J. Physiol. (1986), **379**, *pp.* 495–514
With 13 text-figures
Printed in Great Britain

DUAL EFFECTS OF DIHYDROPYRIDINES ON WHOLE CELL AND UNITARY CALCIUM CURRENTS IN SINGLE VENTRICULAR CELLS OF GUINEA-PIG

BY A. M. BROWN*, D. L. KUNZE* AND A. YATANI*

From the Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77550, U.S.A.

(*Received 17 July 1985*)

SUMMARY

1. We studied the effects of dihydropyridine Ca channel ligands (DHPs), mainly nitrendipine and Bay K8644, on whole cell and single channel Ca currents on single myocytes isolated from the adult guinea-pig ventricle.

2. Nitrendipine had dual effects, stimulatory or inhibitory, depending upon the membrane potential. At low frequencies (less than 0·03 Hz) and negative holding potentials (−90 mV or more), nitrendipine increased the Ca currents in a dose-dependent manner. The dose–response curve was best fitted by a Langmuir adsorption isotherm model which was the sum of two independent one-to-one drug–receptor sites with median effective doses (ED_{50}s) of $1{\cdot}0 \times 10^{-9}$ M and $1{\cdot}4 \times 10^{-6}$ M respectively.

3. When the membrane potential was held at −30 mV or less, nitrendipine inhibited the Ca currents, also in a dose-dependent manner. The dose–response curve was fitted by a single binding site model having a median inhibitor concentration (IC_{50}) of $1{\cdot}5 \times 10^{-9}$ M. At holding potentials between −70 and −40 mV, nitrendipine produced mixed effects on Ca currents; an increase occurred initially and this was followed by a decrease.

4. When rundown was excluded, Bay K8644 showed only stimulatory effects on the Ca currents between holding potentials of −120 and −30 mV. When the test potential was zero or +10 mV the Ca currents reached peak values and the dose–response curve was best fitted by a single binding site model having an ED_{50} of 3×10^{-8} M. When the effects were measured at negative test potentials of −30 to −10 mV, the curve was best fitted by a two-site model with ED_{50}s of 3×10^{-9} and 9×10^{-7} M.

5. At the single Ca channel level the stimulatory effect of nitrendipine was due to an increased probability that a Ca channel which had opened once would reopen, a reduction in records without activity and an increase in the mean open time. There were no changes in unit conductance. Inhibitory effects were due to a large increase in nulls. At lower concentrations the main effect of Bay K8644 was an increase in

* Present address, to which reprint requests should be sent: Department of Physiology and Molecular Biophysics, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, U.S.A.

the probability of opening. At doses above 10^{-6} M, a pronounced increase in the open time was observed.

6. The effects we observed are attributed to at least two sites for DHP related to Ca channels; one with high affinity and one with a lower affinity. The low affinity site mediates a stimulatory effect due to greatly prolonged openings. The high affinity site mediates two effects, stimulatory or inhibitory, depending upon the membrane potential. An alternative possibility is the existence of two distinct high affinity sites.

INTRODUCTION

The dihydropyridines (DHPs) are a class of organic compounds numerous members of which are important calcium antagonists (Triggle & Janis, 1984; Schwartz & Triggle, 1984; Schwartz, Grupp, Grupp, Williams & Vaghy, 1984). Several DHPs, e.g. nifedipine, nimodipine and nitrendipine, inhibit Ca currents (Bean, 1984; Sanguinetti & Kass, 1984*a*; Brown, Kunze & Yatani, 1984*a*; Hess, Lansman & Tsien, 1984) and two DHPs, Bay K8664 and CGP 28392, stimulate Ca currents (Brown *et al.* 1984*a*; Hess *et al.* 1984; Kokubun & Reuter, 1984; Ochi, Hino & Niimi, 1984). In so far as work on cardiac sarcolemmal (s.l.) Ca channels is concerned nitrendipine has been a favourite DHP and has been used extensively in pharmacological and binding experiments. It has been shown to bind with high affinity (apparent dissociation constant, K_D about 0·1 nM) to cardiac s.l. and membrane preparations from other excitable tissues such as brain, smooth muscle and the T-tubules of skeletal muscle (Bellemann, Ferry, Lübbecke & Glossmann, 1981; Glossmann, Ferry, Lübbecke, Meves & Hofman, 1982; Gould, Murphy & Snyder, 1982; Triggle & Janis, 1984; Schwartz *et al.* 1984; Schilling, Hamilton, Yatani, Brush & Brown, 1985). There is a reasonable correlation between the K_D and the median inhibitory concentration (IC_{50}) for Ca channel block in smooth muscle but the correlation was found to be less satisfactory in cardiac muscle (Lee & Tsien, 1983). These authors raised the possibility of state-dependent binding and Sanguinetti & Kass (1984*a*) and Bean (1984) subsequently demonstrated that the IC_{50} is much smaller when the cardiac membrane is held at depolarized potentials. However, the experimental situation is more complicated since it has been shown that nitrendipine has stimulatory as well as inhibitory effects on Ca currents (Brown *et al.* 1984*a*) and cardiac contractility (Thomas, Gross & Schramm, 1984; Schwartz *et al.* 1984). A possible explanation for these dual effects is that the DHPs are modulators of Ca channel function (Schramm, Thomas, Towart & Franckowiak, 1983) and a specific single-site model based on DHP modulation of gating among different channel modes has been proposed (Hess *et al.* 1984). This model does not deal with the possible contribution of a second DHP binding site with low affinity and large capacity which has been found in cardiac s.l. and other membrane preparations (Belleman *et al.* 1981; Marsh, Loh, Lachance, Barry & Smith, 1983; Schwartz *et al.* 1984; Vaghy, Grupp, Grupp, Balwierczak, Williams & Schwartz, 1984). An effect on Ca channels mediated by the low affinity site has been inferred from studies on single Ca channels (Brown, Kunze & Yatani, 1984*b*) and cardiac contractility (Marsh *et al.* 1983; Schwartz *et al.* 1984). In the present experiments we examined the effects of membrane potential on the behaviour of the high affinity site and the role played by the low affinity site in Ca

channel function. We found that the high affinity site is either stimulatory or inhibitory for Ca channels depending upon the membrane potential and that the low affinity site is stimulatory. The results are interpreted by a model in which DHPs act as agonists whose affinities and effects are voltage dependent.

METHODS

Cells, solutions and electrophysiological measurements

Single ventricular cells were isolated from adult guinea-pig hearts by enzymatic dispersion (Brown *et al.* 1984*b*). Whole cell voltage clamp currents were recorded according to Hamill, Marty, Neher, Sakmann & Sigworth (1981). The cells were placed in a small chamber (0·2 ml) which was perfused (2 ml/min) with a Tyrode solution containing (mM): $CaCl_2$, 1·8; NaCl, 135; KCl, 5·4; $MgCl_2$, 1·0; glucose, 10; HEPES, 5 (pH 7·4). In some experiments 5×10^{-5} M-tetrodotoxin was added to the Tyrode solution. For more complete separation, Ca currents were isolated by suppressing Na and K currents. Na- and K-free external solutions contained (mM): $CaCl_2$, 5; tetramethylammonium chloride, 135; $MgCl_2$, 2; glucose, 10; 4-aminopyridine, 5; HEPES, 5 (pH 7·4). Patch pipettes contained (mM): K aspartate, 130; EGTA, 5; adenosine 5′-triphosphate (ATP), 2; HEPES, 5 (pH 7·3) and had resistances ranging from 0·7 to 2·0 MΩ. For Na- and K-free solution experiments, Cs aspartate was substituted for K aspartate. Liquid junction potentials between these solutions and normal Tyrode solution were 10–15 mV. Correction of the membrane potentials was made as described by Hagiwara & Ohmori (1982). For cell-attached single channel recording the patch pipette contained (mM): $CaCl_2$, 40; tetraethylammonium chloride, 20; KCl, 5·5; glucose 20; 4-aminopyridine, 5; Tris-HCl, 55 (pH 7·4) or $BaCl_2$, 96; HEPES, 10 (pH 7·4). The Tyrode solution in these experiments contained 2·0 mM-$CaCl_2$. The patch electrodes were coated with Sylgard 182 and fire polished. The currents were digitized at 10–20 kHz and filtered at 5 or 10 kHz. The single channel currents were analysed using methods described by Lux & Brown (1984). Nitrendipine and Bay K8664 were dissolved in ethanol or polyethylene glycol to 10 mM and then serially diluted in the bathing solutions; they were applied by bath perfusion. The solvent concentration was less than 0·1 %, a concentration which by itself had no effect on the Ca currents. The drugs were obtained as gifts from Miles Laboratories (New Haven, CT, U.S.A.). The batch number for nitrendipine was 828288 and for Bay K8644 was 957020. All experiments were performed at room temperature (20–22 °C).

Fitting the dose–response and current availability–potential curves

The dose–response data were fitted to models consisting of one or two independent one-to-one drug receptor sites. The computer program used a non-linear least-squares Marquardt method (Bevington, 1969) to estimate the free parameters which were B_{max}s (B_{max} is maximum number of binding sites) and K_Ds. The 'F' test (Munson & Robard, 1980) was used to differentiate between single- and two-site models. The inactivation curves were fitted using the standard h_∞–(V) relation based upon a Boltzmann distribution for the inactivating particle. Parameter estimation was approached in the same way as described above.

Stimulation of Ca current kinetics

Estimates of the rate constant parameters used for the scheme in Fig. 13 were obtained from whole cell current relaxations following voltage step perturbations and from single channel mean open times. The whole cell currents were then generated using a numerical modal expansion technique (Colquhoun & Hawkes, 1982).

RESULTS

Whole cell Ca currents

In Na- and K-free solutions the input resistance of adult guinea-pig ventricular myocytes was greater than 10^{10} Ω and could be as large as 10^{11} Ω (Matsuda & Noma, 1984). Under these conditions the inward current required to hyperpolarize the

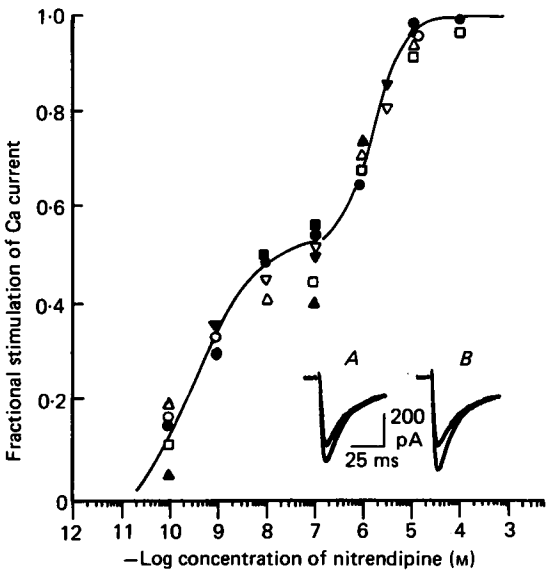

Fig. 1. Dose–response relation of the stimulatory effect of nitrendipine on Ca currents of adult guinea-pig ventricular cells as measured in eight cells. Each symbol represents data from a different cell. Open symbols show the results in normal Tyrode solution containing 5×10^{-5} M tetrodotoxin. Filled symbols show those results obtained in Na- and K-free solutions. The holding potential was between −90 and −120 mV and the test pulse, 50 ms in duration, was applied to 0 or +10 mV every 30 or 45 s. Changes of Ca current 3 min after drug application were plotted as a fraction of the maximal increase above the Ca current in the control drug-free solution. Thus the effect was proportional to occupancy with the maximal effect occurring at maximum occupancy. The maximum response was equal to 1·4 times the control. The Ca current was measured from the zero current level. In Na- and K-free solutions, the holding current level represented the zero current level, as leak current was negligibly small compared to the Ca current amplitude. The smooth curve is best least-squares fitted to an occupancy model having two independent binding sites. The model is based on one-to-one stoichiometry at each binding site and is given by

$$Y = \frac{\text{Effect}_{\max_1}}{1+\text{ED}_{50,1}/X} + \frac{\text{Effect}_{\max_2}}{1+\text{ED}_{50,2}/X}$$

where X is the free-drug concentration, Y is the fractional effect and $\text{Effect}_{\max}$s are the fractions of the combined maximum effect. The best least-square fit gave $\text{ED}_{50,1} = 1{\cdot}0 \times 10^{-9}$ M, $\text{ED}_{50,2} = 1{\cdot}4 \times 10^{-6}$ M, $\text{Effect}_{\max_1} = 50\,\%$ of the total effect. Inset, activation of Ca current by 10^{-6} M (*a*) and 10^{-5} M (*b*) nitrendipine. Control currents and currents 3 min after addition of each concentration of nitrendipine were superimposed. In this experiment, tetrodotoxin-containing normal Tyrode solution was used. The holding potential was −90 mV and the holding current represented the zero current level. The test potential was +10 mV and 50 ms pulses were applied every 30 s.

membrane to −90 mV was less than 10 pA, which was below the noise level in the current record. Hence the zero current level was used as the reference current. In Tyrode solution the zero current potential was usually −90 mV and the current–voltage relation around this potential has been described previously (Brown *et al.* 1984*b*). When the holding potential was −50 mV, the reference current was outward through the inwardly rectifying K channel. This was much smaller when the pipette solution contained Cs rather than K. This current was negligible for depolarizing steps

more positive than −30 mV and we therefore used zero current rather than the holding current as the reference level (Brown *et al.* 1984*b*).

When the holding potential was between −90 and −120 mV and when the test pulse was 0 or +10 mV, 50 ms in duration and delivered at intervals of 30 or 45 s, nitrendipine increased the Ca currents in guinea-pig ventricular myocytes by as much as 40 % (Fig. 1, inset). The effect was present over a dose range that extended from

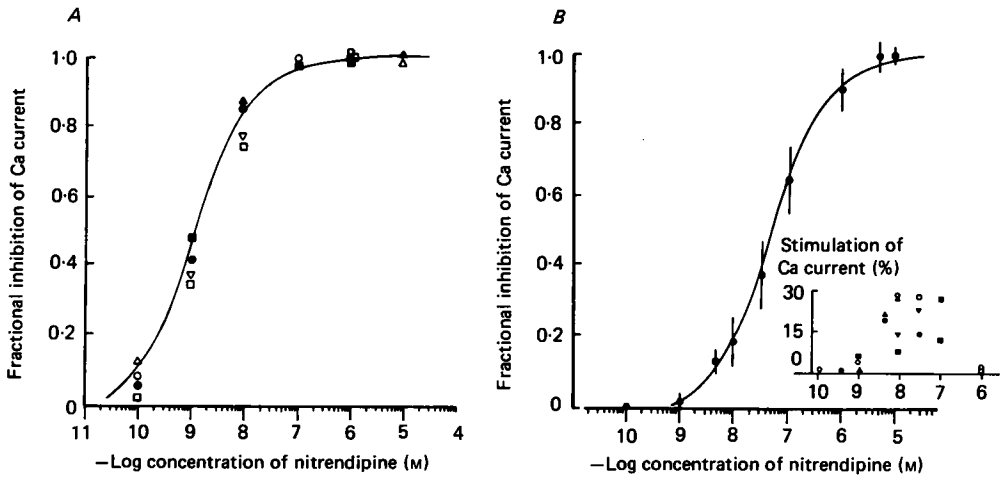

Fig. 2. Dose–response relation of the inhibitory effect (*A*) and the dual stimulatory and inhibitory effects (*B*) of nitrendipine on the Ca current. Data in *A* obtained from six cells as indicated by the different symbols. The duration of exposure to the drug was 5–8 min. The holding potential was −30 mV and a test pulse of 50 ms duration was applied to 0 or +10 mV every 10 s. Open symbols show the results in Na- and K-free solution and filled symbols show those in normal Tyrode solution, with or without tetrodotoxin. At −30 mV, the proportion of activatable Ca channels was about 0·3. The curve is the least-squares fit to a one-site model with $IC_{50} = 1{\cdot}5 \times 10^{-9}$ M. In *B* the holding potential was between −40 and −50 mV. A test pulse of 100 ms duration was applied to 0 or +10 mV every 10 s. These results were obtained in normal Tyrode solution with or without tetrodotoxin. The inhibitory effects of nitrendipine represent the mean ± S.E. of mean for eight to ten cells. The amplitude of the Ca current 5–8 min after application of the drug was plotted as a fraction of the Ca current measured in the control. The curve is the least-squares fit to a one-site model with $IC_{50} = 5 \times 10^{-8}$ M. The inset shows the stimulatory effects of nitrendipine in twelve cells 3 min after exposure to the drug. The effects were plotted as the percentage increase of the Ca current relative to the Ca current in the control.

10^{-10} to 10^{-4} M. The dose–response curve had a clear inflexion and was best fitted by the sum of two independent dose–response curves each with one-to-one occupancy (Fig. 1). This was a significantly better fit statistically than a single-site model provided ($P < 0{\cdot}001$). The two median effective doses (ED_{50}s) were 1×10^{-9} and $1{\cdot}4 \times 10^{-6}$ M. These values approximate the K_Ds of $1{\cdot}3 \times 10^{-10}$ and $1{\cdot}6 \times 10^{-6}$ M for the two nitrendipine binding sites observed in isolated guinea-pig ventricular membrane preparations (Schilling *et al.* 1985). Thus a high affinity site was identified. It produced a stimulatory effect when the holding potential was about −90 mV and when there was little or no inactivation being produced by the test protocols. In addition the dose–response relation at higher concentrations provides clear evidence

for the presence of a low affinity site that is also stimulatory for Ca channels. The functional behaviour of this site is discussed below.

When the membrane potential was held at −30 mV or less nitrendipine inhibited the Ca currents and there was no evidence of any stimulatory effect. At these holding potentials the proportion of activatable Ca channels, denoted by h_∞, was reduced to about 0·3. The inhibitory dose–response curve increased continuously and was best fitted by a single binding site model having an IC_{50} of $1{\cdot}5 \times 10^{-9}$ M (Fig. 2*A*). This is about three times the value obtained by Bean (1984) but the experimental preparations and conditions were different (Bean's experiments were on dog myocytes at holding potentials of −20 mV).

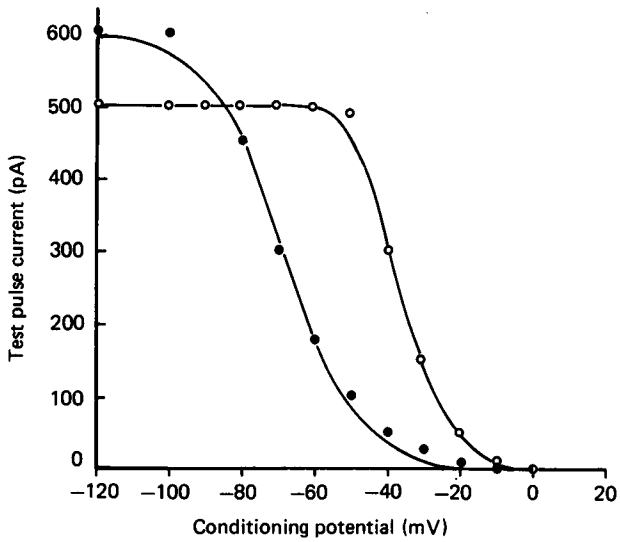

Fig. 3. Effects of 10^{-8} M-nitrendipine on the voltage dependence of Ca current availability. The test pulse current measured from zero current level was plotted *vs.* the conditioning potential. The duration of the latter was 2 s for the control and 3 min in nitrendipine. In the control longer pre-pulses had no further effect. The holding potential was −80 mV and a test pulse of 50 ms duration was applied to 0 mV. Continuous lines are drawn according to $I_{max} = [1+\exp(V-V_{\frac{1}{2}})/k]^{-1}$, where V is the conditioning potential, $V_{\frac{1}{2}}$ is the conditioning potential giving 50 % of the maximum test current (I_{max}) and k is the slope factor. For the control (open symbols) the parameters for the fitted data were $V_{\frac{1}{2}} = -35$ mV, $k = 6{\cdot}3$; in nitrendipine (filled symbols), $V_{\frac{1}{2}} = -68$ mV, $k = 9{\cdot}4$. In this experiment, tetrodotoxin-containing Tyrode solution was used. Similar effects of nitrendipine on the curve showing the voltage dependence of Ca current availability were obtained in the Na- and K-free solution.

Using holding potentials that were between −70 and −40 mV the effects for the pulse protocols were mixed. Following exposure to the drug, the first effect produced by the test pulse was an increase in current. Between 5 and 8 min later, the current produced by the test pulse was decreased when compared to the control, i.e. an inhibitory effect was present. It became constant at these times. Under these circumstances the dose–response curves were plotted as shown in Fig. 2*B*. The initial stimulatory effects became less obvious when higher concentrations of nitrendipine

were used. The dual stimulatory and inhibitory effects of nitrendipine are well shown in a plot of the number of Ca channels available for activation when the holding potential was varied over a wide range (Fig. 3). The results show that the unnormalized control (open symbols) and drug (filled symbols) curves cross each other. Clearly, the sign of the drug's effects was voltage dependent, stimulation occurring at hyperpolarized holding potentials and inhibition at depolarized holding potentials. When dual effects were observed, the onset of the inhibitory effects occurred with half-times of 3–4 min whereas the stimulatory effects occurred about five times more quickly (Fig. 4). Under these conditions recovery times were longer but this may be related to the fact that the drugs contaminate the bath chambers and the perfusion tubing. At the high doses used the time of onset of drug action was too fast to be measured using the pulse protocols employed in our experiments. At these doses recovery was often incomplete.

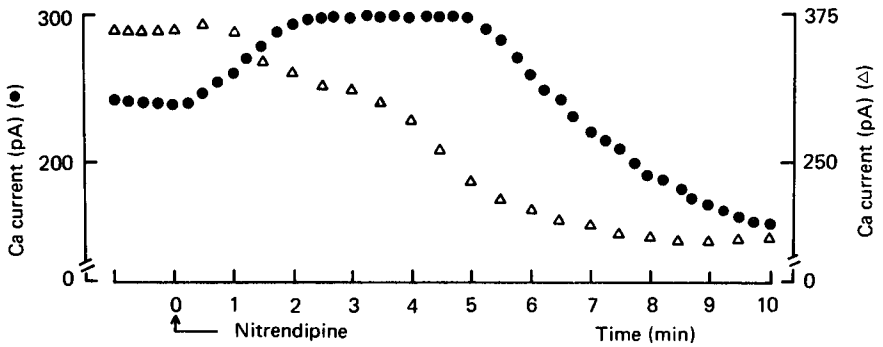

Fig. 4. The time course of changes of Ca current induced by nitrendipine. Results are from two different cells, In one, test pulses of 100 ms duration (using 3×10^{-8} M-nitrendipine) (circles) were applied to 0 mV from a holding potential of −50 mV every 15 s. In the other, pulses of 1000 ms duration (using 10^{-7} M-nitrendipine) (triangles) were applied to 0 mV from a holding potential of −40 to +10 mV every 30 s. The control peak currents were larger in this cell. Tetrodotoxin-containing Tyrode solution was used in both experiments. The Ca current was measured from zero current level.

We also examined the recovery from inactivation in the presence of nitrendipine. For short test pulses, the recovery in the control could be fitted by a single exponential function and in the presence of the drug a prolongation of recovery occurred. For a 1 s test pulse the recovery process was best fitted by the sum of two exponentials the larger of which was about three times greater after exposure to the drug (Fig. 5). This recovery was voltage dependent and was faster at hyperpolarized potentials of −80 mV.

While the effects of nitrendipine depended upon the holding potential the effects of Bay K8644 were clearly stimulatory at holding potentials between −120 and −30 mV. To reach this conclusion it was important that Ca current rundown be evaluated. Rundown may be expressed as an absolute reduction in the current–voltage curve but it may also appear as a shift of the availability curves (Fig. 3) to hyperpolarized potentials as has been reported for Na channels (Fernandez, Fox & Krasne, 1984; Kunze, Lacerda, Wilson & Brown, 1985). Cells showing either

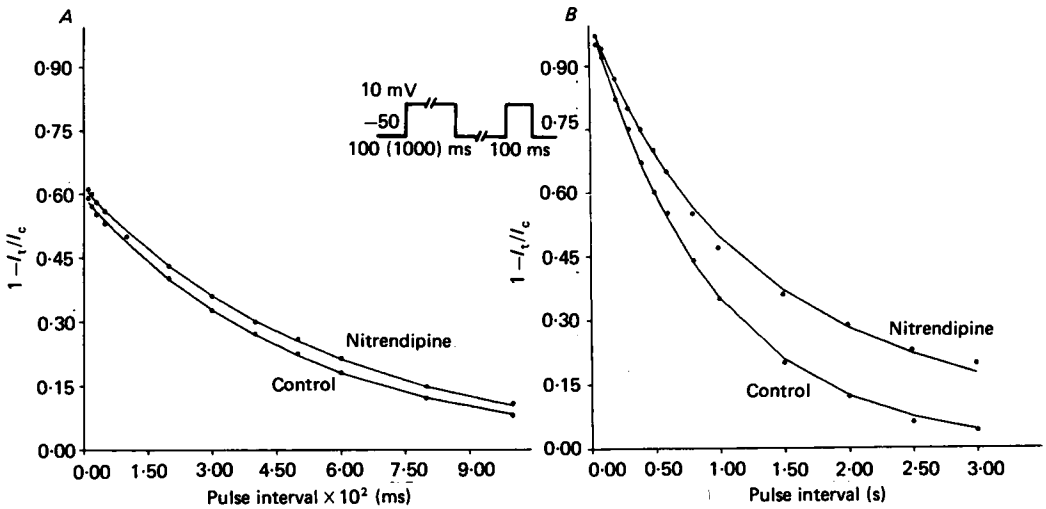

Fig. 5. Effect of nitrendipine (10^{-8} M) on Ca current repriming as studied by a double-pulse procedure (see inset). The Ca current during the test pulse (I_t) was normalized by the Ca current during the immediately preceding pre-pulse (I_c). $1-I_t/I_c$ was plotted as a function of the pulse interval. The duration of the pre-pulse was 100 ms in *A* and the curves are drawn according to $1-\exp(t/\tau)$, where τ is the time constant, with $\tau = 504$ ms for the control and 568 ms for nitrendipine. In *B*, the duration of the pre-pulse was 1000 ms. The curve through the control points gave $\tau = 1061$ ms. The curve through the nitrendipine points is drawn according to $1-A_1 \exp(t/\tau_1)-A_2 \exp(t/\tau_2)$, where A_1 and A_2 are the amplitudes of the first and second components of the fit, τ_1 and τ_2 are the time constants of the first and second components and t is time. The parameter values are: $\tau_1 = 825$ ms, $A_1 = 0{\cdot}5$; $\tau_2 = 2680$ ms, $A_2 = 0{\cdot}5$.

phenomenon were excluded from our analysis. The dose–response curve was measured using test potentials of 0 or +10 mV where the peak current was largest. The curve increased continuously and at a test potential of +10 or 0 mV was fitted by a single-site binding model having an ED_{50} of 3×10^{-8} M (Fig. 6). The stimulatory effects of Bay K8644 were reduced by prior exposure to nitrendipine for 5–8 min. The dose–response curves were shifted in a parallel and dose-dependent manner indicating that the effect was competitive (Fig. 7). The calculated K_I (concentration reducing current by one-half) from a Schild plot having a slope of 1 was 7×10^{-9} M. A two-site model did not significantly improve the fit ($P < 0{\cdot}05$).

We also found that the Bay K8644 effects at the lower test potentials of −30 or −10 mV revealed evidence of dose–response relations that extended over five log units (Fig. 8). At concentrations greater than 5×10^{-7} M this produced a 10 mV shift in the hyperpolarizing direction of the nadir of the current–voltage curve. Under these conditions the dose–response curve was fitted by the sum of two independent binding sites each with one-to-one occupancy and the ED_{50}s were 3×10^{-9} and 9×10^{-7} M ($P < 0{\cdot}001$). At a test potential of −30 mV the absolute increases produced by Bay K8644 could be as much as six times greater than the control values. At 0 or +10 mV the current was three times greater than the control level. This was about ten times greater than the stimulatory effect produced by nitrendipine. We did not find an inhibitory effect at depolarized holding potentials up to −30 mV. This is consistent

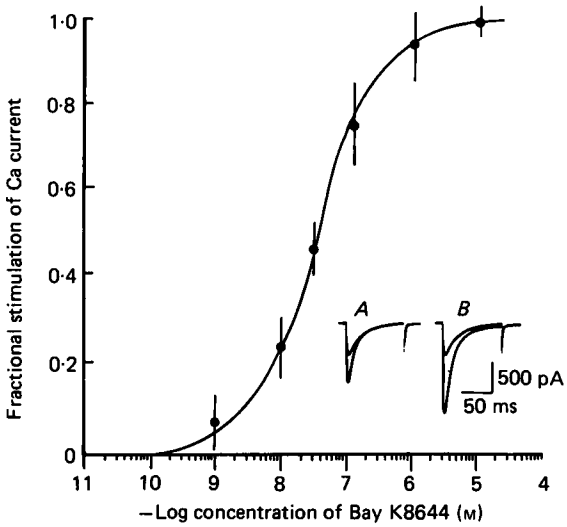

Fig. 6. Dose–response relation of the effects of Bay K8644 on the Ca current. Points represent the mean ± S.D. for four to eight cells. Data were obtained in tetrodotoxin-containing Tyrode or Na- and K-free solutions. The Ca current was plotted 5 min after the drug application as the fractional increase above the control peak Ca current. The increase was to three times the control value. The holding potential was between −120 and −40 mV. The Ca current was measured from zero current level. The test pulse to 0 or +10 mV, 100 ms in duration, was applied every 10 s. There was no voltage-dependent decrease of the Ca current by Bay K8644. The curve is the best least-squares fit to a one-site model with an ED_{50} of 3×10^{-8} M. The inset figure shows activation of the Ca current by 10^{-7} M (*A*) and 10^{-5} M (*B*) Bay K8644. The control current in Na- and K-free solution and 3 min after addition of each concentration of Bay K8644 were superimposed. The holding potential was −90 mV and the holding current was the zero current level. The test pulse was applied to +10 mV.

with our observation that the curve relating activatability to holding potential did not cross the control curve obtained in the absence of drug (Fig. 9). Rather, the normalized activatability curve overlaid the curve obtained in the presence of Bay K8644. At potentials more depolarized than −30 mV the results were variable.

At concentrations less than 10^{-7} M the increased whole cell Ca currents produced by test pulses to 0 mV in both nitrendipine and Bay K8644 had similar wave forms. Compared to the control wave form, the peak currents were exaggerated (more so for Bay K8644 than for nitrendipine, as noted earlier) while the currents at the end of the 100 ms pulse were much less increased above control levels (Figs. 1 and 6). The decreased currents produced by nitrendipine at concentrations below 10^{-7} M were changed in a similar way and when scaled the control and drug-decreased currents approximately overlaid each other (Fig. 10). At higher concentrations the rate of inactivation appeared to increase.

Single channel currents

The effects of nitrendipine in concentrations of 10^{-7} and 10^{-6} M on single channel behaviour are shown in Fig. 11. The membrane patch holding potential, V_H, was equal

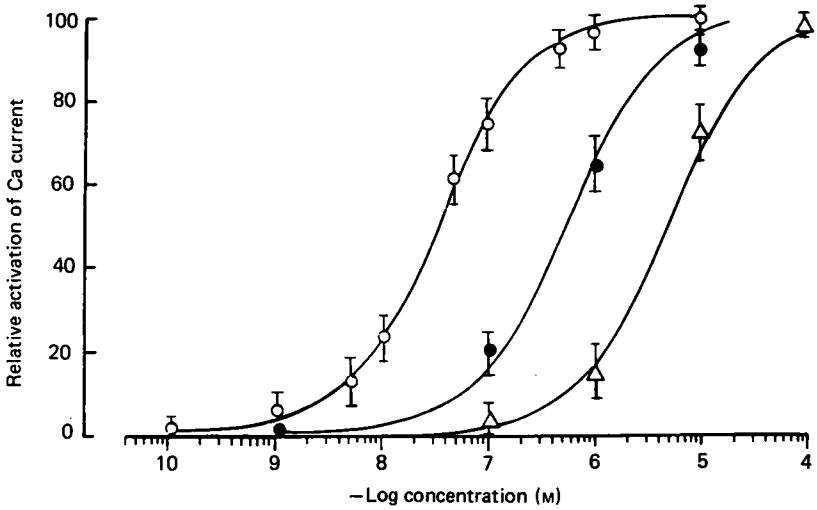

Fig. 7. Dose-dependence of Bay K8644 on peak Ca current in the absence (open circles) and presence of nitrendipine (filled circles, 10^{-7} M; triangles, 10^{-6} M). Nitrendipine was applied for 5–8 min before the Bay K8644 dose–response effects were examined with nitrendipine present. The holding potential was −50 mV and the test pulse of 100 ms duration was applied to 0 or +10 mV every 10 s. Each point is the mean value of data from at least six cells. Note that nitrendipine causes a dose-dependent shift to the right. The continuous lines are the theoretical curves for one-to-one drug–receptor interactions. The ED_{50} for Bay K8644 was shifted by nitrendipine at the two different concentrations from 3×10^{-8} M to 7×10^{-7} M and 4×10^{-6} M.

to V_R −30 mV, where V_R is the resting potential. The test potential was V_H +60 mV. The activity per sample or record as given by the proportion of open time varied from zero (seventeen nulls or failures out of seventy samples) to 0·45 during the control as the records of activity per sample shows (Fig. 11*C*). At the cell's resting potential, the test potentials normally produced slightly less activity than that observed in the sixty control trials shown in Fig. 11*C*. The effects of changing the holding potential were not associated with any change in the unitary current amplitudes. After application of the drug to the bath there was an increase in activity. The increase was gradual, possibly as a result of slow mixing. The frequency of nulls dropped and peak activities rose to values as great as 80 % of a single record's duration. The unitary amplitude was unchanged at both concentrations. The mean open time and the number of openings per record were increased to approximately double the control values at the highest concentration of the drug. At 10^{-6} M a specific enhancement of events with very prolonged open durations (about ten times the control) as described by Hess *et al.* (1984) was not observed.

The effects of changing the holding potential on the action of nitrendipine were also examined. When the membrane was depolarized by removing the 30 mV hyperpolarizing potential it required some twenty-six samples before any effects were observed. There was then an increase in nulls. However, those samples in which activity subsequently recurred had a similar behaviour to the samples observed at hyperpolarized holding potentials, i.e. an increased probability of opening, p_o, and

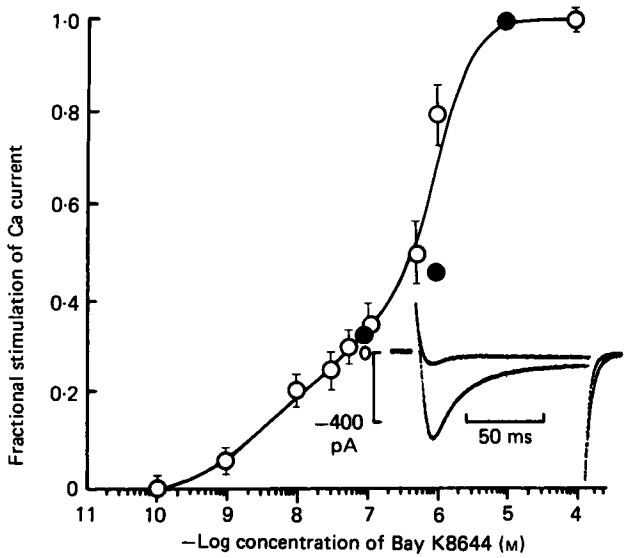

Fig. 8. Dose–response relation of the effects of Bay K8644 on the Ca current at more negative test potentials than were used in Fig. 6. Points represent the mean value obtained from six to eight cells. The Ca current 5 min after the drug application was plotted as the fractional increase above the control Ca current. At high concentrations, the current–voltage curve shifted to negative potentials and the relative increase of the Ca current by Bay K8644 was larger near the threshold potential. The results shown in this Figure give the relative values at test potentials between −30 and −10 mV. The maximum increase of Ca current by the drug was 6- to 10-fold. The test pulse of 100 ms duration was applied every 10 s. The solution used was either normal Tyrode solution containing tetrodotoxin or the Na- and K-free solution. The smooth curve is the best least-squares fit to an occupancy model having two independent binding sites with $ED_{50,1} = 0{\cdot}3 \times 10^{-8}$ M, $ED_{50,2} = 0{\cdot}9 \times 10^{-6}$ M. The filled symbols are the fractional increases of the peak values measured from summed single channel current records of 70–170 records at each concentration during a depolarizing test potential from a holding potential equal to the cell's resting potential. The resting potential value may be assumed to be −80 mV in 2 mM-Ca Tyrode solution. The inset figure shows the activation of the Ca current by 10^{-5} M-Bay K8644. The control current and the current 5 min after addition of the drug were superimposed. The Na- and K-free solution was used. The holding potential was −80 mV and the test potential was −30 mV. A 100 ms duration pulse was applied every 10 s.

an increase in mean open time. In fact records 265–273 showed a substantially increased proportion of open time. After record 300 activity was greatly reduced but was restored when the membrane was again hyperpolarized (not shown).

Bay K8644 had effects of the type previously reported (Brown *et al.* 1984*b*). At concentrations of 10^{-7} and 10^{-6} M an increase in p_o was the main effect. This was due to an increase in the number of openings per record. At doses above 10^{-6} M the effects on open time became pronounced (Fig. 12) and were similar to the effects on open times reported by Hess *et al.* (1984), Kokubun & Reuter (1984) and Ochi *et al.* (1984). At a Bay K8644 concentration of 10^{-5} M, open time distributions were fitted by two exponential functions, one having a mean open time equivalent to that of the control, and that of the other being ten to twenty times greater. At lower concentrations of

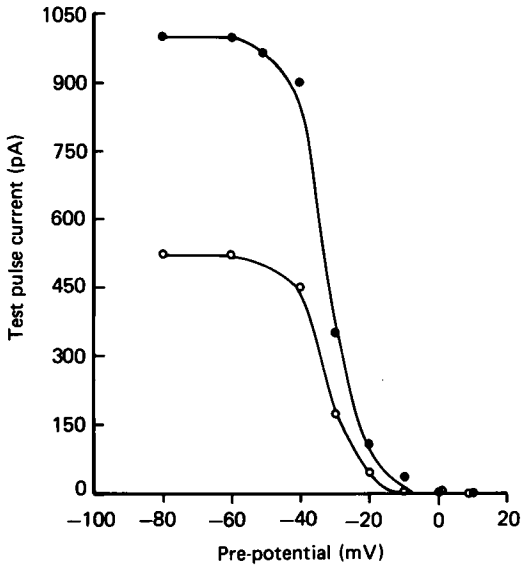

Fig. 9. The effects of Bay K8644 (10^{-6} M) on the steady-state inactivation of Ca current. Tetrodotoxin-containing Tyrode solution was used. The test pulse current measured from zero current level was plotted *vs.* the pre-potential. The cell was clamped to various potentials for 3 min, long enough to reach a steady state. The holding potential was −80 mV and a test pulse of 100 ms duration was applied to 0 mV. The continuous lines are drawn according to $I_{max} = [1+\exp(V-V_{\frac{1}{2}})/k]^{-1}$. For the control (open symbols), $I_{max} = 520$ pA, $V_{\frac{1}{2}} = -32{\cdot}6$ mV, $k = 4{\cdot}30$. With Bay K8644 (filled symbols), $I_{max} = 1007$ pA, $V_{\frac{1}{2}} = -32{\cdot}9$ mV, $k = 5{\cdot}46$. Similar results were obtained in Na- and K-free solution. In neither case was a significant change in $V_{\frac{1}{2}}$ observed in six cells.

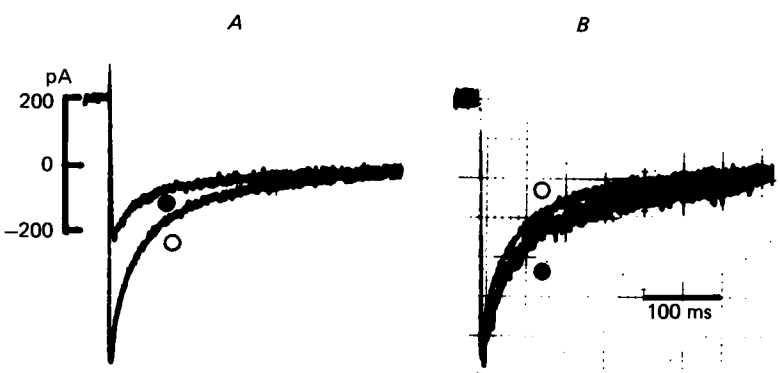

Fig. 10. Effect of nitrendipine (10^{-7} M) on Ca current wave forms. *A*, current recorded on depolarization from −50 to 0 mV in control (open circle) and 10 min after nitrendipine (filled circle). *B*, the control current trace (open circle) and the trace nitrendipine (filled circle) scaled and superimposed to compare the inactivation time course of the Ca current. The data were obtained in a tetrodotoxin-containing Tyrode solution, thus the current amplitudes were standardized from zero current level.

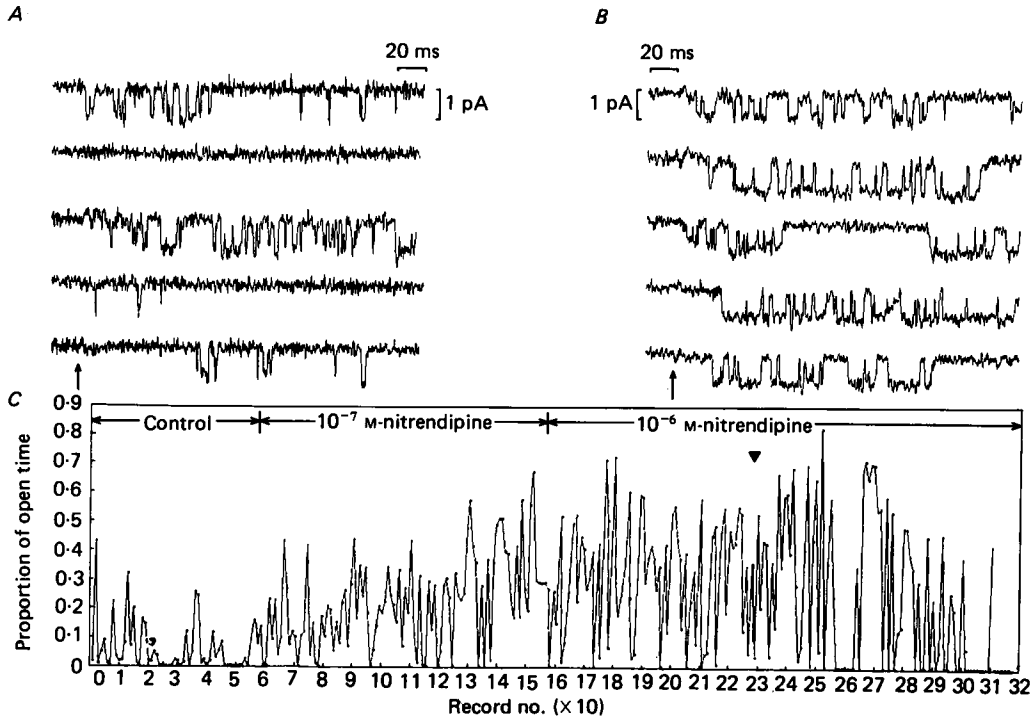

Fig. 11. Effects of nitrendipine on single channel activity in a cell-attached patch containing one channel. *A* and *B*, single channel recording in response to a 60 mV depolarizing pulse delivered at 5 s intervals. The holding potential (V_H) was 30 mV more negative than the resting potential (V_R). The pipette contained 96 mM-$BaCl_2$. The records in *A* were taken in the absence of nitrendipine. The records in *B* show the response to nitrendipine (10^{-6} M) under the same voltage conditions as in *A*. The arrow indicates the potential step. Linear components of capacitance leakage have been substracted. The records were filtered at 1 kHz. *C*, the proportion of open time in each successive record for the control and as nitrendipine was added to a concentration of 10^{-7} M and then of 10^{-6} M. In 10^{-6} M-nitrendipine the hyperpolarizing holding potential of −30 mV was removed at the symbol and the depolarizing test potential was reduced to 30 mV.

Bay K8644 longer openings were present as Fig. 12 shows but their numbers were not sufficient to provide a good fit to the open time distributions with a sum of two exponentials. The dose–response curve for the peak p_o of the summed single channel currents showed an increase at drug concentrations below 10^{-6} M and then a much larger increase at 10^{-5} M (Fig. 8). This is consistent with the finding that the average open time per opening given by the slope of the curve relating the proportion of open time during a record to the number of units during the record (Fig. 12) was only slightly increased at the lower concentrations. The main effect was an increase in the number of units per record. However, at the high concentrations the records were dominated by single openings or by several openings each of very long duration. These results fit well with the dose–response curve for whole cell currents at comparable test potentials of about −30 mV (Fig. 8) and the single channel effects are also consistent with a two binding-site model for drug action. Another point of

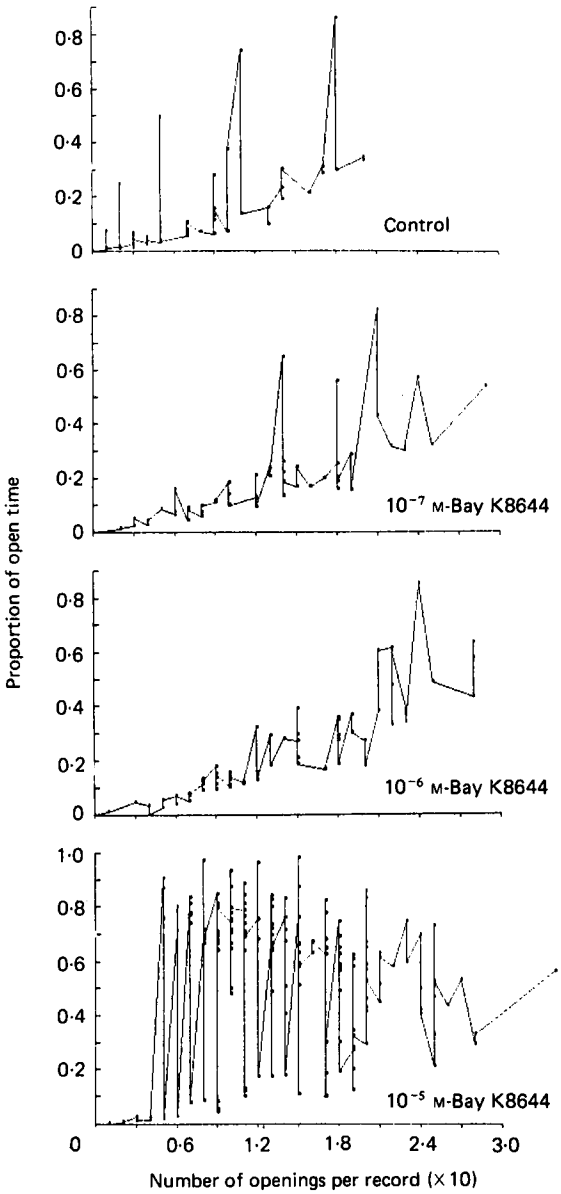

Fig. 12. The proportion of open time in each record is plotted against the number of openings in that record in the absence and presence of 10^{-7}, 10^{-6} and 10^{-5} M-Bay K8644. The slope of such a relation is indicative of the mean open time. An increase in the maximum number of openings/trace in 10^{-6} and 10^{-7} M-Bay K8644 is seen by the extension of the relation beyond that for the control. The cell-attached patch was depolarized by 40 mV from the resting potential. The pipette contained 96 mM-$BaCl_2$. In the presence of 10^{-5} M-Bay K8644 (bottom panel) long duration openings caused an increase in the proportion of open time in traces with few openings.

interest is that the long open times appeared to be less prominent when isotonic Ca was used instead of isotonic Ba. However, this result has not been examined systematically.

The effects of nifedipine and nimodipine were tested in an analogous manner to the tests used to investigate the actions of nitrendipine and Bay K8644. We found equivocal evidence for a stimulatory effect of nifedipine at hyperpolarized holding potentials (-80 mV or more). In two experiments a small transient increase was observed but in two other experiments no such increase was observed. At a holding potential of -50 mV, where the dual stimulatory and inhibitory effects of nitrendipine were apparent, nifedipine produced only an inhibitory effect. Nimodipine never showed stimulatory effects. At -50 mV holding potential the effect was also exclusively inhibitory.

DISCUSSION

The results showing a stimulatory dose–response curve for nitrendipine were interpreted to indicate that both the high and the low affinity nitrendipine binding sites in cardiac s.l. are related to Ca channels. In addition the two ED_{50}s and the two apparent equilibrium K_Ds of binding studies correspond reasonably well. The requirement for two binding sites rather than one arises from the clear inflexion of the dose–response curve and the five to six log units of concentration over which it extends. For similar reasons the present Bay K8644 results on whole cell currents at test potentials of -30 mV support the idea of high and low affinity sites as do the single channel results. The high affinity sites seem to be related exclusively to Ca channel function since these concentrations appear to have no effects on other cardiac s.l. voltage-gated channels (Yatani & Brown, 1985). Previous evidence that the low affinity sites may be pharmacologically relevant to Ca channel function has been presented (Marsh *et al.* 1983; Schwartz *et al.* 1984) and we came to similar conclusions from our observations of the effects of Bay K8644 on single Ca channel behaviour (Brown *et al.* 1984*b*). In the experiments presented here more quantitative evidence shows that the marked increase in the average open time per opening only became evident at drug concentrations of 10^{-6} M or greater (Fig. 12). The low affinity sites are not exclusively related to Ca channels, however, since we have already shown that Na channels are blocked by micromolar doses of nitrendipine (Yatani & Brown, 1985) and Hume (1985) has shown that nisoldipine reduced K currents at these concentrations as well. The low affinity sites may also involve non-excitable membrane proteins (Glossmann, 1985).

The stimulatory and inhibitory effects were attributed to a high affinity site(s) on resting and inactivated channels respectively. A comparison of the half-maximal concentrations required to produce stimulation and inhibition shows a slightly higher apparent affinity of nitrendipine for the resting as compared to the inactivated channel. This was unexpected because our model (see below) indicates a higher affinity for the inactivated state. The result may be explained by the fact that when testing for inhibitory effects it was necessary for accurate measurements of peak currents to have about 30% of the channels in the resting state. Hence the full voltage dependence of block to the inactivated state could not be expressed and the

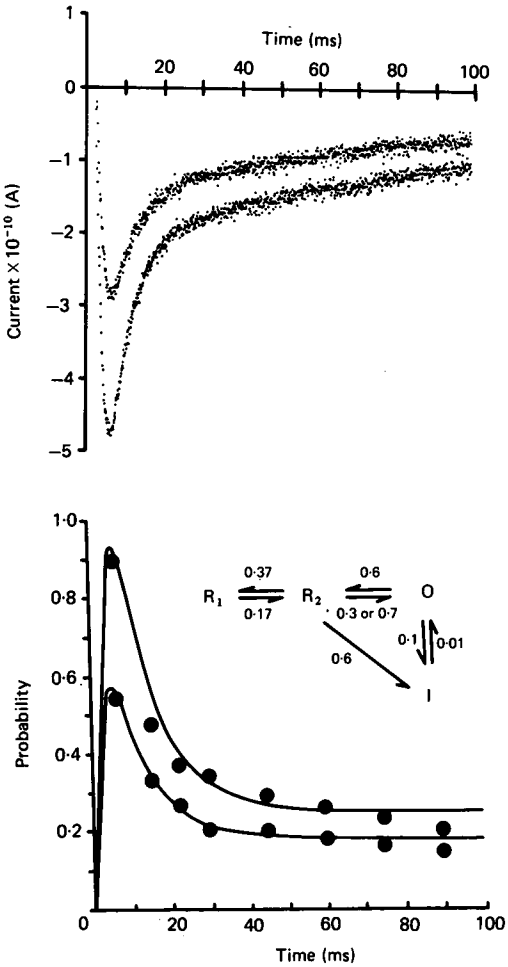

Fig. 13. State diagram of a Ca channel in the control and after nitrendipine. The upper Figure shows original current traces before and 5 min after applying 5×10^{-8} M-nitrendipine. The holding potential was −50 mV and the test potential was +10 mV. Data were obtained using Na- and K-free solution. The lower Figure shows the model that accounts for the effects of nitrendipine on the Ca current. The filled circles represent data points from the current traces in the upper Figure, normalized to peak value. The smooth curve is the fit of the model using the k_{R_2O} values of 0·7 and 0·3 ms^{-1}. R_1 and R_2 are resting states; O and I are open and inactivated states respectively. The sequence is a Markov chain in which the occupancy of a given state is probabilistic and memoryless (Brown, Lux & Wilson, 1984). Numbers given are rate constants in ms^{-1}. In this illustration a reduction in k_{R_2O} from 0·7 to 0·3 ms^{-1} can reproduce the experimental results (inhibition by nitrendipine). An equivalent increase in k_{R_2I} will have the same effect on macroscopic current. These changes will also increase the number of single channel records with nulls, which was actually observed. Other sites of drug action are described in the text. k_{IR_2} was very small, 0·01 and 0·03 ms^{-1} respectively, and was omitted from the Figure.

stimulatory effect on resting channels (demonstrated in the single channel experiments) could not be evaluated in the whole cell currents. By comparison, at a holding potential of −90 mV all the channels were in the resting state and none were in the inactivated state so that a full expression of the stimulatory effect could be produced. Following this line of reasoning, the occurrence of dual effects at a holding potential of −50 mV would represent, in part, a shift with time from a majority of channels in the resting state being occupied by drug to a majority of channels in the inactivated state being occupied by drug. This would require that the drug have a higher affinity for inactivated channels. We could find no convincing evidence that an inhibitory effect was mediated by a low affinity site and we attributed any inhibition at a holding potential of −90 mV either to a slow leakage of channels to the inactivated state or to rundown. The latter is the reduction in Ca currents with time that has been observed in many single cells including cardiac cells (Bean, 1984). In the experiments reported here the data were obtained using cells which had stable Ca currents for at least 3 min prior to experimental manipulations.

The net stimulatory response that we observed for nitrendipine on Ca currents (this paper and Brown *et al.* 1984*a*) has also been reported in studies of cardiac contractility (Schwartz *et al.* 1984; Thomas *et al.* 1984). We cannot account for the fact that it was not observed in other studies of whole cell Ca currents in which nitrendipine was used (Lee & Tsien, 1983; Bean, 1984; Sanguinetti & Kass, 1984*a*) except for the fact that the resting channel state has to be highly favoured as an initial condition. However, single channel studies (Hess *et al.* 1984; Kokubun & Reuter, 1984) have already shown that nitrendipine can have a Bay K8644 type of effect on Ca channels. In addition we did not observe any inhibitory effect of the Bay K8644 compound at holding potentials up to −30 mV such as that reported as occurring in syncytial cardiac Purkinje fibre preparations held at depolarized potentials (Sanguinetti & Kass, 1984*b*) or in studies of cardiac contractility (Schwartz *et al.* 1984). In our experiments we excluded cells that showed Ca current rundown and this might explain the difference. Some voltage dependence for the Bay K8644 effect is present as noted earlier, since the response was greatest at lower test potentials. This is a different phenomenon, however, from the inhibitory effect referred to above.

The single channel results showed that the stimulatory or inhibitory effects of DHPs at concentrations less than 10^{-6} M were not due to changes in conductance. The effects were due primarily to changes in the probability of opening. At higher concentrations, open times were prolonged markedly in the case of Bay K8644 and some increase in conductance with the latter drug may also have occurred (Brown *et al.* 1984*b*).

Our results can be explained in a qualitative way as follows. There are two classes of DHP binding sites: one with high affinity that is voltage dependent and is associated with states R_2 and I, where R_2 is a resting state and I is an inactivated state (see Fig. 13) and one with low affinity which is associated with an open state, O. Binding to I is stronger than binding to R_2 and the drug acts at both sites. The inhibitory effect produced by binding to I is sufficiently slow that it would not be detected during a 50–100 ms test pulse. However, the inhibition is revealed in recovery from inactivation curves such as those shown in Fig. 5. As noted the recovery was faster at more hyperpolarized potentials, further supporting the idea that the

effect at the high affinity site is voltage dependent. Binding to the low affinity site produces a second open state, which is greatly prolonged, as the Bay K8644 data at high concentrations require. This would change the current wave form as discussed in the next paragraph.

We have done some simulations with a simple model (Fig. 13) and have found that varying certain rate constants one at a time can exclude some possibilities and is consistent with others. Decreasing k_{R_2I} increases the opening probability by decreasing failures and increasing the probability of reopening. The time to peak current is reduced but this may be so small as not to be resolvable experimentally. This result is consistent with the stimulatory effects mediated by the high affinity site. Increases of k_{R_2O} have equivalent effects. However, changes in these two rate constants do not effect recovery from inactivation or the relief of steady-state block by hyperpolarization. For this the drug has to bind to I and recovery from drug-bound I occurs at a slower rate than k_{IO}. Decreasing k_{OR_2} can prolong the open times but the reduction necessary to account for the prolonged open times (factor of ten for Bay K8644 and factor of two for nitrendipine) does not reproduce the simple scaling of wave form seen at lower concentrations of these drugs. Hence a minimum model would seem to require effects on at least three states. We have not at this time examined the full range of effects on single channels and whole cell currents that might be produced by changing all three rate constants at one time.

At the concentrations of nitrendipine used, the stimulation shown in Fig. 11*C* was produced by increased reopening mediated by the high affinity site and increased open time mediated by the low affinity site. The effects produced by subsequent changes in membrane potential are complicated. When the membrane holding potential was depolarized the increase in nulls obtained probably reflects slowed recovery from the drug-bound inactivated state, which is favoured by the test pulses. However, recovery is clearly not limited by the rate at which drug leaves the inactivated channel because this would take minutes whereas the sequence of nulls from records 267 to 273 (Fig. 11*C*) was followed by a sequence of records showing an increase in open time per sample. It appears that at the holding potential we used, the affinity of the drug for the inactivated state is high but its potency for inhibition is low. The large number of nulls after record 300 may reflect the dispersion of recovery times for the drug-bound inactivated state. The effect was not due to rundown because hyperpolarization restored activity.

We have used a simple model to explain our results, but other models could undoubtedly be applied (Hondeghem & Katzung, 1977; Starmer, Grant & Straus, 1984; Hess *et al.* 1984). The evidence in this paper and in our previous work (Brown *et al.* 1984*b*) favours the presence of an additional low affinity site that affects Ca channel function. The dual agonist effects (stimulatory and inhibitory) could be mediated by two distinct high affinity sites having similar apparent K_Ds or by one site whose affinity is voltage dependent. The latter possibility has been selected mainly because Occam's razor was used. We conclude by noting that the DHPs we used were in the form of racemic mixtures. Perhaps the use of stereoisomers instead of the racemic mixtures will help to separate stimulatory from inhibitory effects (Hof, Püegg, Hof & Vogel, 1985; Williams *et al.* 1985) although nifedipine, which is not optically active, may have produced dual effects as well. The complicated effects

of the DHPs also presage difficulties in using them as labels for the biochemical isolation of Ca channels. Larger membrane-impermeable toxin molecules may be better candidates (Hamilton, Yatani, Hawkes, Redding & Brown, 1985).

We would like to acknowledge with pleasure our extensive discussions with Dr A. Schwartz on dihydropyridine models and the sharing of unpublished data and information. We thank Ms Lynette Morgan for her excellent secretarial work. These studies were supported by W.I.H. grants HL25145 and NS1143 and a grant from Miles Laboratory to Dr A. M. Brown, N.I.H. grant HL27116 to Dr D. L. Kunze and American Heart Association grant AHA 851159 to Dr A. Yatani.

REFERENCES

BEAN, B. P. (1984). Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 6388–6392.

BELLEMANN, P., FERRY, D., LÜBBECKE, F. & GLOSSMANN, H. (1981). [^{3}H]-Nitrendipine, a potent calcium antagonist, binds with high affinity to cardiac membranes. *Drug Research* **31**, 2064–2067.

BEVINGTON, P. R. (1969). *Data Reduction and Error Analysis for the Physical Sciences.* New York: McGraw Hill.

BROWN, A. M., KUNZE, D. L. & YATANI, A. (1984*a*). Agonist effect of a dihydropyridine Ca channel blocker on guinea-pig and rat ventricular myocytes. *Journal of Physiology* **357**, 59*P*.

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

BROWN, A. M., LUX, H. D. & WILSON, D. L. (1984). Activation and inactivation of single calcium channels in snail neurons. *Journal of General Physiology* **83**, 751–769.

COLQUHOUN, D. & HAWKES, A. G. (1982). On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philosophical Transactions of the Royal Society of London B*, **300**, 1–59.

FERNANDEZ, J. M., FOX, A. P. & KRASNE, S. (1984). Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH_3) cells. *Journal of Physiology* **356**, 565–585.

GLOSSMANN, H. (1984). Activation of chromaffin cell Ca^{2+} channels by a novel dihydropyridine. *Nature* **313**, 504–504.

GLOSSMANN, H., FERRY, D. R., LÜBBECKE, F., MEVES, R. & HOFMAN, F. (1982). Calcium channel: direct identification with radioligand binding studies. *Trends in Pharmacological Sciences* **3**, 431–437.

GOULD, R. J., MURPHY, K. M. & SNYDER, S. H. (1982). [^{3}H]-nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 3656–3660.

HAGIWARA, S. & OHMORI, H. (1982). Studies of calcium channels in rat clonal pituitary cells with patch electrode voltage clamp. *Journal of Physiology* **331**, 231–252.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch clamp technique for high resolution recordings from cells and cell free membranes. *Pflügers Archiv* **391**, 85–100.

HAMILTON, S. L., YATANI, A., HAWKES, M. J., REDDING, K., & BROWN, A. M. (1985). Atrotoxin: A new specific agonist for calcium currents in heart. *Science* **229**, 182–184.

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.

HOF, R. P., PÜEGG, U. T., HOF, A. & VOGEL, A. (1985). Stereoselectivity at the calcium channel: Opposite action of enantiomers of 1,4-dihydropyridine. *Journal of Cardiovascular Pharmacology* **7**, 689–693.

HONDEGHEM, L. M. & KATZUNG, B. G. (1977). Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochimica et biophysica acta* **472**, 373–398.

HUME, J. R. (1985). Comparative interactions of organic Ca^{++} channel antagonists with myocardial Ca^{++} and K^+ channels. *Journal of Pharmacology and Experimental Therapeutics* **234**, 134–140.

KOKUBUN, S. & REUTER, H. (1984). Dihydropyridine derivatives prolong the open state of Ca

channels in cultured cardiac cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 4824–4827.

KUNZE, D. L., LACERDA, A. E., WILSON, D. L. & BROWN, A. M. (1985). Cardiac Na currents and the inactivating, reopening and waiting properties of single cardiac Na channels. *Journal of General Physiology* **86**, 691–719.

LEE, K. S. & TSIEN, R. W. (1983). Mechanisms of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialyzed heart cells. *Nature* **302**, 790–794.

LUX, H. D. & BROWN, A. M. (1984). Patch and whole cell calcium current recorded simultaneously in snail neurons. *Journal of General Physiology* **83**, 727–750.

MARSH, J. D., LOH, E., LACHANCE, D., BARRY, W. H. & SMITH, T. W. (1983). Relationship of binding of a calcium channel blocker to inhibition of contraction in intact cultured embryonic chick ventricular cells. *Circulation Research* **53**, 539–543.

MATSUDA, H. & NOMA, A. (1984). Isolation of calcium current and its sensitivity to monovalent cations in dialyzed ventricular cells of guinea-pig. *Journal of Physiology* **357**, 553–573.

MUNSON, P. J. & ROBARD, D. (1980). Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Analytical Biochemistry* **107**, 202–239.

OCHI, R., HINO, R. & NIIMI, Y. (1984). Prolongation of calcium channel open time by the dihydropyridine derivative Bay K8644 in cardiac myocytes. *Proceedings of the Japan Academy* **60**, 153–156.

SANGUINETTI, M. C. & KASS, R. S. (1984*a*). Voltage-dependent block of calcium channel current in the calf cardiac purkinje fiber by dihydropyridine calcium channel antagonists. *Circulation Research* **55**, 336–349.

SANGUINETTI, M. C. & KASS, R. S. (1984*b*). Regulation of cardiac calcium channel current and contractile activity by the dihydropyridine Bay K8644 is voltage-dependent. *Journal of Molecular and Cell Cardiology* **16**, 667–670.

SCHILLING, W. P., HAMILTON, S. L., YATANI, A., BRUSH, K. & BROWN, A. M. (1985). High and low affinity binding in membrane preparations from guinea pig ventricle: correlation with inhibition of calcium current. *Biophysical Journal* **47**, 265a.

SCHRAMM, M., THOMAS, G., TOWART, T. & FRANCKOWIAK, G. (1983). Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature* **303**, 535–536.

SCHWARTZ, A., GRUPP, I., GRUPP, G., WILLIAMS, J. & VAGHY, P. (1984). Effects of dihydropyridine calcium channel modulators in the heart: pharmacological and radioligand binding correlations. *Biochemical and Biophysical Research Communications* **125**, 387–394.

SCHWARTZ, A. & TRIGGLE, D. J. (1984). Cellular action of calcium channel blocking drugs. *Annual Review of Medicine* **35**, 325–339.

STARMER, C. F., GRANT, A. O. & STRAUSS, H. C. (1984). Mechanisms of use-dependent block of sodium channels in excitable membranes by local anaesthetics. *Biophysical Journal* **46**, 15–27.

THOMAS, G., GROSS, R. & SCHRAMM, M. (1984). Calcium channel modulation: the ability to inhibit or promote calcium influx resides in the same dihydropyridine molecule. *Journal of Cardiovascular Pharmacology* **6**, 1170–1176.

TRIGGLE, D. J. & JANIS, R. A. (1984). Nitrendipine: binding sites and mechanisms of action. In *Nitrendipine*, ed. SCRIABINE, A., VANOV, S. & DECK, K., pp. 33–52. Baltimore, MD: Urban and Schwarzenberg.

VAGHY, P. L., GRUPP, I. L., GRUPP, G., BALWIERCZAK, J., WILLIAMS, J. S. & SCHWARTZ, A. (1984). Correlation of nitrendipine and Bay K8644 binding to isolated canine heart sarcolemma with their pharmacology effects on the canine heart. *European Journal of Pharmacology* **102**, 373–374.

WILLIAMS, J. S., GRUPP, I. L., GRUPP, G., VAGHY, P. L., DUMONT, L., SCHWARTZ, A., YATANI, A., HAMILTON, S. & BROWN, A. M. (1985). Profile of the oppositely acting stereoisomers of the dihydropyridine 202–791 in cardiac preparations. *Biochemical and Biophysical Research Communications* **131**, 13–21.

YATANI, A. & BROWN, A. M. (1985). The calcium channel blocker nitrendipine blocks sodium channels in neonatal rat cardiac myocytes. *Circulation Research* **56**, 868–875.