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Short communication

Effects of metoprolol and ramipril on action potentials after myocardial infarction in rats

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Abstract

The effects of chronic treatment with the β-adrenoceptor antagonist metoprolol, the angiotensin converting enzyme inhibitor ramipril, their combination, or placebo on action potential configuration 6 weeks after myocardial infarction in rats were studied. Action potentials were measured in isolated left ventricular posterior papillary muscles and compared with action potentials from a sham operated group without infarction. After infarction, the action potential amplitude was reduced and this phenomenon was partially reversed by metoprolol- and ramipril-treatment. Prolonged repolarisation after infarction compared to sham operated animals was additionally delayed after metoprolol treatment. Thus, metoprolol extends the refractory period, which may counteract tachyarrhythmia.

Keywords: Metoprolol; Ramipril; Myocardial infarction; Action potential; Papillary muscle

1. Introduction

Myocardial infarction causes hypertrophy, associated with phenotypic modulation of cardiomyocytes, electrical abnormalities, and rhythm disturbances (Hart, 1994). Beneficial actions of β-adrenoceptor antagonists and angiotensin converting enzyme inhibitors after myocardial infarction have been demonstrated in clinical and in experimental studies (Hjalmarson et al., 1983; Acute Infarction Ramipril Efficacy (AIRE) study investigators, 1993). The combination of both drugs, blocking different signalling pathways of cardiac hypertrophy and remodelling, has been found to exert additional positive effects on contractile function after infarction (Wagner et al., 1997). However, little is known about alterations in electrophysiological parameters in response to chronic angiotensin converting enzyme inhibition and β-adrenoceptor antagonism. Changes in cardiac electrophysiology may account at least in part for the antiarrhythmic actions of these drugs. Therefore, we studied action potentials from rat myocardium with experimental infarction after 6 weeks of treatment with the β-adrenoceptor antagonist metoprolol, the angiotensin converting enzyme inhibitor ramipril, or a combination of both.

2. Methods

The animals were cared in accordance with the German Federal Animal Welfare Legislation. Myocardial infarction was induced by ligation of the left coronary artery in 12-week-old males Wistar rats as previously described (Pfeffer et al., 1979). Sham operated animals, where the ligation was placed near the coronary artery (group SO, $n = 13$), served as controls. Rats with infarction were randomised for treatment. Metoprolol (350 mg kg^{-1} day^{-1}, $n = 20$), ramipril (1 mg kg^{-1} day^{-1}, $n = 23$), a combination of both drugs ($n = 21$), or placebo (P, $n = 18$) were given for 6 weeks, starting 1 day after surgery. Treatment was stopped 36 h before the start of the experiments. Isolated left ventricular posterior papillary muscles were electrically stimulated at 0.5 Hz in a solution of the following composition (in mmol/l): NaCl 140.0, KCl 5.0, $CaCl_2$ 1.5, $MgCl_2$ 1.1, Tris–HCl 10.0, glucose 11.1 (pH 7.4 at 31°C). To avoid the build up of a hypoxic core, the papillary muscles were allowed to equilibrate for 25 min at a preload of 50 mN and a stimulation frequency of 0.25 Hz

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in 0.75 mmol/l $CaCl_2$. To test whether the oxygen supply to the hypertrophied preparations was reduced after equilibration, the stimulation frequency was increased four-fold for 5 min. This maneuver did not impair contractile function, suggesting that tissue oxygen delivery was sufficient. Only macroscopically and microscopically unscarred posterior papillary muscles were used for action potential measurements. Cellular electrical activity was recorded using a current clamp scheme as previously described (Kiseleva et al., 1998). Briefly, an Ag/AgCl wire in a glass micropipette filled with 1.5 mol/l KCl served as reference electrode in the perfusion chamber. A short Ag/AgCl pin, connected to the headstage of the amplifier via a 30 μm platinum–iridium wire, was inserted into a floating glass microelectrode filled with 1.5 mol/l KCl. The amplifier was connected to an oscilloscope, a DAT-recorder and a personal computer. Data were digitised, stored, and analysed off-line using software developed in our laboratory. Recovery of action potentials was characterised by their amplitude after extra-stimulations with an interval between the beginning of the extra-stimulus and the onset of the preceding stimulus ranging from 180 to 350 ms. Values are given as means ± SEM. Intergroup comparisons were performed via one-way analysis of variance (ANOVA) with the Bonferroni-test as post-hoc test. Significance was assumed at $P < 0.05$. Metoprolol and ramipril were provided by Astra Hässle, Mölndal, Sweden.

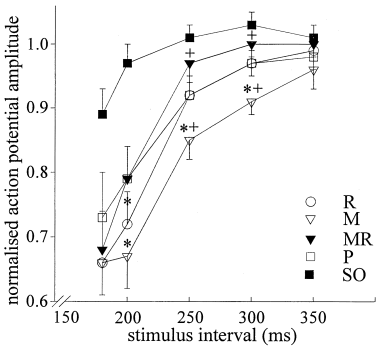

Fig. 1. Recovery of action potential amplitude after extrastimulation intervals between 180 and 350 ms in the sham operated group without myocardial infarction (SO, $n = 13$), in posterior left ventricular papillary muscles from rats with myocardial infarction after 6 weeks of treatment with metoprolol (M, $n = 20$), ramipril (R, $n = 23$), combined treatment (MR, $n = 21$), and in the placebo group (P, $n = 18$). Values are given as means ± SEM. Intergroup comparisons were performed by one-way ANOVA with the Bonferroni-test as post-hoc test. * indicates significance ($P < 0.05$) vs. SO and + vs. alternative treatment.

3. Results

The infarct sizes were 41 ± 2% of the left ventricular endocardial surface in the metoprolol group, 38 ± 3% in the ramipril group, 33 ± 4% after combined treatment, and 37 ± 5% in the placebo group, and not significantly different between the groups. The heart weight/body weight ratios (mg/g) were significantly higher compared to the sham operated group (2.7 ± 0.0) after metoprolol (3.6 ± 0.1), ramipril (3.6 ± 0.1), and placebo treatment (3.7 ± 0.1), but not after combined treatment (3.3 ± 0.2). Also, the papillary cross-section areas were significantly higher in the placebo (3.1 ± 0.3 mm^2) and metoprolol (2.7 ± 0.2 mm^2) groups, but not after ramipril (2.6 ± 0.2 mm^2) and combined (2.4 ± 0.2 mm^2) treatment when compared to sham operated controls (2.2 ± 0.2 mm^2). Mean values of resting membrane potentials ranging between −85 and −90 mV were comparable in all groups. Action potential amplitudes were clearly reduced in the placebo group when compared to sham operated animals. Treatment with metoprolol or ramipril partially restored the action potential amplitudes (Table 1). Chronic treatment with ramipril for 3 ($n = 12$) or 6 ($n = 6$) weeks in rats without infarction, however, had no effect on action potential characteristics when compared to age-matched untreated controls ($n = 13$ each, data not shown).

Table 1
Action potential characteristics from sham operated hearts, and after treatment with ramipril, metoprolol, their combination, or placebo after myocardial infarction
Values are given as means ± SEM. Intergroup comparisons were performed via one-way ANOVA with the Bonferroni-test as post-hoc test.
APD_{35}, APD_{50}, APD_{95}: action potential duration at 35, 50, and 95% of repolarization, respectively.

Group	R ($n = 23$)[a]	M ($n = 20$)[b]	MR ($n = 21$)[c]	P ($n = 18$)[d]	SO ($n = 13$)[e]
AP-amplitude (mV)	106 ± 4[†]	106 ± 5[†]	105 ± 6	91 ± 6*	118 ± 6
APD35 (ms)	36 ± 5*	64 ± 7[†]*	37 ± 4*	44 ± 6*	14 ± 2
APD50 (ms)	69 ± 8*	101 ± 1[†]*	63 ± 6*	72 ± 7*	25 ± 3
APD95 (ms)	259 ± 13*	312 ± 11[†]*	243 ± 11*	254 ± 12*	185 ± 12

[a] Ramipril treatment.
[b] Metoprolol treatment.
[c] Combined treatment with metoprolol and ramipril.
[d] Placebo.
[e] Sham operation without myocardial infarction.
[†] $P < 0.05$ pharmacological treatment vs. placebo.
* $P < 0.05$ vs. the sham operated group.

In the placebo group, all phases of repolarisation were delayed when compared to sham operation as indicated by higher action potential durations at 35% (APD_{35}), 50% (APD_{50}), and 95% (APD_{95}) of repolarisation (Table 1). Ramipril and combined treatment did not significantly affect the delayed repolarisation after myocardial infarction. Only treatment with metoprolol resulted in a marked prolongation of all mentioned phases of repolarisation studied, especially of the late repolarisation. Hence, recovery of action potential amplitude, indicating reactivation of Na^+ conductance after short extra-stimulation intervals, was clearly delayed after metoprolol compared to sham operation and combined treatment (Fig. 1). On the other hand, the particularly short repolarisation in the sham operated group was associated with very fast recovery of action potential amplitudes.

4. Discussion

Our study demonstrates that myocardial infarction results in the development of cardiac hypertrophy, which is partially prevented by combined treatment with metoprolol and ramipril. Ramipril alone did not markedly change the heart weight to body weight ratios after myocardial infarction, but significantly reduced the cross-sectional areas of papillary muscles. This apparent discrepancy might be explained by the inclusion of a scar tissue in the heart weight measurements, which may underestimate the degree of cardiac hypertrophy and, as a consequence, the anti-hypertrophic effect of ramipril.

Development of hypertrophy after myocardial infarction is associated with phenotypic alterations at the cellular level including changes of ionic conductances (Qin et al., 1996). The reduced action potential amplitude after infarction compared to that of sham-operated non-infarcted animals corresponds to a lower amplitude of I_{Na}, which has been shown for canine myocardium after infarction (Pu and Boyden, 1997). A delayed recovery of I_{Na} from inactivation is less likely to account for the reduced action potential amplitude in the placebo group, since the recovery is already complete after 350 ms of extrastimulation interval. The prolonged action potential duration in the placebo group vs. sham operation may result from decreased I_K in post-infarction rat myocardium (Qin et al., 1996). Furthermore, an increased activity of the Na^+–Ca^{2+} exchanger, which has been described for post-infarct hearts (Yoshiyama et al., 1997), may contribute to the observed action potential lengthening (Wagner et al., 1998).

To test whether the beneficial effects of β-adrenoceptor antagonists and angiotensin converting enzyme inhibitors after myocardial infarction (Hjalmarson et al., 1983; Acute Infarction Ramipril Efficacy (AIRE) study investigators, 1993) could be at least in part due to normalisation of electrophysiological parameters, we measured action potentials after long-term treatment with metoprolol or ramipril. In this study, metoprolol and ramipril tended to normalise the action potential amplitudes, which critically depend upon the relatively high Na^+ conductance. It is likely that this partial normalisation was due to the actions of chronic ACE-inhibition and β-adrenoreceptor blockade on phenotypic alterations after MI, since chronic ramipril treatment in animals without infarction had no effect on action potential characteristics (data not shown) and chronic metoprolol treatment in healthy rabbits did not affect action potential amplitudes either (Raine and Vaughan Williams, 1981).

Ramipril did not significantly influence the time course of repolarisation, but metoprolol treatment clearly prolonged the action potential duration in all phases of repolarisation. It is unclear why the combined treatment slightly decreased the action potential duration. Synergistic effects between the renin–angiotensin system and catecholamines in the development of hypertrophy have been reported (Vogt et al., 1991). It is possible, therefore, that angiotensin converting enzyme inhibition reduced the influence of cellular signals required for action potential prolongation after chronic metoprolol treatment.

The delayed recovery of action potential amplitudes after extra-stimulation in the metoprolol group indicates overlapping of the prolonged repolarisation with very early action potential amplitude recovery. The prolonged repolarisation after myocardial infarction, especially in the metoprolol group, may not simply be related to the degree of hypertrophy because ramipril treatment reduced hypertrophy without normalising the action potential duration. Furthermore, it is possible that long-term application of metoprolol and ramipril may have caused ultrastructural alterations and changes of ion conductances of the preparations. It seems less likely, however, that changes in passive or electrotonic properties may account for the observed alterations in action potential characteristics, since ramipril, which is known to reduce fibrosis (Nagasawa et al., 1995) did not normalise the recovery of action potential amplitudes after extrastimulation. Tissue hypoxia of the hypertrophied preparations can be excluded as a reason for reduced action potential amplitude and prolonged action potential duration because electron microscopic examination of representative hypertrophied papillary muscles did not reveal morphological features of hypoxic damage (data not shown). Taken together, we can assume that altered action potential characteristics after myocardial infarction and the additional action potential prolongation after chronic β-adrenoceptor antagonism may result from modified ion conductances. However, a definite answer regarding passive and electrotonic properties and altered ion conductances can only be obtained from patch–clamp experiments, which is impossible to perform in the contracting multicellular preparations.

In summary, the prolonged action potential duration and refractory period after β-adrenoceptor antagonism is suggested to contribute to an antiarrhythmic effect after infarc-

tion. The beneficial effects of β-adrenoceptor antagonists after myocardial infarction reported from clinical studies may be related to suppression of tachyarrhythmia due to action potential prolongation and the recently reported higher Ca^{2+} sensitivity of the myofilaments resulting in improved contractile function (Wagner et al., 1997).

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