Br. J. Pharmac. (1987), **91**, 61–67

Inhibition of myocardial Ca^{2+} channels by three dihydropyridines with different structural features: potential-dependent blockade by Ro 18-3981

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1 Inhibition of myocardial Ca^{2+} channels was investigated for three dihydropyridines with different structural features: Ro 18-3981, darodipine (PY 108-068) and nifedipine. Ro 18-3981 contains a sulphamoyl acetyl side-chain.

2 In voltage-clamp experiments with isolated cardiac myocytes of guinea-pig, Ro 18-3981 caused a concentration-dependent inhibition of the Ca^{2+} current, which was influenced by the membrane holding potential. A markedly greater inhibition by Ro 18-3981 was observed when myocytes were depolarized (to + 10 mV) from a holding potential (V_h) of −20 mV (IC_{50} = 2.3 nM) than at −50 mV (IC_{50} = 100 nM).

3 The three dihydropyridines caused a concentration-dependent reduction in contractile force of isolated, electrically-stimulated left atria of the guinea-pig. Elevation of the extracellular K^+ concentration from 5.9 to 24 mM resulted in a significant reduction in negative inotropic IC_{50} values for Ro 18-3981 (137 fold), darodipine (8 fold) and nifedipine (20 fold).

4 The affinity of these drugs for the high-affinity (+)-[^{3}H]-PN 200–110 binding site was determined in guinea-pig cardiac membranes. The K_D value of Ro 18-3981 (1.0 nM) was similar to the IC_{50} value for blockade of I_{Ca} at a V_h of −20 mV (2.3 nM), i.e. at a level of near-maximal depolarization.

5 Thus, structurally-different dihydropyridines exert potential-dependent inhibition of myocardial Ca^{2+} channel activity consistent with the modulated receptor hypothesis. These results demonstrate that blockade of myocardial excitation-contraction coupling by Ca^{2+} entry blockers is also potential-dependent.

Introduction

Dihydropyridine (DHP) Ca^{2+} channel blockers such as nifedipine and nitrendipine are presumed to inhibit the myocardial slow inward Ca^{2+} current (I_{Ca}) by interacting with receptors located on the Ca^{2+} channel (Janis & Triggle, 1984). Extensive investigations have documented the existence of high-affinity DHP binding sites in cardiac membranes (Bellemann *et al.*, 1981; Holck *et al.*, 1982; Triggle & Janis, 1984a). However, when comparing results of pharmacological and electrophysiological studies (Lee & Tsien, 1983; Janis & Triggle, 1984) with those from binding studies (Holck *et al.*, 1982; 1984), a large discrepancy (> 10 fold) is encountered in the concentrations of DHPs required to suppress myocardial I_{Ca} and to inhibit [^{3}H]-nitrendipine binding. Recent investigations suggest that the Ca^{2+} channel blocking potencies of DHPs such as nitrendipine (Bean, 1984; Reuter *et al.*, 1985) or nisoldipine (Sanguinetti & Kass, 1984) are significantly increased upon reduction of the cardiac cell membrane potential. This potential-dependent blockade of I_{Ca} by DHPs may account for discrepancies between binding and pharmacological actions described in the literature.

It was, therefore, of interest to study potential-dependence of Ca^{2+} channel blockade for structurally-different DHP derivatives. In this study, we have investigated effects of three DHPs on myocardial contractility and (+)-[^{3}H]-PN 200–110 binding. Of the DHPs tested, two are non-chiral compounds present as single isomers: nifedipine and darodipine (PY 108-068; Hof, 1985). Ro 18-3981, 2-propoxyethyl-1,4-dihydro-5-[(isopropyl-sulphamoyl) acetyl]-2,6-dimethyl-4-(*m*-nitrophenyl)nicotinate, contains a sulphamoyl acetyl side-chain distinct from the ester substituents normally found on most derivatives of this chemical class (Figure 1). The effects of this compound have been investigated

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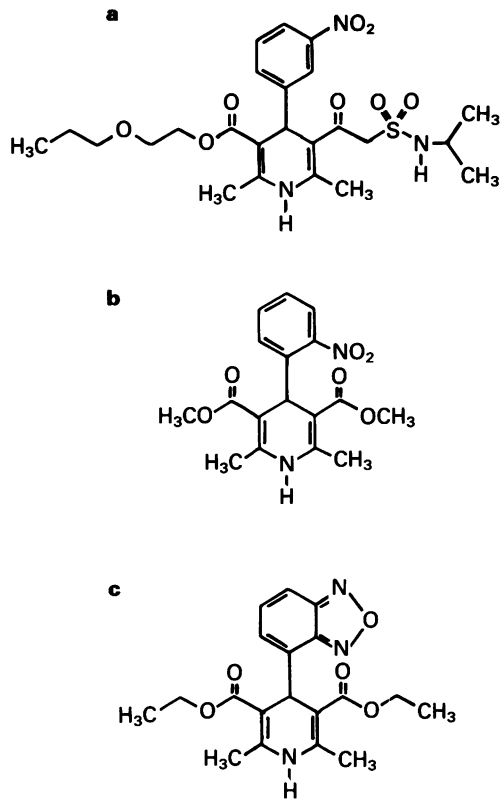

Figure 1 Structural formulae of 3 dihydropyridine Ca^{2+} channel blockers: (a) Ro 18-3981, (b) nifedipine and (c) darodipine (PY 108-068).

in detail on myocardial Ca^{2+} currents in relation to different membrane holding potentials.

Methods

Voltage-clamp experiments with guinea-pig isolated ventricular myocytes

Adult guinea-pigs of either sex (strain, Ibm: GOHI), weighing 200–300 g, were killed by cervical dislocation and the chest opened by midline incision. The hearts were quickly removed and mounted in a Langendorff apparatus for retrograde perfusion at a pressure of 75 cmH_2O. The hearts were perfused with solutions (gassed with 95% O_2 plus 5% CO_2 and warmed to 36°C) in the following sequence: (a) 3 min with Krebs-Henseleit solution (composition in mM: NaCl 120, KCl 5.9, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15, glucose 11 and $CaCl_2$ 1.8; (b) 3 min with (nominally) Ca^{2+}-free Krebs-Henseleit solution; (c) 30 min recirculation with collagenase (type II, Worthington, Freehold, NJ, U.S.A.; 30 mg collagenase 100 ml^{-1}) in Ca^{2+}-free Krebs-Henseleit solution; and (d) washout of collagenase from the heart by perfusion with 50 ml of a 'storage solution' (Isenberg & Klöckner, 1982). The 'storage solution' contained (in mM): glutamic acid 70, taurine 10, KCl 25, KH_2PO_4 10, glucose 11, EGTA 0.5 and HEPES 10 (pH adjusted to 7.3 with KOH). The ventricles were then cut off and dissected into small pieces which were gently shaken in a beaker containing 'storage solution'. The resulting dissociated myocytes were filtered on a cell sieve and stored for at least 1 h in 'storage solution' at room temperature before use. This method of storage has been shown to increase the number of Ca^{2+}-tolerant (viable) cells (Isenberg & Klöckner, 1982) upon reperfusion with Ca^{2+}-containing Krebs-Henseleit solution.

Myocytes were allowed to settle at the glass bottom of a perspex recording chamber which was rapidly perfused with Krebs-Henseleit solution (37°C). Measurement of the Ca^{2+} current (I_{Ca}) was made using the whole-cell voltage clamp technique as described by Hamill *et al.* (1981). Patch clamp electrodes (resistance 2–3 MΩ) were pulled from Pyrex glass (Hilgenberg, Malsfeld, FRG) according to Sakmann & Neher (1983). They were filled with (in mM) KCl 30, K-aspartate 100, Na_2ATP 3, $MgCl_2$ 1, KH_2PO_4 10, glucose 5.5, EGTA 0.5 and HEPES 5 (pH adjusted to 7.3 with KOH). An electrode was moved onto the surface of a cell and, by gentle suction, a GΩ -seal was formed. Thereafter, the membrane under the electrode tip was disrupted by stronger suction. The electrodes were connected by a silver wire to the headstage of a potential follower with current injection (Ehrler, Homburg/Saar, FRG) in order to record the membrane potential. The output of the potential follower was fed into one of 3 additive command inputs of a voltage clamp amplifier (Ehrler, Homburg/Saar, FRG). The output of the voltage clamp amplifier was connected to a current injecting device of the potential follower, thus enabling the potential of the cell membrane to be clamped at a constant level. Another command input of the voltage clamp amplifer was connected to the output of a digital pulse generator (Ehrler, Homburg/Saar, FRG) which elicited the wave forms necessary to elicit the Ca^{2+} current (Figure 2). Analog signals were stored on an 8-channel PCM tape (Heim, Bergisch-Gladbach, FRG) and plotted after digitisation (sampling intervals 122 μs) on a DATALAB 4000 B microprocessor system (Data Laboratories, Mitcham, UK).

Contractility measurements in guinea-pig left atria

Guinea-pigs weighing about 300 g were killed by cervical dislocation. The thorax was opened by

midline incision, the left atrium was removed and mounted in a 1 ml organ bath containing Krebs-Henseleit solution (3.6 mM $CaCl_2$). In the experiments with high KCl (24 mM), NaCl was reduced such that the solution remained isotonic. The solution was kept at 37°C, gassed with 95% O_2 plus 5% CO_2 and continuously changed at a rate of 1 ml min^{-1}. The muscles were stimulated electrically (S5 stimulator, Grass, Quincy, Mass. U.S.A.) at a frequency of 0.33 Hz by square-wave pulses of 2 ms duration and intensity twice threshold. Contractile force was recorded isometrically with a Swema force transducer (sensitivity 10 g; Swema, Stockholm, Sweden) and displayed on a chart recorder (Hellige, Freiburg i. Br., FRG). The resting tension was adjusted to 1 g at the beginning of the experiment. The control developed tension usually ranged from 0.5 to 1.5 g. An equilibration period of at least 30 min preceded a first drug application. Test compounds were infused into the organ bath at a rate of 0.1 ml min^{-1}. For each concentration of a test compound, 10 min were allowed to reach a steady-state drug effect. Concentration-response curves were determined by application of the drug in a cumulative manner. Only one concentration-response relationship at either KCl concentration was determined on each atrium.

(+)-[^{3}H]-PN 200–110 binding assay

(+)-[^{3}H]-PN 200–110 ((+)-[^{3}H]-isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxy-carbonylpyridine-3-carboxylate; specific activity = 83 Ci $mmol^{-1}$) was used as the radioligand. Binding was performed on a microsomal fraction of guinea-pig heart ventricles. A homogenate, suspended in the incubation buffer (Tris-HCl, 50 mM, pH 7.4), was centrifuged at 1,000 *g* (20 min) and the supernatant centrifuged twice at 100,000 *g* (40 min). The resulting pellet was suspended in incubation buffer at a concentration of approximately 1 mg ml^{-1} protein. The binding assay was carried out in a volume of 300 µl with 0.1–0.2 mg protein, varying concentrations of test drug (or water) and 0.2 nM (+)-[^{3}H]-PN 200–110, a concentration equivalent to approximately four times the equilibrium K_D. Incubations proceeded at 37°C for 60 min, a sufficient time to attain steady-state binding conditions. Incubations were terminated by dilution with buffer and bound tracer was separated from free by rapid filtration through Whatman GF/C filters. Filters were counted at 50% efficiency in a liquid scintillation counter. Non-specific binding was defined as that in the presence of nifedipine (10^{-6} M) and amounted to less than 10% of the total bound radioactivity.

The affinity of compounds for the specific (+)-[^{3}H]-PN 200–110 binding site was assessed by their ability to compete with radioligand binding. The radioligand competition curves were subjected to Hill plot analysis (Weiland & Molinoff, 1981). In this procedure, data were plotted as log (bound/$bound_{max}$ − bound) vs log (drug concentration). Linear regression analysis of the central portion of the plot (between 8% and 92% control binding) yielded an intercept on the abscissa scale equal to the IC_{50} (concentration of drug causing 50% inhibition of specific tracer binding).

Drugs

Ro 18-3981, darodipine (PY 108-068) and nifedipine (Figure 1) were synthesized by Profs H. Ramuz and R. Jaunin (F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland). All were prepared as 10 mM stock solutions in ethanol and diluted in Krebs-Henseleit solution or distilled water to the final concentrations. (+)-[^{3}H]-PN 200–110 (specific activity = 83 Ci $mmol^{-1}$) was purchased from Amersham Corp. Tetrodotoxin was from Sigma (St. Louis, MO, U.S.A.). All experiments were carried out in the absence of overhead lighting to prevent degradation of the DHP derivatives.

Results

Voltage clamp experiments

The effect of one of the three DHPs, Ro 18-3981, was investigated in detail on the slow inward Ca^{2+} current (I_{Ca}) of isolated myocardial cells at different holding potentials (V_h). In the first group of experiments, the cell membrane potential was clamped at −80 mV and transiently depolarized to +10 mV at 3 s intervals. Tetrodotoxin (TTX, 20 µM) was included in the perfusion medium to block completely the fast inward Na^+ current. This procedure was repeated from V_h of −30 mV. Figure 2a shows superimposed current traces in response to a 200 ms test pulse recorded from a cell before and during perfusion with 1 µM Ro 18-3981. At this V_h (−80 mV), peak I_{Ca} in the presence of drug was 97 ± 6% (n = 3 different cells) of control levels. Reduction of the V_h to −30 mV resulted in a considerable decrease in the magnitude of control I_{Ca} (Figure 2b). This resulted in a marked increase in the inhibitory effect of 1 µM Ro 18-3981, with peak I_{Ca} being reduced to 13 ± 3% (n = 3) of control levels.

The second series of experiments involved determining the concentration-response relationship of the Ca^{2+} current-blocking effects of Ro 18-3981 at different V_hs. The experimental protocol was as described above, except that only one concentration of drug was applied to a cell at any V_h. Figure 3 shows the concentration-dependent inhibition of I_{Ca} by Ro 18-3981 at V_hs of −50, −35 and −20 mV. There was a shift to the left of the concentration-response curve

accompanying the reduction in V_h. The extrapolated IC_{50} values (and corresponding V_h) were 100 nM (−50 mV), 29 nM (−35 mV) and 2.3 nM (−20 mV). This represents a 44 fold increase in the inhibitory potency of Ro 18-3981 resulting from a 30 mV reduction in the cell membrane potential.

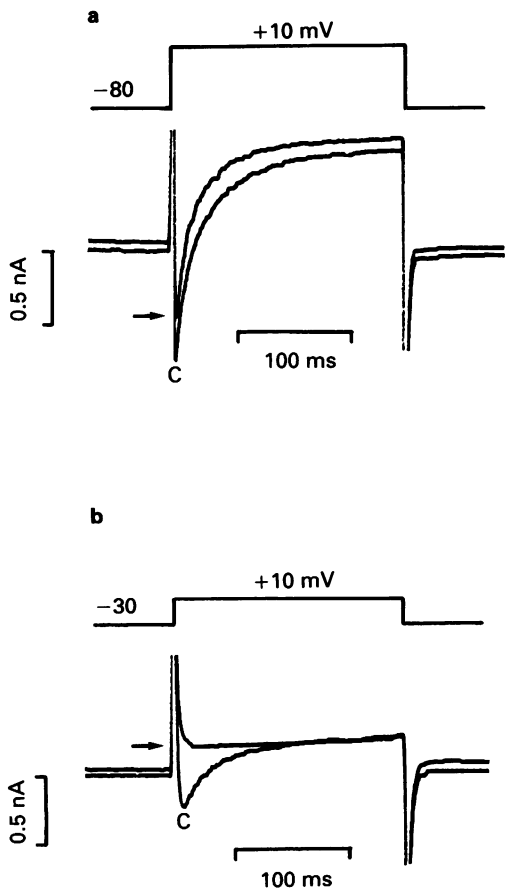

Figure 2 Voltage-clamp recordings of the effects of Ro 18-3981 on membrane currents of isolated myocytes at different holding potentials (V_h). Shown are original tracings of current recordings from the same cell stimulated with 200 ms pulses at 0.33 Hz from V_h of −80 mV (a) or −30 mV (b) to a test potential of +10 mV. Control inward Ca^{2+} currents (I_{Ca}) were recorded from both V_h before exposure to the drug. C is the control inward Ca^{2+} current (I_{Ca}) and the arrow indicates the magnitude of I_{Ca} during exposure to 1 μM Ro 18-3981. Experiments were performed in the presence of 20 μM tetrodotoxin to block the Na^+ current. This experiment was repeated on 3 different cells.

Contractile measurements in guinea-pig isolated left atria

The potential-dependent inhibitory effect of Ro 18-3981 upon the myocardial slow inward Ca^{2+} current prompted us to investigate the influence of membrane potential upon the effects of several DHPs on excitation-contraction coupling in cardiac muscle. In these experiments, the negative inotropic effects of Ro 18-3981, darodipine and nifedipine on contractility of guinea-pig isolated, electrically-stimulated, left atria were studied at normal (5.9 mM) and elevated (24 mM) extracellular K^+ concentrations. Figure 4 shows the concentration-response relationship of the negative inotropic effect of the three DHPs. At 5.9 mM KCl, significant decreases in contractile force were observed at concentrations above 0.1 μM (Figure 4a). Geometric mean IC_{50} values were 7.2 ± 2.3 μM for Ro 18-3981, 0.83 ± 0.2 μM for darodipine and 1.2 ± 0.6 μM for nifedipine. When tested at 24 mM KCl, significant negative inotropic effects were already observed at concentrations as low as 0.01 nM (Figure 4b). Under this experimental condition, geometric mean IC_{50} values for Ro 18-3981 (53 ± 32 nM), darodipine (100 ± 31 nM) and nifedipine (59 ± 23 nM) were 137, 8 and 20 fold lower, respectively, than the values obtained in normal extracellular K^+.

(+)-[^{3}H]-PN 200–110 binding studies

The binding of DHP Ca^{2+} channel blockers is depen-

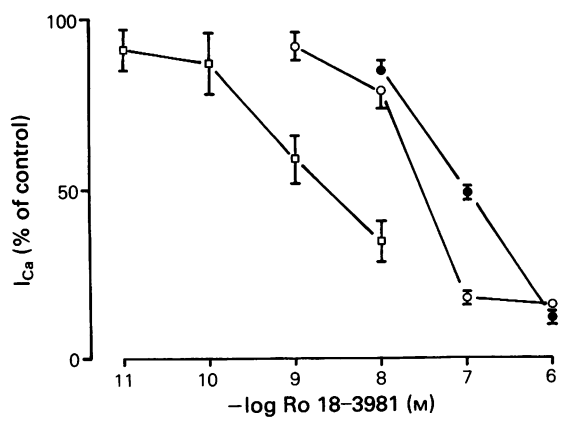

Figure 3 Concentration-dependent decrease on peak Ca^{2+} current (I_{Ca}) by Ro 18-3981 in isolated myocytes at membrane holding potentials (V_h) of −50 mV (●), −35 mV (○) and −20 mV (□). Each point is the mean of pre-drug I_{Ca} from 3–5 different cells recorded under steady-state conditions; vertical lines indicate s.e.mean. Only one concentration of Ro 18-3981 was tested per myocyte at any V_h. Stimulation conditions were as in Figure 2.

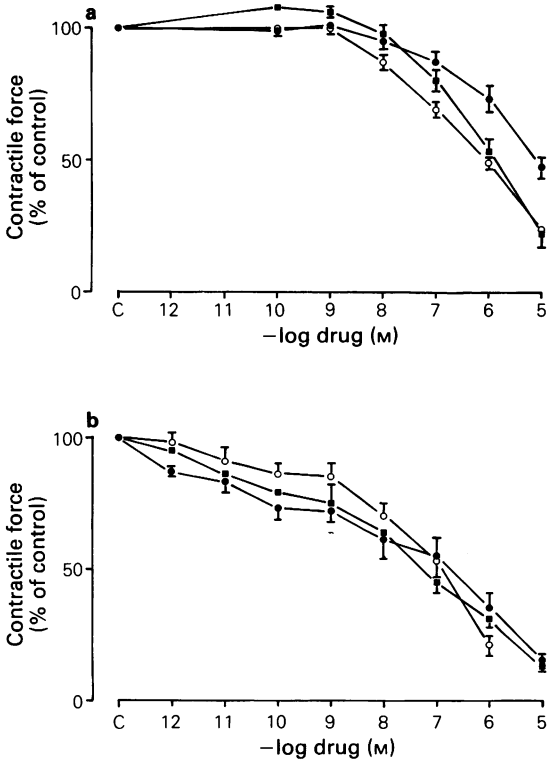

Figure 4 The influence of extracellular K^+ concentration on the negative inotropic effects of Ro 18-3981 (●), darodipine (○) and nifedipine (■) in guinea-pig isolated left atria. The K^+ concentration of the Krebs-Henseleit solution was increased from 5.9 mM (a) to 24 mM (b) with isotonic replacement of Na^+. Stimulation frequency was 0.33 Hz. Each point is the mean of 4–6 separate determinations and vertical lines show s.e.mean.

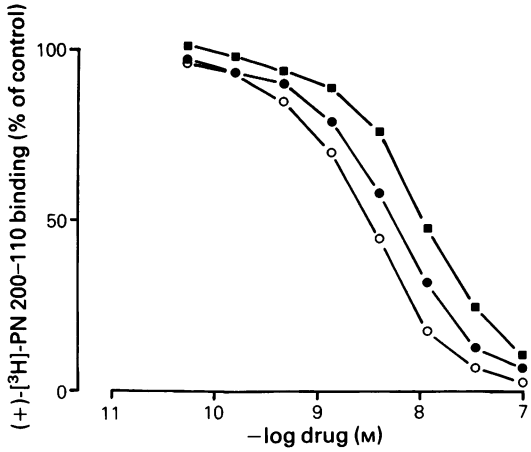

Figure 5 Concentration-dependent inhibition of (+)-[^{3}H]-PN 200–110 binding to cardiac membranes by Ro 18-3981 (●), darodipine (○) and nifedipine (■). One hundred % specific binding was defined by 1 μM nifedipine. Each value is the mean % of control specific binding from 4 different experiments performed in duplicate. The s.e.mean of all points was less than 2.5%.

dent on membrane potential (Green *et al.*, 1985; Reuter *et al.*, 1985). The high affinity [^{3}H]-nitrendipine binding site in cardiac membranes is considered to correspond to the Ca^{2+} channel in its inactivated state (Bean, 1984). We investigated the binding of Ro 18-3981, darodipine and nifedipine to guinea-pig cardiac membranes, to ascertain the affinity of these drugs for the high-affinity DHP receptor as a point of comparison with the electrophysiological and pharmacological data obtained above.

(+)-[^{3}H]-PN 200–110 bound in a saturable manner to guinea-pig cardiac membranes. Scatchard analysis yielded a straight line, consistent with binding to a single population of sites with a K_D of 0.053 ± 0.005 nM and a maximal concentration of 86 ± 11 fmol mg^{-1} of protein ($n = 4$ separate determinations performed in triplicate; data not shown). These results are similar to those obtained for (+)-[^{3}H]-PN 200–110 binding to rat heart membranes (Lee *et al.*, 1984). The affinities of Ro 18-3981, darodipine and nifedipine for the DHP receptor site were determined in competition experiments. Figure 5 illustrates that all three DHPs caused a concentration-dependent inhibition of (+)-[^{3}H]-PN 200–110 (0.2 nM) binding. As determined by the method of Cheng & Prusoff (1973), mean K_D values of 1.0 ± 0.09 nM, 0.59 ± 0.05 nM and 2.1 ± 0.2 nM were determined for Ro 18-3981, darodipine and nifedipine, respectively. For all three compounds, apparent Hill coefficients were not significantly different from unity, implying a competitive interaction with the DHP receptor site.

Discussion

The purpose of this study was to investigate inhibition of the myocardial Ca^{2+} channel by three structurally-different DHPs. The data demonstrate clearly that blockade of I_{Ca} in cardiac myocytes by the DHP containing a sulphamoyl acetyl side-chain, Ro 18-3981, was dependent upon the membrane holding potential (V_h). When cells were clamped at more depolarized V_h, the concentration-response curve for Ro 18-3981 was shifted to the left. At the most depolarized V_h tested (−20 mV), the IC_{50} value for inhibition of I_{Ca} by Ro 18-3981 was nearly identical to its affinity constant (K_D) for the DHP receptor of cardiac membranes. Depolarization of the membrane

potential by elevation of the extracellular K^+ concentration enhanced the ability of Ro 18-3981, darodipine and nifedipine to inhibit excitation-contraction coupling in isolated atria. This was observed as an increased potency in inducing negative inotropic effects. These findings suggest that DHPs of different structure are capable of exerting a qualitatively similar potential-dependent inhibition of myocardial Ca^{2+} channels.

Potential-dependent inhibition of myocardial I_{Ca} has been demonstrated for nitrendipine (Bean, 1984; Sanguinetti & Kass, 1984; Reuter *et al.*, 1985), nifedipine (Uehara & Hume, 1985), nicardipine and nisoldipine (Sanguinetti & Kass, 1984). These compounds contain an *ortho*- or *meta*-nitrophenyl substituent at the C-4 position. Recently, a DHP with a benzoxadiazole moiety at C-4, (+)-PN 200–110, was also shown in voltage-clamp experiments to inhibit I_{Ca} in a potential-dependent manner in cultured skeletal muscle cells of the rat (Cognard *et al.*, 1986). All of these compounds contain ester substituents at C-3 and C-5 of the 1,4-DHP ring. The results of the present study indicate that a DHP containing a sulphamoyl acetyl substituent at the C-3 position is also capable of blocking I_{Ca} in a potent manner. This is in contrast to previous findings that substitution of ester functions at C-3 and C-5 by other groups leads to a markedly reduced Ca^{2+}entry blocking activity (Triggle & Janis, 1984b). Thus, it appears that the 1,4-DHP ring can tolerate non-esteric substituents at the C-3 position with respect to the potential-dependence of I_{Ca} blockade.

In the present study, a 44 fold increase in the inhibitory potency of Ro 18-3981 was observed for a 30 mV reduction in V_h. Bean (1984) demonstrated a more than 1000 fold increase in the inhibitory potency of nitrendipine with an 80 mV reduction in the membrane potential of cardiac myoctyes. An 86 fold increase in the potency to inhibit I_{Ca} of (+)-PN 200–110 was demonstrated for a 35 mV reduction in V_h of skeletal muscle cells (Cognard *et al.*, 1986). Further studies under conditions of controlled stimulation and membrane potential will be required to ascertain quantitative differences in the potential-dependence of I_{Ca} blockade by structurally-diverse DHPs. The difficulty of this task can be appreciated when considering that, according to the modulated receptor hypothesis, DHPs can be expected to possess at least 3 different potencies corresponding to the 3 states of the Ca^{2+} channel: open, resting, inactivated (Hondeghem & Katzung, 1984).

A major point of this study is the observation that potential-dependence of Ca^{2+} channel blockade also extends to myocardial excitation-contraction coupling. To our knowledge this is the first demonstration of a potential-dependent negative inotropic effect by any DHP-type Ca^{2+} channel blocking drug. The development of contractile tension in isolated cardiac preparations has been shown to be closely correlated with activation of the inward Ca^{2+} current (Trautwein *et al.*, 1975). It would be expected, therefore, that DHPs which demonstrate potential-dependence of I_{Ca} blockade would show enhanced negative inotropic activity upon depolarization of the membrane potential.

This was the case for all three DHPs in the isolated atria experiments. A shift to the left of the concentration-response curves, ranging between 8 and 137 fold at the IC_{50} level, was observed when the extracellular K^+ concentration was elevated from 5.9 to 24 mM. The largest and smallest shifts were seen with Ro 18-3981 and darodipine, respectively. While the significance of this difference in potency shift requires further investigation, it appears to support the idea that changes in membrane potential do not affect the potency of all DHPs to the same extent (Hondeghem & Katzung; 1984). Under the conditions used in the isolated atria experiments, neither the duration nor the magnitude of membrane depolarization can be precisely controlled. Thus, an additional influence of use-dependence on drug potency changes induced by K^+ depolarization cannot be excluded. However, use-dependent block by DHPs has been shown to be important only at pulse frequencies greater than 1 Hz (Sanguinetti & Kass, 1984). Therefore, in the present experiments with pulse frequencies of 0.33 Hz, it is unlikely that use-dependence contributed significantly to the potency shifts of the three DHPs following elevation of extracellular K^+.

Recent evidence suggests that DHPs bind to their receptors in intact cardiac myocytes or tightly-sealed vesicles in a potential-dependent manner (Green *et al.*, 1985; Reuter *et al.*, 1985; Schilling & Drewe, 1986), and that high-affinity binding of radioactive DHPs to cardiac membrane labels the Ca^{2+} channel in its inactivated state (Bean, 1984). The results of the present study show that Ro 18-3981, darodipine and nifedipine bound with nanomolar affinity to a single population of high-affinity DHP receptors ((+)-[^{3}H]-PN 200–110 binding sites). Of particular importance are the virtually identical inhibitory constants for Ro 18-3981 in inhibiting (+)-[^{3}H]-PN 200–110 binding (K_D = 1.0 nM) and suppressing I_{Ca} at depolarized membrane holding potentials (IC_{50} = 2.3 nM; V_h of −20 mV). This implies that at a V_h of −20 mV, the cardiac Ca^{2+} channel is in a predominantly inactivated state. Indeed, the steady-state inactivation curve (f_∞) of I_{Ca} in guinea-pig cardiac myocytes predicts > 85% inactivation at this V_h (McDonald *et al.*, 1986). Further reduction of V_h would, therefore, be unlikely to enhance the Ca^{2+} channel blocking potency of Ro 18-3981.

In summary, a DHP with novel structural features, Ro 18-3981, was found to exert a potential-dependent

blockade of myocardial I_{Ca} qualitatively similar to that observed for other drugs of this chemical class. The negative inotropic activity of Ro 18-3981, darodipine and nifedipine was found to increase upon depolarization of the membrane resting potential by elevation of extracellular K^+. These observations support the notion that DHPs exert a potential-dependent inhibition of myocardial Ca^{2+} channel activity according to the modulated receptor hypothesis (cf. Hondeghem & Katzung, 1984).

We wish to acknowledge the technical assistance provided by Mrs B. Holck, Mrs P. Preisendörfer, Mrs U. Sauder and Mr U. Wicki.

References

BEAN, B.P. (1984). Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proc. natn. Acad. Sci. 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. *Arzneim. Forsch.*, **31**, 2064–2067.

CHENG, Y.-C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmac.*, **22**, 3099–3108.

COGNARD, D., ROMEY, G., GALIZZI, J.-P., FOSSET, M. & LAZDUNSKI, M. (1986). Dihydropyridine-sensitive Ca^{2+} channels in mammalian skeletal muscle cells in culture: electrophysiological properties and interactions with Ca^{2+} channel activator (Bay K 8644) and inhibitor (PN 200–110). *Proc. natn. Acad. Sci. U.S.A.*, **83**, 1518–1522.

GREEN, F.J., FARMER, B.B., WISEMAN, G.L., JOSE, M.J.F. & WATANABE, A.M. (1985). Effect of membrane depolarization on binding of [^{3}H]nitrendipine to rat cardiac myocytes. *Circulation Res.*, **56**, 576–585.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

HOF, R.P. (1985). PY 108-068, a calcium antagonist with an unusual pattern of cardiovascular activity. *Gen. Pharmac.*, **16**, 1–6.

HOLCK, M., THORENS, S. & HAEUSLER, G. (1982). Characterization of ^{3}H-nifedipine binding sites in rabbit myocardium. *Eur. J. Pharmac.*, **85**, 305–315.

HOLCK, M., FISCHLI, W. & HENGARTNER, U. (1984). Effects of temperature and allosteric modulators on [^{3}H]nitrendipine binding: methods for detecting potential Ca^{2+} channel blockers. *J. receptor Res.*, **4**, 557–569.

HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *A. Rev. Pharmac. Tox.*, **24**, 387–423.

ISENBERG, G. & KLÖCKNER, U. (1982). Calcium tolerant ventricular myocytes prepared by incubation in a "KB" medium. *Pflügers Arch.*, **395**, 6–18.

JANIS, R.A. & TRIGGLE, D.J. (1984). 1,4-Dihydropyridine Ca^{2+} channel antagonists and activators: a comparison of binding characteristics with pharmacology. *Drug Dev. Res.*, **4**, 257–274.

LEE, H.R., ROESKE, W.R. & YAMAMURA, H.I. (1984). High affinity specific [^{3}H] (+)PN 200–110 binding to dihydropyridine receptors associated with calcium channels in rat cerebral cortex and heart. *Life Sci.*, **35**, 721–732.

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

McDONALD, T.F., CAVALIÉ, A., TRAUTWEIN, W. & PELZER, D. (1986). Voltage-dependent properties of macroscopic and elementary calcium channel currents in guinea pig ventricular myocytes. *Pflügers Arch.*, **406**, 437–448.

REUTER, H., PORZIG, H., KOKUBUN, S. & PROD'HOM, B. (1985). 1,4-Dihydropyridines as tools in the study of Ca^{2+} channels. *Trends Neurosci.*, **8**, 396–400.

SAKMANN, B. & NEHER, E. (1983). Geometric parameters of pipettes and membrane patches. In *Single Channel Recording* ed. Sakmann, B. & Neher, E. pp. 37–51. New York: Plenum Press.

SANGUINETTI, M.C. & KASS, R.S. (1984). Voltage-dependent block of calcium channel current in the calf Purkinje fiber by dihydropyridine calcium channel antagonists. *Circulation Res.*, **55**, 336–348.

SCHILLING, W.P. & DREWE, J.A. (1986). Voltage-sensitive nitrendipine binding in an isolated cardiac sarcolemma preparation. *J. biol. Chem.*, **261**, 2750–2758.

TRAUTWEIN, W., McDONALD, T.F. & TRIPATHI, O. (1975). Calcium conductance and tension in mammalian ventricular muscle. *Pflügers Arch.*, **354**, 55–74.

TRIGGLE, D.J. & JANIS, R.A. (1984a). Calcium channel antagonists: new perspectives from the radioligand binding assay. In *Modern Methods in Pharmacology*. pp. 1–28. New York: Alan R. Liss, Inc.

TRIGGLE, D.J. & JANIS, R.A. (1984b). Nitrendipine: binding sites and mechanism of action. In *Nitrendipine* ed Scriabine, A., Vanov, S. & Deck, K. Baltimore: Urban & Schwarzenberg.

UEHARA, A. & HUME, J.R. (1985). Interactions of organic calcium channel antagonists with calcium channels in single frog atrial cells. *J. gen. Physiol.*, **85**, 621–647.

WEILAND, G.A. & MOLINOFF, P.B. (1981). Quantitative analysis of drug-receptor interactions: I. Determination of kinetic and equilibrium properties. *Life Sci.*, **29**, 313–330.

(Received September 4, 1986.
Revised January 16, 1987.
Accepted January 23, 1987.)