Effects of captopril on membrane current and contraction in single ventricular myocytes from guinea-pig

S.M. Bryant, Kathryn O. Ryder & ¹G. Hart

Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU

- 1 Single guinea-pig ventricular myocytes were voltage-clamped and cell length was measured with a photodiode array.
- 2 Captopril (1×10^{-5} M) reduced both peak early current and active shortening in response to a depolarizing clamp pulse along a similar time course.
- 3 From a holding potential of around -45 mV peak early inward current was reduced by $37 \pm 9\%$ ($P < 0.001$) on exposure to captopril. The early current-voltage relationship was shifted outwards by captopril indicating a reduction in membrane conductance through the L-type calcium channel (I_{Ca}).
- 4 The amplitude of cell shortening in response to depolarizing voltage steps was reduced but the voltage-dependence of contraction after captopril was unchanged.
- 5 A small negative shift of the potential at which I_{Ca} was half-activated was observed after captopril. There was no change in the voltage-dependence of the inactivation variable or in the time-dependence of repriming for I_{Ca} .
- 6 The actions of captopril on I_{Ca} and developed shortening were dose-dependent and took place in the same proportion when I_{Ca} was increased by isoprenaline.
- 7 These results are discussed in relation to the effects of captopril on I_{Ca} and contraction and to its clinical usage.

Introduction

Captopril is a proline derivative whose principal mode of action is inhibition of angiotensin converting enzyme. The possibility that the drug may exert a direct action on the myocardium is of considerable interest given that captopril is extensively prescribed for treatment of heart failure and hypertension. It has recently been reported that captopril reduces developed force in isolated heart muscle of guinea-pig and man in a dose-dependent fashion (Walker *et al.*, 1988). Possible explanations for this negative inotropic effect include inhibition of endogenous angiotensin release (Lindpaintner *et al.*, 1988), interference with the action of catecholamines released by field stimulation in isolated tissue (Koch-Weser, 1965; Collis & Keddle, 1982), or alternatively a direct action of the drug on the myocytes themselves. We have set out to investigate the latter possibility using isolated single ventricular myocytes, and after confirming an effect of the drug on developed length we have sought to define the action of captopril on membrane currents using the voltage-clamp technique. A preliminary account has appeared (Bryant *et al.*, 1989).

Methods

Cells were isolated from both ventricles of female Dunkin-Hartley guinea-pigs weighing 400–600 g by an enzyme technique (Mitchell *et al.*, 1984) and were layered on a glass coverslip which formed the base of a perfusion chamber situated on the stage of an inverted microscope. Micropipettes were filled with 3 M KCl giving a resistance of 18–25 M Ω . Voltage-clamping was achieved by a single micro-electrode switch-clamp technique (Axoclamp II-A, Axon Instruments, Inc., Foster City, CA). The clamp switching frequency was 3.5–6 kHz. An agar bridge in the bath was used as the indifferent electrode. Cell length was measured with an S-series photodiode array (E.G. & G. Reticon, Wokingham, Berks.; Boyett *et al.*, 1988). Superfusate flow rate was 1–2 ml min⁻¹

and solution lines were switched by solenoid valves (Lee Products Ltd., Gerrards Cross, Bucks.) just before entry to the bath. The temperature was maintained at $32 \pm 1^\circ\text{C}$ by a heating coil surrounding the input line which was controlled by a feed-back circuit using a sensing thermocouple positioned between the heating coil and the chamber.

Voltage, current and length signals were recorded on magnetic tape (Racal Store 7 recorder, Hythe, Southampton) for later analysis. The signals were played back through a 6-pole Bessel filter and sampled at 1 kHz by an A to D converter (CED 1401, Cambridge Electronic Design Ltd., Cambridge). Analysis of the digitized records was performed on a Compaq Deskpro 286 computer using software provided by Dr J. Dempster, Strathclyde University.

Superfusate composition was as follows (mM): Na⁺ 136, K⁺ 5.4, Mg²⁺ 1.2, Ca²⁺ 1.8, glucose 11.1, HEPES 5.0, titrated with NaOH to pH 7.4. Captopril was dissolved in water before being added to the superfusate in appropriate dilution.

Results were compared by Student's *t* test for paired samples and are expressed as mean $\pm$ standard error of the mean (s.e.mean).

Results

Time-dependence of captopril action

The holding potential was set between -40 and -45 mV in order to inactivate the sodium channel and the T-type calcium channel. Depolarizing pulses of 500 ms duration were applied from this potential to 0 mV at a frequency of 0.2 Hz. Figure 1 shows the effects of captopril 1×10^{-5} M on the change in cell length associated with contraction (a) and on peak inward current (b) using this protocol. Captopril reduced the amplitude of inward current and active shortening which both decreased along a similar time course. The drug has a rapid onset of action and the changes were complete within 90 s. On removal of captopril there was recovery of both current and contraction in some cells but this was not a consistent finding at the higher concentrations used.

Peak inward current at this potential is composed chiefly of Ca²⁺ current (Mitchell *et al.*, 1987) and it seems possible that

¹ Author for correspondence.

Figure 1 Effects of captopril on the reduction in cell length (a) and on peak inward current (b) in a single cardiac myocyte. Step depolarizations to 0 mV from a holding potential of -40 mV were elicited at a frequency of 0.2 Hz . Application of the drug is indicated by the arrow. The changes are complete in approximately 90 s.

a reduction in this current could account for the fall in developed length after captopril. We therefore set out to look at the effects of the drug on the L-type Ca^{2+} current in more detail.

Current-voltage relationships

Figure 2 shows current records from a single cell obtained by depolarizing from a holding potential of -45 mV to the potentials shown. There is an inward peak of membrane current at the onset of the depolarizing step as L-type calcium current (I_{Ca}) activates rapidly (Fox *et al.*, 1987). Inactivation of this current follows a much slower time course and at the more positive potentials shown an increasing outward current,

Figure 2 Membrane current records showing the effects of depolarizations lasting 500 ms from a holding potential of -45 mV to the potentials shown by the records. The left-hand records were obtained in control solution, the right-hand after captopril $1 \times 10^{-5} \text{ M}$. Identical clamp steps were used for each sequence of depolarizations. The labelled tickmarks on the abscissa scale refer to the lowest pair of records. For clarity the remaining records have been shifted outwards by 5 nA and the unlabelled tickmarks indicate the zero current level for these records.

I_K , develops during the later part of the clamp step. The right-hand records were obtained from the same cell a few minutes after exposure to captopril ($1 \times 10^{-5} \text{ M}$) and it can be seen that captopril has reduced the peak early current at each potential.

Current-voltage relationships for this cell are shown in Figure 3. The circles represent peak early current at increments of approximately 10 mV from the holding potential and show that I_{Ca} has been reduced by the drug (cf., for example, Kameyama *et al.*, 1986). The potential at which inward current was maximal did not change in the presence of captopril. In 18 cells, a concentration of $1 \times 10^{-5} \text{ M}$ -captopril reduced mean maximum inward current from 2.3 ± 0.3 to $1.5 \pm 0.2 \text{ nA}$ ($P < 0.001$). The reversal potential for I_{Ca} showed no significant change after captopril. There was also no significant change in the holding current or in the current at the end of the 500 ms depolarizing clamp steps (shown as squares in the graph), indicating that there is no significant action of the drug on the magnitude of the delayed rectifier I_K .

Length-voltage relationships

The changes in length associated with depolarizing voltage-clamp steps in this cell are illustrated in Figure 4. It can be seen from the original length records in Figure 4a that the amplitude of the phasic component of contraction, produced by the depolarization, increases with voltage and then declines as the potential becomes more positive. Figure 4b shows that after captopril the voltage-dependence of this U-shaped relationship is maintained but the amplitude of contraction at each potential is reduced. The peak shortening has been reduced from 6.4 to 3.9 μm , amounting to a 40% reduction in developed length. In 5 cells the peak reduction in developed length seen at this concentration of captopril ($1 \times 10^{-5} \text{ M}$) was $47 \pm 3\%$ ($P < 0.01$).

Kinetic parameters for I_{Ca}

In order to find out whether the reduction in I_{Ca} is attributable to a reduction in conductance for I_{Ca} or to a shift in the kinetics of this channel we have measured the voltage-dependence of the kinetic variables for the current. The activation variable for the current was calculated from the chord conductance for peak early current at each potential step, expressed as a fraction of peak conductance. Figure 5 shows a typical result from one cell. The voltage at which I_{Ca} was half-activated moved $-3.8 \pm 0.6 \text{ mV}$ after exposure to captopril 10^{-5} M ($P < 0.01$, $n = 5$). The inactivation variable was measured by stepping the membrane potential from approx-

Figure 3 Current-voltage relationships for peak early current (circles) and current at the end of 500 ms depolarizing steps (squares) obtained from a holding potential of -45 mV . Open symbols represent control, closed symbols, captopril, $1 \times 10^{-5} \text{ M}$.

Figure 4 (a) Changes in cell length on depolarization to different potentials. The upper records in this panel are controls; the lower set were recorded after captopril. The potentials to which the cells were depolarized are shown at the top of each vertical pair of records. The top of each length calibration bar represents resting cell length ($129 \mu\text{m}$). (b) Relationships between the change in cell length on depolarization and clamp potential before ($\circ$) and after ($\bullet$) captopril ($1 \times 10^{-5} \text{ M}$), plotted from the data used to illustrate the upper panel. The experimental protocol was the same as in Figure 3.

Figure 5 Voltage-dependence of the steady-state activation (d_{∞}) and inactivation (f_{∞}) variables from a representative myocyte before ($\circ$) and after captopril $1 \times 10^{-5} \text{ M}$ ($\bullet$). Curves drawn by eye. The inactivation curve is the same after captopril; the activation curve is shifted to the left by a small amount. Note that the inactivation curve was constructed from a holding potential of -40 mV so that by default $f_{\infty} = 1$ at this potential and the bottom end of this curve may therefore have been abbreviated (Fox *et al.*, 1987).

imately -40 mV to different conditioning potentials for 200 ms followed by a test pulse to 0 mV for 100 ms . There was no significant shift in the voltage dependence of the inactivation variable (mean of differences $-1.7 \pm 1.1 \text{ mV}$, $P < 0.5$).

The recovery of I_{Ca} from inactivation was studied by use of a two-pulse protocol. Both pulses were to 0 mV and the interpulse interval was increased from 10 to 500 ms . The ratio of currents produced by the two pulses as a function of interpulse interval is shown for the mean data in Figure 6. The time for 50% recovery from inactivation was $81 \pm 13 \text{ ms}$ before and $83 \pm 11 \text{ ms}$ after captopril (NS, $n = 4$).

Dose-response curve for captopril

In order to show that the changes in I_{Ca} and contraction which we have described are attributable to a pharmacological effect of the drug it is also necessary to define the concentration-dependence of these actions. Figure 7 shows the

Figure 6 Time-dependence of restitution of I_{Ca} in 4 cells before ($\circ$) and after ($\bullet$) captopril $5 \times 10^{-5} \text{ M}$. Recovery of early peak current is expressed as a fraction of the current seen in the absence of a prepulse, as a function of interpulse interval. There was no significant shift in the time for 50% recovery after captopril.

Figure 7 Incremental dose-response curves for change in developed length ($\blacksquare$) and peak early inward current ($\blacktriangle$) expressed as fractional reduction of control values for each parameter, plotted against captopril concentration. The voltage-clamp protocol involved depolarizing steps from a holding potential of -40 mV to 0 mV lasting 500 ms , applied every 5 s . Standard error bars are shown for $n = 4$ cells. The stippled area represents the therapeutic range of plasma concentrations of the drug in man.

effects of incremental concentrations of captopril on the mean change in length associated with step depolarization to 0 mV and on mean I_{Ca} recorded simultaneously, from completed protocols in 4 cells. There was a significant reduction in both contraction and I_{Ca} at the lowest concentration of captopril used (10^{-6} M). It appears that the decrease in contraction is relatively greater than the decrease in I_{Ca} at each concentration tested. Maximal inhibition does not appear to have been achieved. Further increases in concentration of the drug resulted in transient inward currents and cell death followed rapidly.

Further experiments on the mechanism of captopril action on I_{Ca}

The effect of captopril on the early current-voltage relationship (Figure 3) strongly suggests an action on the L-type calcium channel, either directly or by a secondary action on a mechanism controlling I_{Ca} amplitude. When I_{Ca} was blocked with gallopamil (D600), subsequent addition of captopril (1×10^{-5} M) had no effect on the remaining membrane currents and current-voltage relationship. In order to confirm that the effect is specific to the calcium channel we increased the calcium current with isoprenaline and then exposed the myocytes to captopril in the presence of isoprenaline. Isoprenaline 1×10^{-7} M increased I_{Ca} seen on depolarization from -45 mV to 0 mV by $173 \pm 17\%$. Exposure to captopril, 1×10^{-5} M in the presence of isoprenaline reduced I_{Ca} by $21 \pm 4\%$ ($n = 4$). The drug therefore reduced I_{Ca} amplitude by a similar extent to that seen in the absence of the β -adrenoceptor agonist.

Discussion

These experiments show that captopril reduces developed length in isolated mammalian ventricular myocytes in a time-dependent and concentration-dependent fashion and that this negative inotropic effect appears to be linked to a reduction in L-type calcium current. The change in I_{Ca} is attributable to a reduction in membrane conductance rather than to a shift in the kinetic variables. The basis for this action may be a reduction in open-channel probability after captopril which would be detectable in patch-clamp studies of this channel. We have carried out some provisional patch-clamp experiments which indicate an effect of the drug directly on the outer surface of the channel, rather than via intracellular second messenger systems. A small negative shift of the activation variable *per se* would be expected to give rise to an increase in current at potentials between the holding potential and the top of the activation curve. Clearly this effect is offset by the fall in I_{Ca} conductance produced by the drug.

Possible mechanisms of action of the drug on the L-type calcium channel may be considered. The shift of the d_{∞} variable is small and is in the opposite direction to that reported to occur on exposure to the phenylalkylamine calcium antagonists. For example Cohen & Lederer (1987) found that D600 produced a positive shift of this variable in neonatal rat ventricular myocytes. On the other hand these workers and Fox *et al.* (1987) found that the dihydropyridine agonist Bay K 8644 gives rise to a negative shift of the activation curve. We may rule out the possibility that inhibition of neuronal catecholamine release is involved since isolated myocytes are effectively denervated (Dow *et al.*, 1981) and the effects of the drug on the activation variable are not those which would be expected from reversal of β -adrenoceptor stimulation (Cohen & Lederer, 1987). Angiotensin II increases the calcium current in neonatal rat ventricular myocytes (Allen *et al.*, 1987). Could the drug be inhibiting endogenous angiotensin II synthesis, which has been demonstrated to occur in cardiac muscle (Nakashima *et al.*, 1982)? In isolated hearts, no angiotensin II synthesis has been demonstrated without the presence of angiotensin I in the perfusate (De Graeff *et al.*, 1986; Lindpaintner *et al.*, 1988). This explanation is therefore unlikely for the *in vitro* effects here described since there was no angiotensin I in

the perfusate. That the effect of captopril on I_{Ca} is proportionately similar when this current has been increased by catecholamines suggests that the drug is having a fairly direct effect on the channel rather than acting via some less specific mechanism.

We have consistently observed a good temporal relationship between the onset of negative inotropy and the decline in I_{Ca} . Even though the amplitude is reduced, the time course and voltage-dependence of the developed shortening response are unchanged after captopril. Nevertheless it is unlikely that a decrease in I_{Ca} is the sole mechanism of the reduced contractile response to captopril. From Figure 3 it appears that the magnitude of I_{Ca} at -10 mV is close to that at 0 mV in the presence of captopril. However the length changes accompanying these currents (Figure 4) are different, the shortening at 0 mV after captopril being nearly $2 \mu\text{m}$ less than that at -10 mV in control solution. Terrar & Victory (1988) have shown, in guinea-pig ventricular myocytes under switch-clamp conditions, that on exposure to verapamil or nifedipine, a 60% fall in I_{Ca} gives a reduction in contraction of about 35%. From the data in Figure 7 we may estimate that a 35% reduction in developed shortening after captopril would be accompanied by a decrease in I_{Ca} of only 13%. The relationship between I_{Ca} and the contractile response is not linear, but even allowing for this it is necessary to postulate an action of captopril on excitation-contraction coupling distal to I_{Ca} , which may involve a reduction in Ca^{2+} release from the sarcoplasmic reticulum in response to a given increment in intracellular Ca^{2+} . Further work will be necessary to explore these additional effects.

We may consider briefly possible implications of this work which are relevant to the clinical use of the drug. Plasma levels of captopril in human subjects following therapeutic administration of the drug have been in the range $1.7\text{--}8.9 \times 10^{-6}$ M, at which concentrations there is a significant effect of the drug in reducing developed shortening and I_{Ca} in guinea-pig single ventricular myocytes. When treatment with captopril is started in patients with heart failure it is usual to prescribe a small first dose because of the risk of hypotension. The mechanism of this 'first-dose' effect is likely to involve enzyme inhibition as the magnitude of the fall in arterial pressure is related to the resting pretreatment plasma renin activity (Dzau *et al.*, 1980; Faxon *et al.*, 1982). However, the finding that captopril has a direct negative inotropic effect *in vitro*, at concentrations similar to those found in plasma after routine administration of the drug, raises the possibility that a reduction of myocardial calcium current *in vivo* may play a part in the hypotensive effect of the drug, particularly in patients with impaired left ventricular function.

There is good evidence that the vasodilator action of captopril cannot be fully explained in terms of reduction of circulating angiotensin II (Gavras *et al.*, 1978). Inhibition of central and peripheral tissue renin conversion has been demonstrated and may be involved in the hypotensive action of the drug (e.g. Jin *et al.*, 1988). Aaronson *et al.* (1988) have described a dihydropyridine-sensitive calcium current in vascular smooth muscle cells from rabbit ear artery. L-type Ca^{2+} current has been shown to be the predominant Ca^{2+} channel in coronary artery smooth muscle cells from guinea-pig (Ganitkevich & Isenberg, 1990). Given this homology between L-type calcium channels in heart and in vascular smooth muscle it is possible that a reduction of I_{Ca} in vascular smooth muscle is contributing to the vasodilatation produced by captopril and so to the reduction of arterial pressure and afterload which follows its therapeutic administration.

We conclude that captopril reduces I_{Ca} and developed length in guinea-pig single ventricular myocytes.

This work was supported by BHF grant no. 87/63. K.O.R. is an MRC Training Fellow. Captopril was obtained as a gift from E.R. Squibb & Sons Ltd. We are indebted to Dr T. Powell for help with single cell isolation and to Mr A.J. Spindler for making equipment. The preliminary patch-clamp experiments were carried out in collaboration with Dr T.J.A. Allen.

References

- AARONSON, P.I., BOLTON, T.B., LANG, R.J. & MACKENZIE, I. (1988). Calcium currents in isolated smooth muscle cells from the rabbit ear artery in normal-calcium and high-barium solutions. *J. Physiol.*, **405**, 57–75.
- ALLEN, I.S., COHEN, N.M., DHALLAN, R.S. & ROGERS, T.B. (1987). Angiotensin II increases the calcium current in neonatal rat single cardiac myocytes. *J. Physiol.*, **390**, 64P.
- BOYETT, M.R., MOORE, M., JEWELL, B.R., MONTGOMERY, R.A.P., KIRBY, M.S. & ORCHARD, C.H. (1988). An improved apparatus for the optical recording of contraction in single heart cells. *Pflügers Arch.*, **413**, 197–205.
- BRYANT, S.M., HART, G. & RYDER, K.O. (1989). Captopril reduces L-type calcium current in isolated guinea-pig ventricular myocytes. *J. Physiol.*, **418**, 167P.
- COHEN, N.M. & LEDERER, W.J. (1987). Calcium current in isolated neonatal rat ventricular myocytes. *J. Physiol.*, **391**, 169–191.
- COLLIS, M.G. & KEDDIE, J.R. (1981). Captopril attenuates adrenergic vasoconstriction in rat mesenteric arteries by angiotensin-dependent and -independent mechanisms. *Clin. Sci.*, **61**, 281–286.
- DE GRAEFF, P.A., VAN GILST, W.H., DE LANGEN, C.D.J., KINGMA, J.H. & WESSELING, H. (1986). Concentration-dependent protection by captopril against ischaemia-reperfusion injury in the isolated rat heart. *Arch. Int. Pharmacodyn.*, **280**, 181–193.
- DOW, J.W., HARDING, N.G.L. & POWELL, T. (1981). Review: isolated cardiac myocytes: I Preparation of adult myocytes and their homology with the intact tissue. *Cardiovasc. Res.*, **15**, 483–514.
- DZAU, V.J., COLUCCI, W.S., WILLIAMS, G.H., CURFMAN, G., MEGGS, L. & HOLLENBERG, N.K. (1980). Sustained effectiveness of converting-enzyme inhibition in patients with severe congestive heart failure. *New Engl. J. Med.*, **302**, 1373–1379.
- FAXON, D.P., HALPERIN, J.L., CREAGER, M.A., GAVRAS, H., SCHICK, E.C. & RYAN, T.J. (1981). Angiotensin inhibition in severe heart failure: acute central and limb hemodynamic effects of captopril with observations on sustained oral therapy. *Am. Heart J.*, **101**, 548–556.
- FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987). Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol.*, **394**, 173–200.
- GANITKEVICH, V.Y. & ISENBERG, G. (1990). Contribution of two types of calcium channels to membrane conductance of single myocytes from guinea-pig coronary artery. *J. Physiol.*, **426**, 19–42.
- GAVRAS, H., BRUNNE, H. & TURINI, G. (1978). Antihypertensive effect of the oral angiotensin converting enzyme inhibitor, SQ 14,225 in man. *New Engl. J. Med.*, **298**, 991–995.
- JIN, M., WILHELM, M.J., LANG, R.E., UNGER, T., LINDPAINTNER, K. & GANTEN, D. (1988). Endogenous tissue renin-angiotensin systems. *Am. J. Med.*, **84** (suppl 3A), 28–36.
- KAMEYAMA, M., HESCHELER, J., HOFMANN, F. & TRAUTWEIN, W. (1986). Modulation of Ca current during the phosphorylation cycle in the guinea pig heart. *Pflügers Arch.*, **407**, 123–128.
- KOCH-WESER, J. (1965). Nature of the inotropic action of angiotensin on ventricular myocardium. *Circ. Res.*, **16**, 230–237.
- LINDPAINTNER, K., JIN, M., WILHELM, M.J., SUZUKI, F., LINZ, W., SCHOELKENS, B.A. & GANTEN, D. (1988). Intracardiac generation of angiotensin and its physiologic role. *Circulation*, **77** (suppl I), I-18–I-23.
- MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1984). The effects of ryanodine, EGTA and low-sodium on action potentials in rat and guinea pig ventricular myocytes: evidence for two inward currents during the plateau. *Br. J. Pharmacol.*, **81**, 543–550.
- NAKASHIMA, A., ANGUS, J.A. & JOHNSON, C.I. (1982). Chronotropic effects of angiotensin I, angiotensin II, bradykinin and norepinephrine in guinea pig atria. *Eur. J. Pharmacol.*, **81**, 479–485.
- TERRAR, D.A. & VICTORY, J.G.G. (1988). Effects of halothane on membrane currents associated with contraction in single myocytes isolated from guinea-pig ventricle. *Br. J. Pharmacol.*, **94**, 500–508.
- WALKER, J.M., BRYANT, S.M. & WESTABY, S. (1988). Captopril has a direct negative inotropic effect on isolated myocardium. *Br. Heart J.*, **59**, 611–612.

(Received July 23, 1990
 Revised October 10, 1990
 Accepted October 12, 1990)