

S-T segment voltage during sequential coronary occlusions is an unreliable marker of preconditioning

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Birincioglu, Mustafa, Xi-Ming Yang, Stuart D. Critz, Michael V. Cohen, and James M. Downey. S-T segment voltage during sequential coronary occlusions is an unreliable marker of preconditioning. *Am. J. Physiol.* 277 (Heart Circ. Physiol. 46): H2435–H2441, 1999.—During coronary angioplasty, a stair-step decrease in peak S-T segment elevation from the first to the second coronary occlusion has been assumed to indicate a preconditioning (PC) effect. This association was evaluated with myocardial electrograms in rabbits, which revealed that two sequential 5-min coronary occlusions resulted in a marked decrease in the area under the S-T segment voltage-time curve ($P < 0.05$) with no change during a third occlusion. Pretreatment with either 5-hydroxydecanoate, a mitochondrial ATP-sensitive potassium (K_{ATP}) channel blocker, or anisomycin, an activator of stress-activated protein kinases, had no effect on the stair-step decline in the S-T segment voltage between the first two occlusions. HMR-1883, a potent closer of sarcolemmal K_{ATP} channels, abolished changes in S-T segment elevation after brief coronary occlusions but had no effect on the infarct-sparing property of the two preconditioning 5-min occlusions. Interestingly, HMR-1883 blocked myocardial protection from diazoxide, raising doubt that the latter opens only mitochondrial channels. Therefore, myocardial protection and S-T segment changes during ischemia are dissociated. These data suggest that it is the mitochondrial K_{ATP} channel that protects the myocardium, and it is the sarcolemmal channel that is responsible for changes in S-T elevation. Therefore, it cannot always be inferred that changes in S-T segment elevation reflect the state of myocardial protection.

anisomycin; diazoxide; 5-hydroxydecanoate; HMR-1883; adenosine 5'-triphosphate-sensitive potassium channel

ISCHEMIC PRECONDITIONING protects the heart through an as yet, poorly understood mechanism. Considerable evidence points to a role for the ATP-sensitive potassium channel (K_{ATP}) in this protection. In many animal models K_{ATP} channel antagonists block and K_{ATP} channel openers mimic the protection of ischemic preconditioning (10, 11, 24). More recently it has been demonstrated that the K_{ATP} channel in the mitochondrial inner membrane of the heart differs from that in the sarcolemma, and that the former channel is the one responsible for protection (7, 14). A large number of agents have been proposed to be preconditioning mimetics. A popular method for testing these compounds in humans has been by monitoring electrocardiographic

changes in the setting of coronary angioplasty when coronary flow is transiently interrupted during inflation of the angioplasty balloon. Deutsch et al. (6) reported in 1990 that there was much greater S-T segment elevation during the first inflation than was seen during subsequent inflations. They proposed that the decline in S-T segment voltage during the second coronary occlusion represented less myocardial ischemia and was the result of preconditioning of the heart by the first inflation. Tomai and colleagues (22) extended this reasoning to develop a clinical test for preconditioning mimetics and blockers. In 1994 they showed that the K_{ATP} blocker glibenclamide could abort this stair-step decrease of S-T segment voltage in patients during successive coronary occlusions. Because this agent also blocks the infarct-sparing action of ischemic preconditioning in animal models, it was reasoned that the absence of a stair-step fall in maximal S-T segment voltage during serial occlusions reflected an unvarying degree of myocardial ischemia during each occlusion because of pharmacological blockade of the protection of preconditioning. Subsequently, Tomai et al. (23) showed that an adenosine receptor blocker could also abort the decline of the S-T segment signal during sequential coronary occlusions. Kerensky et al. (13) demonstrated that the method could be adapted to investigate a pharmacological preconditioning agent such as intracoronary adenosine as well.

The validity of the assumption that changes in S-T segments during sequential coronary occlusions somehow reflected the preconditioning state was critically examined in animal models. Using open-chest pigs, Shattock et al. (19) showed a similar pattern of diminishing S-T segment elevation during successive coronary occlusions. Because pig hearts lack preformed collateral vessels, it was evident that opening of collaterals was not the cause. A similar observation was made in collateral-deficient rabbit hearts by Birnbaum et al. (3). In these studies the multiple-occlusion protocol naturally preconditioned the hearts, thus suggesting a correlation between diminution in S-T segment voltage during sequential occlusions and appearance of the protection of preconditioning. In a subsequent study, Cohen et al. (4) showed that the S-T segment test was valid for pharmacological preconditioning as well. Activation of adenosine A_1 or α_1 -adrenergic receptors before the first occlusion abolished the stair-step decrease in the magnitude of S-T segment voltage expected during the second occlusion. It was reasoned that because preconditioning had occurred before the first occlusion, the S-T segment voltage had already been minimized during that first occlusion and was

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therefore unchanged during the second and third occlusions. Additionally, abolition of protection with an adenosine receptor antagonist blocked the stair-step changes in the S-T segment voltage during sequential occlusions. If there were no cardiac protection, it was felt that there would also be no diminution in the S-T segment voltage during later coronary occlusions. These observations supported a direct association between protection and the response of S-T segments to myocardial ischemia. It was concluded that the S-T segment could be relied upon to indicate whether a drug could mimic or abort the protection of preconditioning.

In the present study we wanted to determine whether manipulation of the protection of preconditioning at the level of the mitochondrial K_{ATP} channel would also be reflected in changes in the S-T segment during serial coronary occlusions. There are several tools available for this manipulation. Diazoxide is a selective opener of the mitochondrial K_{ATP} channel. Conversely, 5-hydroxydecanoate (5-HD) is a highly selective blocker of the mitochondrial K_{ATP} channel, whereas HMR-1883 is a selective blocker of the surface K_{ATP} channel. Whereas diazoxide mimics preconditioning (1), 5-HD (1) but not HMR-1883 (see RESULTS) will abort the protection of preconditioning.

METHODS

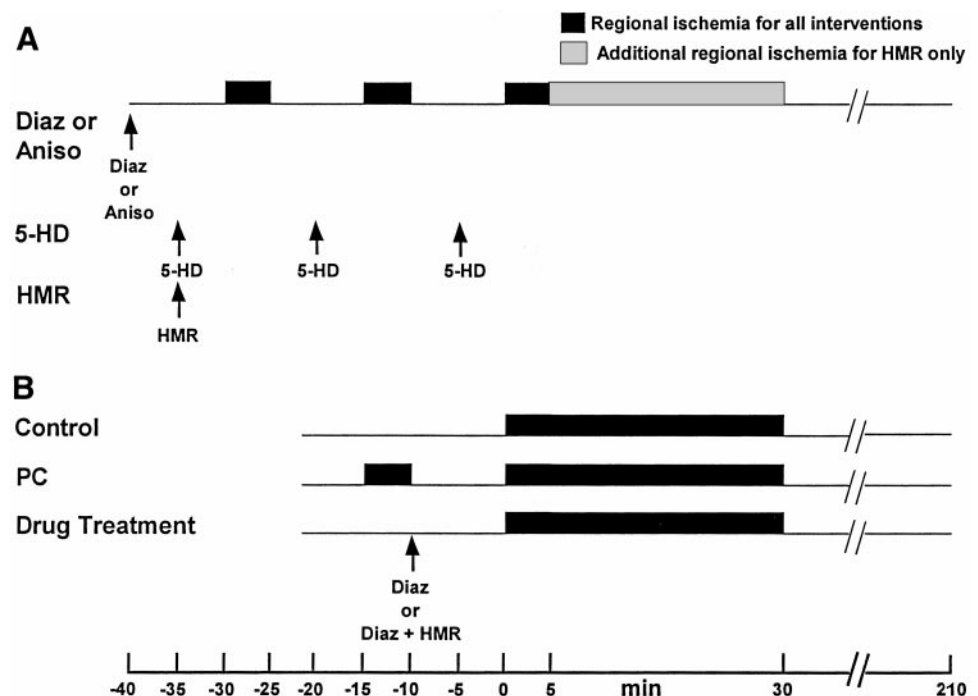
Animal surgery. All procedures were approved by the institutional animal care and use committee and are in compliance with the *Guide for the Care and Use of Laboratory Animals* (18). New Zealand White rabbits weighing between 2 and 3 kg were anesthetized with intravenous pentobarbital sodium (30 mg/kg), intubated through a tracheotomy, and mechanically ventilated with a positive-pressure respirator (MD Industries, Mobile, AL) and 100% oxygen. Ventilator settings were adjusted throughout the protocol to maintain arterial pH in the range of 7.35–7.45. A jugular vein and

carotid artery were cannulated for administration of additional anesthetic agent and other drugs and for measurement of arterial pressure, respectively. A left thoracotomy was performed in the third intercostal space. The pericardium was incised, and a prominent epicardial branch of the left coronary artery was encircled with a silk suture to form a snare. Proper functioning of the snare was confirmed by noting cyanosis of the distal myocardium and cessation of effective contraction after pulling it and hyperemia and resumption of contraction after its release. A fine (30 gauge) copper wire was passed superficially through the epicardium near the center of the vascular territory served by the snared coronary artery as defined by the area of cyanosis upon vessel occlusion and attached to the positive lead of an electrocardiogram amplifier. A reference electrode was created by attaching leads to the two forelegs and the left rear leg. The right rear leg was grounded.

Protocol. Insertion of the myocardial electrode typically caused S-T segment elevation, which slowly disappeared over the course of 45 min–2 h. No experimental manipulations or observations were made until the S-T segment elevation had abated. Thereafter, a baseline epicardial electrogram was recorded. All animals underwent two 5-min coronary occlusions, each followed by 10 min of reperfusion. A third occlusion was then performed. In some groups the study was terminated after only 5 min of that final occlusion, whereas other groups underwent 30 min of regional ischemia followed by 3 h of reperfusion so that infarct size could be measured by triphenyltetrazolium staining. After the onset of each coronary occlusion, epicardial electrograms were recorded every minute for 5 min.

Preconditioned rabbits experienced only three cycles of 5 min of ischemia with intervening 10-min reperfusion periods (Fig. 1). A second group of rabbits was treated 10 min before the first coronary occlusion with diazoxide (10 mg/kg iv), a selective mitochondrial K_{ATP} channel opener (8). 5-HD (5 mg/kg iv), a selective mitochondrial K_{ATP} channel blocker (7, 17), was administered to a third group of rabbits 5 min before each of the three coronary occlusions. In the fourth group, anisomycin (100 μ g/kg iv), an activator of stress-activated

Fig. 1. Diagrammatic representation of protocols for S-T segment (A) and infarct size (B) studies. Arrows indicate timing of administration of diazoxide (Diaz), 5-hydroxydecanoate (5-HD), or HMR-1883 (HMR). For S-T segment studies, hearts experienced 3 sequential periods of 5 min of ischemia except those hearts treated with HMR-1883 in which the third 5-min coronary occlusion was extended to 30 min followed by 3 h of reperfusion so that infarct size could also be quantitated. PC, ischemic preconditioning.



protein kinases (26), was given 10 min before the first ischemic period. In the fifth group HMR-1883 (3.14 mg/kg iv), a selective blocker of the sarcolemmal K_{ATP} channels (9) (Marban, E., personal communication), was infused 5 min before the first occlusion (HMR-1883 was a generous gift of Hoechst Marion Roussel). In this latter group of rabbits, the third occlusion was extended to 30 min, and after 3 h of reperfusion infarct size was measured. The sixth (control) and seventh (preconditioning, PC) groups of rabbits studied in parallel with the fifth group experienced either only 30 min of regional ischemia followed by 3 h of reperfusion or a cycle of 5-min coronary occlusion/10-min reperfusion before the longer occlusion. Two other groups of rabbits were treated with 5 mg/kg diazoxide iv 10 min before the 30-min coronary artery occlusion and 3 h of reperfusion. One of these groups also received a simultaneous intravenous infusion of 3.14 mg/kg HMR-1883. Electrograms were not measured in these latter four groups, but infarct size was quantitated.

Risk zone and infarct size assessment. After we completed the 3-h reperfusion period, we excised the hearts and retroperfused them through the aortic roots with Krebs buffer. The snare was again pulled to reocclude the coronary artery branch. The aortic root was perfused with saline for 2 min, and then 2 ml of a 1% solution of 1–10 μ m ZnCd sulfide fluorescent microspheres (Duke Scientific, Palo Alto, CA) were added to the perfusate to mark the region at risk. The heart was frozen for 24 h and then cut perpendicular to the long axis of the heart into 2-mm slices that were incubated for ~15 min at 37°C in 1% triphenyltetrazolium chloride, which stains viable tissue a deep red color. The slices were compressed between clear plastic plates and illuminated with ultraviolet light. Outlines of the nonfluorescent areas (risk regions) were traced on plastic overlays. The slices were then illuminated with white light, and the outlines of the unstained regions (infarcts) were traced on the same overlays. Risk and infarct areas were quantitated by planimetry with a digitizer (SAC, Norwalk, CT) interfaced to a computer. Volumes were calculated by multiplying the sum of the areas by slice thickness.

Data analysis. As previously described (4), S-T segment shifts were measured 40 ms after the termination of the QRS complex. Typically there was a plateau making the timing of peak S-T segment deflection less critical. Baseline for S-T segment shifts was the T-P segment. In rabbits treated with diazoxide, the Q-T interval from the beginning of the Q wave to the end of the T wave was measured every 2 min during the 10-min interval before the first coronary occlusion. All electrograms were calibrated so that 1 mm was equivalent to 0.5 mV.

During all recordings paper speed was 25 mm/s. S-T segment shifts were plotted against time for all 5-min occlusions and the first 5 min of the 30-min coronary occlusion. The S-T segment was usually seen to progressively increase during each 5-min coronary occlusion, although there was sometimes individual variability from 1 min to the next. Because of the problems associated with statistical analysis of sequentially obtained data (15), we reduced the multiple sequential measurements during each occlusion to a single number by simply calculating the area under the S-T segment voltage versus time plot. Areas for each occlusion were calculated and compared.

Statistics. All data are presented as means $\pm$ SE. Statistical significance of differences during sequential coronary occlusions and reperfusion were determined by ANOVA with replication and the post hoc Scheffé's test. A probability value of $P < 0.05$ was considered to be significant. After ANOVA, post hoc analysis with a Student's *t*-test with Dunn-Sidak correction was used to determine the significance of differences in infarct size in treatment groups compared with the control group. Because the area under the S-T segment voltage-time plot is heavily influenced by anatomical factors and quite variable from animal to animal, the data were normalized by dividing the area for a given 5-min occlusion by the area under the curve for the first occlusion.

RESULTS

In all rabbits the initial S-T segment elevation that accompanied epicardial electrode placement resolved after 45–120 min. There was never any macroscopic infarction at the electrode attachment site. Coronary occlusion characteristically elevated the S-T segment, but the shift predictably disappeared completely during the 10-min reperfusion interval.

Hemodynamics are summarized in Table 1. There were no differences in baseline hemodynamic data in any group. However, diazoxide significantly lowered blood pressure and increased heart rate. Risk zone volume was not systematically measured in hearts in which infarct size was not quantitated. However, when measured, risk zone volume was 10–15% of the combined right and left ventricular mass. These measurements are consistent with our prior experience as well as the risk zone volumes carefully measured in hearts in the infarct studies described below. Areas of cyanosis

Table 1. Hemodynamic data

	<i>n</i>	Baseline		Intervention*		1st Occl*		Intervention*		2nd Occl*		Intervention*		3rd Occl*	
		HR	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR	BP
PC	8	260 ± 22	56.3 ± 4.3			271 ± 23	54.4 ± 4.5			270 ± 21	56.4 ± 3.7			265 ± 16	55.8 ± 2.7
Diazoxide	8	250 ± 14	58.9 ± 3.1	273† ± 14	45.6† ± 3.2	276† ± 16	49.1† ± 3.2			284† ± 16	53.8 ± 2.7			279† ± 15	51.6† ± 3.1
5-HD	8	224 ± 10	52.6 ± 1.2	228 ± 10	54.0 ± 1.7	231 ± 7	52.4 ± 1.8	229 ± 10	51 ± 1.1	232 ± 9	52.5 ± 1.3	233 ± 11	51.5 ± 0.9	233 ± 11	52.6 ± 1.4
Anisomycin	6	229 ± 11	53.2 ± 3.8	242 ± 9	46.2 ± 4.5	242 ± 9	54.5 ± 5.7			235 ± 10	50.0 ± 4.1			235 ± 10	53.5 ± 4.8
HMR-1883	6	266 ± 9	78.5 ± 3.7	259 ± 11	79.3 ± 5.3	256 ± 9	75.0 ± 4.3			259 ± 9	75.2 ± 4.6			252 ± 9	72.2 ± 4.5

Values are means $\pm$ SE; *n*, number of animals. BP, mean blood pressure (in mmHg); HR, heart rate (in beats/min); Occl, occlusion; PC, preconditioned; 5-HD, 5-hydroxydecanoic acid sodium; PC, preconditioning; 5-HD, 5-hydroxydecanoate. *Intervention measurements made following drug administration and immediately before the next coronary occlusion; 1st, 2nd, and 3rd occl measurements made at the end of the 5-min coronary occlusions. †Statistical significance of difference between observation and baseline: $P < 0.001$.

Table 2. Area under S-T segment voltage-time curve during three sequential 5-min coronary occlusions

	Occlusion 1, mV·min	Occlusion 2, mV·min	Occlusion 3, mV·min
PC	18.8 ± 2.9	11.8 ± 2.7*	11.1 ± 1.8*
Diazoxide	27.4 ± 3.8	23.7 ± 4.2	25.7 ± 4.4
5-HD	34.6 ± 2.8	23.0 ± 2.5†	16.2 ± 2.3†
Anisomycin	31.1 ± 2.6	24.0 ± 3.7†	21.1 ± 4.1†
HMR-1883	14.9 ± 2.6	13.5 ± 2.5	16.7 ± 4.3

Values are means ± SE. Statistical significance of difference between occlusion 2 or 3 and occlusion 1: * $P < 0.05$, † $P < 0.01$.

during coronary occlusion always involved the apical one-half to two-thirds of the anterior wall of the heart and the apex.

S-T segment elevation measurements are summarized in Table 2, and normalized data are presented in Fig. 2. In the preconditioned group the area under the voltage-time curve decreased from the first to the second occlusion without further change during the third occlusion, a pattern believed to reflect the presence of ischemic preconditioning during the second and third occlusions (6, 21). In contrast, pretreatment with diazoxide, a K_{ATP} channel opener with selectivity for mitochondrial channels, was associated with no change in the magnitude of S-T segment elevation during sequential coronary occlusions. Thus constancy of the S-T segment pattern was consistent with diazoxide, putting the heart into a preconditioned state before the first occlusion as seen previously with other preconditioning-mimetic agents (4). As shown in Table 3, Q-T intervals significantly shortened in hearts treated with diazoxide ($P < 0.004$) despite insignificant change in the R-R interval.

The next three groups showed a clear dissociation between protection and the S-T segment voltage. Blocking the mitochondrial K_{ATP} channels before the first occlusion with 5-HD did not change the descending staircase pattern during the three occlusions as seen in the control group. From previous studies we know, however, that 5-HD in this model aborts the anti-infarct effect of preconditioning. Yet the S-T segment changes would suggest that the heart had been preconditioned by the first occlusion.

Anisomycin protects ischemic hearts presumably by stimulating the stress-activated protein kinases, which are thought to constitute the distal signal transduction pathway of ischemic preconditioning (12, 25). Hearts pretreated with anisomycin also demonstrated a significant decline in S-T segment elevation from the first to second occlusion even though they should have been fully protected before the first occlusion. Finally, pretreatment with HMR-1883, a potent selective antagonist of sarcolemmal K_{ATP} channels, abolished the decline in the S-T segment voltage during sequential coronary occlusions. Whereas HMR-1883 blocked the stair-step changes in S-T segment voltage, which have been assumed to reflect ischemic preconditioning, it did not block the protective effect of the two 5-min occlusion/10-min reperfusion cycles on infarct size. Figure 3 reveals that infarct size in the HMR-1883 group averaged only $5.6 \pm 2.1\%$ of the risk zone, which is similar to that in ischemically preconditioned hearts ($8.4 \pm 3.1\%$). Infarct size in both groups is significantly less than that in the control group ($36.8 \pm 2.6\%$, $P < 0.001$). In these groups in which infarction was quantitated as well as those described below, risk zone volumes were comparable.

Because the diazoxide-treated rabbits showed no stair-step decline in S-T segment voltage with serial coronary occlusions, we decided to further test whether diazoxide actually protects the heart exclusively through mitochondrial K_{ATP} channels. As indicated in Fig. 3, diazoxide (5 mg/kg) did protect the heart ($21.7 \pm 2.0\%$ infarction, $P < 0.01$ vs. control), but this modest protection was completely eliminated by HMR-1883, the surface channel blocker ($39.5 \pm 3.3\%$ infarction). This casts doubt on the assumption that diazoxide does not affect surface channels. In these rabbits diazoxide modestly lowered mean blood pressure from 80 to 67 mmHg, a decline that was not seen in animals simultaneously treated with HMR-1883. However, in prior studies we have shown that modest changes in blood pressure cannot account for cardioprotective effects (20).

DISCUSSION

Previous animal studies appeared to support the hypothesis that a transition from a nonpreconditioned

Fig. 2. Average area under S-T segment voltage-time curve for 3 sequential 5-min coronary occlusions after normalization for the area of first occlusion. In PC group there was a significant decline from the first to second occlusion with no further change during the third occlusion. A similar pattern was observed in hearts pretreated with anisomycin (Aniso), an activator of stress-activated protein kinases, before first occlusion or treated with 5-HD, a selective closer of mitochondrial K_{ATP} channels, 5 min before each occlusion. On the other hand, both Diaz, a selective opener of mitochondrial K_{ATP} channels, and HMR, a selective antagonist of sarcolemmal K_{ATP} channels, blocked any change in magnitude of S-T segment elevation from the first to second and third 5-min coronary occlusion. * $P < 0.025$ vs. first occlusion.

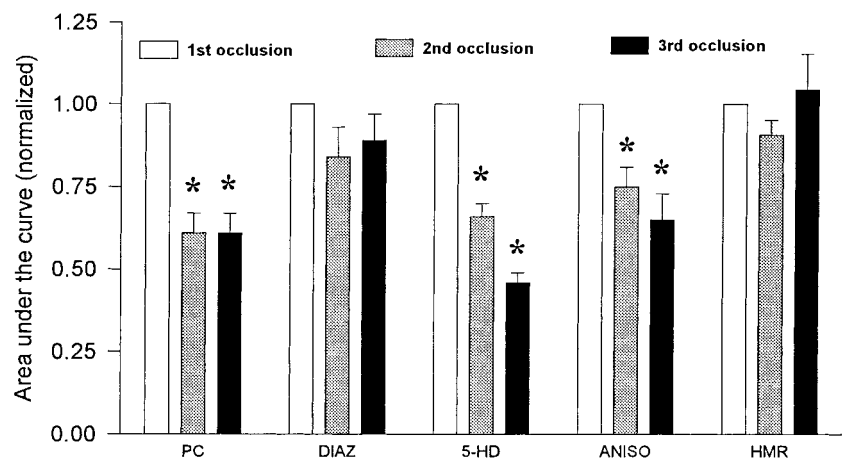


Table 3. Effect of diazoxide on Q-T and R-R intervals in *in situ* rabbit hearts

	Time After Diazoxide Administration, min					
	0	2	4	6	8	10
Q-T interval, ms*	140 ± 7	135 ± 7	135 ± 7	130 ± 7	130 ± 7	130 ± 6
R-R interval, ms	190 ± 9	195 ± 10	195 ± 10	190 ± 12	190 ± 11	190 ± 11

Values are means ± SE. *Significance of change in Q-T interval over the 10-min observation period: $P < 0.004$.

to a preconditioned state effected by brief ischemia could be detected by attenuation of the S-T segment voltage during sequential coronary occlusions (3, 4, 19). Either blocking the protection pharmacologically or preconditioning the heart with pharmacological agents before the first occlusion should and did block the descending stair-step pattern in the magnitude of S-T segment voltage with successive occlusions (4). In the present study, however, we manipulated what is thought to be the distal portion of the signal transduction pathway of preconditioning, the mitochondrial K_{ATP} channel. Whereas opening the channel with diazoxide before the first occlusion did abolish the stair-step pattern, blocking the channel with 5-HD did not. Putting the heart into a protected state with anisomycin also failed to block the stair-step pattern. Finally, selective blockade of surface K_{ATP} channels with HMR-1883 did block the S-T segment changes but did not block the myocardial protection of preconditioning. Is there a unifying hypothesis? Taken together these results suggest that mitochondrial K_{ATP} channel opening leads to protection of the heart without any effect on the extent of S-T segment elevation observed during coronary occlusions, whereas surface K_{ATP} channel

opening causes attenuation of S-T segment elevation without being able to salvage ischemic myocardium.

Ischemic preconditioning apparently opens both populations of channels. If the mitochondrial K_{ATP} channel is the distal pathway of preconditioning, then the S-T segment test fails to be a reliable indicator of the protected state when the distal pathway of preconditioning is manipulated.

The electrocardiogram is generated by transmembrane voltages, which exist across the individual cardiomyocytes. In retrospect it seems reasonable that changes in surface channel activity would be most likely to influence the electrocardiogram. It is less obvious how opening mitochondrial channels could alter the electrogram.

Activation of the stress-activated protein kinases with anisomycin puts the heart into a protected state similar to that seen with ischemic preconditioning (2, 12). Additional evidence suggests that p38 mitogen-activated protein kinase, one of the two stress-activated protein kinases that anisomycin stimulates, is a key step in the signal transduction pathway of preconditioning (5, 16, 25). Furthermore, protection by anisomycin, like that from ischemic preconditioning, can be blocked by the mitochondrial K_{ATP} channel antagonist 5-HD (1). Anisomycin did not block the stair-step diminution in peak S-T segment voltage during the second and third coronary occlusions. This observation would suggest that anisomycin does not affect the sarcolemmal K_{ATP} channels. The pathways leading to opening of surface and mitochondrial channels must diverge somewhere upstream of p38 mitogen-activated protein kinase.

The diazoxide data are more difficult to explain. Diazoxide is reported to be about 2,000-fold more selective for beef cardiac mitochondrial K_{ATP} channels than for myocardial surface channels (8). A similar high affinity of diazoxide for mitochondrial channels was seen in the rabbit heart (14). Unfortunately, because diazoxide was given intravenously, we have no precise knowledge of its concentration at the cardiomyocyte. According to the manufacturer, it is very lipid soluble, and ~90% is bound to plasma proteins. In two rabbits we gave one-tenth of the dose and found large infarcts in both, suggesting that our dose was not far above the threshold for protection (data not shown). If diazoxide does have a 2,000-fold selectivity for mitochondrial channels in the rabbit heart, then the explanation that it may have been given in a concentration that was high enough to affect both surface and mitochondrial channels seems unlikely.

Most disturbing was the finding that HMR-1883 could eliminate the anti-infarct effect of diazoxide. This

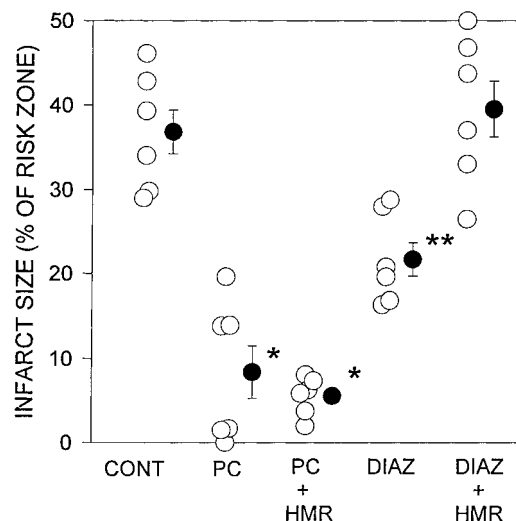


Fig. 3. Infarct size plotted on ordinate as a percentage of risk zone for control rabbit hearts experiencing only 30-min coronary occlusions (Cont); hearts preconditioned with two 5-min occlusion/10-min reperfusion cycles before the longer coronary occlusion (PC); preconditioned hearts also pretreated with HMR, a selective blocker of sarcolemmal K_{ATP} channels; hearts pretreated only with Diaz, the putative mitochondrial K_{ATP} opener, before the long occlusion; and hearts simultaneously pretreated with Diaz and HMR. Open circles, data from individual hearts; filled circles, means ± SE. HMR was unable to block the protection of ischemic preconditioning but did successfully eliminate anti-infarct effect of Diaz. * $P < 0.001$ ** $P < 0.01$ vs. control.

argues against diazoxide acting purely as a mitochondrial K_{ATP} channel opener. The ability of diazoxide to promote surface K_{ATP} channel opening during ischemia when ATP is falling has not been investigated. Had the diazoxide caused the surface channels to open early in the first occlusion, the S-T segment voltage would have been attenuated during that occlusion as is seen in the pharmacologically preconditioned heart. We examined the Q-T interval, an index of action potential duration, and saw that it did fall after diazoxide injection. There was no significant change in the R-R interval in the 10-min period following diazoxide administration. Therefore, the change in Q-T interval does suggest that diazoxide promoted surface K_{ATP} channel opening. Given the data by Garlid et al. (8) and Liu et al. (14), our observations are difficult to explain. But perhaps caution is needed when these drugs are used in future studies. Either diazoxide may not be a pure opener of mitochondrial channels when used in vivo, or the actions of HMR-1883 are not confined to sarcolemmal channels. In either situation it is appropriate to remember that any given pharmacological agent is unlikely to have only one action.

It was not possible to test whether anisomycin or diazoxide protected the hearts in the present study in which S-T segments were monitored, because the hearts would have been protected by the two preconditioning occlusions even in the absence of either of these agents. However, in previous studies from this laboratory both diazoxide (1) and anisomycin (2) have been shown to mimic preconditioning in the open-chest rabbits at the doses used, and the infarct investigations with diazoxide in the present study confirm these earlier conclusions.

If our surface channel hypothesis were valid, then an agent that blocked only mitochondrial K_{ATP} channels should effectively abort the protection of preconditioning against infarction without having any effect on S-T segment changes. Indeed, 5-HD, a highly selective mitochondrial K_{ATP} channel antagonist, did not eliminate the decrement in S-T segment voltage noted between *occlusions 1* and *2* in untreated hearts. Although not tested here, we have previously reported that 5-HD completely aborts the myocardial protection of a single 5-min ischemia/10-min reperfusion cycle of ischemic preconditioning in the open-chest rabbit (1). Therefore, we are confident that myocardial protection was clearly blocked in these hearts, yet the S-T segments during the second occlusion were diminished just as was seen in the preconditioned and protected hearts. Again the dissociation between protection and change in S-T segment deviation was evident.

The final evidence supporting a dissociation between protection and the S-T segment voltage was obtained in the hearts pretreated with HMR-1883, an agent that is known to block only sarcolemmal but not mitochondrial K_{ATP} channels. If the changes in S-T segment voltage are indeed related to alterations in the state of the surface channels, then their blockade should abolish any change in the magnitude of the S-T segment signal. But because the mitochondrial channels responsible for myocardial protection are not being affected, the brief

occlusions should still be able to precondition the heart and result in small infarcts despite closure of the surface channels. The above predictions were borne out: infarcts were still small in hearts treated with HMR-1883 in the face of a blocked S-T segment stair-step response.

The S-T segment data in this study were analyzed by measuring and then comparing areas under the time-voltage curves. The data were also analyzed by considering only the maximal absolute S-T segment voltage in a given 5-min period of coronary occlusion. It is noteworthy that during any 5-min occlusion there was not always a progressively greater S-T segment elevation as the duration of the occlusion increased, but sometimes early measurements were greater than later ones. Nonetheless when the analysis of areas under the curves demonstrated a significant change during sequential occlusions, the same was apparent when peak voltage changes were used, and both approaches equally identified interventions resulting in no change. However, because of increased variability in peak S-T segment measurements, post hoc analysis showed no significant differences between any two occlusions. On the other hand, use of the area method served to diminish variability and allowed successful post hoc analysis, suggesting that the integration approach was superior.

There are several limitations of this study that should be appreciated. First, we used a wire electrode that caused local injury after its insertion into the myocardium. After 45–120 min these injured cells adjacent to the wire became electrically quiescent, presumably because of death, and S-T segments returned to baseline. Subsequent S-T elevation during coronary occlusion represented the response of a considerable mass of myocardium. But reversion of the electrical signal back to baseline during the cycles of reperfusion interposed between occlusions suggests that there was no residual influence of the locally injured cells. Second, we did not measure electrograms and infarct size in the same animals except for the group of rabbits treated with HMR-1883. This was a deliberate move to simplify the protocol, although it might have been useful to correlate the magnitude of S-T segment elevation with the extent of infarction in individual animals. However, the extent of infarction per se had no effect on S-T segment responses. As seen in Fig. 3 the rabbits treated with diazoxide and those preconditioned rabbits treated with HMR-1883 had significantly different infarct sizes, yet the S-T segment responses were virtually identical (Fig. 2). Furthermore, in the one group in which S-T segments were monitored during three occlusions and infarct size measured after a 30-min coronary occlusion and 3 h of reperfusion (HMR in Fig. 2 and PC+ HMR in Fig. 3), infarct size was uniformly small in the face of unchanged S-T segment elevation during the three occlusions. Again the dissociation between S-T segment response and infarct size is emphasized.

As a result of these studies, we suggest that caution be used in interpreting clinical data using S-T segment analysis. For example, Tomai et al. (22) reported that

pretreatment with glibenclamide could block the S-T segment stair-step pattern in patients undergoing angioplasty. We propose that it was blockade of the surface K_{ATP} channels by glibenclamide that resulted in the observed electrocardiographic changes. It would be inappropriate to use these data to imply that mitochondrial channels, and thus myocardial protection, had also been blocked. Similarly, any other agent's effect on S-T segments can be attributed to changes in sarcolemmal K_{ATP} channels, which themselves have no influence on protection. In light of the latter it seems that no inference about mitochondrial channels that do regulate myocardial protection should be drawn from S-T segment data.

In summary, the results suggest that myocardial protection from ischemic preconditioning derives from opening of mitochondrial K_{ATP} channels, whereas attenuation of the S-T segment voltage seen during sequential ischemia in the preconditioned heart is exclusively the result of opening of sarcolemmal K_{ATP} channels. If true, then caution must be exercised in using changes in the S-T segment to evaluate the ability of a compound to confer or block the preconditioned state.

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