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Facilitation of spontaneous defibrillation by moxonidine during regional ischaemia in an isolated working rabbit heart model

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Abstract

Moxonidine has been shown to be antiarrhythmic during ischaemia in vivo. This study aimed to investigate its electrophysiological effects in isolated working rabbit hearts in vitro. Monophasic action potential duration, effective refractory period and conduction delay were measured at three ventricular sites. The hearts were treated before and during ischaemia and reperfusion with vehicle, moxonidine (0.01, 0.1 and 1 μM) or labetalol (1 μM). In all groups, ventricular fibrillation was always induced during ischaemia. Only 0.1 μM moxonidine decreased the incidence of sustained ventricular fibrillation from 86 to 17%, although it did not affect any electrophysiological parameters measured. Similarly, labetolol, an adrenoceptor blocker, facilitated spontaneous defibrillation without any electrophysiological effects. In conclusion, moxonidine directly facilitates spontaneous defibrillation of ventricular fibrillation during ischaemia. Since the same effect is observed with labetalol, it is possible that the defibrillatory action of moxonidine is related to its peripheral antiadrenergic activity, although other mechanisms cannot be excluded.

Keywords: Myocardial ischaemia; Arrhythmia; Moxonidine; Labetalol; Chemical defibrillation

1. Introduction

Moxonidine, an imidazoline I_1 receptor agonist, inhibits central sympathetic cardiovascular outflow and is currently used in the treatment of hypertension (Ernsberger et al., 1992, 1993a; Ollivier and Christen, 1994). Interestingly, an antiarrhythmic action of moxonidine has been reported in ischaemia-reperfusion arrhythmias in vivo (Leprán and Papp, 1994). The authors ascribed this effect to the central action of the drug. This mechanism of antiarrhythmic action has also been implicated to explain the effects of imidazoline I_1 receptor agonists on some non-ischaemic arrhythmias (Hayashi et al., 1993; Mest et al., 1995). The possibility of direct cardiac antiarrhythmic effects of moxonidine during ischaemia has not been studied, although there is some indirect evidence that this could be the case.

First, imidazoline compounds, including clonidine, have been shown to inhibit K_{ATP} channels in guinea pig ventricular myocytes—an effect related to interaction with imidazoline receptors, but not adrenoceptors (Lee et al., 1995). Whereas clonidine displays a 5-fold selectivity for the α_{2B}-adrenoceptors over the imidazoline I_1 receptors, moxonidine shows nearly 600–700-fold selectivity for imidazoline I_1 receptors relative to the α_{2B}-adrenoceptor subtype (Ernsberger et al., 1992, 1993a; Ollivier and Christen, 1994). Therefore, it is possible that moxonidine might also block K_{ATP} channels. Since K_{ATP} channel blockers have been suggested to decrease the magnitude of ischaemia-induced action potential shortening and the incidence of ischaemia-related arrhythmias (Hicks and Cobbe, 1991; Wilde and Janse, 1994), it cannot be excluded that moxonidine would act similarly. Secondly, imidazoline receptors have been shown to exist on postganglionic sympathetic nerves of the rabbit heart and their stimulation has been suggested to inhibit electrically evoked noradrenaline overflow (Fuder and Schwarz, 1993). Also, moxonidine has been shown to inhibit electrically evoked norepinephrine release from the rabbit pulmonary artery and aorta, although the contribution of imidazoline receptors to this

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effect is not clear (Molderings et al., 1991; Göthert and Molderings, 1992). If moxonidine were able to directly inhibit ischaemia-induced catecholamine release in the heart, it could exert its antiarrhythmic effects during myocardial ischaemia through this mechanism. Finally, moxonidine has been found to increase effective refractory period in canine ventricular myocardium and in isolated rabbit papillary muscle, which may be yet another mechanism of its direct antiarrhythmic action (Papp, private communication).

Consequently, the aim of this study was to assess the direct electrophysiological and antiarrhythmic effects of moxonidine in a previously described isolated working rabbit heart model of regional myocardial ischaemia and reperfusion (Wolk et al., 1998a,b). In view of its proposed ability to reduce noradrenaline release, moxonidine was compared with labetalol, which has combined β_1-, β_2- and α_1-adrenoceptor blocking properties (Brittain et al., 1982; Gold et al., 1982).

2. Materials and methods

2.1. Whole heart preparation

The isolated working rabbit heart preparation was used, as described previously (Wolk et al., 1998a,b). Briefly, male New Zealand White rabbits (weight 2.5–3.8 kg) received heparin (2000 IU) and were euthanised with sodium pentobarbitone (100 mg kg^{-1}). The hearts were excised and perfused with oxygenated modified Tyrode solution (pH = 7.4; composition in mM: Na^+ 142.0, K^+ 4.0, Ca^{2+} 1.8, Mg^{2+} 1.0, Cl^- 121.0, HCO_3^- 28.0, $H_2PO_4^-$ 0.4, glucose 11.0) in a working heart mode (preload: 10 cm H_2O; afterload: 75 cm H_2O). The right atrium was paced at 3.3 Hz and epicardial temperature was maintained at 35°C. In order for a preparation to be included in the study, a minimum baseline aortic forward flow of 80 ml min^{-1}, measured using an inline flow meter, was required.

2.2. Electrophysiological and other measurements

Monophasic action potentials were recorded continuously using three custom-made suction electrodes. One electrode was located in the apical region of the left ventricle that was subsequently made ischaemic by coronary occlusion (area at risk), and the other electrodes were placed in a non-ischaemic area above the area at risk (area above occlusion) and in the lower half of the right ventricular free wall. A pair of platinum electrodes was inserted not more than 1–1.5 mm from each monophasic action potential electrode in all three areas. Local effective refractory periods were determined during local ventricular pacing by the extrastimulus technique, using square wave impulses of 2 ms duration at twice diastolic threshold. Following a train of eight regular stimuli with a cycle length of 300 ms, an early 'ineffective' extrastimulus was introduced; during consecutive stimulation cycles the extrastimulus was introduced at progressively longer coupling intervals in 5 ms steps until it triggered an action potential. The effective refractory period was defined as the longest coupling interval at which an extrastimulus failed to produce a propagated ventricular response.

Ventricular fibrillation threshold was determined during atrial pacing by local application of a train of 10 consecutive stimuli (all with a duration of 2 ms and at 10 ms intervals) generated by a constant current stimulator (model DS7, Digitimer, UK). This train of stimuli was visually monitored on an oscilloscope to ensure that it was present during the vulnerable period. The local stimulus strength was set at 5 mA and then it was increased at 5 mA steps until ventricular fibrillation was produced or until 100 mA was achieved.

Haemodynamic variables from the working hearts were recorded continuously. These were mean aortic forward flow using an inline flow meter (model T106, Transonic Systems, Ithaca NY, USA) and left intraventricular pressure which was measured with a venflon catheter inserted into the left ventricle and connected to a pressure transducer (model: P23XL, Gould, UK). In addition, haemodynamic variables from the working hearts (mean aortic forward flow, left ventricular peak systolic and end diastolic pressures) were recorded continuously throughout the duration of each experiment. At the end of the reperfusion period the coronary artery was occluded again at the same site as previously and Evans blue dye was injected into the perfusate to distinguish the area at risk (calculated as a percentage of the left ventricular mass).

2.3. Experimental protocol

The hearts were perfused in a working heart mode for 50–60 min during which time all electrodes and other measuring devices were applied. At the end of this period the perfusate was replaced by fresh Tyrode solution and an equilibration period of 15–20 min was allowed, after which two series of measurements were made with a 30 min interval between them. Monophasic action potentials and conduction delays were recorded simultaneously at all 3 sites. Effective refractory periods and diastolic stimulation thresholds were measured first in the right ventricle, followed immediately by measurements in the area above occlusion and then in the area at risk. After the second series of measurements had been completed, the solution was changed for a second time. The new solution contained either vehicle or a drug. Once a new equilibrium had been established, all electrophysiological measurements were repeated at another two time points, 30 min apart.

Subsequently, a period of 30 min of ischaemia was induced by tightening of the snare around the coronary artery. Local monophasic action potentials were measured

continuously together with haemodynamic parameters. Effective refractory periods were measured 15 min into ischaemia in the same order as described previously. When any sustained arrhythmia appeared spontaneously or was induced by the stimulation protocol, direct electrical defibrillation was used after 30 s to restore normal rhythm and time was allowed for stability to be re-established. If no arrhythmia was induced during the measurement of effective refractory periods, ventricular fibrillation threshold was measured and any induced arrhythmia was terminated after 30 s. Any arrhythmia lasting 30 s or more was considered sustained (Ryan et al., 1996).

Following the 30 min period of regional ischaemia, the snare on the coronary artery was released and reperfusion was allowed for 15 min. Local monophasic action potentials as well as haemodynamic recordings were measured continuously and effective refractory periods were measured at 15 min of reperfusion. If no arrhythmia was induced during the measurement of effective refractory periods, ventricular fibrillation threshold was measured and any induced arrhythmia was terminated after 30 s.

2.4. Drugs

The hearts were randomly assigned to the following groups: vehicle control (0.9% NaCl) ($n = 7$), 0.01 μM moxonidine ($n = 6$), 0.1 μM moxonidine ($n = 6$), 1 μM moxonidine ($n = 6$) and 1 μM labetalol ($n = 6$). Moxonidine was diluted in 0.9% NaCl to a range of different concentrations and frozen in 1 ml aliquots. During the experiments, the appropriate 1 ml aliquot was thawed and added to circulating Tyrode solution to obtain the desired final concentration. Labetalol was made up fresh daily and added to Tyrode solution to achieve the concentration of 1 μM. In the control experiments 1 ml of 0.9% NaCl was added to circulating Tyrode solution.

2.5. Data analysis

The monophasic action potential signals were recorded on a videotape recorder and were analysed off-line, as previously described (Wolk et al., 1998a,b). The action potential duration was measured at 90% repolarisation and the conduction delay was taken as the time from the atrial pacing trigger to the onset of the monophasic action potential. Dispersion of monophasic action potentials was calculated as the difference between monophasic action potential duration in the area at risk and the area above occlusion and between the area at risk and the right ventricle. In addition, dispersion of repolarisation was calculated as: (monophasic action potential duration + delay in the control area) − (monophasic action potential duration + delay in the area at risk). Dispersion of effective refractory periods and dispersion of refractoriness were calculated in a manner similar to that for dispersion of monophasic action potentials and dispersion of repolarisation, respectively. Post-repolarisation refractoriness was calculated as local effective refractory period minus monophasic action potential duration.

For statistical analysis a Student's paired 2-tailed *t*-test was used to assess changes within each group produced by a drug/vehicle, 15 min of ischaemia or 15 min of reperfusion. Analysis of variance (ANOVA) followed by *t*-tests with Bonferroni's correction were employed for comparisons between the groups at baseline, after drug/vehicle administration, 15 min of ischaemia and 15 min of reperfusion, as well as for comparison between the magnitudes of changes in different parameters produced by a drug/vehicle. A chi-square test followed (when appropriate) by Fisher's exact test were used for comparison of the incidence of different arrhythmic events. A P value of less than 0.05 was considered statistically significant. All data are expressed as mean ± S.E.M.

3. Results

3.1. Electrophysiological effects of moxonidine and labetalol before ischaemia

In non-ischaemic conditions, there were no statistically significant differences in monophasic action potential duration and effective refractory period between experimental groups in any area. Basal values of monophasic action potential duration and effective refractory period in the area at risk were 119 ± 1 and 126 ± 4 ms, respectively. Neither monophasic action potential duration nor effective refractory period were affected by moxonidine or labetalol in any area. For example, in the area at risk the magnitudes of changes in monophasic action potential duration and effective refractory period following vehicle/drug administration were 5 ± 2, 6 ± 3, 12 ± 3, 11 ± 2 and 12 ± 2 ms for changes in monophasic action potential duration (not significant) and 7 ± 3, 4 ± 5, 9 ± 2, 11 ± 4 and 14 ± 2 ms for changes in effective refractory period (not significant) in the vehicle, 0.01 μM moxonidine, 0.1 μM moxonidine, 1 μM moxonidine and 1 μM labetalol groups, respectively. Both conduction delay and diastolic stimulation threshold were also similar in all groups and were not markedly affected by any drug intervention, the respective baseline values in the area at risk in the vehicle-treated hearts being 102 ± 3 ms and 0.57 ± 0.1 V.

3.2. Electrophysiological effects of moxonidine and labetalol during ischaemia / reperfusion

Ischaemia/reperfusion-induced changes in monophasic action potential duration and effective refractory period in the vehicle, 0.1 μM moxonidine and 1 μM labetalol group are illustrated in Figs. 1 and 2, respectively. In the vehicle-treated hearts, regional ischaemia shortened monophasic action potential duration (from 123 ± 1 to

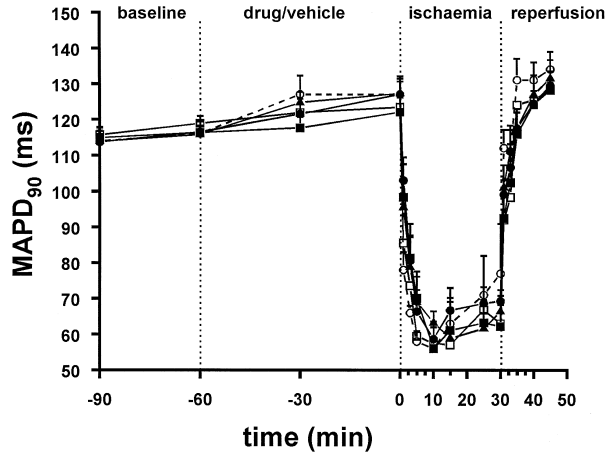

Fig. 1. Monophasic action potential duration ($MAPD_{90}$) in the area at risk before and during ischaemia and reperfusion in the vehicle (□), 0.01 μM moxonidine (■), 0.1 μM moxonidine (△), 1 μM moxonidine (•) and 1 μM labetalol (– ◦ –) groups. In the vehicle group $n = 7$ before and until the 15th min of ischaemia and $n = 5$ thereafter. In all the other groups $n = 6$ throughout the experimental protocol. Ischaemia shortened monophasic action potential duration in each group ($P < 0.05$), but analysis of variance did not reveal any statistically significant differences between the groups.

57 ± 2 ms, $P < 0.05$) and effective refractory period (from 132 ± 3 to 76 ± 4 ms, $P < 0.05$), without any changes in the area above occlusion or in the right ventricle. The magnitude of monophasic action potential shortening at 15 min was similar in the vehicle, 0.01 μM moxonidine, 0.1 μM moxonidine, 1 μM moxonidine and 1 μM labetalol groups (66 ± 2, 61 ± 8, 68 ± 5, 60 ± 5 and 65 ± 8 ms, respectively, not significant). Also, the magnitude of effective refractory period shortening was similar in all the groups, the respective values being 56 ± 4, 51 ± 7, 56 ± 6, 59 ± 3 and 57 ± 5 ms (not significant). All these changes were reversible upon reperfusion. Neither postrepolarisation refractoriness nor diastolic stimulation threshold were significantly affected by myocardial ischaemia in any group, the respective values before and during ischaemia in the vehicle-treated hearts being 8 ± 3 vs. 19 ± 6 ms (not significant) for the postrepolarisation refractoriness and 0.57 ± 0.1 vs. 0.5 ± 0.1 V (not significant) for the diastolic stimulation threshold.

Ischaemia-induced changes in dispersion of monophasic action potential durations and effective refractory periods in the vehicle, 0.1 μM moxonidine and 1 μM labetalol group are illustrated in Figs. 3 and 4. Dispersion of monophasic action potential durations and effective refractory periods between the area at risk and the area above occlusion increased during ischaemia in the vehicle treated hearts from 5 ± 2 to 72 ± 4 ms ($P < 0.05$) and from 4 ± 2 to 61 ± 5 ms ($P < 0.05$), respectively. The magnitude of the ischaemia-induced increase in dispersion of monophasic action potential durations and effective refractory periods was not affected by any drug intervention. Although conduction delay increased in the area at risk during ischaemia (by 10 ± 2 ms in the vehicle group), the magnitude of this effect was not influenced by any drug intervention and, consequently, there were no significant differences between the groups with regards to dispersion of repolarisation or refractoriness (not shown). Similarly, none of the pharmacological interventions used had any effect

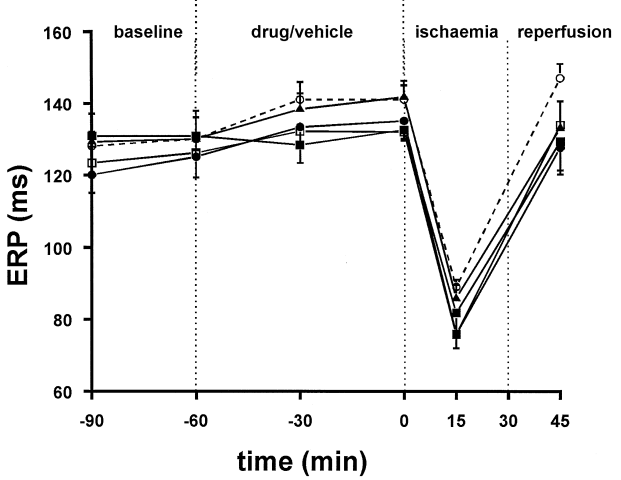

Fig. 2. Effective refractory period (ERP) in the area at risk before and during ischaemia and reperfusion in the vehicle (□), 0.01 μM moxonidine (■), 0.1 μM moxonidine (△), 1 μM moxonidine (•) and 1 μM labetalol (– ◦ –) groups. In the vehicle group $n = 7$ before and during ischaemia and $n = 5$ thereafter. In all the other groups $n = 6$ throughout the experimental protocol. Ischaemia shortened effective refractory period in each group ($P < 0.05$), but analysis of variance did not reveal any statistically significant differences between the groups.

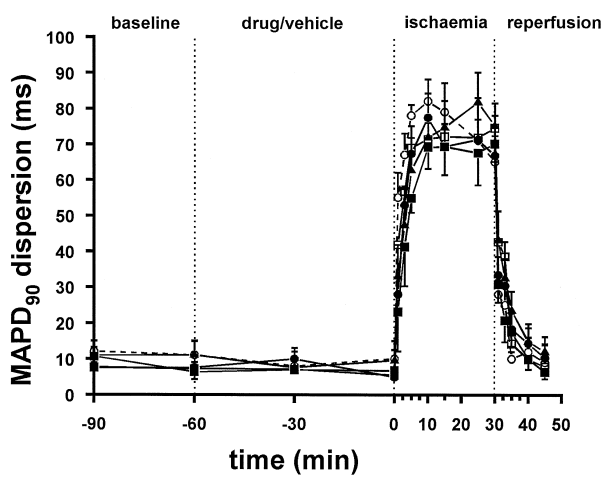

Fig. 3. Dispersion of monophasic action potential duration ($MAPD_{90}$) between the area at risk and the area above occlusion before and during ischaemia and reperfusion in the vehicle (□), 0.01 μM moxonidine (■), 0.1 μM moxonidine (△), 1 μM moxonidine (•) and 1 μM labetalol (– ◦ –) groups. In the vehicle group $n = 7$ before and until the 15th min of ischaemia and $n = 5$ thereafter. In all the other groups $n = 6$ throughout the experimental protocol. Ischaemia increased dispersion of monophasic action potential duration in each group ($P < 0.05$), but analysis of variance did not reveal any statistically significant differences between the groups.

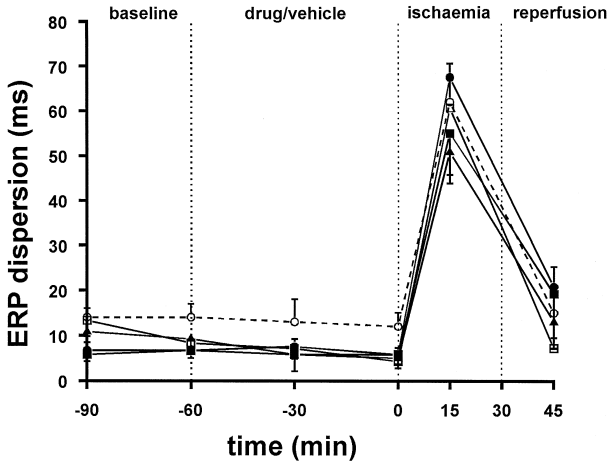

Fig. 4. Dispersion of effective refractory period (ERP) between the area at risk and the area above occlusion before and during ischaemia and reperfusion in the vehicle (□), 0.01 μM moxonidine (■), 0.1 μM moxonidine (△), 1 μM moxonidine (●) and 1 μM labetalol (– ◦ –) groups. In the vehicle group $n = 7$ before and during ischaemia and $n = 5$ thereafter. In all the other groups $n = 6$ throughout the experimental protocol. Ischaemia increased dispersion of effective refractory period in each group ($P < 0.05$), but analysis of variance did not reveal any statistically significant differences between the groups.

on the magnitude of electrical dispersion between the right and the left ventricle (not shown).

3.3. Ischaemia- and reperfusion-induced arrhythmias

No spontaneous arrhythmias were observed before or during myocardial ischaemia in any of the groups. In the vehicle-treated hearts during ischaemia, application of an extrastimulus during the measurement of effective refractory period always resulted in ventricular fibrillation (100%). Neither moxonidine nor labetalol had any effect on inducibility of ventricular fibrillation by single extrastimuli during ischaemia, the observed incidence being 100% of hearts in the 0.01, 0.1 and 1 μM moxonidine groups and 83% of hearts in the 1 μM labetalol group (not significant). However, both moxonidine and labetalol facilitated spontaneous termination of ventricular fibrillation, so that the incidence of sustained ventricular fibrillation (≥ 30 s) was significantly reduced in the 0.1 μM moxonidine and in the 1 μM labetalol group (Table 1).

During reperfusion, spontaneous ventricular fibrillation occurred in 60% of vehicle-treated hearts and its incidence tended to be decreased by the highest concentration of moxonidine and labetalol (Table 1). The failure to achieve statistical significance for this effect is probably related to a smaller number of observations in the vehicle-treated group during reperfusion ($n = 5$), since 2 hearts in this group did not recover after defibrillation during ischaemia. The incidence of transient idioventricular rhythm was not significantly affected by any drug intervention and at 15 min of reperfusion single extrastimuli during the measurements of effective refractory periods did not induce any arrhythmia in any of the groups (Table 1). Ventricular fibrillation threshold protocol produced ventricular fibrillation in all hearts in the vehicle group (at a current of 35 ± 15 mA), 67% of hearts in the 0.01 μM moxonidine group (at a current of 26 ± 11 mA), 80% of hearts in the 0.1 μM moxonidine group (at a current of 31 ± 4 mA), 83% of hearts in the 1 μM moxonidine group (at a current of 32 ± 3 mA) and 25% of hearts ($P < 0.05$) in the 1 μM labetalol group (at a current of 27 ± 12 mA).

3.4. Haemodynamic effects

Neither drug had any effect on mean forward flow and intraventricular systolic or end diastolic pressures throughout the experimental protocol. In the vehicle-treated group, prior to coronary artery occlusion, there was a gradual decline in mean forward flow (from 131 ± 12 to 90 ± 14 ml/min, $P < 0.05$) and in intraventricular peak systolic pressure (from 106 ± 4 to 94 ± 4 mmHg, $P < 0.05$) in the vehicle treated group. Coronary artery occlusion resulted in an immediate further fall in both flow and systolic pressure (from 90 ± 14 to 48 ± 11 ml/min and from 94 ± 4 to 81 ± 3 ml/min, respectively, $P < 0.05$), which did not fully recover during reperfusion (relative to their

Table 1
Incidence of ventricular arrhythmias during ischaemia (first two columns) and reperfusion in vehicle, moxonidine and labetalol treated, isolated working rabbit hearts

	Ischaemia (%)		Reperfusion (%)		
	Induced VF	Sustained VF	Spontaneous VF	Idioventricular rhythm	Induced VF
Vehicle	100	86	60	40	0
Moxonidine 0.01 μM	100	83	67	50	0
Moxonidine 0.1 μM	100	17[a]	33	17	0
Moxonidine 1 μM	100	50	0[b]	17	0
Labetalol 1 μM	83	0[a]	0[b]	80	0

VF–ventricular fibrillation; Induced VF–VF induced during ERP measurements at 15 min of ischaemia or reperfusion; Sustained VF–a proportion of induced VF lasting ≥ 30 s.
[a] Indicates a statistically significant difference from the vehicle group ($P < 0.05$).
[b] Indicates a P value of 0.06 in relation to the vehicle group.

ischaemic values). Intraventricular end diastolic pressure was 9 ± 2 mmHg at baseline and it did not change significantly throughout the experimental protocol.

3.5. Left ventricular weight and area at risk

The left ventricular weight and the area at risk were similar in the vehicle-treated, 0.01 μM moxonidine, 0.1 μM moxonidine, 1 μM moxonidine and 1 μM labetalol groups, the respective values being 6.5 ± 0.2, 5.7 ± 0.9, 6.9 ± 0.4, 6.1 ± 0.3 and 5.9 ± 0.3 g for the left ventricular weight, and 25 ± 1.1, 26 ± 2.8, 25 ± 1.7, 26 ± 1.8 and 26 ± 1.4% for the area at risk. The area at risk comprised: the anterior lower third of the left ventricle, the apex, the lower fifth of the septum and the anterior or both papillary muscles.

4. Discussion

4.1. Antifibrillatory action of moxonidine

In the present study, moxonidine did not have any direct electrophysiological or antifibrillatory activity (defined as the ability to prevent induction of ventricular fibrillation). This observation is in contrast with the ability of moxonidine to decrease the incidence of ischaemia-induced ventricular fibrillation in in vivo preparations (Leprán and Papp, 1994) and suggests that the antifibrillatory properties of moxonidine are related to its known central sympatho-inhibitory action. Re-entry has been suggested to be the major mechanism leading to ventricular fibrillation during acute ischaemia (Janse and Wit, 1989), and prevention of ventricular fibrillation may result from the modification of certain 'vulnerable parameters' for the induction of re-entry (such as conduction velocity, excitability and refractoriness) (Janse, 1992). The lack of an effect of moxonidine on any of these parameters in the present study is consistent with the lack of an antifibrillatory action.

Our results do not agree with the findings of Papp's group that moxonidine can increase effective refractory period in isolated canine ventricular myocardium or in isolated rabbit papillary muscle, in which 1 μM moxonidine prolonged effective refractory period by 30% (Papp, personal communication). The reason for this discrepancy is unknown. It may be related to differences in the models used, different stimulation frequencies or tissue specificity of the electrophysiological effects of moxonidine. The observation that moxonidine did not exert any significant electrophysiological effects on ischaemic myocardium does not support our hypothesis that moxonidine could modify arrhythmogenesis through K_{ATP} channels. However, we have also found in this model that monophasic action potential duration is not modified by classical K_{ATP} channel blockers (glibenclamide, 5-hydroxydecanoate) during ischaemia (Wolk et al., 1998b). Consequently, the lack of any effect of moxonidine on ischaemia-induced monophasic action potential shortening is probably not a reliable index of its effect on K_{ATP} channels in this model.

4.2. Defibrillatory action of moxonidine

The new finding of this study is the ability of moxonidine to bring about spontaneous defibrillation in vitro once ventricular fibrillation has been initiated during ischaemia, in spite of the lack of antifibrillatory properties. This observation is particularly interesting in the view of the findings of Leprán and Papp, who reported that in vivo, apart from its antifibrillatory activity, moxonidine also increased the possibility of spontaneous defibrillations after coronary ligation (Leprán and Papp, 1994). The present report is the first to suggest direct peripheral effects of moxonidine as a mechanism of spontaneous defibrillations during ischaemia.

The actual mechanism whereby moxonidine could facilitate spontaneous defibrillations in the present model is unknown. It is of note, however, that labetalol also had defibrillatory effects in the present study, in spite of the fact that (similar to moxonidine) it did not affect any electrophysiological parameters measured during ischaemia and was not antifibrillatory. Since labetalol is a combined β_1-, β_2- and α_1-adrenoceptor blocker (Brittain et al., 1982; Gold et al., 1982), it is possible that its defibrillatory activity was related to its adrenolytic properties. Specifically, electrophysiological effects of locally released catecholamines in the heart are heterogeneous and may facilitate re-entry (Schwartz and Priori, 1990). Consequently, any sympatholytic intervention (e.g., a decrease in catecholamine release or a blockade of effector adrenoceptors) should facilitate homogeneous electrical activity during ischaemia and spontaneous termination of arrhythmias based on re-entry. Although at this point the mechanisms underlying the defibrillatory action of moxonidine can only be speculated upon, one of the plausible explanations is a decrease in local ischaemia-induced catecholamine release. As mentioned earlier, imidazoline receptors have been shown to exist on postganglionic sympathetic nerves of the rabbit heart, and their stimulation has been suggested to inhibit electrically evoked noradrenaline overflow (Fuder and Schwarz, 1993). In addition, moxonidine has been shown to inhibit electrically evoked norepinephrine release from the rabbit pulmonary artery and aorta (Molderings et al., 1991; Göthert and Molderings, 1992). If moxonidine were able to inhibit ischaemia-induced catecholamine release in the heart, it could explain its defibrillatory action.

It has to be emphasized, however, that the similar effects of labetalol and moxonidine on arrhythmias do not prove that their mechanism of action is the same and the proposed 'catecholamine' hypothesis of the defibrillatory action of moxonidine is speculative at this stage. It will

have to be tested more directly in future experiments. In addition, other possible mechanisms have to be addressed. For example, moxonidine has been shown to have a direct effect against aconitine-induced arrhythmias (associated with calcium overload) (Mest et al., 1995) and a similar mechanism of action during ischaemia has to be excluded. It is also unclear whether moxonidine exerts any use-dependent electrophysiological effects, that would play a role during rapid ventricular rhythms (such as ventricular fibrillation) rather than at physiological heart rates.

The lack of a concentration-dependent effect of moxonidine is difficult to explain on the basis of the present series of experiments. The observation that only the middle concentration (0.1 μM) of moxonidine was defibrillatory may suggest a bell-shaped relationship. It may be related to complex pharmacological effects of moxonidine or its tissue and receptor specificity at different concentrations. For example, it is known that at all three concentrations used moxonidine is an imidazoline I_1 receptor agonist, but the higher concentrations are also likely to have a significant α_2-adrenoceptor agonist effect (Ernsberger et al., 1992; Ernsberger et al., 1993b). Also, as mentioned earlier, the 1 μM concentration of moxonidine has been suggested to exert a direct electrophysiological effects on isolated rabbit papillary muscle (Papp, private communication). Interestingly, in the quoted in vivo study of Leprán and Papp, the antiarrhythmic effect of moxonidine during ischaemia was also not concentration-dependent. At present, the exact mechanisms of action of moxonidine are still under investigation.

4.3. Conclusions

In the present study, we have demonstrated that moxonidine does not have any major direct electrophysiological effects in either non-ischaemic or ischaemic isolated rabbit hearts. The comparison with the in vivo results (Leprán and Papp, 1994) suggests that, whilst antifibrillatory properties of moxonidine during ischaemia may be related to its central sympatholytic activity, the defibrillatory action may be due to some direct action on the myocardium. The same defibrillatory effect is observed with labetalol at the concentration known to block cardiac adrenoceptors. Consequently, it is possible that the peripheral antiadrenergic properties of moxonidine underlie its defibrillatory action observed in the present experiments, although other mechanisms cannot be excluded.

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