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ORIGINAL CONTRIBUTION

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Antiarrhythmic effect of ischemic preconditioning during low-flow ischemia

The role of bradykinin and sarcolemmal versus mitochondrial ATP-sensitive K^+ channels

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Abstract Short episodes of ischemia (ischemic preconditioning) protect the heart against ventricular arrhythmias during zero-flow ischemia and reperfusion. However, in clinics, many episodes of ischemia present a residual flow (low-flow ischemia). Here we examined whether ischemic preconditioning protects against ventricular arrhythmias during and after a low-flow ischemia and, if so, by what mechanism(s).

Isolated rat hearts were subjected to 60 min of low-flow ischemia (12% residual coronary flow) followed by 60 min of reperfusion. Ischemic preconditioning was induced by two cycles of 5 min of zero-flow ischemia followed by 5 and 15 min of reperfusion, respectively. Arrhythmias were evaluated as numbers of ventricular premature beats (VPBs) as well as incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF) during low-flow ischemia and reperfusion. Ischemic preconditioning significantly reduced the number of VPBs and the incidence of VT and of VF during low-flow ischemia. This antiarrhythmic effect of preconditioning was abolished by HOE 140 (100 nM), a bradykinin B_2 receptor blocker. Similar to preconditioning, exogenous bradykinin (10 nM) reduced the number of VPBs and the incidence of VT and of VF during low-flow ischemia. Furthermore, the antiarrhythmic effects of both ischemic preconditioning and bradykinin were abolished by glibenclamide (1 μM), a non-specific blocker of ATP-sensitive K^+ (K_{ATP}) channels. Finally, the antiarrhythmic effects of both ischemic preconditioning and bradykinin were abolished by HMR 1098 (10 μM), a sarcolemmal K_{ATP} channel blocker but not by 5-hydroxydecanoate (100 μM), a mitochondrial K_{ATP} channel blocker. In conclusion, ischemic preconditioning protects against ventricular arrhythmias induced by low-flow ischemia, and this protection involves activation of bradykinin B_2 receptors and subsequent opening of sarcolemmal but not of mitochondrial K_{ATP} channels.

Key words Preconditioning – ischemia/reperfusion – bradykinin – K_{ATP} channels – arrhythmias

Introduction

Brief episodes of ischemia and reperfusion (ischemic preconditioning) protect the heart against a subsequent ischemic injury [21]. Accordingly, ischemic preconditioning confers protection against myocardial necrosis, contractile dysfunction and ventricular arrhythmias occurring during and/or after a subsequent ischemia [3, 20, 21, 29]. Most studies on preconditioning, however, have focused on the protection against ischemia without residual myocardial blood flow (zero-flow ischemia). Far

fewer studies have focused on the protection against ischemia with a residual flow (low-flow ischemia). From a clinical point of view, low-flow ischemia is just as relevant to study because ischemia can vary from mild to severe and because life-threatening ventricular arrhythmias occur not only during zero-flow ischemia but also during low-flow ischemia [8]. Previous studies on low-flow ischemia suggest that ischemic preconditioning confers protection against myocardial necrosis [26] but not against post-ischemic contractile dysfunction [4]. So far, few studies [29, 30] have investigated the effect of ischemic preconditioning on arrhythmias induced by low-flow ischemia and reperfusion. In these two studies in dogs, low-flow ischemia presumably occurred despite complete coronary occlusion because of the collateral circulation providing some residual myocardial blood flow in the ischemic area in this species. The precise degree of ischemia in these studies [29, 30], however, is not known.

The aim of the present study was to determine whether ischemic preconditioning protects against ventricular arrhythmias induced by a perfusion-controlled low-flow ischemia and subsequent reperfusion and, if so, to elucidate the underlying mechanism(s). For this purpose, we tested whether ischemic preconditioning reduces the number of ventricular premature beats (VPBs) and the incidence of ventricular tachycardia (VT) and of ventricular fibrillation (VF) during low-flow ischemia and reperfusion in isolated rat hearts.

Previous studies [23, 30] on antiarrhythmic effects of preconditioning proposed a role for bradykinin and bradykinin B_2 receptors that have also been implicated in antinecrotic effects of preconditioning [2, 27] and in cardioprotection in general [13]. In a recent report on limitation of myocardial necrosis, B_2 receptors were suggested to be linked to the opening of mitochondrial (mito) but not sarcolemmal (sarc) ATP-sensitive K^+ (K_{ATP}) channels [15]. In addition, recent reports have implicated mitoK_{ATP} channels as the end effectors in the downstream signaling pathways of early and delayed preconditioning against myocardial necrosis [11, 22]. In the signaling pathway of preconditioning against arrhythmias, however, a relationship between B_2 receptors and sarcK_{ATP} and/or mitoK_{ATP} channels has not yet been defined.

Therefore, to elucidate underlying mechanism(s) of potential antiarrhythmic effects of preconditioning during low-flow ischemia and reperfusion, we treated preconditioned rat hearts with HOE 140 (100 nM), a selective blocker of B_2 receptors. Additionally, we perfused the hearts with exogenous bradykinin (10 nM) during low-flow ischemia to reproduce (endogenous) bradykinin-mediated cardioprotection after preconditioning. To link bradykinin to K_{ATP} channels, we tested whether glibenclamide (1 μM), a non-specific blocker of K_{ATP} channels [14], abolishes the effects of both ischemic preconditioning and exogenous bradykinin. Finally, to identify the K_{ATP} channel subtype mediating the antiarrhythmic effects of preconditioning during low-flow ischemia, we treated preconditioned hearts as well as bradykinin-perfused heart with HMR 1098 (10 μM), a selective sarcK_{ATP} channel blocker [6, 10], or with 5-hydroxydecanoate (5-HD; 100 μM), a selective mitoK_{ATP} channel blocker [12, 18].

Methods

Isolated rat heart preparation

Treatment of animals conformed to the rules of the Swiss Federal Act on Animal Protection (1998), and was approved by the veterinary department of Basel (Switzerland). Male Sprague Dawley rats (RCC Ltd., Füllinsdorf, Switzerland), weighing 300 – 350 g were anesthetized using intraperitoneal injection of 30 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL, USA). After midline sternotomy, hearts were excised rapidly and perfused within 30 sec as previously described [35]. All hearts were perfused at a constant pressure of 80 mm Hg with a filtered (pore size 0.65 μm) nonrecirculating modified Krebs-Henseleit buffer containing (in mM) NaCl 117.0, KCl 4.3, $MgCl_2$ 1.2, $CaCl_2$ 2.0, $NaHCO_3$ 25.0, EDTA 0.5, glucose 15.0, and albumin 10.0 mg/L at pH 7.4. This buffer was saturated with 95 % O_2/5 % CO_2 ensuring a minimal pO_2 of 550 mm Hg. During all experiments, the hearts were immersed in perfusate maintained at exactly 37.0 °C [35] preventing bradycardia due to cardiac cooling.

Experimental protocols

The experimental protocols are presented in Fig. 1. Low-flow ischemia was induced for 60 min by reducing the perfusion pressure from 80 to 15 mm Hg leading to a reduction of coronary flow of ≈90 %. To prevent bradycardia and asystole during low-flow ischemia, hearts were paced (as recommended by [5]) at 300 beats per min via a pair of platinum pacemaker wires implanted in the right ventricular free wall and connected to a pulse generator (Grass SD 5, Grass Instruments, Quincy, MA, USA). Reperfusion was induced by normalizing the perfusion pressure to 80 mm Hg for 60 min. This protocol did not cause any macroscopically visible myocardial necrosis in our experiments as evaluated by 2,3,5 triphenyltetrazolium chloride (TTC) staining [1] after 60 min of low-flow ischemia and prolonged reperfusion (120 min) in additional experiments. Additionally, this protocol did not cause contractile dysfunction in control hearts (Table 1).

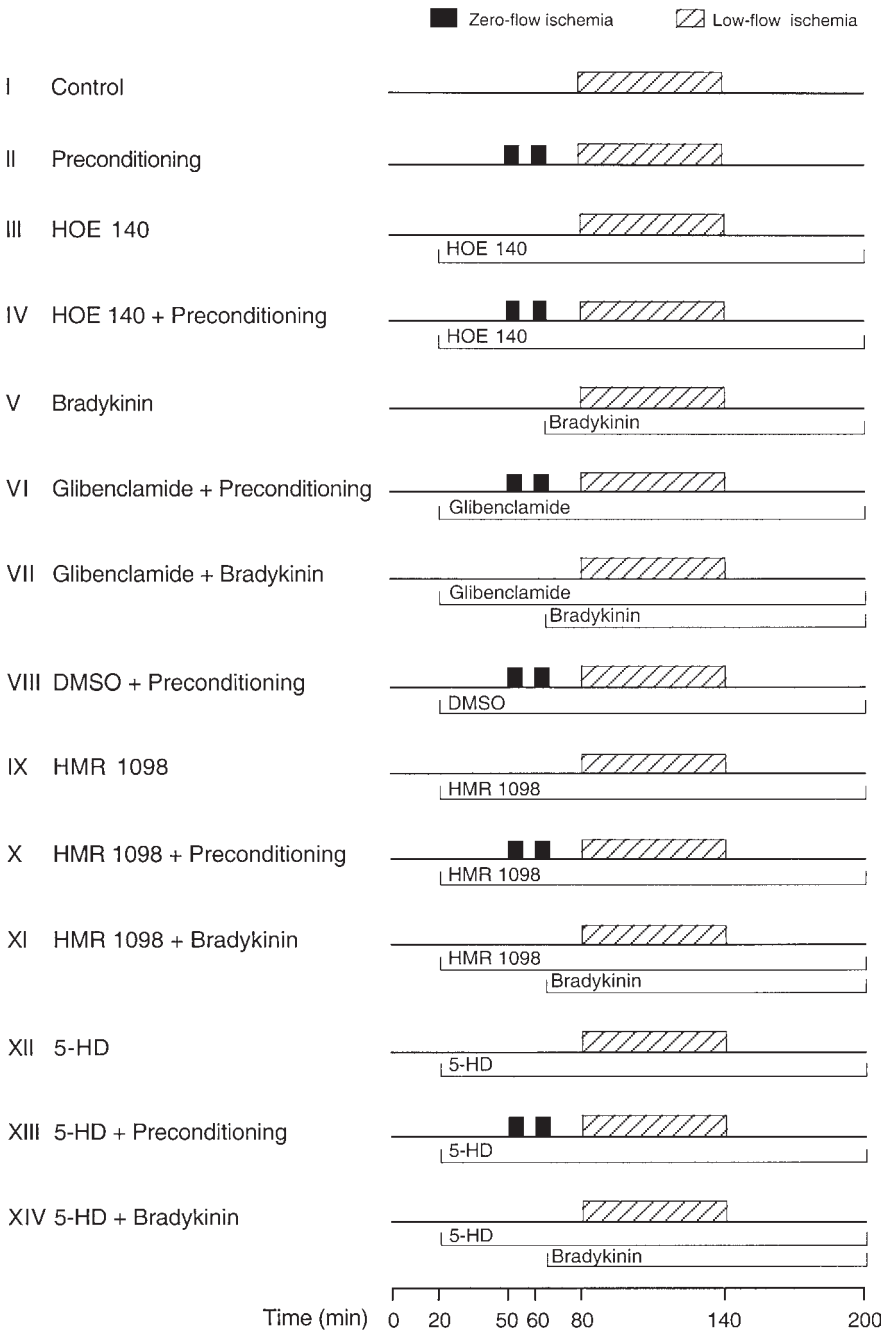

Fig. 1 Experimental protocols. All hearts were submitted to 60 min of low-flow ischemia (12% residual coronary flow) followed by 60 min of reperfusion. Drug treatment is indicated by brackets and timing of interventions is indicated at the bottom. DMSO, dimethylsulfoxide; 5-HD, 5-hydroxydecanoate

Hearts were randomly assigned to one of 14 groups: (I) Control: hearts subjected to low-flow ischemia and reperfusion without drug treatment or ischemic preconditioning (n = 10); (II) Preconditioning: hearts subjected to ischemic preconditioning consisting of two cycles of 5 min of global zero-flow ischemia followed by 5 and 15 min of reperfusion, respectively before low-flow ischemia (n = 11); (III) HOE 140: hearts treated with HOE 140 (100 nM) 60 min before low-flow ischemia until the end of the experiment (n = 10); (IV) HOE 140 + Preconditioning: hearts treated with HOE 140 (100 nM) 30 min before the preconditioning ischemia until the end of the experiment (n = 10); (V) Bradykinin: hearts treated with bradykinin (10 nM) 15 min before low-flow ischemia until the end of the experiment (n = 11); (VI) Glibenclamide + Preconditioning: hearts treated with glibenclamide (1 μM, a non-specific blocker of K_{ATP} channels [14]) 30 min before the preconditioning ischemia until the end of the experiment (n = 10); (VII) Glibenclamide + Bradykinin: hearts treated with glibenclamide (1 μM)

Table 1 Hemodynamic results

		Coronary flow (ml/min)				Left ventricular developed pressure (mm Hg)			
	n (hearts)	Baseline	Before ischemia	Low-flow Ischemia	End Reperfusion	Baseline	Before ischemia	Low-flow Ischemia	End Reperfusion
Control	10	22.6 ± 6.0	19.1 ± 5.6	2.5 ± 1.2	14.7 ± 7.0	79 ± 5	79 ± 5	11 ± 5	70 ± 16
Preconditioning	11	24.0 ± 6.7	23.0 ± 6.2	3.0 ± 0.7	14.0 ± 4.2	81 ± 8	79 ± 12	13 ± 5	72 ± 10
HOE 140	10	23.2 ± 3.7	20.0 ± 4.5	3.0 ± 1.0	15.5 ± 4.3	79 ± 3	79 ± 4	13 ± 4	74 ± 5
HOE 140 + Preconditiong	10	23.8 ± 2.6	21.7 ± 3.2	2.8 ± 0.8	16.4 ± 5.8	78 ± 4	77 ± 4	12 ± 3	70 ± 6
Bradykinin	11	24.1 ± 5.2	24.1 ± 3.4*	3.1 ± 0.5	18.9 ± 5.8	76 ± 5	77 ± 4	13 ± 2	70 ± 9
Glibenclamide + Preconditioning	10	25.4 ± 2.6	16.9 ± 1.8	2.7 ± 0.7	10.0 ± 2.2	78 ± 7	72 ± 8	11 ± 3	66 ± 9
Glibenclamide + Bradykinin	10	25.2 ± 3.7	19.7 ± 5.9	2.4 ± 0.9	11.2 ± 4.8	82 ± 6	80 ± 9	10 ± 2	66 ± 14
HMR 1098	8	23.1 ± 5.1	20.0 ± 3.6	2.4 ± 0.7	14.5 ± 5.0	82 ± 6	81 ± 4	14 ± 4	78 ± 23
HMR 1098 + Preconditioning	10	23.2 ± 3.5	15.7 ± 2.1	3.2 ± 0.8	10.0 ± 1.4	76 ± 5	72 ± 11	9 ± 2	62 ± 8
HMR 1098 + Bradykinin	9	24.8 ± 5.4	20.0 ± 4.0	2.7 ± 0.6	16.8 ± 4.1	81 ± 8	80 ± 6	12 ± 4	72 ± 10
5-HD	9	23.5 ± 4.2	21.3 ± 3.6	2.2 ± 0.7	15.1 ± 3.4	80 ± 5	81 ± 4	12 ± 3	63 ± 12
5-HD + Preconditioning	10	22.9 ± 2.7	21.9 ± 3.0	2.9 ± 0.4	14.2 ± 3.2	79 ± 2	80 ± 2	11 ± 3	70 ± 4
5-HD + Bradykinin	12	23.2 ± 5.8	23.8 ± 5.8	2.7 ± 0.7	15.7 ± 3.5	80 ± 6	79 ± 6	13 ± 3	72 ± 11

Values are mean ± SD. Low-flow ischemia values are averaged over the entire 60-min period. Note that at baseline, during, and after low-flow ischemia, neither coronary flow nor left ventricular (LV) developed pressure significantly differed among groups. *$P < 0.05$ vs. control

and bradykinin (10 nM) 60 and 15 min before low-flow ischemia, respectively, until the end of the experiment (n = 10); (VIII) DMSO + Preconditioning: hearts treated with DMSO (0.01 %, the vehicle of glibenclamide) 30 min before the preconditioning ischemia until the end of the experiment (n = 3); (IX) HMR 1098: hearts treated with HMR 1098 (10 μM, a selective sarcK_{ATP} channel blocker [6, 10] in a concentration known to block >90 % of the sarcolemmal K_{ATP} channel current [17]) 60 min before low-flow ischemia until the end of the experiment (n = 8); (X) HMR 1098 + Preconditioning: hearts treated with HMR 1098 (10 μM) 30 min before the preconditioning ischemia until the end of the experiment (n =10); (XI) HMR 1098 + Bradykinin: hearts treated with HMR 1098 (10 μM) and bradykinin (10 nM) 60 and 15 min before low-flow ischemia, respectively, until the end of the experiment (n = 9) ; (XII) 5-HD: hearts treated with 5-HD (100 μM, a selective mitoK_{ATP} channel blocker [12, 18]) 60 min before low-flow ischemia until the end of the experiment (n = 9); (XIII) 5-HD + Preconditioning: hearts treated with 5-HD (100 μM) 30 min before the preconditioning ischemia until the end of the experiment (n = 10); and (XIV) 5-HD + Bradykinin: hearts treated with 5-HD (100 μM) and bradykinin (10 nM) 60 and 15 min before low-flow ischemia, respectively, until the end of the experiment (n = 12).

Measurement of hemodynamic variables

Coronary flow was measured within the aortic canula using an inline flowprobe (Transonic 2N) connected to a transit time flowmeter (Transonic TTFM-SA type 700, Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Left ventricular (LV) pressure was measured by a fluid-filled polyethylene catheter inserted through the left atrial appendage into the LV cavity. The catheter was connected to an Isotec pressure transducer (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). LV developed pressure was defined as the difference between systolic and diastolic values of LV pressure. Simultaneously, a bipolar electrocardiogram (ECG) was recorded from electrodes implanted superficially in the right atrium and the apex. A digitized readout of the LV pressure and the ECG was recorded at 200 Hz sampling rate throughout the experiment using PowerLab 8e (AD Instruments, Castle Hill, Australia) connected to a Macintosh computer (Apple, Cupertino, CA, USA) running Chart software (AD Instruments, Castle Hill, Australia).

Analysis of arrhythmias

Analysis of ventricular arrhythmias was based on the Lambeth Conventions [32]. Accordingly, arrhythmias were categorized as single ventricular premature beat (VPB), ventricular tachycardia (VT, run of four or more consecutive VPBs with corresponding effective LV pressure) or ventricular fibrillation (VF, ECG waves of irregular morphology without corresponding effective LV pressure). Salvos (VPB doublet or triplet) were included in the analysis of VPB and, therefore, not analyzed separately. However, persistent bigeminies (alternating normal and premature beats) were not included in the analysis as they occurred in only three hearts. Sustained and

spontaneously reverted VF were not analyzed separately, and VF persisting longer than 30 sec was terminated by a bolus of 0.25 mg lidocaine hydrochloride (Sintetica, Mendrisio, Switzerland) injected into the perfusion canula proximal to the aorta [36]. After 5-min washout, the contractile and electrophysiological properties were restored [36] and the experiment was continued to avoid censoring of the data as recommended by the Lambeth Conventions [32].

Chemicals

HOE 140 and HMR 1098 were gifts from Aventis (Frankfurt, Germany). Bradykinin, glibenclamide (= glyburide), and 5-HD were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glibenclamide was dissolved in dimethylsulfoxide (DMSO) before it was added to the perfusate, and the final concentration of DMSO was 0.01 %.

Evaluation and statistical analysis

Coronary flow and LV developed pressure were expressed as mean ± SD. The number of VPBs was evaluated on the digitized ECG at 5-min intervals. Because of non-Gaussian distribution, the number of VPBs was expressed as median with interquartile range that is the distance between 25th and 75th percentile [34]. Finally, the incidence of VT and VF during low-flow ischemia and reperfusion was evaluated on the digitized ECG and pressure readouts. This analysis was to represent the presence or absence or ventricular tachyarrhythmias (disregarding the number and duration of VT or VF episodes).

Statistical comparisons of coronary flow and LV developed pressure among groups were performed by one-way analysis of variance (ANOVA) followed by Dunett's test to detect groups different from control. Comparisons of the incidence of VT and of VF among groups were performed by chi-squared analysis followed by Fisher's Exact test. Differences were considered significant for p values <0.05. For comparison of VPBs among groups, the number of VPBs were pooled during low-flow ischemia and during reperfusion for each experiment and analyzed by the Kruskal Wallis test followed by multiple Mann-Whitney U test with downward adjustment of the a level for 14 groups (0.004). Early reperfusion VPBs were analyzed separately during the first 5 min of reperfusion using Kruskal Wallis test followed by Mann-Whitney U test with downward adjustment. Statistical computations were done using Prism software (GraphPad, San Diego, CA, USA; version 3.0a) and Statview (SAS Institute, Cary, NC, USA; version 5.0). The sample size of the various groups was chosen to statistically detect a difference in the incidence of VF of at least 50 % [34].

Results

Exclusions

We used a total of 141 isolated rat hearts for this study. Of these, eight hearts were excluded due to unstable LV pressure readings during stabilization.

Hemodynamic variables

At baseline, no significant differences of coronary flow and LV developed pressure could be detected among the groups (Table 1; $p = 0.95$ and 0.31, respectively). However, after bradykinin treatment, coronary flow was slightly, but significantly, higher than in control hearts. Additionally, glibenclamide and HMR 1098 caused a slight decrease in coronary flow that was not significantly different from control. Nevertheless, LV developed pressure was similar after drug treatment and before low-flow ischemia ($p > 0.26$). Inducing low-flow ischemia by reducing the perfusion pressure led to a reduction of coronary flow and LV developed pressure from 23.9 ± 4.4 to 2.8 ± 0.7 mL/min (= 11.8 % residual coronary flow) and from 79.1 ± 5.1 to 11.6 ± 3.3 mm Hg, respectively (Table 1). Throughout this low-flow ischemia, coronary flow and LV developed pressure values were not significantly different among the groups ($p = 0.14$ and 0.16, respectively). During reperfusion, coronary flow was similar in all groups except in the first 15 min of reperfusion where flow was reduced in glibenclamide- and HMR 1098-treated hearts. In the other groups, coronary flow returned to near baseline levels in the first 5 min of reperfusion and subsequently fell slightly to ≈60 % of baseline levels. At the end of reperfusion, however, no group significantly differed from control hearts. Similarly, LV developed pressure did not differ among the groups at the end of reperfusion ($p = 0.13$). Finally, coronary flow and LV developed pressure in preconditioned hearts receiving DMSO (the vehicle of glibenclamide) were not different from preconditioned hearts without DMSO throughout the experiments, excluding potential hemodynamic effects of DMSO.

Ventricular premature beats

At baseline, the number of VPBs was similarly low in all groups (Fig. 2A and 2B, $p = 0.99$). During low-flow ischemia, ischemic preconditioning significantly reduced the number of VPBs. Specifically, the median number of VPBs was significantly lower in preconditioned hearts than in control hearts during low-flow ischemia (Fig. 2A). This protection was abolished when hearts were pretreated with HOE 140. However, the

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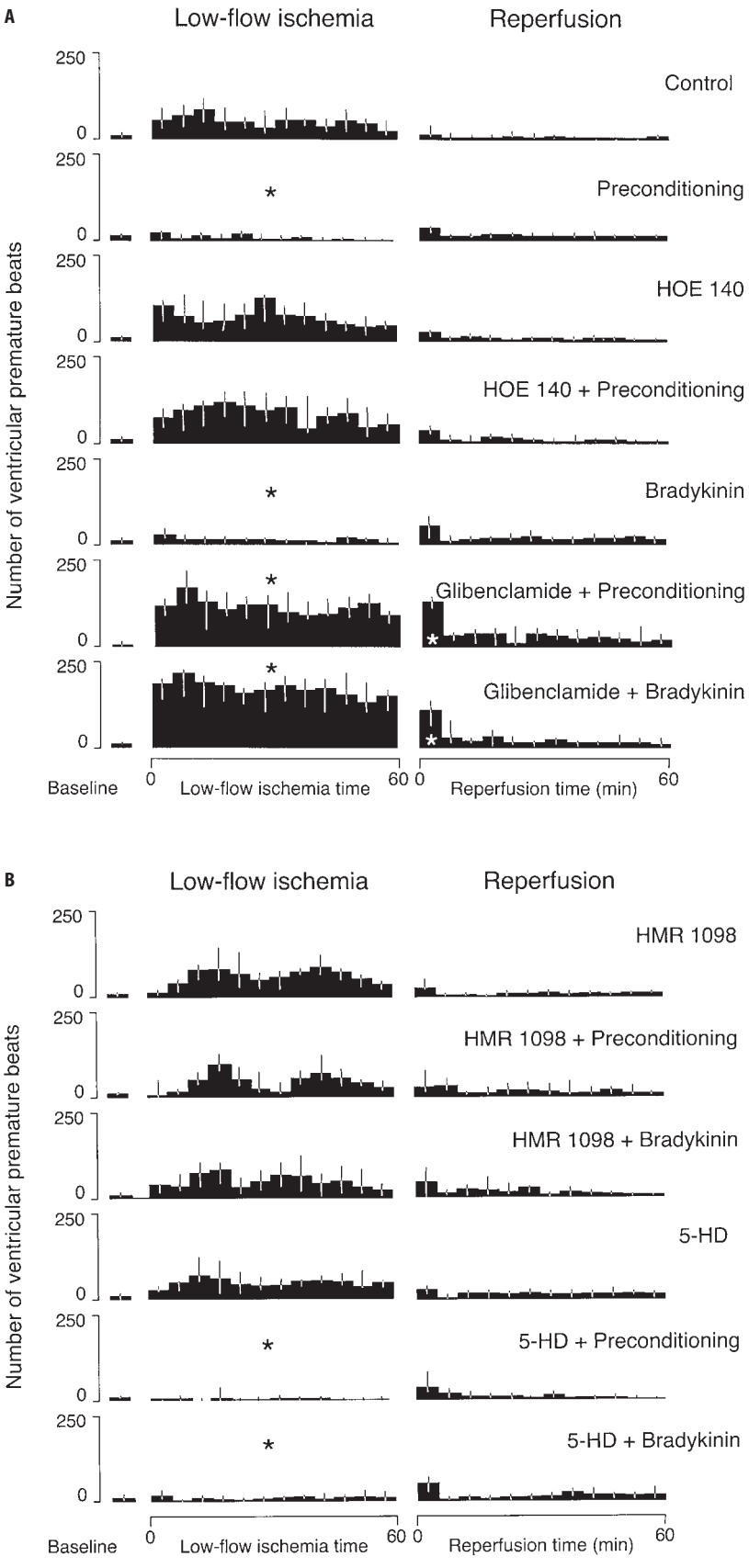

Fig. 2 **A** Number of ventricular premature beats of groups I to VII during 60 min of low-flow ischemia and 60 min of reperfusion. Values are medians with quartiles (25th and 75th percentile) evaluated at 5-min intervals for 10 – 11 rat hearts per group. *P < 0.05 vs. control. **B** Number of ventricular premature beats of the groups IX to XIV receiving HMR 1098 or 5-HD, the selective sarcolemmal and mitochondrial K^+_{ATP} channel blockers, respectively during 60 min of low-flow ischemia and 60 min of reperfusion. Values are medians with quartiles (25th and 75th percentile) evaluated at 5-min intervals for 8-12 rat hearts per group. *P < 0.05 vs. control (shown on Fig. 2A)

number of VPBs in HOE 140-treated hearts was not higher than in control hearts ($p = 0.17$). Similar to preconditioning, the number of VPBs in bradykinin-treated hearts was significantly reduced during low-flow ischemia. The protective effects of both preconditioning and exogenous bradykinin were abolished by glibenclamide increasing the number of VPBs beyond that of control hearts. Similarly, the protective effect of both preconditioning and bradykinin was abolished by HMR 1098, and was not modified by 5-HD (Fig. 2B). However, in hearts treated with HMR 1098 or 5-HD alone (without preconditioning or bradykinin), the number of VPBs was not different from control during low-flow ischemia ($p = 0.47$).

During reperfusion, far fewer VPBs occurred than during low-flow ischemia in all groups (Fig. 2A and 2B). In addition, the median number of VPBs was similar in all groups except in glibenclamide-treated hearts where the median number of VPBs was significantly higher than in control hearts. This difference was particularly pronounced during the first 5 min of reperfusion. Finally, the number of VPBs in preconditioned hearts receiving DMSO was not different from preconditioned hearts without DMSO throughout the experiments ($p = 0.25$; data not shown), excluding potential electrophysiologic effects of DMSO.

Incidence of VT and VF

During low-flow ischemia, similar findings as for VPBs were observed for the incidence of VT and VF (Fig. 3). Accordingly, ischemic preconditioning significantly reduced the incidence of both VT and VF from 80% in control to 27%. This protection, however, was significantly attenuated by HOE 140 since HOE 140 given before preconditioning increased the incidence of VT and VF to 70%. Nevertheless, treatment with HOE 140 alone (without preconditioning) did not affect the incidence of VT and VF. Similar to preconditioning, bradykinin treatment significantly reduced the incidence of both VT and VF during low-flow ischemia to 27% and 18%. As for the number of VPBs, glibenclamide abolished the protective effects of both preconditioning and bradykinin, increasing the incidence of VT and VF during low-flow ischemia similar to that of control hearts. Similarly, treatment with HMR 1098 abolished the protective effect of both preconditioning and bradykinin increasing the incidence of both VT and VF during low-flow ischemia to 90%. In contrast, treatment with 5-HD did not alter the effect of both preconditioning and bradykinin on the incidence of both VT and VF. However, in hearts treated with HMR 1098 or 5-HD alone

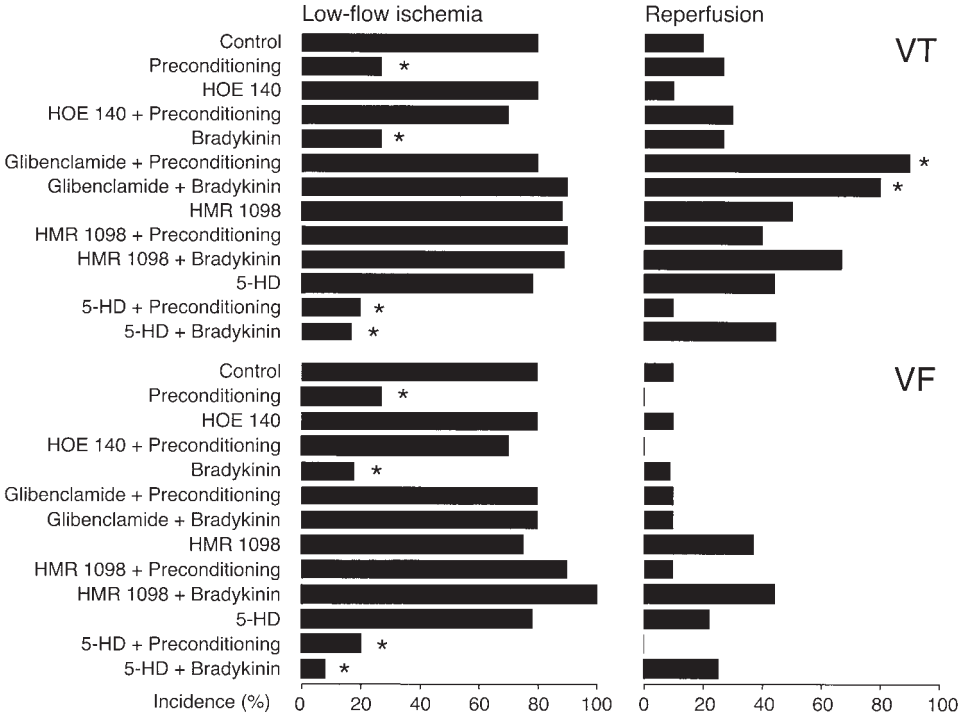

Fig. 3 Incidence of ventricular tachycardia (VT) and of ventricular fibrillation (VF) during 60 min of low-flow ischemia and during 60 min of reperfusion representing the presence or absence or ventricular tachyarrhythmias in a particular heart (independent on number and duration of VT and of VF). *$P < 0.05$ vs. control

(without preconditioning or bradykinin), the incidence of VT and VF was not different from control.

During reperfusion, the incidence of VT and VF was lower than during low-flow ischemia. However, glibenclamide significantly increased the incidence of VT during reperfusion. Nevertheless, the incidence of VF in all groups was not significantly different from control hearts. Finally, the incidence of VT and VF in preconditioned hearts receiving DMSO was not different from preconditioned hearts without DMSO during low-flow ischemia and reperfusion ($p > 0.51$; data not shown).

Discussion

In this study in isolated rat hearts, we demonstrate that ischemic preconditioning protects against ventricular arrhythmias induced by a low-flow ischemia and that this protection involves activation of bradykinin B_2 receptors and subsequent opening of sarcK_{ATP} but not of mitoK_{ATP} channels. Evidence for an antiarrhythmic effect of ischemic preconditioning during low-flow ischemia is that two cycles of 5 min of global zero-flow ischemia significantly reduced both the number of VPBs and the incidence of VT and of VF during a 60-min low-flow ischemia at a defined residual coronary flow of 12%. Furthermore, evidence that bradykinin B_2 receptors are involved in this protection is that the B_2 blocker HOE 140 abolished the effect of preconditioning and that exogenous bradykinin reproduced the antiarrhythmic effects of preconditioning during low-flow ischemia. Moreover, evidence for bradykinin B_2 receptor-linked opening of K_{ATP} channels arises from the finding that glibenclamide abolished the antiarrhythmic effects of both preconditioning and exogenous bradykinin in our experiments. Finally, evidence for a role of sarcK_{ATP} rather than mitoK_{ATP} channels in this form of protection is that HMR 1098, but not 5-HD, abolished the protective effects of both preconditioning and exogenous bradykinin against arrhythmias during low-flow ischemia. Exclusive administration of either selective K_{ATP} channel blocker (without preconditioning or bradykinin) did not affect the number of VPBs or the incidence of VT and of VF during low-flow ischemia or reperfusion. This excludes potential proarrhythmic effects of HMR 1098 or 5-HD that would have undermined our conclusion. During reperfusion, too few arrhythmias occurred in control hearts for any antiarrhythmic effect of preconditioning to be detectable.

As described previously [29, 30], the antiarrhythmic effects of ischemic preconditioning were not dependent on the degree of ischemia in our experiments and preconditioning can, thus, protect against subsequent ischemic episodes when myocardial blood flow is not completely interrupted. Similar to our findings in low-flow ischemia, bradykinin has been suggested to underlie antiarrhythmic effects of preconditioning during zero-flow ischemia [30]. As in our experiments, HOE 140 abolished the antiarrhythmic effects of preconditioning in these studies. Additionally, antiarrhythmic effects of exogenous bradykinin have been reported in a previous study in dogs [31]. Furthermore, a recent report showed a link between bradykinin B_2 receptor activation and K_{ATP} channel opening in the effect of preconditioning against myocardial necrosis [15]. Additionally, reperfusion-induced arrhythmias after brief regional zero-flow ischemia have been shown to be suppressed after preconditioning via K_{ATP} channel opening [16]. The present study extends these studies by showing for the first time that antiarrhythmic effects of ischemic preconditioning can be mediated by bradykinin B_2 receptor activation linked to sarcK_{ATP} channel opening.

Surprisingly, glibenclamide not only abolished preconditioning and bradykinin-induced protection against arrhythmias, but it significantly increased the number of VPBs beyond that of control hearts. However, glibenclamide did not increase the incidence of VT and of VF beyond that of control hearts during low-flow ischemia (as a limitation of our study, such an increase would have been impossible to detect with statistical significance because of the high incidence of VT and of VF in control hearts during low-flow ischemia). Still, because of the effects of glibenclamide on VPBs and on VT during reperfusion, this drug may be favoring VPBs without providing a substrate to maintain re-entrant tachyarrhythmias. K_{ATP} channels blockade by glibenclamide can prolong action potential duration [9] and may give rise to early and delayed afterdepolarizations, triggered activity and, thus, VPBs [7]. Alternatively, it may be that blocking solely K_{ATP} channels does not sufficiently alter the dispersion of repolarization to cause re-entrant excitations and, thus, to induce and maintain VT or VF.

The signal transduction of bradykinin B_2 receptors to K_{ATP} channel opening in preconditioned hearts remains speculative. Bradykinin released from endothelial cells during preconditioning can stimulate endothelial release of nitric oxide and prostanoids, which have been shown to activate the mitoK_{ATP} channel in rabbit ventricular myocytes [2, 24]. However, B_2 receptors are also present on rat cardiomyocyte [19] where they lead to protein kinase C (PKC) activation. Of interest, PKC potentiated the opening of the mitoK_{ATP} channel in rabbit ventricular myocytes [25] and in isolated rat hearts [33]. Additionally, PKC phosphorylated the pore forming subunit Kir6.2 [28] that has been proposed to be the sole pore forming subunit of the sarcK_{ATP} channel in rabbit ventricular myocytes [28]. Alternatively, bradykinin-induced prostanoid release may account for sarcolemmal K_{ATP} channel activation [2]. Taken together, these studies suggest a role for both mitoK_{ATP} and sarcK_{ATP} channels in the cardioprotection afforded by precondi-

tioning. However, recent reports have implicated the mitoK_{ATP} rather than the sarcK_{ATP} channels in the early and delayed antinecrotic effect of preconditioning [11, 22]. By contrast, our results showed that the sarcK_{ATP}, but not the mitoK_{ATP}, channels mediate the antiarrhythmic effects of preconditioning during low-flow ischemia. Possible mechanisms responsible for this antiarrhythmic effect of sarcK_{ATP} channel activation might include shortening of the cardiac action potential duration and membrane hyperpolarization, which would lead to a reduction in Ca^{2+} overload and a preservation of ATP.

During reperfusion, too few arrhythmias occurred in control hearts for any antiarrhythmic effect of preconditioning to be detectable. However, in glibenclamide-treated hearts, the number of VPBs and the incidence of VT were higher than in control hearts. This indicates a pro-arrhythmic effect of glibenclamide during reperfusion after a low-flow ischemia. Nevertheless, for unknown reasons, the incidence of VF was not altered in glibenclamide-treated hearts during reperfusion. As discussed above, it may be speculated that this drug can cause runs of VPBs (thus leading to *non-sustained* VT, as observed in our experiments), but does not provide a substrate for *sustained* VT or VF during reperfusion.

During low-flow ischemia, the residual coronary flow was 12% of baseline coronary flow in our experiments. This flow is comparable to a previous study on preconditioning against contractile dysfunction [4]. In that study [4], however, low-flow ischemia lasted longer (90 min) than in our experiments (60 min). In our experiments, severe ischemia was absent as functional recovery was complete after reperfusion and as myocardial necrosis (potentially confounding the analysis of arrhythmias) was excluded by TTC staining. Finally, pretreatment periods and concentrations of HOE 140, bradykinin, glibenclamide, HMR 1098, and 5-HD were based on the literature to ensure efficacy of the drugs and not to distinguish trigger and mediator mechanisms of preconditioning.

In conclusion, ischemic preconditioning protects against ventricular arrhythmias induced by low-flow ischemia in isolated rat hearts and this protection involves activation of bradykinin B_2 receptors and subsequent opening of sarcK_{ATP}, but not of mitoK_{ATP}, channels. These findings support the view that antiarrhythmic effects of ischemic preconditioning are brought about by different mechanisms than antinecrotic effects.

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