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Cardiac electrophysiological actions of captopril: lack of direct antiarrhythmic effects

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1 Standard microelectrode techniques were used to study the effects of captopril (1, 10 and 100 μM) on action potentials recorded from guinea-pig ventricular cells and sinoatrial node cells.

2 Captopril had no effect on the maximum rate of depolarization ($\dot{V}_{max}$) of ventricular action potentials in cells exposed to either normal Locke solution or 'simulated ischaemic' solution (K^+ = 11.2 mM; pH = 6.4; Po_2 < 80 mmHg), nor was there any augmentation of the normal small decline in $\dot{V}_{max}$ with increasing stimulation rate (range of interstimulus intervals = 2400 ms to 300 ms).

3 Captopril had no effect on the duration of ventricular action potentials, nor did it alter the shortening seen on exposure to simulated ischaemia.

4 Captopril did not alter spontaneous sinus cycle length or any recorded parameter of sinus node action potentials.

5 It is concluded that any antiarrhythmic effects observed during clinical use of captopril are most unlikely to be due to direct actions of the drug on cardiac cell membrane properties.

Introduction

Cardiac failure is a frequent cause of mortality and morbidity. The angiotensin converting enzyme (ACE) inhibitors appear to be of value in alleviating morbidity (Edwards & Padfield, 1985), and at least one of them (enalapril), has now been proven to reduce mortality in this condition (Consensus Trial Study Group, 1987).

In view of the fact that some 35–50% of deaths in chronic cardiac failure are due to ventricular arrhythmias rather than pump failure (Massie & Conway, 1987), it is of interest that enalapril was reported to have made no impact at all on arrhythmic deaths in the Consensus study. There is, however, circumstantial evidence from a number of studies that captopril and possibly enalapril may possess antiarrhythmic properties. Van Gilst *et al.* (1984, 1986) reported that captopril but not enalapril reduced reperfusion arrhythmias in Langendorff-perfused rat hearts following 15 min coronary artery occlusion. Furthermore, at least two controlled, clinical trials of captopril have also shown statistically significant reductions of ventricular arrhythmias (Cleland *et al.*, 1984a,b; Captopril-Digoxin Multicenter Research Group, 1988), and a similar claim has been made for enalapril (Webster *et al.*, 1985).

There are several possible mechanisms by which ACE inhibition might be antiarrhythmic (see Discussion). In addition, captopril but not enalapril, has significant free-radical scavenging ability (Westlin & Mullane, 1988) which could certainly contribute to such an effect. The purpose of the present study, however, was to ascertain whether captopril possesses any direct cellular electrophysiological actions which might potentially be antiarrythmic. To this end, we have used microelectrodes to record action potentials from guinea-pig myocardium during superfusion with normal oxygenated physiological saline and during simulated ischaemia.

Methods

Guinea-pigs were stunned by a blow to the head, their hearts rapidly removed and placed in cold Locke solution. Ventricular myocardial preparations consisted of small strips cut from the right ventricular free wall, pinned to the base of a tissue bath, and superfused with Locke solution (at 36°C) gassed with 95% O_2 and 5% CO_2. The tissue was stimulated by

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constant current pulses of 4 ms duration, from a Grass S11 stimulator, delivered through a bipolar platinum electrode. The stimuli were of sufficient magnitude to maintain a constant latency between the stimulus and the action potential upstroke (Walton & Fozzard, 1979). Sinus node preparations consisted of a small area of the posterior wall of the right atrium, with its borders formed by the crista terminalis, the interatrial septum, and the superior and inferior vena cavae. This preparation beat spontaneously and the area adjacent to the crista terminalis was explored to locate a typical sinoatrial node cell (maximum diastolic potential less than -70 mV, spontaneous diastolic depolarization, and an upstroke dV/dt of less than $10\,V\,s^{-1}$; Campbell, 1987). Once a stable impalement had been maintained for at least 15 min, the experiment was started.

Action potentials were recorded by glass microelectrodes filled with 3 M KCl (resistance 20–30 Mohm) and connected to a high input impedance d.c. amplifier (WPI 750). Action potentials were monitored on an oscilloscope and analyzed by digital techniques modified from previously described methods (Campbell, 1987).

The effects of three concentrations of captopril (supplied by Squibb Australia) on action potential duration and resting and rate-dependent depression of $\dot{V}_{max}$ were investigated, under control and simulated ischaemic conditions. The control Locke solution contained (mM): NaCl 125, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 25, $MgCl_2$ 1.0, NaH_2PO_4 0.44, glucose 11 (pH 7.4). To simulate ischaemia, the concentration of KCl was doubled to 11.2 mM, glucose was omitted from the solution, and bicarbonate concentration was decreased to 2.5 mM, which reduced pH to 6.4. The solution was gassed with 95% N_2 and 5% CO_2, which decreased the Po_2 of the superfusate from >500 mmHg to <80 mmHg. To eliminate any possible 'order' effect, the sequence of exposure to normal Locke solution and simulated ischaemia, and to captopril-containing and control solutions was randomized.

Data are expressed as mean $\pm$ s.d., and Student's t test was used to test the significance of difference of means of individual pairs of samples.

Results

Effects on maximum rate of depolarization ($\dot{V}_{max}$)

In this series of experiments, continuous impalement was maintained to study $\dot{V}_{max}$ in 'normal' Locke solution and under conditions of simulated ischaemia.

Under ischaemic conditions, the cells depolarized from a control resting potential of -93 ± 3.8 mV to -74 ± 6.2 mV in drug-free solution. The presence of captopril in the superfusate in concentrations of 1, 10 and 100 μM had no effect on this parameter, with the ischaemic solution still causing approximately a 20 mV depolarization (from 90.7 ± 1.2 mV to 71.8 ± 6.2 mV at 100 μM captopril; $n = 5$). Neither the change in action potential amplitude (123 ± 3.8 to 95.4 ± 12.8 mV) nor decrease in the rate of rise of the action potential upstroke ($\dot{V}_{max}$) (reduced from $217 \pm 62\,V\,s^{-1}$ to $150 \pm 68\,V\,s^{-1}$; $n = 5$) caused by the depolarizing effect of the ischaemic conditions was altered by the presence of up to 100 μM captopril in the superfusate. The corresponding values (at 100 μM captopril) were a reduction of action potential amplitude from 115 ± 6.6 mV to 89 ± 12.4 mV, and a decrease in $\dot{V}_{max}$ from 235 ± 47.2 to $111 \pm 29.9\,V\,s^{-1}$. The differences in mean values for control and drug solutions did not reach significance. All the differences between 'nonischaemic' and ischaemic solutions were highly significant ($P < 0.001$).

The depression of V_{max} in the absence of prior stimulation is known as resting block (Campbell, 1983), and was measured by recording $\dot{V}_{max}$ of the first action potential of a train following 30 min exposure of unstimulated tissue to captopril (100 μM). No significant resting block was produced by captopril in either normal Locke solution or in simulated ischaemia.

Rate-dependent depression of $\dot{V}_{max}$ was measured by driving the tissue at a series of interstimulus intervals ranging from 2400 ms down to 300 ms, in both control and ischaemic solutions, and measuring the percentage depression of $\dot{V}_{max}$ during trains of 100 action potentials, In control (non-ischaemic) solution, the presence of captopril had no effect on the depression of $\dot{V}_{max}$ seen during the train of action potentials, with the largest decline occurring at an interstimulus interval of 300 ms. This value was $5.6 \pm 1.8\%$ for drug-free solution, and $5.0 \pm 2.5\%$ in the presence of captopril 100 μM ($n = 5$). The values for depression of $\dot{V}_{max}$ in ischaemic conditions were higher than control due to depolarization of the membrane. At an interstimulus interval of 300 ms, $\dot{V}_{max}$ declined by $35.1 \pm 8.9\%$ in the absence of drug, with a corresponding value for the captopril solution of $37.8 \pm 9.4\%$. There were no significant differences between these values, nor the means of the values at the slower driving rates.

Effects on action potential duration

A separate series of experiments was done to monitor any effects on action potential duration. In these studies, impalement of one ventricular cell was maintained while the effects of captopril in control and ischaemic conditions were studied. Action potential duration was measured at both 50% repol-

Table 1 Effects of captopril (100 μM) on sinus node potentials

	Control	*Captopril*	*30 min Wash*
Cycle length (ms)	345 ± 17.1	347 ± 14.8	345 ± 16.9
Amplitude (mV)	76 ± 10.2	79.3 ± 8.7	77 ± 9.1
Maximum diastolic potential (mV)	−63 ± 12.4	−64.3 ± 8.0	−64.6 ± 9.1
$\dot{V}_{max}$ ($V s^{-1}$)	6.4 ± 3.3	6.7 ± 3.4	6.8 ± 2.2
Repolarization rate ($V s^{-1}$)	−0.95 ± 0.18	−0.96 ± 0.11	−0.98 ± 0.15
Phase 4 slope ($mV s^{-1}$)	88 ± 31	92 ± 38	93 ± 36
Action potential duration (ms)	204 ± 8.3	206 ± 12.9	207 ± 10.1

Exposure times = 30–60 min; $n = 6$, $\dot{V}_{max}$ = maximum rate of depolarization.

arization (APD_{50}) and 90% repolarization (APD_{90}). In the absence of drug there was a marked and rapid (maximal in 15–20 min) reduction in both parameters during simulated ischaemia. APD_{50} fell from 141 ± 20.3 ms to 74 ± 27 ms, and APD_{90} from 162 ± 20.6 ms to 90 ± 31.3 ms ($n = 5$; $P < 0.001$). In the presence of captopril 100 μM, the corresponding values for the same 5 cells in normal Locke solution and simulated ischaemia were APD_{50}: 140 ± 20.0 ms to 64 ± 23.5 ms ($P < 0.001$); APD_{90}: 165 ± 19 ms and 83.2 ± 27.2 ms ($P < 0.001$). None of these values was significantly different from the respective value recorded in the absence of drug.

Sinus node potentials

The effects of 30–60 min exposure to captopril 100 μM were studied during continuous impalement of six sinus node cells exposed to normal Locke solution. No drug effects were noted on any parameter recorded (Table 1). In particular the spontaneous cycle length, and rates of depolarization and repolarization were unaltered, suggesting no effect on either inward calcium or outward potassium currents.

Discussion

The evidence suggesting that ACE inhibitors (and particularly captopril) might be antiarrhythmic has been outlined in the Introduction. It is based partly on a series of *in vitro* experiments showing reduced post-ischaemic reperfusion arrhythmias in rat hearts (van Gilst *et al.*, 1984, 1986; Rochette *et al.*, 1987), and partly on the results of three clinical trials involving patients with moderate to severe congestive cardiac failure (Cleland *et al.*, 1984a,b; Webster *et al.*, 1985; Captopril-Digoxin Group, 1988). In Langendorff-perfused rat hearts, pretreatment with a very high concentration of captopril (369 μM) both abolished serious reperfusion arrhythmias after 15 min of coronary artery ligation and markedly reduced noradrenaline overflow (van Gilst *et al.*, 1986). Enalapril had no such effect, although its active metabolite, enalaprilat was not tested. Rochette *et al.* (1987) confirmed this antiarrhythmic action in a very similar preparation using a lower concentration of captopril (50 μM). At this concentration, which is still well above even peak plasma concentrations seen in therapeutic use (< 10 μM; Kripilani *et al.*, 1980; Singhvi *et al.*, 1982), captopril did not alter the large scale release of noradrenaline accompanying reperfusion.

Two of the clinical trials suggesting antiarrhythmic actions for ACE inhibitors have been extremely small, involving only 14 patients and 19 patients respectively (Cleland *et al.*, 1984a,b; Webster *et al.*, 1985). The Captopril-Digoxin Multicenter study (1988) was significantly larger with approximately 100 patients in each of the captopril, digoxin and placebo groups. The mean number of ventricular premature beats per hour fell 29.4% for the captopril patients ($n = 104$), rose 2.3% for the digoxin group ($n = 96$) and fell 16.1% in the placebo-treated patients ($n = 100$). The reduction with captopril was statistically significant when compared to the digoxin patients but not the placebo group.

However, it is open to question whether the modest reductions in ventricular arrhythmias claimed in these studies truly indicate an antiarrhythmic effect (Morganroth, 1984; Bigger & Rolnitzky, 1985). Certainly no evidence for a reduction in sudden death rates can be gleaned from any of these trials, nor was such an effect noted in the Consensus Study (1987). This may, of course, be simply due to a lack of adequate statistical power in the reports to date (Packer, 1987).

There are certainly a number of theoretical grounds for supposing that ACE inhibitors (and captopril in particular) might be antiarrhythmic. Patients in chronic heart failure treated with these agents generally experience a reduction in sympathetic activity. This is at least partly due to withdrawal of reflexly increased tone, but may also result

from prevention of direct and indirect inotropic and arrhythmogenic properties of angiotensin II (Dzau, 1988; Lindpaintner *et al.*, 1988). Noradrenaline levels in blood may come down initially (Cleland *et al.*, 1984a,b) but this effect seems not to persist (Francis *et al.*, 1986). The improvement in total body potassium balance frequently seen after the addition of ACE inhibitors to patients already on diuretic therapy might also reduce the likelihood of arrhythmias. Finally, captopril, but not enalaprilat, has been shown in dog studies to have significant free-radical scavenging properties at clinically relevant concentrations. Pretreatment with captopril led to marked enhancement of recovery of contractile function during 3 h of reperfusion following 15 min of coronary ischaemia (Westlin & Mullane, 1988). Of more relevance to the present study, the incidence of ventricular fibrillation on reperfusion fell from 6 out of 16 (control) to 0 out of 13 (captopril; $P < 0.05$). Oxygen-free radical production is causally implicated in reperfusion arrhythmias (Pallandi *et al.*, 1987) and other free-radical scavengers have been found to be of benefit in these circumstances (Bernier *et al.*, 1986).

The experiments described in the present study however, were performed to ascertain whether the ACE inhibitor captopril showed evidence of any direct cellular electrophysiological actions which might be antiarrhythmic. In particular, we were interested in identifying any effects on $\dot{V}_{max}$ on action potential duration, or on sinus node potentials. In concentrations up to 100 μM, well above the usual levels found during clinical use, captopril had no significant influence on any of these parameters. Nor were any effects seen in the presence of the 'ischaemic' superfusate, which is probably more representative of the extracellular fluid in the immediate vicinity of arrhythmogenic foci.

It seems reasonable to assume then, that if captopril is exerting any beneficial effect on arrhythmic morbidity and mortality in patients with congestive cardiac failure, such an action is not mediated by any of the established, direct antiarrhythmic mechanisms (Vaughan Williams, 1984). It must be stated, however, that with the possible exception of amiodarone (Dargie *et al.*, 1987; Neri *et al.*, 1987), the available 'traditional' antiarrhythmic agents appear to be of little if any value in preventing or reducing the very high incidence of ventricular tachycardia and sudden death in such patients (Wilson, 1987; Prystowsky, 1988). Indeed the problem of possible proarrhythmic effects of these drugs is a matter for concern (Podrid, 1985).

It may be that the indirect antiarrhythmic properties listed above for captopril and related drugs are ultimately of greater therapeutic benefit in heart failure patients than the direct actions of the traditional antiarrhythmic compounds.

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