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Effects of the $I_{K.ATP}$ blockers glibenclamide and HMR1883 on cardiac electrophysiology during ischemia and reperfusion

Stefan Dhein [a,*], Pantea Pejman [b], Kathi Krüsemann [b]

[a] *Institute of Pharmacology, University of Halle, Magdeburger Str.4, 06097 Halle (Saale) Germany*
[b] *Institute of Pharmacology, University of Köln, Gleueler Str.24, 50931 Cologne, Germany*

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Abstract

Clinical evidence indicates an antiarrhythmic effect of sulfonylureas, which might be blunted by their vascular action. We wanted to investigate the effect of glibenclamide and the new sulfonylthiourea compound 1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]-sulfonyl]-3-methylthiourea (HMR1883) on cardiac electrophysiology in the course of regional ischemia and reperfusion. Isolated rabbit hearts (Langendorff-technique) were pretreated with either vehicle ($n = 14$), 3 μmol/l glibenclamide ($n = 7$) or 3 μmol/l HMR1883 ($n = 7$) before regional ischemia was induced by left coronary artery branch occlusion (45 min) followed by 45 min reperfusion. Unipolar epicardial electrocardiograms were recorded from 256 epicardial AgCl electrodes. Coronary ligation resulted in a decrease in coronary flow (CF) by 35% and in left ventricular pressure (LVP) by 40% in all series. The occluded zone was 23 ± 3% in all series. Ischemia led to shortening of the epicardial activation–recovery interval (ARI) in the ischemic area, which was inhibited by both drugs especially in the early phase. In the non-ischemic area, ARIs remained stable and there was no effect of the drugs. Ischemia led to an increase in the regional difference in ARI between ischemic center and border zone. This increase was significantly inhibited by both substances during late ischemia and early reperfusion (until 15 min reperfusion). In addition, the dispersion of ARIs was reduced by both drugs during late ischemia and reperfusion. Ventricular fibrillation was observed in 7/14 (control), 0/7 (glibenclamide), and 0/7 (HMR1883). All ventricular fibrillation occurred during reperfusion. In glibenclamide but not in HMR1883-treated hearts recovery of CF upon reperfusion was significantly depressed (control: 25.5 ± 4; HMR1883: 23 ± 2.5; glibenclamide: 16 ± 1 ml/min, values at 2 min reperfusion), while the elevation of ST-segments of the electrograms in early ischemia was fully prevented by both treatments. We conclude that both glibenclamide and HMR1883 exert an antiarrhythmic effect in this model, and reduce the shortening of the ARIs in the ischemic area, thus attenuating regional differences in ARIs between ischemic and non-ischemic area. Furthermore, unlike glibenclamide HMR1883 does not interfere with postischemic hyperemia.

Keywords: $I_{K.ATP}$; Glibenclamide; HMR1883; Sulfonylurea; Cardiac ischemia; Dispersion; Arrhythmia; Sulfonylurea

1. Introduction

Sulfonylureas are widely used in type II diabetic patients and their possible effects on heart have been discussed controversely. Regarding cardiac effects of sulfonylureas, there is clinical evidence that $I_{K.ATP}$ blockers ($I_{K.ATP}$ = ATP sensitive K^+ channel) in diabetic patients can reduce the incidence of arrhythmia and of ventricular fibrillation in acute myocardial infarction (Cacciapuoti et al., 1991; Lomuscio et al., 1994; Lomuscio and Fiorentini, 1996; Davis et al., 1996, 1998). Furthermore, it was shown in dogs that the susceptibility to ventricular fibrillation can be reduced by the sulfonylurea glyburide (Billman et al., 1993). However, these positive actions of glibenclamide or of some other sulfonylureas are blunted by the vascular effect of the drug, which counteracts postischemic hyperemia and antagonizes the cardioprotective effects of preconditioning (Munch-Ellingsen et al., 1996). However, this vascular glibenclamide-effect is time-dependent: it was also shown, that glibenclamide did not affect preconditioning effects if it were administered shortly, i.e. 5 min instead of 30 min before ischemia (Schultz et al., 1997).

Sulfonylureas bind to certain sulfonylurea-receptors (SUR) which are coupled to a K^+ channel forming a functional $I_{K.ATP}$ (for review, see Schotborgh and Wilde, 1997). There are two isoforms of SUR namely SUR1,

* Corresponding author. Tel.: +49-345-557-4420; fax: +49-345-557-1835.
E-mail address: stefan.dhein@medizin.uni-halle.de (S. Dhein).

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which is found on pancreatic β-cells, and SUR2 (Gribble et al., 1998). Two subtypes of SUR2 have been identified, namely SUR2A (only expressed in heart and skeletal muscle) and SUR2B (expressed more abundantly in extrapancreatic tissues such as smooth muscle cells) (for review, see Schotborgh and Wilde, 1997).

Most of the studies performed on cardiac effects of sulfonylureas were carried out using glibenclamide, which blocks both SUR1 and SUR2 sulfonylurea-receptors (Gribble et al., 1998). With the sulfonylthiourea 1-[[5-[2-(5-chloro-*o*-anisamido) ethyl]-2-methoxyphenyl]-sulfonyl]-3-methylthiourea (HMR1883) a new type of $I_{K.ATP}$ blocker became available, which has a significantly lower affinity to pancreatic or vascular $I_{K.ATP}$ channels as compared to cardiac (Gögelein et al., 1998b), which are regulated via type 2 SUR (Gribble et al., 1998). In concentrations up to 100 μmol/l, HMR1883 did not inhibit other potassium channels such as I_{K1}, I_{Kr} or I_{Ks}, while the rilmakalim-induced action potential shortening was antagonized with an IC_{50} of 1.8 μmol/l and hypoxia-induced action potential shortening was inhibited in concentrations ranging from 2 to 20 μmol/l. (Gögelein et al., 1998b). The chemical structure of HMR1883 in comparison to glibenclamide is given in Fig. 1. Regarding the metabolic effects of HMR1883, Billman et al. (1998) showed that HMR1883 (3 mg/kg) did not affect blood glucose or plasma insulin in anaesthetized dogs.

Although an antiarrhythmic effect in ischemia was recently shown (Barrett and Walker, 1998; Gögelein et al., 1998b; Billman et al., 1998), only little is known on the underlying electrophysiological effects of $I_{K.ATP}$ blockers in the center and border zone of a regional ischemia regarding regional differences in action potential durations. Thus, the aim of our study was to investigate the effect of glibenclamide and the new compound HMR1883 on cardiac electrophysiology in the course of regional ischemia and reperfusion using a high resolution mapping technique which allows simultaneous registration of epicardial potentials from ischemic center and border zone as well as from the non-ischemic area.

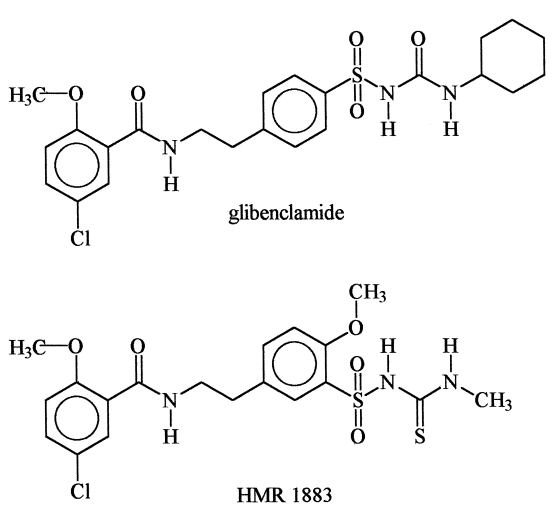


Fig. 1. Chemical structure of glibenclamide and HMR1883.

2. Methods

All experiments were performed in accordance to the ethical rules of the Council for International Organisation of Medical Science and the German laws for animal welfare. The method of heart preparation and epicardial potential mapping has been described in more detail previously (Dhein et al., 1993) and will be explained only briefly in the following paragraph.

Male white New Zealand rabbits (conventional, normally fed ad libitum, 1500–1800 g, Rollié, Lengerich, FRG) were treated with 1000 IU/kg heparin i.v. 5 min before they were stunned by a sharp blow on the neck and rapidly killed by subsequent exsanguination. The heart was excised, prepared and perfused according to the Langendorff-technique at constant pressure (70 cm H_2O) with Tyrode's solution of the following composition: Na^+ 161.02, K^+ 5.36, Ca^{2+} 1.8, Mg^{2+} 1.05, Cl^- 147.86, HCO_3^- 23.8, PO_4^{2-} 0.42, and glucose 11.1 mmol/l, equilibrated with 95% O_2 and 5% CO_2. The surface temperature of the heart was 37°C. The hearts were connected to a 256 channel mapping system HAL3 (temporal resolution: 4 kHz/channel; amplitude resolution: 0.04 mV, interchannel coupling < -60 dB; bandwidth of the system: 0.5–20 kHz, data were not filtered) as described previously (Dhein et al., 1988). A total of 256 AgCl electrodes were cast in four polyester plates (in 8×8 orthogonal matrices with 1 mm interelectrode distance), which were attached to the heart surface in an elastic manner, so that they could follow the heart movements easily without dislocation, covering the right, front, left, and back ventricular wall. The hearts were spontaneously beating during the entire experimental protocol.

We tested the influence of regional ischemia on the parameters as well as the effect of 3.0 μmol/l glibenclamide or HMR1883 on the ischemia-induced alterations. Concentrations of 2 or 20 μmol/l glibenclamide have been reported to be effective (Gögelein et al., 1998b) in preventing from hypoxic action potential shortening. Similarly, in the same study hypoxia-induced action potential shortening was antagonized with 2 to 20 μmol/l HMR1883 (see Fig. 5 of the cited study), while both drugs were ineffective in submicromolar concentrations.

Before starting the present study, we carried out concentration–response curves for both glibenclamide ($n = 3$) and HMR1883 ($n = 3$) ranging from 0.1 to 10 μmol/l in isolated hearts paced at 2.7 Hz [so that the ST-segments were slightly elevated (ST see below) and the activation recovery intervals (ARI, see below) became slightly shortened]. We found that under these conditions, an activation–recovery interval (ARI) of 128 ± 4 ms (control, $n = 3$), which was slightly prolonged by both drugs (HMR1883:

$+5 \pm 1\%$, $n = 3$; glibenclamide: $+3 \pm 0.3\%$, $n = 3$; 3 μmol/l). In addition, ST was elevated to 42 ± 27 arbitrary units [a.u.] ($n = 3$) and was reduced by both drugs (HMR1883: $-49 \pm 10\%$, $n = 3$; glibenclamide: $-36 \pm 12\%$, $n = 3$; 3 μmol/l). In concentrations below 3 μmol/l, we observed no effect of both drugs on ARI and only a slight effect on ST. Thus, 3 μmol/l seemed to represent a threshold concentration for both drugs in this model.

Because of these experiments and the cited literature, we decided to use 3 μmol/l as a test concentration for each drug. After 1 h of equilibration under standard conditions and after subsequent 10 min pretreatment with either Tyrode solution (control, $n = 14$) or 3.0 μmol/l glibenclamide ($n = 7$) or 3 μmol/l HMR1883 ($n = 7$), a branch of the left anterior descending coronary artery was occluded for 45 min by ligation. Thereafter, the ligation was released and the heart was reperfused for another 45 min. The treatment with the drug was continued throughout the entire ischemia and reperfusion period. At the end of the experiment, a second occlusion at the same location was performed, Evans blue was injected in the coronary circulation so that the occluded zone could be seen as the non-stained area. After weighing the whole heart, this occluded zone was excized and weighted and after cutting into small cubes of 0.5 mm length incubated in 0.5% nitro blue tetrazolium for 30 min. All tissue samples, which were stained yellowish after this incubation, were weighed again giving the infarct size, which was expressed as the percentage of the occluded zone. Ischemia was confirmed during the experiment by (1) the reduction in coronary flow (CF), (2) the area of the ventricle in which ST-elevation was observed, (3) the intensity of ST-elevation, and finally (4) the reduction in left ventricular pressure (LVP), and after the experiment by the infarct size.

Epicardial potential mapping was carried out in each experimental phase during periods of constant cycle length of at least 4 min, in order to make it possible to compare the activation patterns (of single heart beats) or their alterations. In addition, the functional parameters maximum systolic LVP, end-diastolic LVP (= EDP), heart rate (HR), and CF were assessed continuously as described (Dhein et al., 1993).

2.1. Evaluation of mapping data

For evaluation of the mapping data, the activation time points at each electrode were determined as $t(\mathrm{d}U/\mathrm{d}t_{\min})$ (Dhein et al., 1993; Durrer and Van der Tweel, 1954). Next, the repolarization time points were determined as $t(\mathrm{d}U/\mathrm{d}t_{\max})$ during the T-wave as described (Dhein et al., 1993; Millar et al., 1985). After automatical determination activation and repolarization timepoints were verified (or corrected if necessary) manually by the experimentator. From these data for each electrode, an ARI was calculated as the difference between the timepoint of activation and the timepoint of repolarization indicating the epicardial action potential duration. ARIs were only calculated if the QRS complex showed both a positive and a negative component and if there was a well defined T-wave as was published for analysis of ARIs in ischemic tissue by Gottwald et al. (1998) and in accordance to Janse (1993).

ARIs were determined for each region of the heart (right, front, left and back wall) as well as for the ischemic center and border zone. Center and border were determined from ST-elevation and dye injection, which gave the localization of the occluded zone. In addition, the distribution of the ARIs was analyzed for each area of the heart (i.e. front, left, right or back wall) calculating the standard deviation of the ARIs at 64 electrodes and expressed as ARI-dispersion.

From the activation time points an activation sequence was determined. We determined those electrodes which were activated before any of the neighbouring ones and defined them as ''breakthrough-points'' (BTPs) which can be considered as the origins of epicardial activation (Arisi et al., 1983). These BTPs were determined for heartbeats under control conditions and for heart beats under treatment. Heartbeats in the various phases of ischemia and reperfusion were compared to those under control conditions by calculating the percentage of BTPs with identical location as compared to their location under control conditions (identical = deviating not more than 1 mm from their location under control conditions). That means that two identical heartbeats should reveal a similarity in BTP location of 100%. It is, however known from previous studies, that identical heartbeats do occur only rarely and that arrhythmogenic stimuli can reduce BTP similarity (Dhein et al., 1988, 1993). Decreasing values for this BTP similarity indicate progressive deviation from the initial (control) activation pattern.

In addition, the ST-segments (see also Fig. 2) of the 256 ECGs were analyzed. We summed up all deviations from the isoelectrical level at the time point 50% of mean ARI and calculated the total ST-deviation of 256 leads in a.u. An increase in that parameter points to an increase in efflux of positively charged ions during the action potential and is indicative for ischemic regions (Coronel et al., 1988, Gottwald et al., 1998).

2.2. Statistics

All values are given as means ± S.E.M. Significance was analyzed using analysis of variance (ANOVA) for comparison of multiple groups. If ANOVA indicated significant differences Wilcoxon rank test for paired observations or Mann–Whitney–*U*-test for unpaired observations were performed. The level of significance was $P < 0.05$.

2.3. Chemicals

All chemicals used in this study were of analytical grade. All chemicals were purchased from Sigma (St.

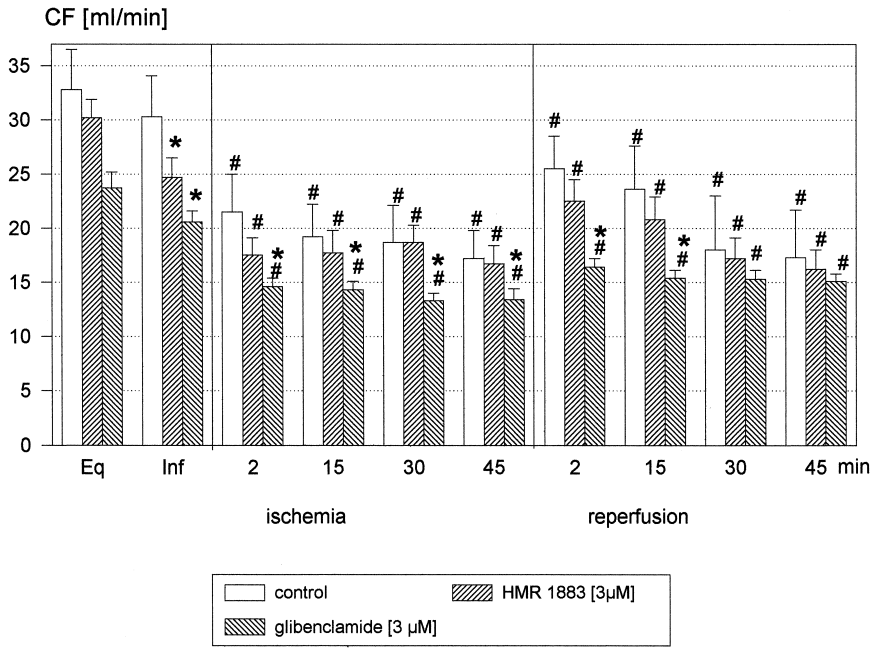


Fig. 2. Changes in CF (equilibration ''EQ'') in the course of ischemia and reperfusion in untreated control heart ($n = 14$) and in glibenclamide (3 μmol/l; $n = 7$) and in HMR1883 (3 μmol/l, $n = 7$) treated hearts. The ''Inf'' values give the change of the parameter after 10 min preischemic infusion of either the drug or the vehicle. Values are given as means ± S.E.M. Significant differences to the control series are indicated by an asterisk. Significant differences against the preischemic equilibrium value (''EQ'') are indicated by #.

Louis, USA), except heparin, which was from Serva (Heidelberg, FRG). The sodium salt of HMR1883 (Batch: HT1), was supplied by Hoechst Marion Roussel (Frankfurt, BRD).

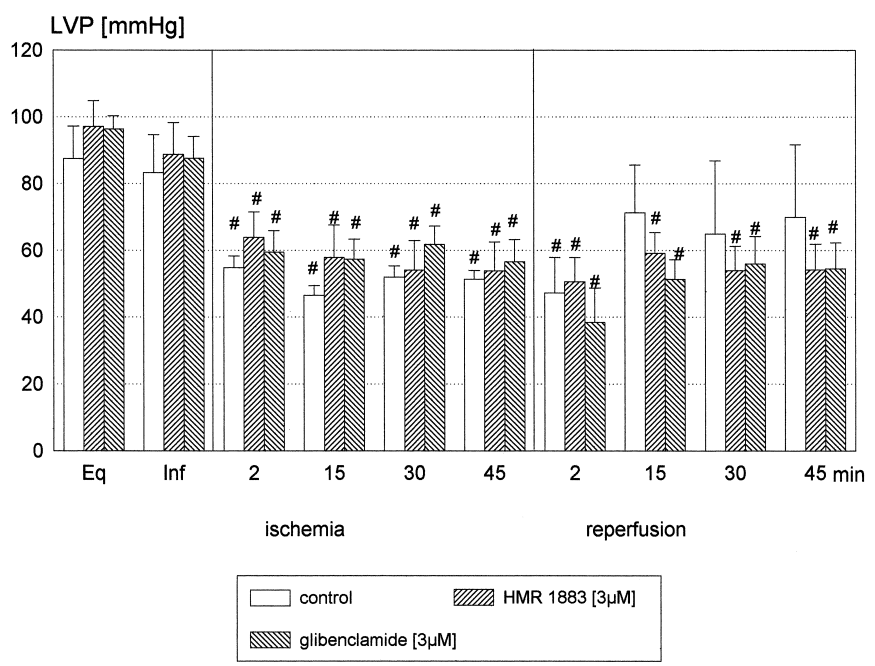


Fig. 3. Changes in systolic LVP (equilibration ''EQ'') in the course of ischemia and reperfusion in untreated control heart ($n = 14$) and in glibenclamide (3 μmol/l, $n = 7$) and in HMR1883 (3 μmol/l, $n = 7$) treated hearts. The ''Inf'' values give the change of the parameter after 10 min preischemic infusion of either the drug or the vehicle. Values are given as means ± S.E.M. Significant differences to the control series are indicated by an asterisk. Significant differences against the preischemic equilibrium value (''EQ'') are indicated by #.

3. Results

3.1. Arrhythmias, functional parameters

Heart wet weight was 7.8 ± 0.3 g ($n = 28$). The occluded zone was comparable in all experimental series with an average of $23 \pm 3\%$ (control series: $25 \pm 5\%$, $n = 14$; HMR1883 series: $21 \pm 7\%$, $n = 7$; glibenclamide series: $22 \pm 7\%$, $n = 7$). There were no significant differences between the three experimental series regarding heart weight and occluded zone. However, the infarct size was significantly smaller in HMR1883 treated hearts: $8.5 \pm 3.7\%$ ($n = 7$, HMR1883) versus $23.7 \pm 9.6\%$ ($n = 14$, control) ($P < 0.05$). In glibenclamide treated hearts, however, we found a higher variability of the infarct size ($18.6 \pm 12.5\%$, $n = 7$).

Ventricular fibrillation occurred in 7/14 hearts in the untreated controls. In one case it was self-terminating, in the six other cases it was sustained ventricular fibrillation. In all 7/14 cases, ventricular fibrillation occurred during reperfusion period, i.e. in one case at 2 min reperfusion (not sustained), in three cases at 10–15 min reperfusion (sustained), in two more cases at 20–30 min reperfusion (sustained), and in one case at 40 min reperfusion (sustained); thus, 57% of all ventricular fibrillation occurred within the first 15 min of reperfusion. In contrast, in glibenclamide treated hearts, we did not observe ventricular fibrillation (0/7) and in HMR1883 treated hearts, we also did not find ventricular fibrillation in any case (0/7). While there was a significantly reduced incidence of ventricular fibrillation (7/14 versus 0/7; $P < 0.05$), we found monomorphic extrasystoles during early reperfusion in all hearts (all series).

Regarding the functional parameters, we found that the CF was reduced about equally in all three series during ischemia (CF at 2 min ischemia: control series: $65 \pm 8\%$, $n = 14$; HMR1883 series: $58 \pm 5\%$, $n = 7$; glibenclamide series: $63 \pm 5\%$ of initial value, $n = 7$). After 2 min reperfusion, CF recovered but did not completely reach preischemic values. Thereafter, CF declined with time (in control and HMR1883 series). In contrast, in hearts receiving glibenclamide we did not (or only minimally) find enhanced CF after release of the occlusion (Fig. 2), i.e. the postischemic hyperemia was suppressed by glibenclamide but not by HMR1883.

LVP was reduced by nearly 40% and partly recovered after 15 min reperfusion. The postischemic values for LVP in the treatment series were slightly but not significantly lower as compared to the control series (Fig. 3).

3.2. ST-elevation

In order to characterize the underlying electrophysiology, we carried out a 256 channel epicardial potential mapping. Typically, in unipolar electrocardiograms an elevation of the ST-segment can be observed in the ischemic

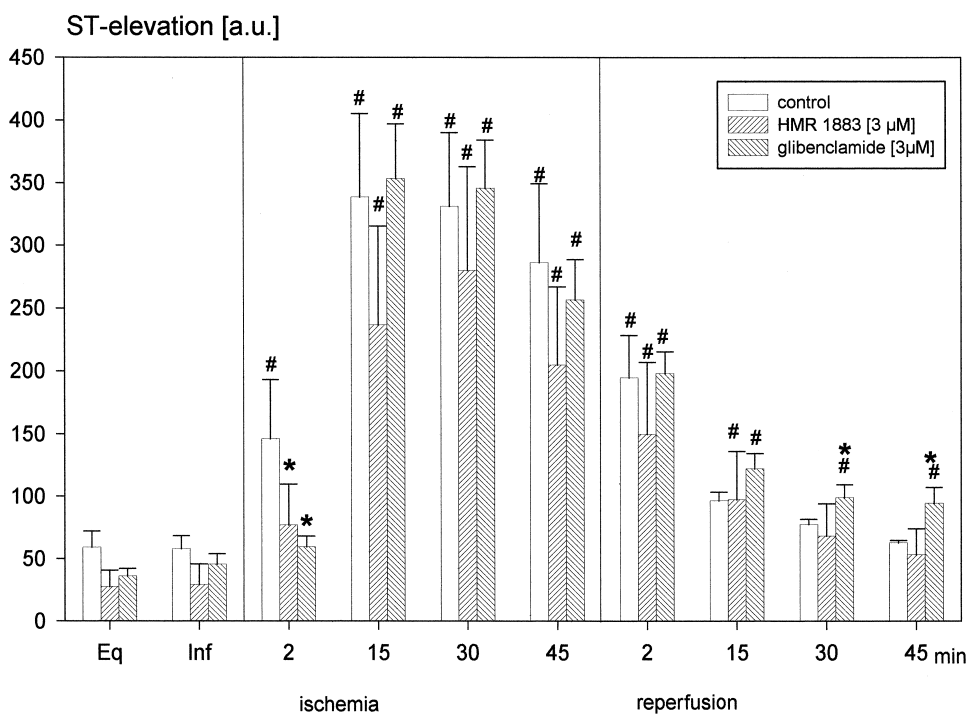


Fig. 4. Changes in ST-elevation (preischemic equilibration value = ''EQ'') in the course of ischemia and reperfusion in untreated control heart ($n = 14$) and in glibenclamide (3 µmol/l, $n = 7$) and in HMR1883 (3 µmol/l, $n = 7$) treated hearts. The ''Inf'' values give the change of the parameter after 10 min preischemic infusion of either the drug or the vehicle. Values are given as means ± S.E.M. Significant differences to the control series are indicated by an asterisk. Significant differences against the preischemic equilibrium value (''EQ'') are indicated by #.

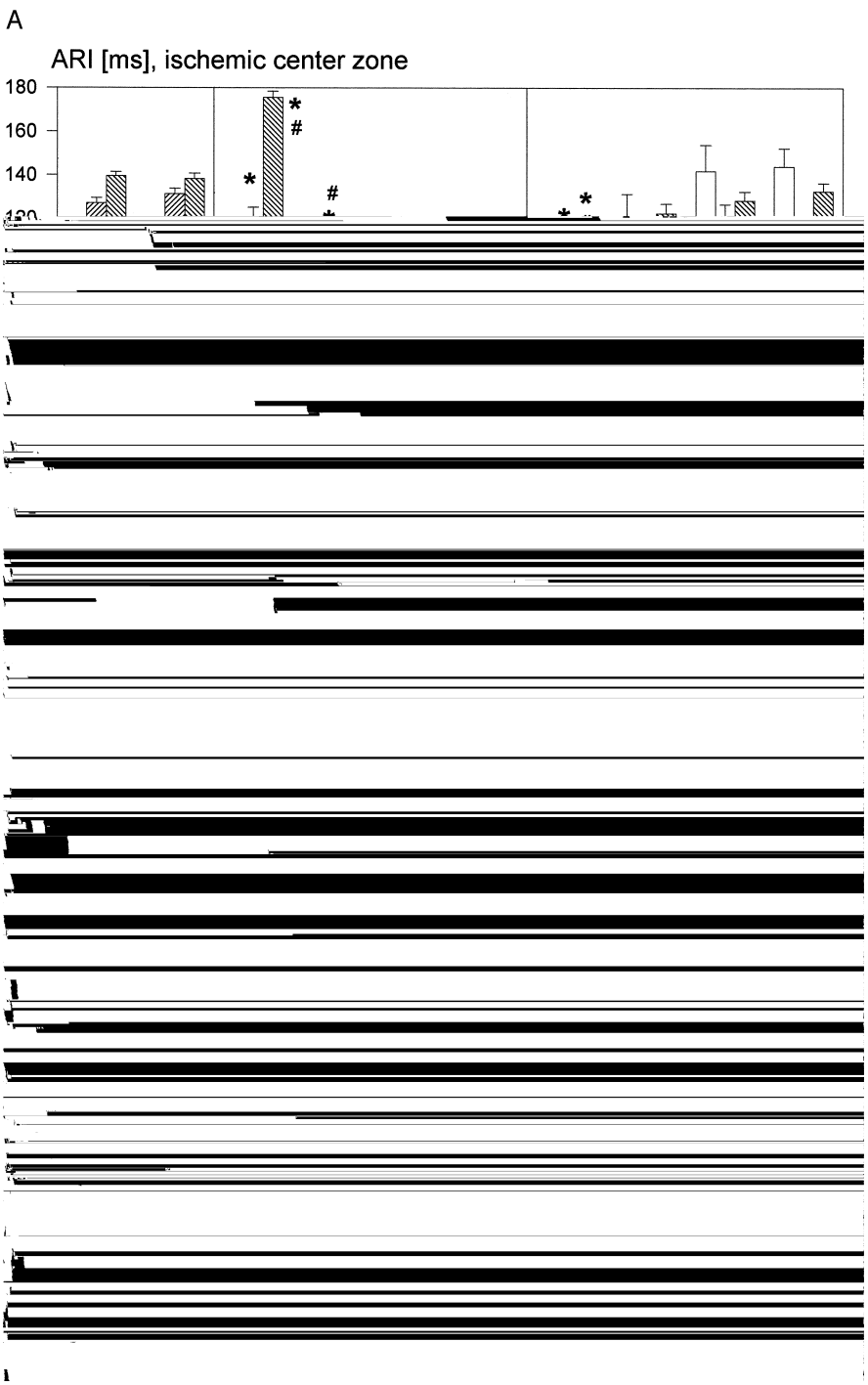
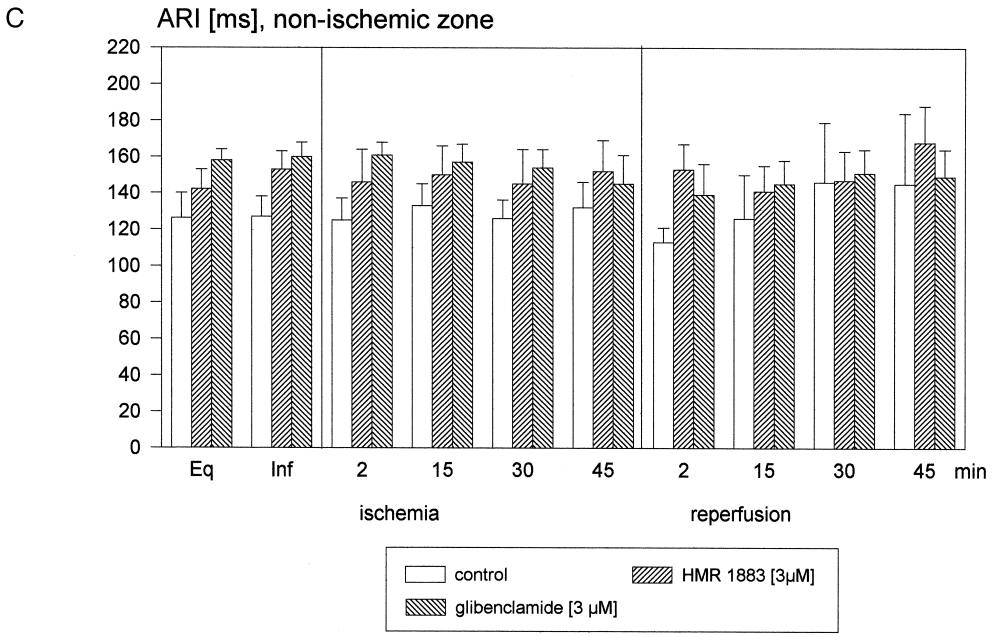


Fig. 5. (A) Changes in epicardial activation recovery interval ARI in the ischemic center at the left ventricular wall, assessed as the epicardial activation–recovery interval, (preischemic equilibration value = ''EQ'') in the course of ischemia and reperfusion in untreated control heart ($n = 14$) and in glibenclamide (3 μmol/l, $n = 7$) and in HMR1883 (3 μmol/l, $n = 7$) treated hearts. The ''Inf'' values gives the change of the parameter after 10 min preischemic infusion of either the drug or the vehicle. Values are given as means ± S.E.M. Significant differences between treated and untreated hearts are indicated by an asterisk (*). Significant differences against the preischemic equilibrium value (''EQ'') are indicated by a #. (B) Changes in ARI in the ischemic border zone. Explanations see above. Note that the changes in ARI are smaller as compared to those observed in the center (A). (C) Changes in ARI in the non-ischemic area. Please note that ARI is not affected by both drugs in the non-ischemic area and (if comparing to (A) and (B)) that effects of glibenclamide and HMR1883 can only be observed in the ischemic region. (D) Original registrations of unipolar epicardial potentials from the ischemic center and border zone under control conditions and under drug treatment before and after 2 min of ischemia. The vertical bar is 2 mV, the horizontal bar 125 ms for reference. Please note that ST-elevation is most pronounced in the center zone and that this ST-elevation is suppressed by both drugs. Moreover, please note that ischemic potential shortening is attenuated in treated hearts. (E) The figure gives the difference in epicardial activation recovery intervals (ARI, [ms]) between ischemic center and border zone in untreated and treated (HMR1883 or glibenclamide) hearts occurring during ischemia. Significant differences are indicated by an asterisk ($P < 0.05$).



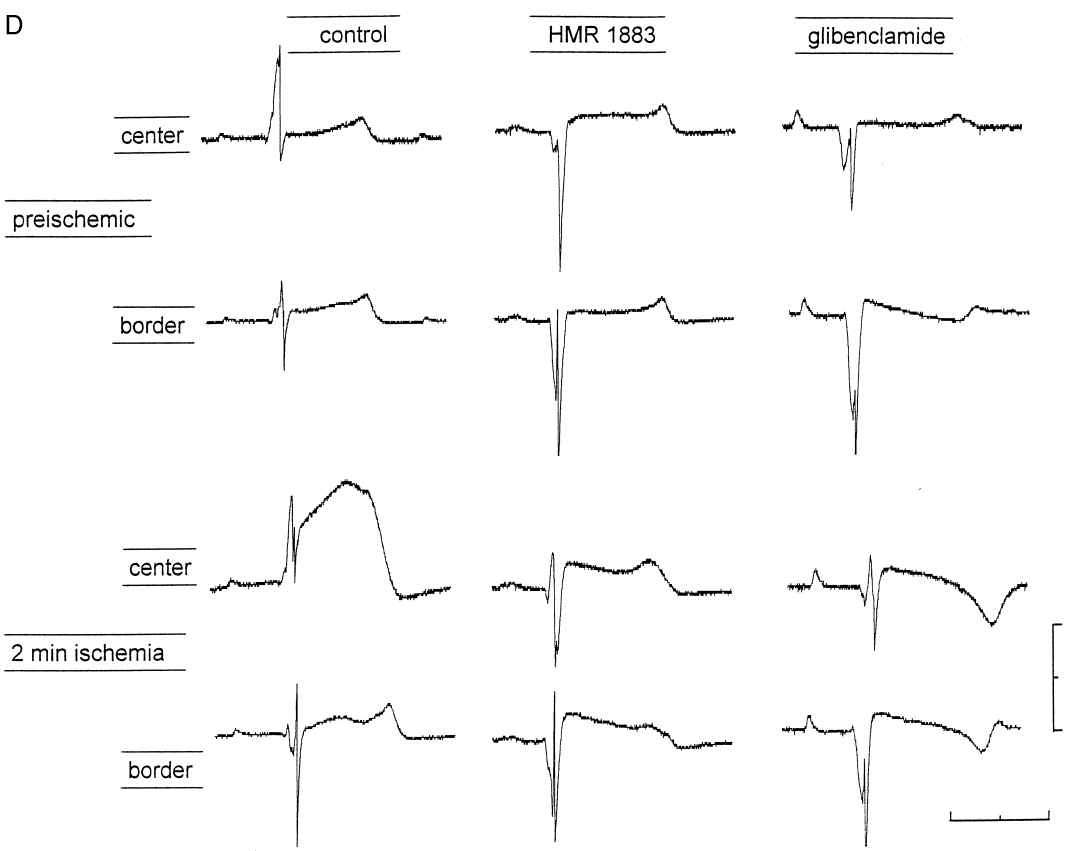


Fig. 5 (*continued*).

area due to the efflux of K^+ (Coronel et al., 1988, 1992; Dhein et al., 1990). The potential at 50% ARI, i.e. ST-segment, was maximally elevated during ischemia, from preischemic control values of 59 ± 13 ($n = 14$)–340 ± 55 a.u. ($n = 14$) after 15 min ischemia, declining to the preischemic control values upon reperfusion. This rise in ST-elevation at 2 min ischemia was significantly suppressed by both $I_{K.ATP}$ blockers (for original registration see Fig. 5D). However, the maximum values for ST-elevation were somewhat, although not significant, reduced

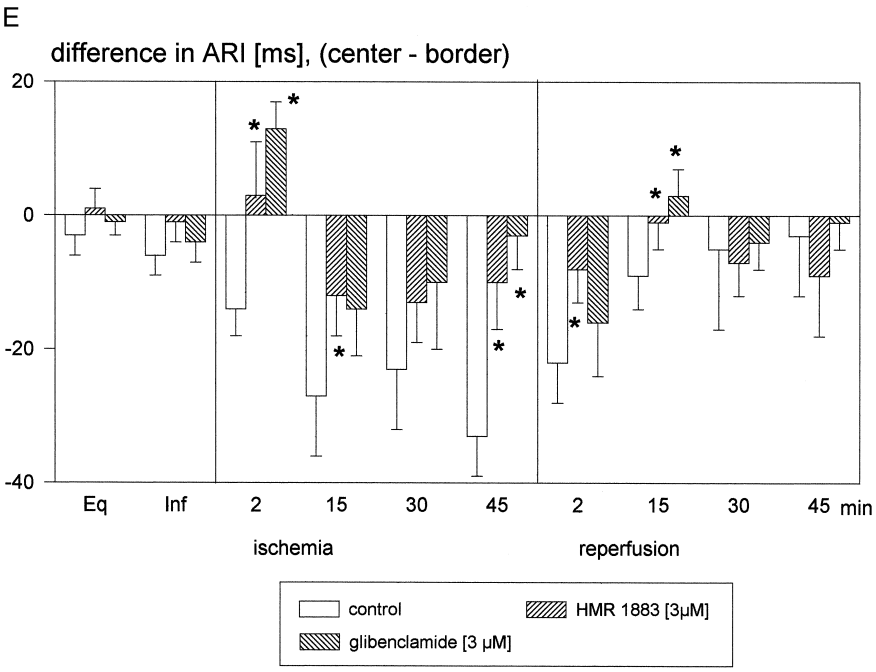


Fig. 5 (*continued*).

under the influence of HMR1883, but not under glibenclamide. While after 45 min reperfusion, the value for ST-elevation in HMR1883 treated hearts returned to values not significantly different from its preischemic control value, it remained still elevated at that timepoint in glibenclamide treated hearts (Fig. 4).

3.3. Activation–recovery intervals

In the following analysis, we have evaluated the duration of the epicardial ARI in the ischemic center and border zone as well as on the whole cardiac surface. The epicardial ARIs were reduced in the course of ischemia and recovered to preischemic values within 15 min of reperfusion. This reduction in ARI was significantly less pronounced in the presence HMR1883 and of glibenclamide (at early ischemia and early reperfusion). The effect seemed time-dependent since this drug effect was most prominent in early ischemia. After 2 min reperfusion, the ARIs again were significantly longer in treated than in untreated hearts. Thereafter, all hearts recovered in a similar manner.

In order to investigate these phenomena in detail, we examined the differential effects of ischemia and drug treatments on the ischemic center and border zone by selecting five electrodes from the ischemic center and five electrodes from the ischemic border as defined by ST-segment changes and dye injection. It became obvious that ischemia led to a shortening of the ARIs which was most pronounced in the ischemic center. This shortening was significantly antagonized by both treatments. The effect of the drug treatment was also more prominent in the ischemic center zone (Fig. 5A) as compared to the border zone (Fig. 5B) (for original registrations see Fig. 5D).

Interestingly, in the prolonging drug effect on the ARIs was restricted to the ischemic area while in the non-ischemic area neither glibenclamide nor HMR1883 exerted any influence on ARIs (Fig. 5C), i.e. an prolonging effect was only observed in regions with ischemia-induced shortening of the ARIs. Thus, the difference in epicardial action potential duration between ischemic center and border was lower in treated hearts (Fig. 5E) especially during early (2 min) and late (45 min) ischemia and during the first 15 min of reperfusion ($P < 0.05$), so that the drug-treatment seemed to attenuate regional differences in ARIs between ischemic and non-ischemic area (Fig. 5E).

In order to quantify this effect, we assessed the global dispersion of the ARIs at the whole left ventricular wall. The dispersion of the epicardial action potential durations significantly increased in the course of ischemia from preischemic control values between 7.88 ± 1.8 ms to a maximum of 40 ± 6 ms (45 min ischemia, untreated hearts, $n = 14$). Dispersion partially recovered upon reperfusion in the control series and remained at elevated values around 20 ms (21 ± 3 ms, 45 min reperfusion, $n = 7$). This increase in dispersion was significantly reduced by both, glibenclamide and HMR1883, in late ischemia (HMR1883 ($n = 7$): 29 ± 4 ms; Glib. ($n = 7$): 29 ± 5.5 versus 40 ± 6 ms, 45 min ischemia; $P < 0.05$). In addition, there was a better recovery of dispersion upon reperfusion under the influence of the drugs so that dispersion was (although still elevated) significantly lower at the end of the reperfusion period in glibenclamide or HMR1883 treated hearts

(HMR1883 ($n = 7$): 15 ± 2 ms; Glib. ($n = 7$): 15 ± 2.5 versus 21 ± 3 ms, 45 min reperfusion; $P < 0.05$).

3.4. Activation pattern

Regarding the activation process and the geometry of activation, we found in accordance with previous investigations (Gottwald et al., 1998; Dhein et al., 1990) that ischemia resulted in a significant change in the location of the BTPs so that only $45 \pm 5\%$ (45 min ischemia, $n = 14$) were found to exhibit a similar location as compared to the preischemic, while normally, i.e. during the preischemic equilibration period, two succeeding heart beats revealed a BTP similarity of $72 \pm 6\%$ ($n = 14$). In glibenclamide treated hearts, we found a BTP similarity of $37 \pm 8\%$ ($n = 7$) at the end of the ischemic period, while in HMR1883 treated hearts $64.9 \pm 9\%$ ($n = 7$) of the BTPs remained stable (45 min ischemia). After reperfusion we found $56 \pm 14\%$ (control, $n = 7$), $67 \pm 6\%$ (HMR1883, $n = 7$) and $39 \pm 8\%$ (glibenclamide, $n = 7$) of the BTPs with similar location as during preischemic equilibration.

It should be noted that during the preischemic infusion of the drugs, we found $74 \pm 5\%$ (vehicle, control series, $n = 14$), $73 \pm 4\%$ (HMR1883, $n = 7$) and $80 \pm 3.3\%$ (glibenclamide, $n = 7$) of the BTPs at the same location as before infusion. Thus, there was no significant drug-effect on the activation pattern during the preischemic infusion.

Regarding the velocity of the epicardial activation process, we found an ischemia-induced prolongation of the total activation time, i.e. the time delay between activation of the first and of the last electrode of the left ventricular wall, indicating the well known slowing of conduction during ischemia (from a preischemic value of 9 ± 1–24 ± 5 ms at 45 min ischemia, control series, $n = 14$). The prolongation of the total activation time recovered only slightly and almost incomplete during reperfusion. This ischemia-induced increase in the total activation time was not influenced by the drug treatment. Similarly, there was no effect of the drugs on the atrioventricular conduction time (control: 58 ± 5 ms, $n = 14$; glibenclamide 3 μmol/l: 56 ± 2 ms, $n = 7$ and HMR1883 3 μmol/l: 59 ± 3 ms, $n = 7$; values after 10 min pretreatment under non-ischemic conditions).

The preischemic infusion itself of the substances or of the vehicle did not exhibit any effect on the parameters under investigation during preischemic control period as can be seen from the ''Infusion-values'' given in the figures (indicated as ''Inf'').

4. Discussion

This study demonstrated antiarrhythmic action of HMR1883 and glibenclamide. Ischemia led to ventricular fibrillation during reperfusion (7/14), with a peak during the first 15 min. During this time, the most prominent and significant effect of the drug treatment was a suppression of regional differences in ARIs between ischemic center and border zone. Thus, we suppose that the attenuation of regional differences in ARI during reperfusion may be related to the antiarrhythmic effect of the drugs. Besides this, other significant drug effects were observed, such as suppresion of ST-elevation and shortening of ARIs during early ischemia, which do not seem to be related to the antiarrhythmic effect in this model, since at that time no arrhythmia occurred in this rabbit model. Both drugs differed with regard to their vascular action since glibenclamide but not HMR1883 prevented from reactive hyperemia at early reperfusion. However in this model, this difference in vascular action does not seem to affect the antiarrhythmic properties of the treatment.

The reduction in ARIs as well as the increase in ST-elevation during untreated ischemia can be explained by the opening of potassium channels (Coronel et al., 1988) especially by opening of $I_{K.ATP}$ channels (Noma, 1983; Wilde et al., 1990). This is further supported by the inhibitory effect of both $I_{K.ATP}$ blockers on this shortening of ARIs. However, since we observed an incomplete antagonization, other potassium channels may also be involved in ischemic shortening of ARIs (Kim and Clampham, 1989; Kameyama et al., 1984) or K^+-efflux coupled to lactate and inorganic phosphate efflux (Kléber, 1984). In parallel, ST-segments are elevated due to the accumulation of positive charges in the extracellular space beneath the unipolar electrode (Coronel et al., 1988).

The shortening of the ARIs was most pronounced in the ischemic center, so that marked differences in ARI (as an indicator of action potential duration) occurred between center and border zone. Such regional differences in ARI in concert with slowing of conduction are generally considered to favor arrhythmia (Kuo et al., 1983; Kléber et al., 1978; Han and Moe, 1964), especially if the action potential duration is shortened and conduction is slowed (slowing of conduction is indicated in our study by the prolongation of the total activation time) (Smeets et al., 1986; Reensma et al., 1988) and if the activation pattern becomes altered (as indicated in our study by a reduced BTP similarity) (Gottwald et al., 1998). The importance of regional differences in ARIs for arrhythmogenesis has also been demonstrated in regional ischemia (Gottwald et al., 1998). Since these regional differences in ARIs were attenuated by both drugs not only during early and late ischemia but also during the first 15 min of reperfusion (when most arrhythmia occurred) this drug effect may be involved in the antiarrhythmic action, as the inhibition of the increase in ARI-dispersion by both drugs.

There was almost no drug effect on ARIs in non-ischemic tissue, but a clear prolonging effect in the ischemic center, and a mild effect in the border zone. Thus, the drug effect seems to depend on the degree of shortening of the ARIs. As was recently reported (Barrett and Walker, 1998), glibenclamide does not inhibit the action

potential shortening per se but slows the rate of shortening. This is compatible with our findings of a predominant effect in early ischemia seen with both drugs leading to a slower shortening of ARI. In the study mentioned above, the authors found an antiarrhythmic action of glibenclamide as well. However, they could not explain this antiarrhythmic effect.

The increase in dispersion of the ARIs in the course of ischemia can be ascribed to regional differences in potassium efflux depending on the localization with regard to the center of ischemia and secondly to cellular uncoupling (Dekker et al., 1996). This increase was antagonized by both drugs during late ischemia and reperfusion and thus this drug action may be involved in the antiarrhythmic effect. Since this dispersion was calculated from all 64 electrodes of the whole left ventricular wall, it does not only reflect the ischemic center and border zone. In an earlier study by DiDiego and Antzelevitch (1993), it was shown that glibenclamide could antagonize electrical heterogeneity induced by the $I_{K.ATP}$-opener pinacidil. Thus, an inhomogeneous distribution of $I_{K.ATP}$ may also contribute to the enhanced dispersion during ischemia.

One could imagine that HMR1883 and glibenclamide may exert their antiarrhythmic effect also via a blockade of other channels such as I_{Na} or I_{Ca}. However, our data do not indicate such an action on I_{Na} since there was no drug effect on the velocity of epicardial activation spreading, and not on I_{Ca} since there was no effect on LVP or on the atrioventricular conduction time.

Since a significant reduction of infarct size was only seen with HMR1883, the antiarrhythmic action of both drugs seen in our study does not seem to be related to the reduction in infarct size, although this may play — at least in parts — a role in the effects of HMR1883. The basis of this effect remains unclear. One could imagine an effect on the mitochondrial ATP-modulated channels, but this is hypothetical at present. However, it might be concluded from this finding that a reduction in farct size is not per se necessarily antiarrhythmic. On the other hand, it should be stated that 45 min reperfusion, although used by several investigators, may be too short for the tetrazolium method, so that the infarct size data should not be over-interpreted.

With regard to their vascular effects only glibenclamide, but not HMR1883, inhibited the postischemic hyperemia. This can be explained by the different affinities of the drugs to vascular sulfonylurea receptors or vascular $I_{K.ATP}$ channels. Gögelein et al. (1998a) showed that HMR1883 (below 10 μmol/l) did not affect enhancement of CF during global hypoxia. This is in good accordance with our finding of a normal hyperemic response after release of coronary ligation. However, this seems unrelated to the antiarrhythmic effect, which was seen with both drugs, but it might be involved in the lower or missing effect of glibenclamide on infarct size.

It is known that $I_{K.ATP}$ plays a role in cardioprotection by preconditioning (Morita et al., 1997). The vasoconstrictor effect of sulfonylureas can counteract preconditioning (Thornton et al., 1993; for review see: Wilde, 1994) and may exert proarrhythmic effects (D'Alonzo et al., 1995; Schultz et al., 1997; Munch-Ellingsen et al., 1996) depending on the model (preconditioning or not) and the anaesthetics used (Morita et al., 1997). These apparently conflicting results may be related to the different electrophysiological mechanisms underlying a particular arrhythmia. The vasoconstrictor action and the inhibition of postischemic hyperemia seen in our study may account for the longer lasting ST-elevation in glibenclamide treated hearts in our study, which was not seen with HMR1883.

With regard to the ongoing discussion about a possible proarrythmic potential of $I_{K.ATP}$ blockers, it should be noted that in a recent clinical investigation in 188 diabetics on sulfonylurea, who suffered from acute myocardial infarction, early mortality seemed to be increased while the late survival was not influenced (Garrat et al., 1999). In 874 patients with myocardial infarction including 102 diabetics, it was found that the use of sulfonylureas was not associated with an increased long-term mortality (Brady et al., 1998). It should be added that in another study, second generation sulfonylureas like glibenclamide or glipizide reduced the number of ventricular ectopic beats in ischemic rat hearts while first generation sulfonylureas like tolbutamide increased arrhythmias (Ballagi-Pordany et al., 1990).

Another point worth for discussion is the concentration used for both drugs. We decided to use 3 μmol/l of each drug, since concentrations of 2–20 μmol/l of both glibenclamide and HMR1883 were shown to prevent from hypoxic action potential shortening (Gögelein et al., 1998b), which is a situation somehow related to our study, while 0.2 or 0.5 μmol/l were ineffective. However, regarding the antagonization of a rilmakalim induced action potential shortening, the IC_{50} was 0.33 μmol/l (glibenclamide) and 1.8 μmol/l HMR1883 in that study. Since we were interested in the effects against hypoxia/ischemia-induced shortening, which may be pathophysiologically somewhat different, we used 3 μmol/l. Furthermore, we wanted to know, whether in this higher concentration HMR1883 might exert vascular effects like glibenclamide. In preliminary concentration–response curves, we established that the threshold concentration for an ARI-prolonging effect in hearts which are paced higher than their physiological rate (2.7 Hz instead of 3–3.5 Hz) was 3 μmol/l for both drugs. Interestingly, we found similar effects regarding ARI-shortening or inhibition of ST-elevation but diverging vascular effects.

From these results, it is concluded that (a) both drugs, HMR1883 and glibenclamide can exert antiarrhythmic effects in this model, and that (b) this antiarrhythmic effect seems to be — at least in parts — related to the attenuation of regional inhomogeneities in ARIs and the antagonization of the increase in dispersion. These results may help to explain the antiarrhythmic effects of glibenclamide

seen in diabetic patients (Cacciapuoti et al., 1991; Lomuscio et al., 1994; Lomuscio and Fiorentini, 1996; Davis et al., 1996, 1998).

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