings. The best approximation of the Ba^{2+} currents (I_{Ba}) were achieved by use of

$$I_{Ba} = \bar{g}_{Ba}\, d^2(t) f(t)\,(V - V_R), \tag{4}$$

where $\bar{g}_{Ba}$ describes the maximal conductance of the membrane for Ba^{2+} ions, V is the membrane potential and V_R the apparent reversal potential. The term $d(t)$ describes the activation of Ba^{2+} currents by the conventional Hodgkin–Huxley kinetics:

$$d(t) = d_\infty - (d_0 - d_\infty)\exp(-t/\tau_d), \tag{5}$$

with d_0 and d_∞ being the fraction of open channels at zero and infinite time, respectively. The term τ_d is the time constant of activation. A d^2 model was chosen because (i) it described the measured currents with a high reliability and (ii) it is similar to the conventionally used C_1–C_2–O (closed–closed–open) sequential scheme for the activation of Ca^{2+} channels as revealed in single-channel measurements

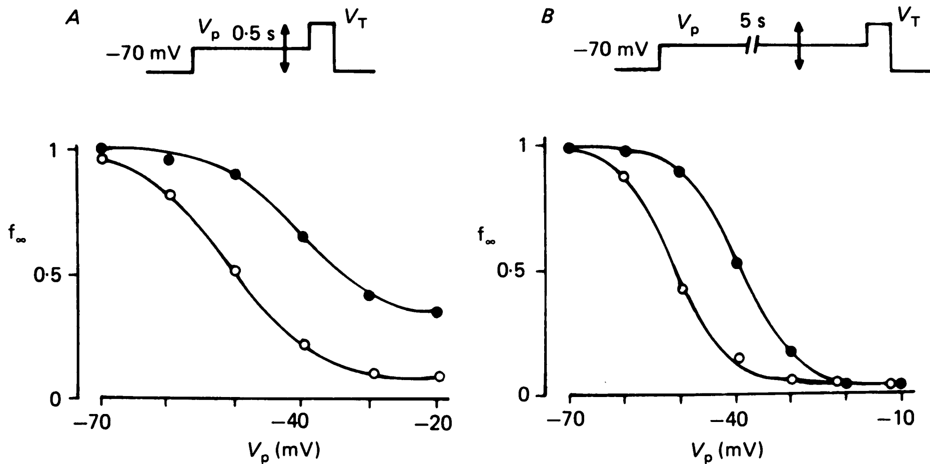


Fig. 7. Effects of Bay K 8644 on the 'steady-state' inactivation. Control: ●; Bay K 8644: ○. *A*: inactivation curves were constructed from the normalized peak currents after different pre-pulses to the potential V_p. Pre-pulse duration 500 ms, same protocol and same cell as in Fig. 6*A* and *B*. The curves were fitted by the equation

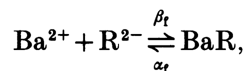
$$f_\infty = (1-b_3)/(1+\exp((V_p-b_1)/b_2))+b_3 \quad \text{(eqn (3))}$$

	Control	Bay K 8644
b_1 (mV)	−40·3	−50·9
b_2 (mV)	5·7	6·1
b_3	0·31	0·07

B: same protocol as in *A* but 5 s pre-pulse (same cell as in Fig. 6*C* and *D*). Note the nearly complete inactivation.

	Control	Bay K 8644
b_1 (mV)	−39·2	−51·4
b_2 (mV)	5·1	4·6
b_3	0·03	0·04

(Cavalie, Ochi, Pelzer & Trautwein, 1983; Tsien *et al.* 1986). The term $f(t)$ in eqn (4) describes the current-dependent inactivation. Assuming the reaction scheme



$$Ba^{2+} + R^{2-} \underset{\alpha_f}{\overset{\beta_f}{\rightleftharpoons}} BaR,$$

where R represents the Ca^{2+} (Ba^{2+}) binding site, $f(t)$ was calculated by:

$$-\frac{df(t)}{dt} = \alpha_f(1-f(t)) - \beta_f[Ba^{2+}]_i f(t). \quad (6)$$

The dissociation constant for the binding of Ba^{2+} onto the site R is

$$k_f = \frac{\alpha_f}{\beta_f}.$$

The concentration of Ba^{2+} at the inner side of the membrane $[Ba^{2+}]_i$ was determined by

$$\frac{d[Ba^{2+}]_i}{dt} = \frac{j_{Ba}\,a}{2F\sigma} - k_s[Ba^{2+}]_i, \tag{7}$$

where j_{Ba} equals the flow of charge per unit volume of the cell (C ms^{-1} cm^{-3}) which is determined from I_{Ba}, based on a cell volume of 40 pl. The symbol σ is the volume fraction of the myocyte in which Ba^{2+} accumulation takes part, a means the fraction

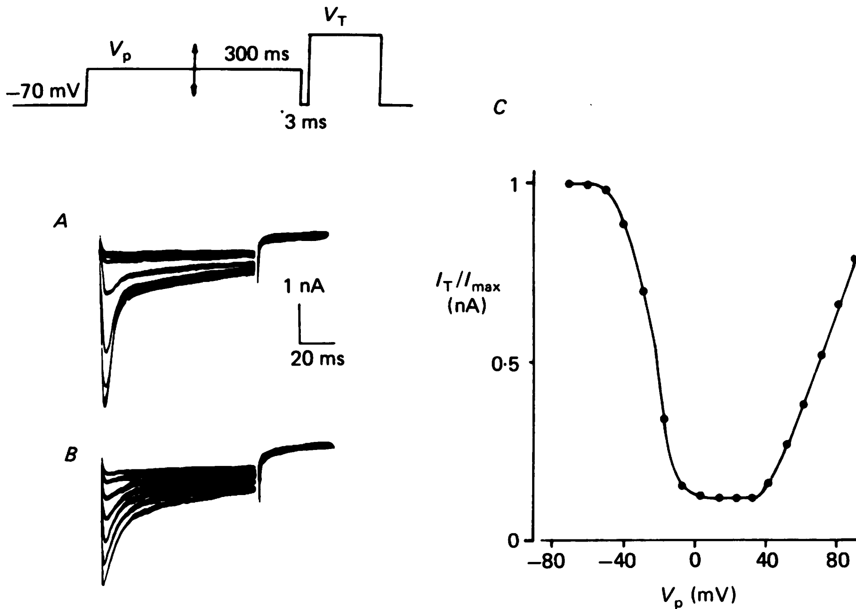


Fig. 8. Strong depolarizing pre-pulses do not inactivate Ba^{2+} currents. *A*: $V_p = -70$ to $+20$ mV. *B*: $V_p = +30$ to $+90$ mV, 10 mV spaced. 200 μM-TTX added to the external solution had no influence on the currents. *C*: dependence of the peak test current I_T at the test potential V_T divided by the maximal peak test current I_{max} (occurred after application of $V_p = -70$ mV) on pre-pulse potential V_p. The increasing test currents following successively stronger depolarizing pre-pulses beyond $+30$ mV is an indication of current-dependent inactivation of the Ba^{2+} currents.

of Ba^{2+} unbound by a Ca^{2+} (Ba^{2+}) buffer, and F is the Faraday constant. The term $1/k_s$ means the time constant of Ba^{2+} removal from the accumulation compartment by diffusion and dialysis. Equations (6) and (7) were solved using a Runge–Kutta algorithm for a system of first-order differential equations. Taking into account our experimental conditions (internal dialysis, weak affinity of Ba^{2+} for an intracellular Ca^{2+} binding site; e.g. Kostyuk, Mironov & Shuba, 1983; Stephenson & Thieleczek, 1986) we used a set of parameters different from that already described by Standen & Stanfield (1982):

$$k_f = 80\ \mu\text{M},\quad k_s = 0{\cdot}005\ \text{ms}^{-1},$$
$$\alpha_f = 0{\cdot}8\ \text{ms}^{-1},$$
$$a = 0{\cdot}3,$$
$$\sigma = 0{\cdot}2.$$

The values of $\bar{g}_{Ba}$ and d_{∞} were calculated from the measured current–voltage relationships using eqn (1). The τ_d values were derived from the measured peak time (t_p). With these parameters we calculated families of Ba^{2+} currents before and after application of Bay K 8644. The test potentials varied between −50 and +20 mV in 10 mV steps (Fig. 9).

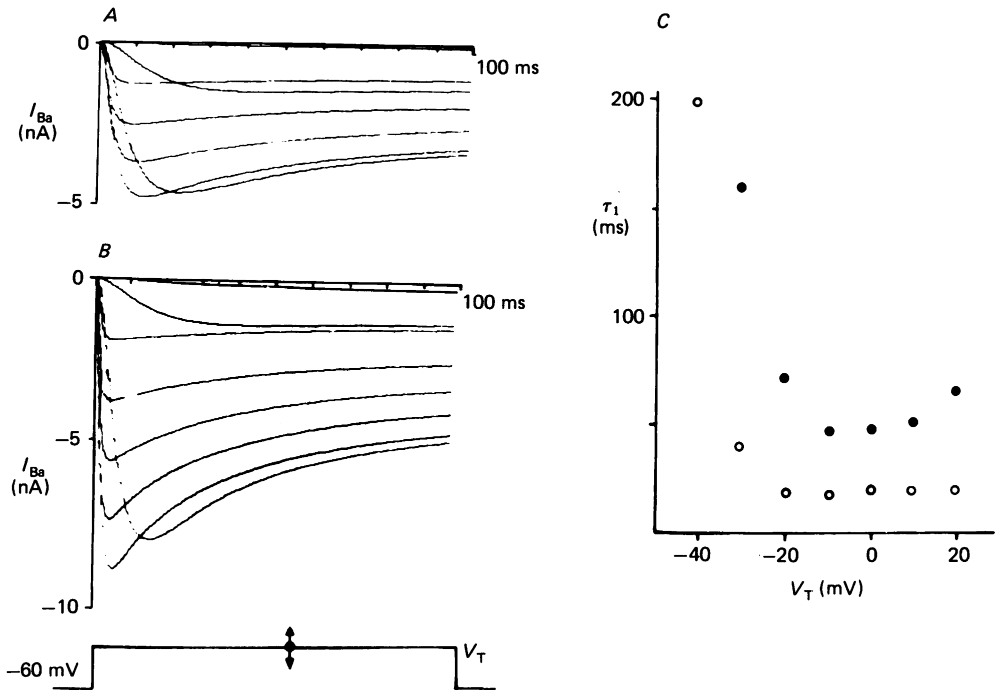


Fig. 9. Ba^{2+} currents at different test potentials simulated by a model of Ba^{2+}-current-dependent inactivation. Holding potential = −60 mV, depolarizing voltage steps spaced 10 mV up to +20 mV. *A*: control, the maximal current being calculated from a voltage step to −10 mV. *B*: currents after application of Bay K 8644, same voltage protocol as in *A*. The activation parameters for *A* and *B* are determined from the *I–V* and t_p–*V* curves (see Figs 2 and 3). *C*: voltage dependence of the time constant of the fast inactivation. The decaying currents were fitted by the same equation as used for fitting the measured time-dependent inactivation. Control: ●; Bay K 8644: ○. Note the coincidence with the measured currents as shown in Figs 1*C* and *D*, 2*A* and *B*, 3*A* and *B*.

The time course of the simulated currents equals the measured currents as depicted in Fig. 1*C* and *D*. This is demonstrated by the calculated time constants for the fast inactivation using eqn (2). The comparison with the corresponding time constants of the measured currents shows the close correlation concerning magnitude and voltage dependence (Fig. 9*C*).

Figure 10 demonstrates that the model used also simulates the inactivation studied with 500 ms pre-pulses of different voltages followed by test steps to −10 (controls) or −20 mV (Bay K 8644). With our experimental data included into the Standen–Stanfield model of a current-dependent inactivation we were able to describe sufficiently both the decrease of the non-inactivated part of the f_{∞}–V_p

relationship (pedestal) and the shift of the f_{∞}–V_p curve towards hyperpolarizing pre-pulse potentials after application of Bay K 8644. Obviously, the model of current-dependent inactivation explains both qualitatively and quantitatively the effects of Bay K 8644 on the steady-state inactivation.

It has been demonstrated in Fig. 6 that besides the effects on the peak currents, depolarizing pre-pulses also cause a slowing of the decay of the test currents. This

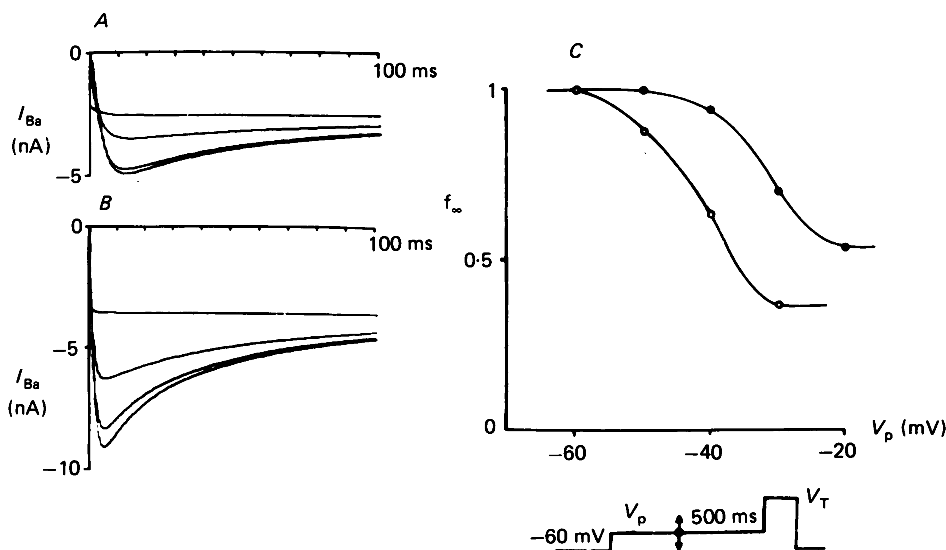


Fig. 10. Steady-state inactivation, calculated by the model of current-dependent inactivation. *A*: $V_T = -10$ mV (control). *B*: $V_T = -20$ mV (Bay K 8644); depolarizing pre-pulses, 500 ms long, starting from holding potential of −60 mV, 10 mV spaced. *C*: steady-state inactivation curves constructed from the normalized peak values of *A* and *B*. Control: ●; Bay K 8644: ○.

finding again contradicts a pure voltage-dependent inactivation. In Fig. 11 the time constants of the fast-inactivating component of the test currents shown in Fig. 6*A* and *B* are compared with the corresponding time constants of the currents calculated by the model (Fig. 10*A* and *B*). The time constants of the model agree well with the time constants of the measured currents with respect to their dependence on the pre-pulse potential and the effects of Bay K 8644. Pre-pulses more positive than −30 (control) or −40 mV (Bay K 8644) caused a nearly complete disappearance of the decay of both the measured and calculated test currents.

DISCUSSION

Modulation of Ca^{2+} channel activity has recently become a subject of wide interest. Among possible modulators, 1,4-dihydropyridines seem to be particularly helpful both for studying the molecular properties of Ca^{2+} channels and for effectively changing the functional properties of these channels (Schramm & Towart, 1985; Reuter *et al.* 1985; Kokobun, Prod'hom, Becker, Porzig & Reuter, 1986). However,

the effects of the most commonly used Ca^{2+} agonistic compound, Bay K 8644, on whole-cell currents through Ca^{2+} channels are not completely understood.

In our experiments with isolated ventricular cells from the guinea-pig we could demonstrate that Bay K 8644 enhances the conductance of the heart cell membrane for Ba^{2+} and shifts both activation and steady-state inactivation of Ba^{2+} currents towards hyperpolarizing potentials. All these findings coincide very well with results

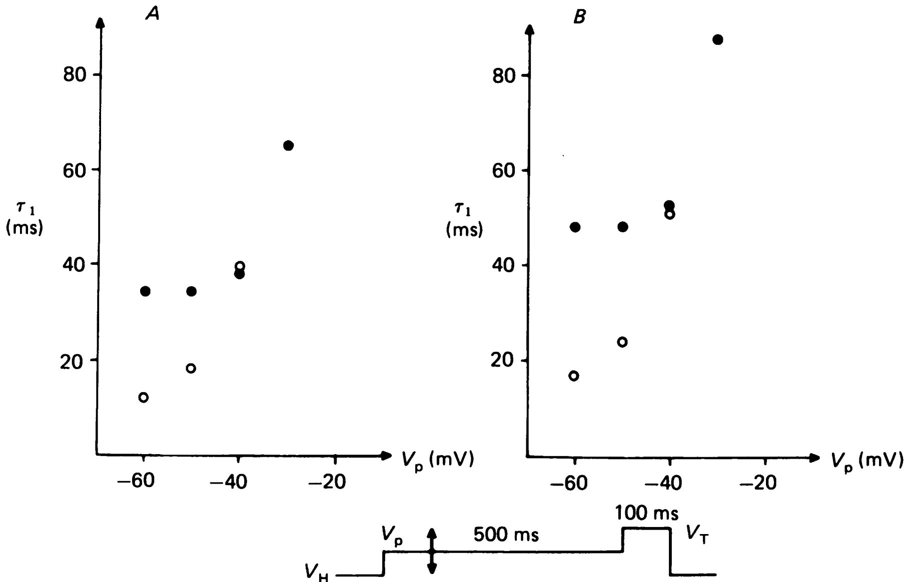


Fig. 11. Dependence of the time constant of the fast inactivation of test currents on pre-pulse potential and application of Bay K 8644. The time constants were obtained *A*: from the measured currents demonstrated in Fig. 6*A* and *B*, and *B*: from the simulated currents as shown in Fig. 9*A* and *B*. At pre-pulses positive to −30 mV (control), −40 mV (Bay K 8644), the decay of both measured and calculated test currents was too slow to be determined with 100 ms test pulse duration. Control: ●; Bay K 8644: ○. V_H is the holding potential.

obtained from other cardiac preparations (Sanguinetti & Kass, 1984; Thomas *et al.* 1985; Sanguinetti *et al.* 1986). As demonstrated in single-Ca^{2+}-channel measurements (Hess *et al.* 1984; Kokubun & Reuter, 1984; Ochi *et al.* 1984; Reuter *et al.* 1985; Tsien *et al.* 1986) the enlargement of the macroscopic currents (or averaged Ca^{2+} channel currents) is mainly due to the prolonged mean open time of the single Ca^{2+} channels caused by Bay K 8644. The increase in the Ca^{2+} channel current induced by Bay K 8644 may be responsible for well-known macroscopic effects of this compound on cardiac tissues, like the positive inotropy, a modestly positive chronotropy and dromotropy (Schramm, Thomas, Towart & Franckowiak, 1983*a*, *b*; Vaghy, Grupp, Grupp & Schwartz, 1984; Böhm, Barmann, Meyer, Nose, Schmitz & Scholz, 1985; Preuss, Brooks, Gross & Warltier, 1985; Rogg, Criscione, Truog & Meier, 1985), the prolonged duration of ventricular action potentials (Brown, Kunze & Yatani, 1984; Thomas *et al.* 1985) and the increased upstroke velocity of Ca^{2+}-mediated action potentials (Nilius, 1984*a*, *b*; Wahler & Sperelakis, 1984).

A further result of our experiments was the acceleration of the activation of the Ba^{2+} currents. This speeding up of activation has not yet been described but agrees very well with data obtained from the single-Ca^{2+}-channel recording by Hess *et al.* (1984). If a single C_1–C_2–O kinetic scheme is assumed to describe the activation of Ca^{2+} channels one can calculate from the histograms shown by Hess *et al.* (1984) that the forward rate coefficient for the transition of the channel from C_1 to C_2 (k_1) and from C_2 to O (k_2) are both strikingly increased by Bay K 8644 (k_1 about 750 s^{-1} in the control but about 1200 s^{-1} with Bay K 8644; k_2 of about 250 s^{-1} to about 1900 s^{-1}). This reflects an accelerated microscopic activation of the Ca^{2+} channels in accordance with the acceleration of the macrosopic activation by Bay K 8644 as shown in our experiments.

The most substantial lack in understanding of the effects of Bay K 8644 concerns the inactivation of whole-cell currents through Ca^{2+} channels. Different explanations have been proposed to interpret the effects of Bay K 8644 on the inactivation of macroscopic currents. The acceleration of inactivation has been supposed to be an inherent Ca^{2+} antagonistic effect of Bay K 8644 (Hess *et al.* 1984). Sanguinetti *et al.* (1986) reconstructed the inactivation of macroscopic Ca^{2+} channel currents using a C–O–I-model (I described the inactivated, non-available state of the channel). They considered a decrease in the rate coefficient from state O back to C to be the only effect of Bay K 8644. We could show that changes in the time course and voltage dependence of inactivation could be explained by a current-dependent mechanism, as already described for various tissues (see Eckert & Chad, 1984, for a review), including cardiac cells (Nilius & Hencek, 1983; Kass & Sanguinetti, 1984; Mentrard *et al.* 1984; Bechem & Pott, 1985; Lee *et al.* 1985; Nilius & Benndorf, 1986). The main objection of Sanguinetti *et al.* (1986) against a contribution of a current-dependent inactivation of Ca^{2+} channel currents was the finding that Bay K 8644 accelerated the inactivation even if the peak current was smaller than under control conditions. However, as shown by the currents calculated with the Standen–Stanfield model, speeding up of activation alone without an increase in the magnitude of the currents can cause an acceleration of inactivation (see Fig. 9).

The main shortcomings of the Sanguinetti–Krafte–Kass model are the prediction of an always monoexponential decay of the Ca^{2+}-channel current, not changing its time constant by depolarizing pre-pulses, and a prolongation of the time to peak by Bay K 8644, opposite to our findings. The decay of the currents calculated by the Standen–Stanfield model can be fitted with high correlation coefficients (> 0.999) by two exponentials including a pedestal. It is, however, worth mentioning that the mechanism of inactivation does not consist of two first-order reactions which could be deduced from the approximation. The model of current-dependent inactivation alone yields an approximately biexponential decay.

The leftward shift of the steady-state inactivation is a consequence of the shift of activation toward hyperpolarizing potentials as in the model of Sanguinetti *et al.* (1986). However, the model of a current-dependent inactivation predicts sufficiently well the slowing of the inactivation due to increased depolarizing pre-pulse potentials which cannot be explained by the proposals given in the paper by Sanguinetti *et al.* (1986).

Because many parameters of the model are not directly measurable they were

chosen to match the experimental data. However, independently of the actual values of the parameters, the Standen–Stanfield model qualitatively predicts an acceleration of the inactivation in the presence of Bay K 8644. This is due to the increased and fast activated currents. Also, other phenomena like the leftward shift of the steady-state inactivation, its depressed pedestal, as well as the pre-pulse-dependent deceleration of the inactivation can be qualitatively predicted by a current-dependent inactivation.

It should be pointed out that only one set of parameters for the current-dependent inactivation can reproduce all the experimental data of both the controls and the currents modified by Bay K 8644 application.

It can be concluded from our findings that changes in the kinetics of the activation of single Ca^{2+} channels induced by the Ca^{2+} agonistic compound Bay K 8644 determine the apparent inactivation of macroscopic currents by a current-dependent mechanism.

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