Basic Research in Cardiology (2019) 114:22
https://doi.org/10.1007/s00395-019-0732-3

ORIGINAL CONTRIBUTION

Attenuation of ST-segment elevation after ischemic conditioning maneuvers reflects cardioprotection online

Georgios Amanakis[1] · Petra Kleinbongard[1] · Gerd Heusch[1] · Andreas Skyschally[1]

Received: 05 December 2018 / Revised: 12 March 2019 / Accepted: 23 March 2019 / Published online: 1 April 2019

Abstract

Ischemic conditioning maneuvers, when induced either locally in the heart or remotely from the heart, reduce infarct size. However, infarct size reduction can be assessed no earlier than hours after established reperfusion. ST-segment elevation and its attenuation might reflect cardioprotection by ischemic conditioning online. Pigs were subjected to regional myocardial ischemia/reperfusion (1 h/3 h). Ischemic conditioning was induced prior to ischemia either locally (preconditioning; IPC; $n=15$) or remotely (remote preconditioning; RIPC; $n=21$), remotely during ischemia (remote perconditioning; RPER; $n=18$), or locally at reperfusion (postconditioning; POCO; $n=9$). Pigs without conditioning served as controls (PLA; $n=29$). Area at risk and infarct size were measured postmortem, and ST-segment elevation was analyzed in a V2-like electrocardiogram lead. Ischemic conditioning reduced infarct size (PLA $42\pm11\%$ of area at risk; IPC $18\pm10\%$; RIPC $22\pm12\%$; RPER $23\pm12\%$, POCO $22\pm11\%$). With PLA, ST-segment elevation was increased at 5 min ischemia, sustained until 55 min ischemia and further increased at 10 min reperfusion. IPC and RIPC did not impact on ST-segment elevation at 5 min ischemia, but attenuated ST-segment elevation at 55 min ischemia. With RPER, ST-segment elevation was not different from that with PLA at 5 min, but attenuated at 55 min ischemia. POCO abolished the further increase of ST-segment elevation with reperfusion. Cardioprotection by ischemic conditioning is robustly reflected by attenuation of ST-segment elevation online.

Keywords Cardioprotection · Infarct size · Ischemia–reperfusion injury · Ischemic conditioning · ST-segment elevation

Introduction

In patients surviving an acute myocardial infarction, infarct size is a major determinant of prognosis [14, 36]. Ischemic conditioning maneuvers attenuate infarct size [12], and such protection can be recruited by short cycles of non-lethal ischemia/reperfusion either locally in the heart or remotely from tissues other than the heart. Cardioprotection by ischemic conditioning can be induced prior to ischemia as local ischemic preconditioning (IPC) [21] or as remote ischemic preconditioning (RIPC) [13, 24], during ischemia as remote ischemic perconditioning (RPER) [28], and locally at the onset of reperfusion as ischemic postconditioning (POCO) [42]. The success of such cardioprotective maneuvers in the acute setting can only be assessed with measurement of infarct size which is, however, no earlier possible than several hours or even days after the injurious event [1]. Experimental studies have reported an impact of local ischemic preconditioning on the electrocardiogram (ECG), but were limited to acute changes in ST-segment elevation during the brief ischemia/reperfusion cycles of a preconditioning maneuver. ST-segment elevation was attenuated during the second and subsequent ischemic cycles of the preconditioning maneuver [4, 29] or the first few minutes of sustained coronary occlusion [8]. Such attenuation of ST-segment elevation has also been observed in patients

B. Ibáñez, Madrid, Spain, served as guest editor for the manuscript and was responsible for all editorial decisions, including the selection of reviewers. The policy applies to all manuscripts with authors from the editor's institution.

Georgios Amanakis and Petra Kleinbongard contributed equally to this manuscript.

✉ Gerd Heusch
gerd.heusch@uk-essen.de

[1] Institut für Pathophysiologie, Westdeutsches Herz- und Gefäßzentrum, Universitätsklinikum Essen, Hufelandstr. 55, 45122 Essen, Germany

undergoing elective percutaneous coronary interventions [6, 7, 39].

We have recently reported that in pigs undergoing RPER, protection is reflected by an attenuated ST-segment elevation already during ongoing ischemia [18], suggesting that cardioprotection can be predicted from the ECG already after the protective stimulus and long before a measurement of final infarct size is possible. Thus, ST-segment elevation analysis might be a useful tool to assess the effectiveness of a cardioprotective maneuver online.

In the present study, we have retrospectively analyzed data from our anesthetized pig model of myocardial infarction [15, 18] to investigate also the impact of other conditioning maneuvers, namely IPC, RIPC, and POCO on ST-segment elevation during ischemia/reperfusion.

Methods

All experimental protocols were approved by the Bioethical Committee of the district of Duesseldorf (LANUV NRW, G1240/11, G1388/13, and G1407/14) and conformed with the "Position of the American Heart Association on Research Animal Use" adopted by the American Heart Association on November 11, 1984. Materials were obtained from Sigma-Aldrich (Deisenhofen, Germany), unless otherwise specified.

Experimental preparation

The experiments were performed between November 2012 and November 2017 and have already been reported in a different context [10, 18, 31, 32, 34]. Male Goettingen minipigs (body weight 30–35 kg, age 14 ± 2 months, Ellegaard, Dalmose, Denmark) were provided standard chow (twice 300 g/day, #V4133, ssniff, Soest, Germany), had free access to water, and were kept in tiled rooms (~ 2 m^2/pig) with straw bedding, at 12 h/12 h light/dark cycles. Only male pigs were used, as sex-based differences were not the subject of our studies. Pigs were sedated with flunitrazepam (0.4 mg/kg i.m., Sigma-Aldrich, Munich, Germany), and anesthesia was induced with etomidate (0.3 mg/kg i.v., Hypnomidat, Janssen-Cilag, Neuss, Germany) and sufentanil (1 µg/kg i.v., Sufenta, Janssen-Cilag, Neuss, Germany) through an ear vein. After tracheotomy, pigs were mechanically ventilated with oxygen-enriched air supplemented with isoflurane (2%, Forene, AbbVie, Ludwigshafen, Germany) to maintain anesthesia. Arterial blood gases and blood glucose levels were regularly monitored and kept within the normal range. Before a left lateral thoracotomy, additional analgesia was provided with sufentanil (10 µg/kg i.v.), and neuromuscular blockade was induced with rocuronium (0.6 mg/kg i.v., Esmeron, MSD, Haar, Germany). This anesthesia protocol is identical to that used in our institution for patients undergoing coronary artery bypass surgery [38]. A jugular vein was cannulated for saline volume replacement. A common carotid artery was cannulated to measure arterial pressure. The heart was exposed by pericardiotomy, and a catheter connected to a micromanometer (CODAN pvb Medical, Lensahn, Germany) was inserted through the apex into the left ventricle to measure left ventricular pressure. A Teflon catheter was placed in the left atrium for injection of colored microspheres [19]. A silk suture was placed around the left anterior descending coronary artery (LAD) distal to its second diagonal branch for later coronary occlusion. Occasionally, visible epicardial collaterals were ligated. During surgery, pigs were placed on a heated table and covered with drapes to keep body temperature (esophageal probe) at 37 ± 1 °C. Ventricular fibrillation was immediately terminated by internal defibrillation [30].

Protocols

A schematic on protocols is given in Fig. 1.

Placebo (PLA; *n* = 29)

Myocardial ischemia was induced for 60 min by tightening of the silk suture around the LAD against a silicone plate. Reperfusion was performed by quick release and removal of the suture and continued for 3 h. Heart rate, maximal left ventricular pressure (LVP_{max}), its first derivative (dP/dt_{max}), and ST-segment elevation were measured at baseline, at 5 and 55 min after the onset of ischemia, and at 10, 30, 60, and 120 min after reperfusion. Area at risk and infarct size were determined postmortem.

Ischemic preconditioning (IPC; *n* = 15)

Ischemic preconditioning was induced by two 3 min LAD occlusions, separated by 2 min reperfusion. The index ischemia was started 15 min later. The subsequent protocol was identical to PLA.

Remote ischemic preconditioning (RIPC; *n* = 21)

For remote ischemic preconditioning, a tourniquet was applied around the left hindlimb. RIPC was induced by four cycles of 5 min ischemia, followed by 5 min reperfusion, starting 40 min before thoracotomy. Skin cyanosis was taken to indicate leg ischemia and blushing to indicate reperfusion. The subsequent protocol was identical to PLA.

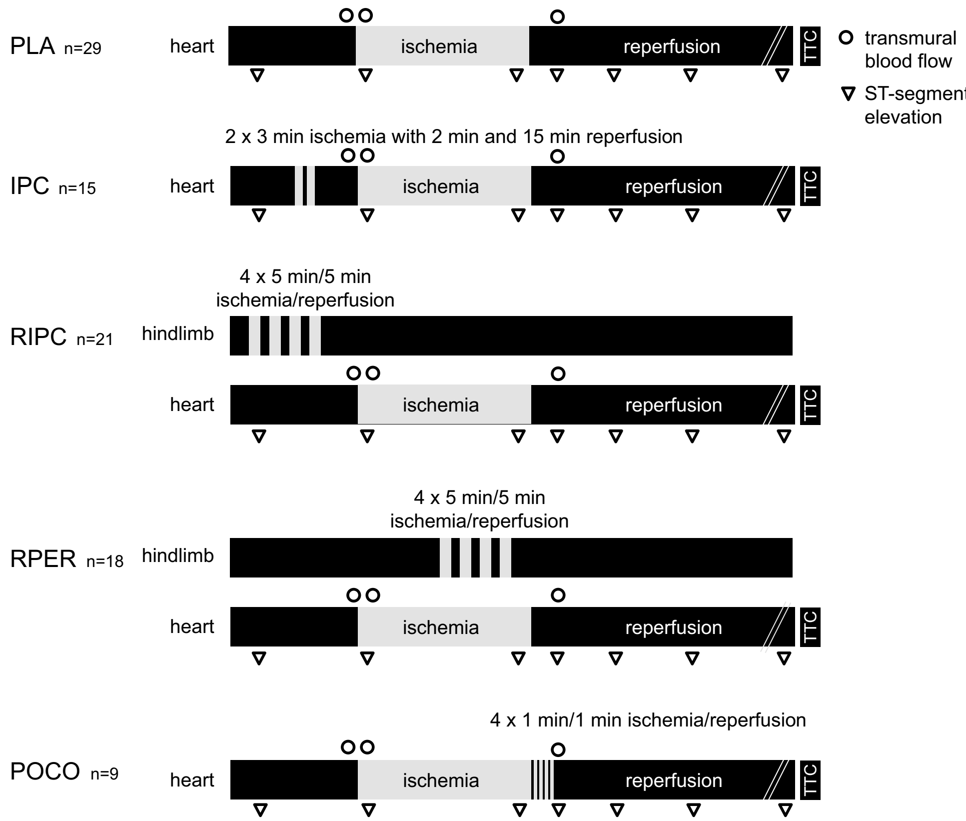

Fig. 1 Conditioning protocols, time points of ST-segment elevation analysis (triangles) and transmural blood flow measurements (circles); 5 and 55 min after the onset of coronary occlusion, 10, 30, 60, and 120 min after the onset of reperfusion. *PLA* placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning

Remote ischemic perconditioning (RPER; *n* = 18)

Remote ischemic perconditioning was performed in the same way as RIPC, starting 20 min after coronary occlusion. The subsequent protocol was identical to PLA.

Ischemic postconditioning (POCO; *n* = 9)

Ischemic postconditioning was induced by four cycles of 1 min LAD occlusion followed by 1 min reperfusion each, starting at 1 min reperfusion. The subsequent protocol was identical to PLA.

For a more sophisticated analysis of the impact of cardioprotection on ST-segment elevation, we have included experiments which have already been published ($n = 19$ PLA, $n = 18$ RPER) [18].

Systemic hemodynamics

Heart rate, LVP_{max}, and dP/dt_{max} were averaged offline over a period of ten subsequent cardiac cycles (CORDAT II) [33] at baseline, 5 and 55 min after the onset of coronary occlusion, and at 10, 30, 60, and 120 min reperfusion. Intervals with premature beats and periods of ventricular tachycardia/fibrillation were excluded from analysis.

Transmural myocardial blood flow

Transmural myocardial blood flow was measured with fluorescent microspheres (FluoSpheres Polystyrene 15 µm, Life technologies/Molecular probes, Darmstadt, Germany) [19]. A reference withdrawal was taken from a catheter in the descending thoracic aorta, and transmural tissue samples from the central area at risk were analyzed. Transmural myocardial blood flow is expressed in ml/min/g of tissue; transmural myocardial blood flow > 0.06 ml/min/g at 5 min of coronary occlusion was considered as non-severe ischemia, and these experiments were excluded from further analysis.

No-reflow, area at risk, and infarct size

At the end of each experiment, 30 ml of warmed 4% thioflavin-S solution (Morphisto, Frankfurt, Germany) was filtered through a 0.2 µm syringe filter to remove particulate debris and slowly infused into the left atrium to demarcate non-perfused areas of the left ventricle after 180 min reperfusion [30]. Thereafter, the LAD was re-occluded and 5 ml blue dye (Patentblau V, Guerbet, Sulzbach, Germany) was injected into the left atrium to delineate the area at risk. The pig was then euthanized by rapid injection of 20 ml potassium chloride solution (2 mol/l), and the heart was excised and sectioned into five slices parallel to the atrioventricular groove. Slice shape and the demarcated areas of no-reflow and the area at risk were traced on transparent film. The slices were immersed in 90 mmol/l sodium phosphate buffer containing 1% triphenyl-tetrazolium-chloride (TTC, Sigma-Aldrich Chemie, Munich, Germany) and 8% dextran for 20 min at 37 °C to demarcate infarcted areas which were traced again on transparent film. Particular care was taken to properly realign the slices using landmarks, such as the position of papillary muscles. Area at risk (expressed as fraction of the left ventricle) and infarct size (expressed as fraction of the area at risk) were determined by computer-assisted planimetry.

ECG analysis

ST-segment elevation was measured, as previously described [18]. A surface ECG was continuously recorded using a single channel, calibrated (1 mV reference) amplifier. Due to the surgical preparation and use of a metal rib retractor, the

Table 1 Systemic hemodynamics

	Time	HR (1/min)	LVP_{max} (mmHg)	dP/dt_{max} (mmHg/s)
PLA ($n=29$)	Baseline	117 ± 16	89 ± 9	1793 ± 370
	5 min ischemia	113 ± 14	80 ± 8*	1508 ± 291*
	55 min ischemia	117 ± 15	82 ± 9*	1564 ± 347*
	10 min reperfusion	118 ± 16	81 ± 9*	1622 ± 432*
	30 min reperfusion	119 ± 14	81 ± 8*	1675 ± 446
	60 min reperfusion	118 ± 13	81 ± 7*	1669 ± 317*
	120 min reperfusion	118 ± 12	78 ± 9*	1520 ± 332*
IPC ($n=15$)	Baseline	112 ± 18	87 ± 8	1629 ± 319
	5 min ischemia	105 ± 17	79 ± 8*	1416 ± 244*
	55 min ischemia	107 ± 13	79 ± 10*	1422 ± 256*
	10 min reperfusion	110 ± 19	80 ± 7*	1525 ± 368
	30 min reperfusion	108 ± 14	78 ± 5*	1429 ± 288*
	60 min reperfusion	107 ± 14	78 ± 5*	1411 ± 268*
	120 min reperfusion	106 ± 15	74 ± 6*	1303 ± 237*
RIPC ($n=21$)	Baseline	112 ± 11	88 ± 7	1669 ± 389
	5 min ischemia	109 ± 12	79 ± 7*	1374 ± 307*
	55 min ischemia	114 ± 18	79 ± 8*	1393 ± 269*
	10 min reperfusion	118 ± 20	77 ± 10*	1418 ± 286*
	30 min reperfusion	117 ± 17	78 ± 9*	1485 ± 309*
	60 min reperfusion	116 ± 16	76 ± 9*	1477 ± 394*
	120 min reperfusion	113 ± 15	76 ± 8*	1437 ± 394*
RPER ($n=18$)	Baseline	112 ± 11	88 ± 10	1733 ± 417
	5 min ischemia	107 ± 12	81 ± 9*	1327 ± 272*
	55 min ischemia	107 ± 13	75 ± 9*	1370 ± 276*
	10 min reperfusion	111 ± 13	78 ± 8*	1456 ± 379*
	30 min reperfusion	109 ± 13	78 ± 9*	1499 ± 414*
	60 min reperfusion	112 ± 13	79 ± 10*	1529 ± 382*
	120 min reperfusion	112 ± 13	78 ± 9*	1499 ± 435*
POCO ($n=9$)	Baseline	117 ± 12	85 ± 10	1833 ± 502
	5 min ischemia	108 ± 15	75 ± 6*	1276 ± 195*
	55 min ischemia	113 ± 11	73 ± 5*	1449 ± 500*
	10 min reperfusion	114 ± 10	74 ± 7*	1545 ± 535*
	30 min reperfusion	117 ± 12	73 ± 6*	1725 ± 585
	60 min reperfusion	118 ± 16	71 ± 6*	1521 ± 351*
	120 min reperfusion	115 ± 10	70 ± 6*	1420 ± 343*

PLA placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning, *HR* heart rate, LVP_{max} maximal left ventricular pressure, dP/dt_{max} maximal rate of rise of left ventricular pressure; mean ± SD

*$p < 0.05$ vs. baseline, two-way repeated measures ANOVA

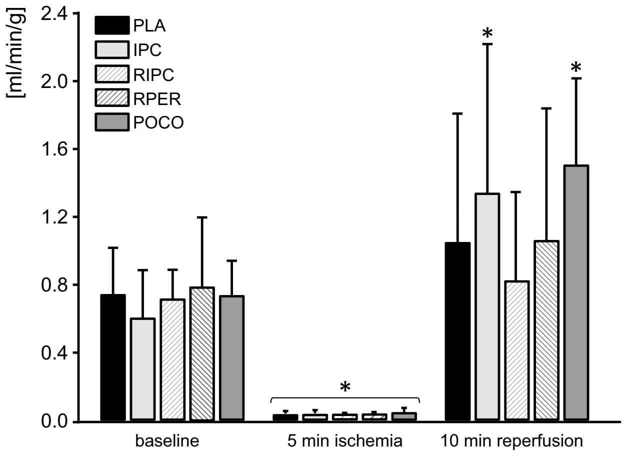

Fig. 2 Transmural myocardial blood flow in the area at risk at baseline, 5 min ischemia, and 10 min reperfusion. No significant differences between groups; $*p<0.05$ vs. baseline (two-way ANOVA and Fisher's least significant difference post hoc test). *PLA* placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning

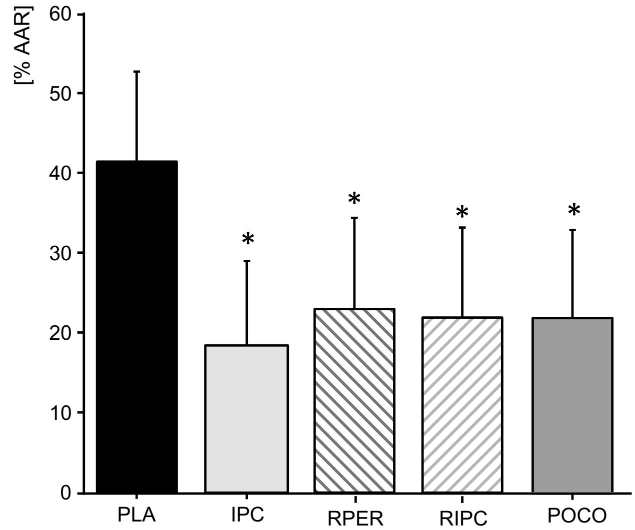

Fig. 4 Infarct size expressed as percent of the area at risk (AAR). All conditioning maneuvers reduced infarct size. $*p<0.05$ vs. PLA (one-way ANOVA and Fisher's least significant difference post hoc test). *PLA* placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning

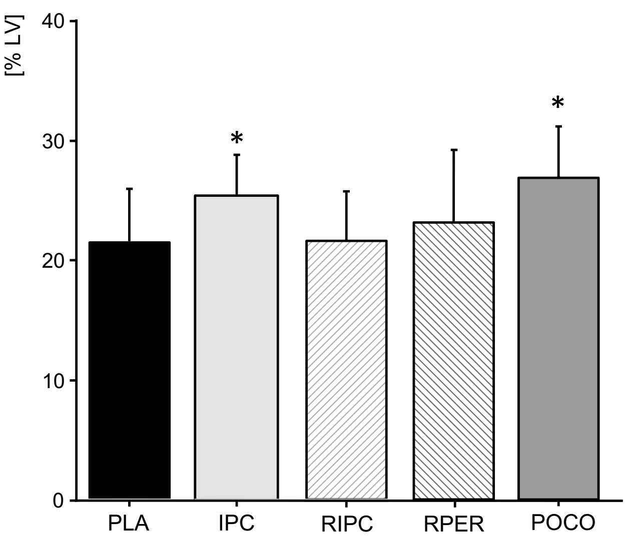

Fig. 3 Area at risk expressed as percent of the left ventricle (LV). $*p<0.05$ vs. PLA (one-way ANOVA and Fisher's least significant difference post hoc test). *PLA* placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning

recorded ECG lead appeared similar to a V2 Wilson lead in humans. The ST-segment elevation was defined as the amplitude difference between a point 30 ms before the P wave and a second point 20 ms after the J-point [5, 22]. In addition, we measured QRS duration, QT interval, and R amplitude [37, 41]. Analysis was performed offline using digital calipers (Labchart 8, AD Instruments Pty Ltd, New South Wales, Australia) at baseline, 5 and 55 min after the onset of ischemia, and at 10, 30, 60, and 120 min reperfusion. For each measurement, the ECG of 30 consecutive cardiac cycles was averaged; premature beats or periods of ventricular tachycardia/fibrillation were excluded. Of note, the position of the animal remained the same throughout the course of ECG registration to avoid changes in ECG amplitude.

Statistical analysis

All data are reported as mean ± SD. Area at risk, area of no-reflow, and infarct size were analyzed by one-way ANOVA with Fisher's least significant difference post hoc tests. Transmural myocardial blood flow, heart rate, LVP_{max}, and dP/dt_{max} were analyzed by two-way ANOVA for repeated measures with Fisher's least significant difference post hoc tests (SigmaStat 3.5; Systat Software, Erkrath, Germany). The incidence of ventricular fibrillation was compared between protocols by a Chi-Square test. Normal distribution of ST-segment elevation data was tested with the Shapiro–Wilk method. Outliers were identified using the Boxplot method and excluded from statistical analysis when off by more than 1.5 times the interquartile range. One pig of the PLA group and two of the IPC group were excluded retrospectively from the analysis, as their ST-segment elevation met the criteria for multiple outliers (≥ 5 time points). ST-segment elevation, QRS duration, QT interval, and R amplitude were analyzed by a mixed model analysis (SAS 9.4, SAS Institute Inc., Cary, NC, USA) for the fixed effects "group", "time", and the interaction "group × time", considering random effects induced by the individual animal. Least square means were computed as post hoc tests to identify differences between single mean values. Differences were considered significant at the level of $p < 0.05$.

We tested whether the magnitude of attenuation of ST-segment elevation was related to the magnitude of infarct size reduction on an individual level. For that, a linear regression between myocardial blood flow in the area at risk at 5 min ischemia and infarct size in pigs subjected to ischemia/

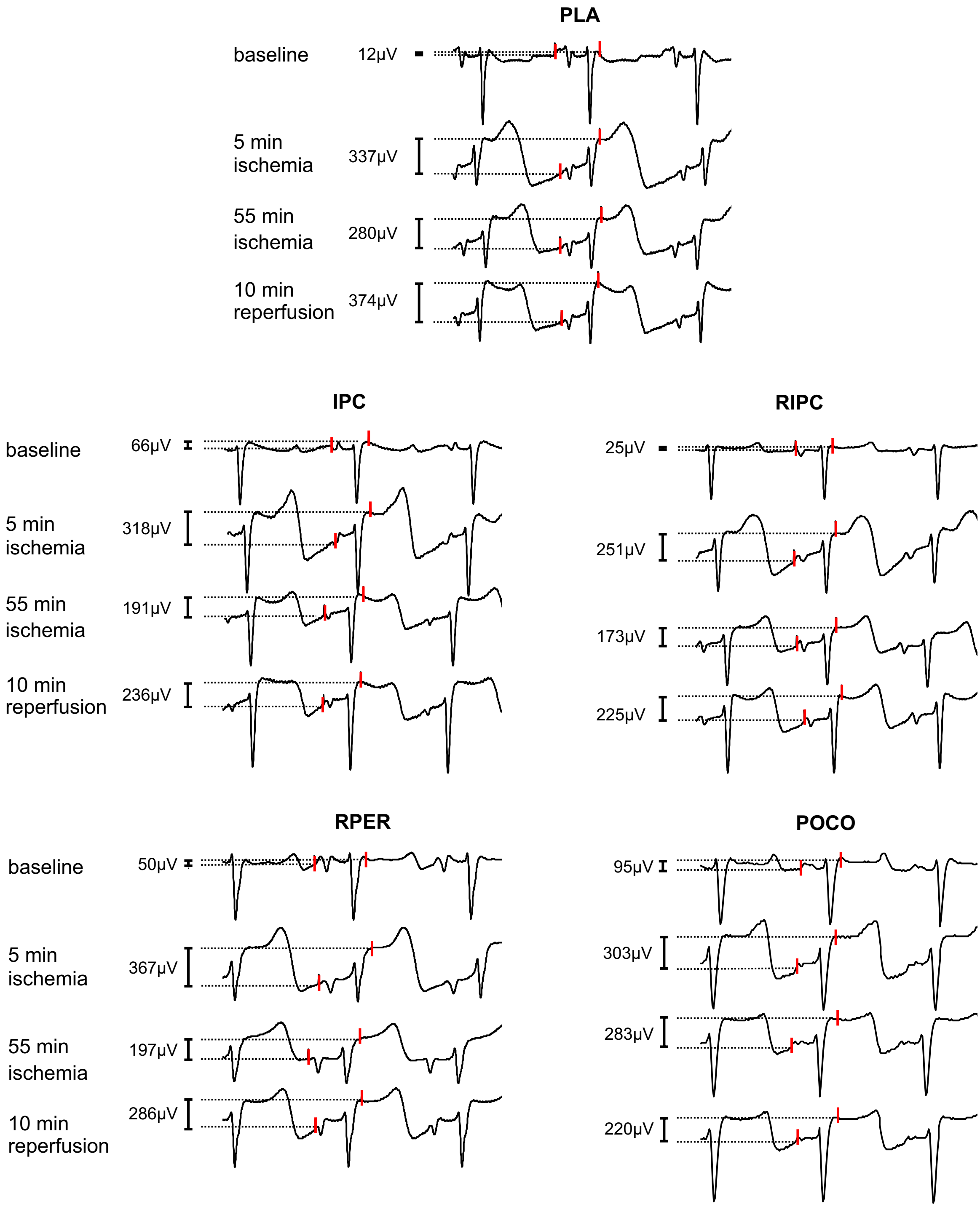

Fig. 5 Original ECG recordings from one pig of each experimental group (*PLA* placebo, *IPC* ischemic preconditioning, *RIPC* remote ischemic preconditioning, *RPER* remote ischemic perconditioning, *POCO* ischemic postconditioning). ST-segment elevation is displayed as amplitude difference between two points (vertical lines) 30 ms before the P wave and 20 ms after the J-point, respectively

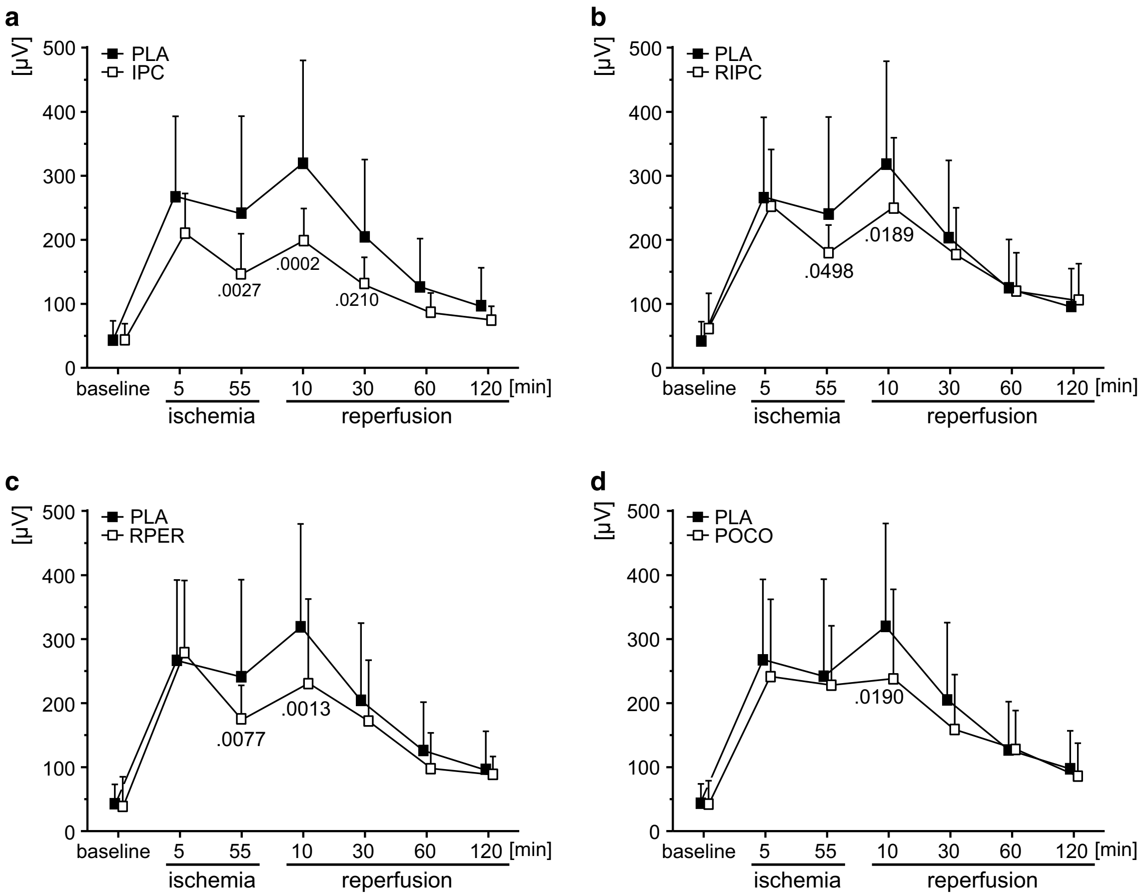

Fig. 6 Time course of ST-segment elevation with ischemic conditioning. **a** Ischemic preconditioning (IPC), **b** remote ischemic preconditioning (RIPC), **c** remote ischemic perconditioning (RPER), and **d** ischemic postconditioning (POCO). Mixed model analysis and least significant means post hoc test (*p* values vs. PLA are given below the respective data point). For comparison, the time course of placebo (PLA) is displayed in each panel

reperfusion only was calculated. Then the individual infarct size reduction for each pig in the IPC, RIPC, and RPER groups was assessed as the difference between the observed infarct size and the expected infarct size without a protective intervention, given the individual blood flow at 5 min ischemia.

Results

Systemic hemodynamics, transmural myocardial blood flow, ventricular fibrillation, area at risk, area of no-reflow, and infarct size

Heart rate, LVP_{max}, and dP/dt_{max} did not differ between the groups (Table 1). Transmural myocardial blood flow in the area at risk was reduced with coronary occlusion in all groups to a similar extent and did not differ between groups at baseline, 5 min ischemia, and 10 min reperfusion (Fig. 2). The incidence of ventricular fibrillation was 38% in PLA, 57% in IPC, 35% in RIPC, 44% in RPER, and 44% in POCO and not different between groups ($p=0.74$). Ventricular fibrillation occurred after 33 ± 9 min of the index ischemia and was terminated by 2.9 ± 2.6 defibrillations. In eight pigs ventricular fibrillation occurred during the initial minutes of reperfusion. We did not further stratify for the time of ventricular fibrillation. The area at risk (Fig. 3) was similar between the PLA, RIPC, and RPER groups, but slightly larger with IPC and POCO. The total area of no-reflow expressed as a fraction of the area at risk was similar with PLA (15 ± 13%), IPC (16 ± 19%), and RIPC (15 ± 15%) and tended to be larger with POCO (23 ± 15%) and smaller with RPER (9 ± 12%), but none of these differences reached statistical significance. All ischemic conditioning maneuvers reduced infarct size from that seen with PLA (Fig. 4).

ECG analysis

Typical original recordings of ST-segment elevation from each experimental group are shown in Fig. 5. The time course of ST-segment elevation differed between protocols (Fig. 6). ST-segment elevation was negligible at baseline and not different between groups. With PLA, ST-segment elevation was increased at 5 min after the onset of ischemia and remained unchanged at 55 min ischemia. At 10 min reperfusion, ST-segment elevation was increased further and then gradually recovered, but remained slightly above baseline at 120 min reperfusion (Fig. 6). IPC and RIPC did not significantly attenuate ST-segment elevation at 5 min ischemia (there was a trend for IPC), but did so at 55 min of ischemia. This attenuation of ST-segment elevation by IPC and RIPC remained apparent up to 30 min reperfusion (Fig. 6a, b). RPER attenuated ST-segment elevation after the conditioning maneuver at 55 min ischemia and up to 10 min reperfusion (Fig. 6c). ST-segment elevation with POCO was not different from that with PLA at baseline and during ischemia, but the further increase in ST-segment elevation at 10 min reperfusion was abrogated (Fig. 6d).

There was no correlation between the individual change in ST-segment elevation between 5 and 55 min ischemia and the calculated individual reduction in infarct size ($r = 0.004$).

QRS duration and QT interval did not change during the experimental protocols and were not different between groups (Figs. 7, 8). R amplitude was markedly decreased at 5 min ischemia and then gradually recovered without differences between groups (Fig. 9).

Limitation of methods

The underlying data were retrospectively analyzed from experiments performed in a single institution over a period of 5 years. Such approach has been used by others before [26] and is in line with the 3R (replace, reduce, refine) principle, since all experiments have been performed and reported previously in another context. The algorithms for the cardioprotective maneuvers have been adapted from published maneuvers in the clinical setting (IPC [3], RIPC [20], RPER [2], POCO [35]) with slight modifications. These maneuvers robustly reduced infarct size in our prior studies [10, 18, 31, 34]. We therefore did not modify the algorithms in number and duration of ischemia/reperfusion cycles to further optimize the magnitude of infarct size reduction [16]. Anesthesia was maintained with

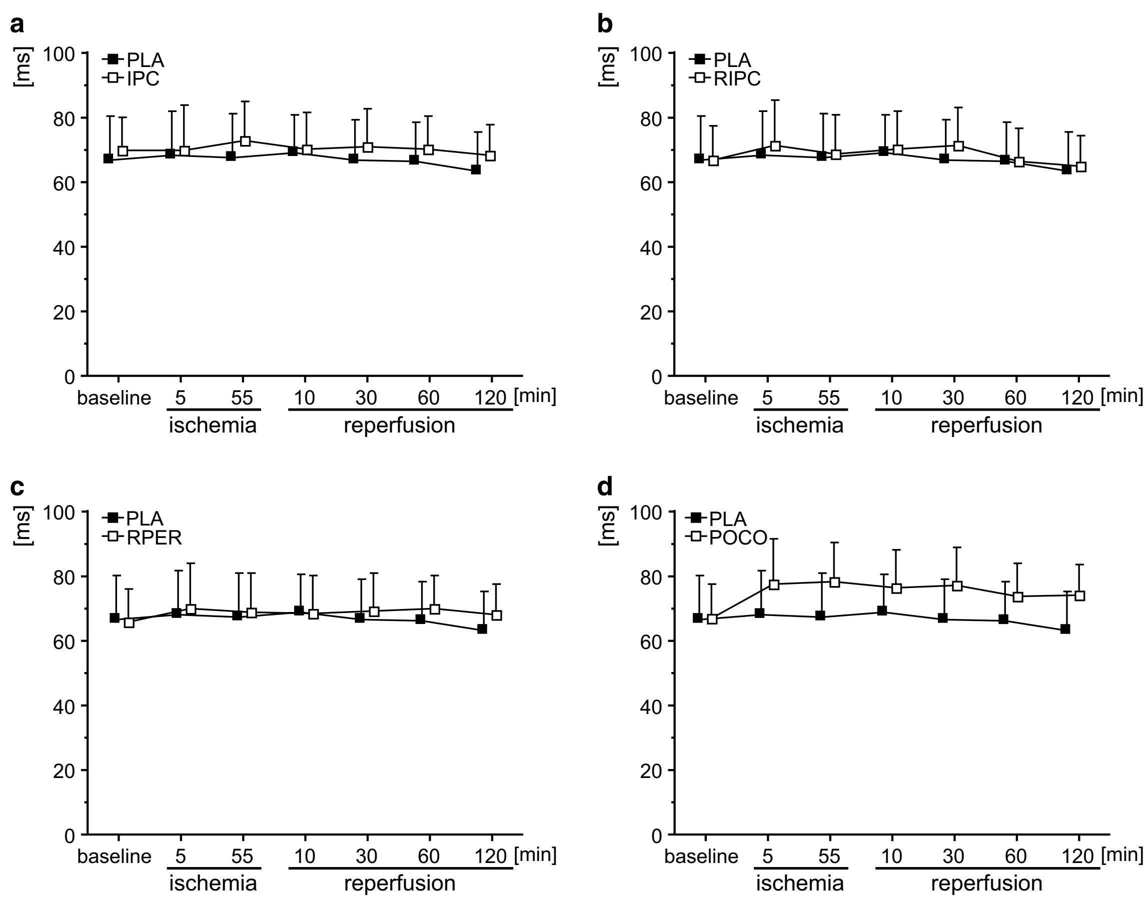

Fig. 7 Time course of QRS duration with ischemic conditioning. **a** Ischemic preconditioning (IPC), **b** remote ischemic preconditioning (RIPC), **c** remote ischemic perconditioning (RPER), and **d** ischemic postconditioning (POCO). Mixed model analysis; no significant differences between groups. For comparison, the time course of placebo (PLA) is displayed in each panel

isoflurane which might facilitate cardioprotection [11]. However, the anesthetic regimen was identical in all pigs. From our retrospectively analyzed experiments no data on biomarkers such as LDH [25], troponin, or CK were available. A reliable analysis of QRS distortion as a predictor of large myocardium at risk and infarct size, as recently described for patients with ST-elevation myocardial infarction [40], was not reasonable with the available single lead ECG recording.

Discussion

The present retrospective analysis of ST-segment elevation in pigs subjected to ischemia/reperfusion without or with conditioning maneuvers revealed that each cardioprotective maneuver can attenuate ST-segment elevation acutely during ischemia/reperfusion, suggesting that an ECG analysis might serve as an acute online marker of cardioprotection. Cardioprotection by conditioning maneuvers prior to ischemia, i.e., IPC and RIPC, did not attenuate ST-segment elevation at early ischemia but, similar to RPER, did so at 55 min ischemia and during early reperfusion. POCO abrogated the further increase in ST-segment elevation seen in PLA between 55 min ischemia and 10 min reperfusion.

At present, the mechanisms behind ST-segment elevation during ischemia/reperfusion and its attenuation are largely unknown. The ionic currents resulting in ST-segment elevation during ischemia/reperfusion are complex [17]. During electric diastole, there is a current away from the ischemic myocardium, which is in a largely depolarized state, to the non-ischemic myocardium. This current is reversed in electric systole. Thus, both non-ischemic and ischemic myocardium contributes to the injury current which is reflected in the ST-segment elevation. It is impossible to attribute the observed attenuation in ST-segment elevation by ischemic conditioning to a specific ionic current in either non-ischemic or ischemic myocardium. An attenuation of ST-segment elevation with repetitive brief periods of ischemia has been reported in patients undergoing elective percutaneous coronary interventions [6, 7, 39], and in experimental studies which reported reduced ST-segment elevation during the brief ischemia/reperfusion cycles of an

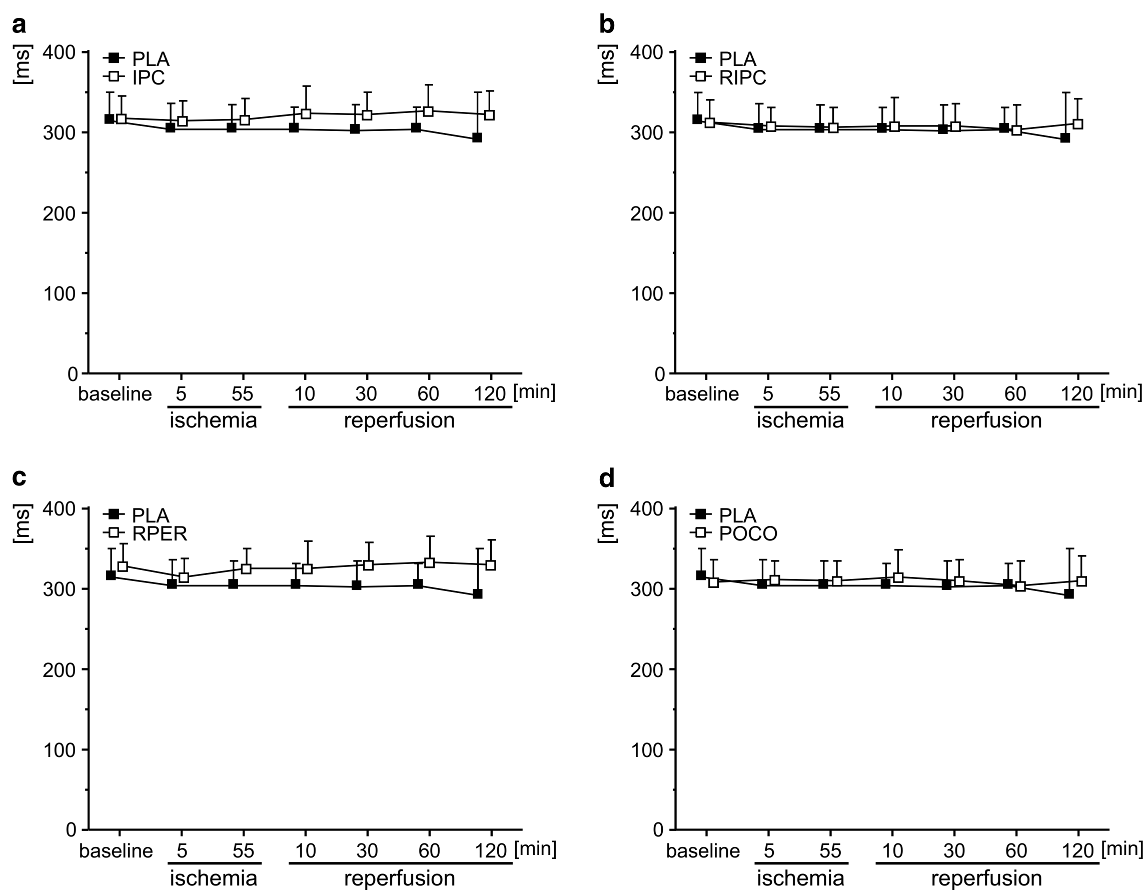

Fig. 8 Time course of QT interval with ischemic conditioning. **a** Ischemic preconditioning (IPC), **b** remote ischemic preconditioning (RIPC), **c** remote ischemic perconditioning (RPER), and **d** ischemic postconditioning (POCO). Mixed model analysis; no significant differences between groups. For comparison, the time course of placebo (PLA) is displayed in each panel

IPC maneuver [4, 29] and during the initial minutes of sustained ischemia [8]. Such trend for attenuated ST-segment elevation was also seen with IPC at 5 min ischemia in the present study. However, in patients undergoing elective PCI, myocardial infarction is not present, and the animal studies did not report infarct size data such that an association between infarct size reduction and attenuated ST-segment elevation in these studies cannot be made.

ST-segment elevation obviously reflects the ongoing ischemia- (during coronary occlusion) and reperfusion- (augmented ST-segment elevation at early reperfusion) induced injury. It does not distinguish between reversible (5 min coronary occlusion) and irreversible (55 min coronary occlusion/10 min reperfusion) injury. It also does not reflect the result of ischemia and reperfusion injury, since it has more or less recovered at 120 min reperfusion when there is larger or less infarct size. Likewise, the attenuation of ST-segment elevation by an ischemic conditioning maneuver obviously reflects the ongoing cardioprotective action, but not the result of it. Accordingly, the magnitude of attenuation of ST-segment elevation did not reflect the magnitude of infarct size reduction on an individual basis.

Nevertheless, attenuation of ST-segment elevation in response to an ischemic conditioning maneuver reflects an ongoing cardioprotective action and can therefore serve as an online marker of it. With remote ischemic perconditioning such ongoing cardioprotective action occurs obviously during ongoing coronary occlusion as seen previously in pigs [18]. Also, such protection during ongoing coronary occlusion is in line with prior clinical studies using remote ischemic perconditioning [23] or metoprolol [9], where patients with acute ST-segment elevation myocardial infarction tolerated longer coronary occlusion and still had less infarction when receiving the cardioprotective intervention early during coronary occlusion. This effect may reflect the potential of conditioning to achieve protection not only from reperfusion, but also from ischemic injury, as recently emphasized [27].

In summary, ongoing cardioprotection by ischemic conditioning is reflected online by attenuated ST-segment elevation.

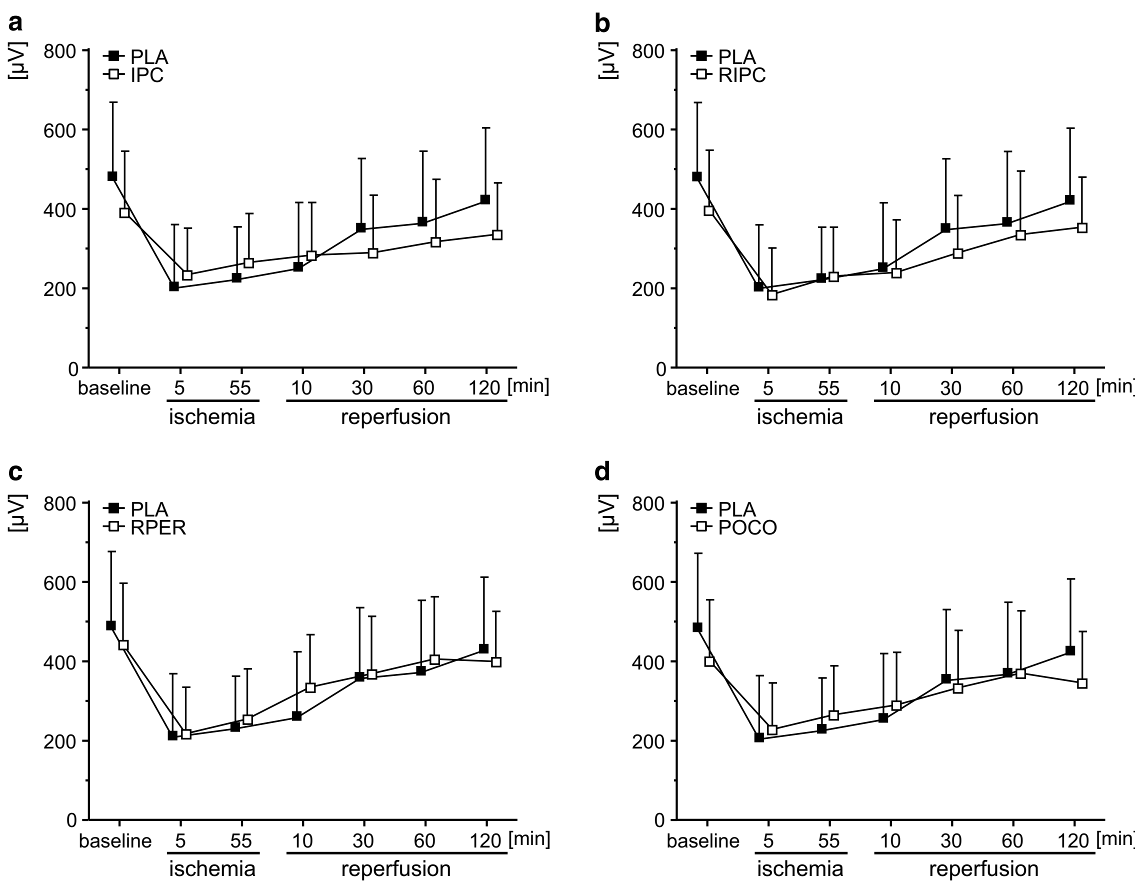

Fig. 9 Time course of R amplitude with ischemic conditioning. **a** Ischemic preconditioning (IPC), **b** remote ischemic preconditioning (RIPC), **c** remote ischemic perconditioning (RPER), and **d** ischemic postconditioning (POCO). Mixed model analysis; no significant differences between groups. For comparison, the time course of placebo (PLA) is displayed in each panel

Acknowledgements The data of the present manuscript are the subject of G. Amanakis's MD thesis. We thank M. Neuhäuser, Department of Mathematics and Technology, Koblenz University of Applied Science, Remagen, Germany, for his advice and revision of statistical analyses.

Author contributions GA: acquisition, analysis, and interpretation of data, drafting of manuscript. PK: analysis and interpretation of data, drafting of manuscript. GH: study design, interpretation of data, final revision of manuscript. AS: study design, acquisition, and interpretation of data, drafting of manuscript.

Sources of funding The present study was supported by the German Research Foundation (SFB 1116 B08).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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