Enhanced cardiac preconditioning in the isolated heart of the transgenic (mREN-2)27 hypertensive rat

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Abstract

Objective: Cardiac preconditioning represents an important cardioprotective mechanism which limits myocardial ischaemic damage. The aim of this investigation was to assess the impact of preconditioning in hypertension, which is a major risk factor for ischaemic heart disease. Methods: Hearts isolated from male transgenic (mREN-227) hypertensive TGH rats, and their normotensive controls (Hannover Sprague-Dawley; SD rats), were perfused at constant flow using the Langendorff technique, and were subjected to either ischaemic preconditioning (3 × 4 min ischaemia) or continuous perfusion. Global ischaemia was then induced for 30 min, followed by 60 min of reperfusion, during which time mechanical performance was assessed (left ventricular developed pressure (LVDP), heart rate, and end-diastolic pressure). Results: In the absence of preconditioning, mechanical performance was substantially depressed on reperfusion, and there was no difference between TGH hearts and SD hearts (area under the curve (AUC) for the LVDP (LVDP0–60) plot against time for reperfusion = 356 ± 115 and 296 ± 206 mmHg·min, respectively). Cardiac preconditioning caused significant protection in both groups, but this was significantly (P < 0.05) greater (3-fold) in the TGH hearts (AUC for LVDP0–60 = 3349 ± 610 mmHg·min) compared to the SD hearts (AUC for LVDP0–60 = 1153 ± 527 mmHg·min). In both groups, preconditioning induced significant protection of diastolic function. The enhanced effects of preconditioning on mechanical performance in the TGH hearts were unaffected by the angiotensin AT1-receptor antagonist, losartan (3 μM). However, losartan did partially reverse the beneficial effects of preconditioning on post-ischaemic diastolic function in the TGH hearts. Conclusions: The results of the present investigation clearly show that cardiac preconditioning is substantially enhanced in hearts from TGH rats. Furthermore, the beneficial effects of preconditioning on diastolic function, but not mechanical performance, in TGH hearts is partially mediated by AT1-receptors.

Keywords: Rat, heart; Preconditioning; Hypertension; Transgenic models; Renin–angiotensin–aldosterone system; Myocardial ischemia; Reperfusion

1. Introduction

Brief periods of transient cardiac ischaemia exert a protective effect on the myocardium, by limiting both ischaemic tissue damage and arrhythmogenesis, following prolonged ischaemia. This phenomenon has been termed ‘cardiac preconditioning’ [1], and protects the heart in all species so far studied, including man [2–4].

The mechanisms underlying cardiac preconditioning have been extensively investigated, and several regulatory systems have been implicated in different species. For example, it is known that adenosine may cause protection via adenosine A1-receptors, in a variety of species including the dog, pig and rabbit [4]. There is further evidence that ATP-sensitive potassium (KATP) channels, coupled to adenosine release via adenosine A1-receptors, may also play a role [5]. Protein kinase C has been identified as an important second messenger in preconditioning mechanisms in several species including the rat [6,7].

Despite the fact that the mechanisms of cardiac preconditioning are reasonably well characterized, our knowledge of the effects of disease states on cardiac preconditioning is limited. This is surprising, considering cardiac events are usually associated with underlying cardiovascular diseases or risk factors, such as hypertension. In the absence
of cardiac preconditioning, ischaemic damage has been shown to be enhanced in various models of hypertension. In this respect, hearts from rats rendered hypertensive through chronic nitric oxide inhibition [8], and hearts from spontaneously hypertensive rats [9], show an increased incidence of arrhythmias on reperfusion after ischaemia, which is related to the degree of left ventricular hypertrophy [9]. Furthermore, in the canine heart, the infarct size on coronary occlusion has been reported to be greater in hypertensive than in normotensive animals [10]. However, this phenomenon was unrelated to left ventricular hypertrophy, but was linked to elevated arterial pressure [11]. To our knowledge only two investigations have so far examined the effects of hypertension on preconditioning. In the first, Speechly-Dick et al. [12] found that the hypertrophied myocardium of rats with mineralocorticoid-induced hypertension was protected by preconditioning in vivo. More recently, Boutros and Wang [13] reported that, in hearts isolated from spontaneously hypertensive rats (SHR), preconditioning induced significant protection of mechanical performance, but to a slightly lesser degree than that in hearts from normotensive controls.

We have now investigated the effects of cardiac preconditioning, ex vivo, using hearts from male, heterozygous, transgenic (mREN-227) hypertensive (TGH) rats, with Hannover Sprague-Dawley (SD) rats as the appropriate normotensive controls [14-17]. Heterozygous TGH rats have substantial elevations in systemic arterial pressure, and have associated cardiovascular changes [14-17], including left ventricular hypertrophy. In the present investigation, all the hearts were perfused and reperfused at constant flow, in order to eliminate complications due to impairment of the reactive hyperaemic response, which contributes to reduced post-ischaemic recovery in the hypertrophied myocardium [18]. Furthermore, the hearts were all perfused at matched flow rates, as there is evidence that coronary flow is similar in normotensive and hypertensive rats, despite the presence of myocardial hypertrophy in the latter [19]. In each case, the effects of preconditioning were assessed by both the recovery of mechanical performance on reperfusion and the changes in end-diastolic pressure.

The TGH rats studied in the present investigation were originally produced by insertion of the mouse renin-2 gene into the rat genome. These TGH rats have enhanced activity of local renin-angiotensin systems (RASs) [14,15,17,20], which is thought to contribute to the cardiovascular changes, including cardiac hypertrophy [17,20,21]. Indeed, cardiac RAS has been implicated in other non-hypertensive rat models of left ventricular hypertrophy [22]. In view of the elevated intracardiac RAS in TGH rats [20], the effects of preconditioning of the selective angiotensin AT1-receptor antagonist, losartan, were investigated. This was of particular interest, since recent evidence from isolated perfused rabbits hearts has shown that treatment with angiotensin II, prior to an ischaemic insult, may mimic cardiac preconditioning, and this effect is mediated via AT1-receptor-dependent activation of protein kinase C [23].

2. Methods

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.1. Rat models of hypertension

TGH and SD rats were bred in the Biomedical Services Unit, University of Nottingham Medical School, from animals supplied by Dr. J.J. Mullins at the Centre for Genome Research, Edinburgh. Male, heterozygous transgenic hypertensive rats (TGH) (4-5 months old) and age-matched Sprague-Dawley (SD) rats (originally from Centralklinik für Versuchstierkunde, Hannover) were studied. The heterozygous TGH rats were bred by crossing male, homozygous TGH rats with female, control SD rats. The homozygous TGH rats were kept on chronic captopril treatment (50 mg·kg⁻¹·day⁻¹ in the drinking water [14]), but the homozygous animals used in this study were untreated.

2.2. Measurement of arterial pressure in conscious rats

The rats were anaesthetized (sodium methohexitone, Brietal, Lilly, Basingstoke; 40-60 mg·kg⁻¹·i.p.) and had polyethylene catheters implanted in the abdominal aorta (via the caudal artery), for blood pressure and heart rate recording, and in the right jugular vein for the administration of heparin and anaesthetic (see below). At least 24 h later, measurements of mean arterial blood pressure and heart rate were made in fully conscious, freely moving animals, using a Gould electrostatic recorder (ES 1000 with SP400 preamplifier), transducer amplifier and rate meter (Biotach model 13-4613-65A) (Gould Electronics, Cleveland, OH, USA).

2.3. Preparation of the isolated Langendorff heart perfused at constant flow

Male TGH (473 ± 9 g; n = 26) and SD (454 ± 7 g; n = 22) rats were heparinized (1000 U·kg⁻¹·i.v. or i.p.) and anaesthetized with sodium pentobarbitone (44 mg·kg⁻¹·i.v. or 60 mg·kg⁻¹·i.p.: Sagatal, Rhône Mérieux, Harlow, Essex). In each case, following a mid-line thoracotomy, the heart was rapidly excised and placed in ice-cold, oxygenated, modified Krebs-Henseleit solution (containing (mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, CaCl₂ 2, d-glucose 10, Na-pyruvate 2) to arrest cardiac contraction. The aortic stump was then cannulated and the heart perfused retrogradely by the Langendorff technique at a constant flow of 20 ml·min⁻¹.
with oxygenated (P_{O_2} = 550–600 mmHg) Krebs-Henseleit buffer [24,25]. A water-filled latex balloon catheter, coupled to a pressure transducer, was inserted through the pulmonary vein and advanced into the left ventricle, in order to measure developed left ventricular pressure (LVDP). In each case, left ventricular end-diastolic pressure (EDP) was initially set to approximately 5 mmHg by adjusting the volume of fluid in the balloon. The pressure transducer was coupled to a MacLab 4e recording system (AD Instruments, New South Wales, Australia), and heart rate (HR) was derived from the pressure signal. Coronary flow was measured by means of a transit time ultrasonic flow meter (model T106, Transonic Systems Incorporated, Ithaca, NY, USA) coupled to an extracorporeal flow probe, placed in series with the aortic cannula. Coronary perfusion pressure was measured by means of a second pressure transducer connected to the aortic cannula and coupled to the recording system.

### 2.4. Experimental protocol

Following a 30 min equilibration period, baseline cardiac variables were recorded and the hearts were then either subjected to a preconditioning cycle [25] or were continuously perfused (i.e., non-preconditioned). To induce preconditioning, perfusion was stopped for 4 min, while the heart was bathed in buffer at 37°C, and the preparation was then reperfused for 6 min, after which the cycle was repeated twice more. At the end of the reperfusion phase of the third cycle, the hearts were subjected to 30 min of homothermic global ischaemia, followed by 60 min of reperfusion. As recovery of cardiac function is optimized at reduced reperfusion flow rates [26], the flow on reflow was, in all cases, reduced to 75% of the initial pre-ischaemic flow (i.e., 15 ml·min^{-1}); throughout this time the cardiac variables were continuously monitored. The non-preconditioned hearts were treated in a similar manner to the preconditioned hearts, except that, instead of the 3 cycles of preconditioning, the hearts were perfused continuously for 30 min immediately prior to the ischaemic insult.

Some preparations were treated with the AT1-receptor antagonist, losartan, which was added to the perfusion fluid to achieve a concentration of 3 μM, and was present throughout the experiment. This concentration of losartan was found, in preliminary experiments, to completely abolish the increases in coronary perfusion pressure (≤ 10 mmHg) produced by bolus administration of angiotensin II (5–500 pmol). In the isolated perfused rabbit heart a similar concentration was found to abolish the cardioprotective effects of angiotensin II [23].

### 2.5. Quantitation and statistical analysis

All data are given as the mean ± s.e.m. and baseline variables were compared by Mann-Whitney U-tests. Cardiac mechanical performance was quantified as the rate × pressure product (RPP, the mathematical product of LVDP and HR). Diastolic function was assessed as the end-diastolic pressure. The areas under the curves (AUCs) for the LVDP–, HR– and EDP–time plots were determined, by means of KaleidaGraph, in order to give a quantitative measure of the magnitude of the recoveries of cardiac performance during the 60 min reperfusion period. The derived AUCs were then compared using the Kruskal-Wallis test with significance being determined by Dunn’s post-test.

### 2.6. Drugs

Losartan, a generous gift from Dr. R.D. Smith (Merck Sharp and Dohme, USA) was initially dissolved as a stock solution in 0.9% (w/v) saline prior to addition to the perfusion fluid.

### 3. Results

#### 3.1. Baseline cardiovascular variables

In conscious, unrestrained TGH rats, the mean arterial pressure (MAP) was 160 ± 5 mmHg (n = 12), which was significantly (P < 0.001) higher than in age-matched normotensive SD rats (108 ± 4 mmHg, n = 11). The cardiac mass to body mass ratio was also significantly (P < 0.001) higher in the TGH rats with a value of 0.55 ± 0.01 g·100 g^{-1} body weight (n = 26) compared with a value of 0.41 ± 0.01 g·100 g^{-1} bw (n = 22) in the SD rats.

#### 3.2. Baseline cardiac variables

In hearts perfused at constant flow there were no significant differences between baseline coronary perfusion pressure, developed left ventricular pressure (LVDP), or the rate × pressure product (RPP) of the hearts from the hypertensive rats and those from their appropriate controls (Table 1). There was, however, significant (P < 0.01) brady-cardia in the hearts from TGH rats compared to those from the SD normotensive controls (Table 1).

In the case of the SD and TGH hearts treated with 3 μM losartan there were no significant differences in either baseline mechanical performance or coronary perfusion pressure compared to preparations not receiving the antagonist (Table 1).

#### 3.3. Recovery of mechanical performance on reperfusion following 30 min of global ischaemia in the SD hearts

Following the 30 min ischaemic period, the recovery of mechanical performance on reperfusion in the SD hearts was substantially greater in the preconditioned, compared to the non-preconditioned, preparations (Fig. 1). Specific
cally, the area under the curve (AUC) for LVDP–time plot between 0 and 60 min of reperfusion (LVDP_{0–60}) was significantly (P < 0.001) greater in the preconditioned than in the non-preconditioned hearts (Fig. 1a; Table 2). The recovery of HR was also significantly (P < 0.05) greater in the preconditioned hearts than in the non-preconditioned hearts (Fig. 1b; Table 2). Accordingly, the recovery of the RPP at 60 min (RPP_{60}) of reperfusion was 6046 ± 2025 mmHg · beats · min⁻¹ in preconditioned hearts, which was significantly (P < 0.05) greater than in the non-preconditioned hearts (891 ± 693 mmHg · beats · min⁻¹). In both cases, coronary perfusion pressure showed a significant (P < 0.05) greater increase from baseline on reperfusion (see Table 1), but this did not differ between the preconditioned (107 ± 8 mmHg at 60 min) and non-preconditioned (97.3 ± 10.8 mmHg at 60 min) hearts.

3.4. Recovery of mechanical performance on reperfusion following 30 min of global ischaemia in the TGH hearts

Recovery of systolic function was also greater in preconditioned hearts from the TGH rats than in the non-preconditioned hearts (Fig. 1) in that the AUC for LVDP_{0–60} in the preconditioned hearts was significantly (P < 0.001) greater than the corresponding value in the non-preconditioned group (Fig. 1a; Table 2). The recovery of HR was also significantly (P < 0.05) greater in the preconditioned hearts than in the non-preconditioned hearts (Fig. 1b; Table 2). Accordingly, in the preconditioned hearts the RPP_{60} was 14 500 ± 2 840 mmHg · beats · min⁻¹, which was significantly (P < 0.01) greater than the corresponding value for the non-preconditioned TGH hearts (1659 ± 816 mmHg · beats · min⁻¹). In both cases, coronary perfusion pressure showed a significant (P < 0.05) increase on reperfusion above baseline (see Table 1) which was slightly higher in the non-preconditioned (147 ± 12 mmHg at 60 min) hearts than in the preconditioned (111 ± 11 mmHg at 60 min) group.

The recovery of the mechanical performance in the preconditioned TGH hearts was substantially and significantly (P < 0.05) greater than in the preconditioned SD control hearts, such that the AUC of LVDP_{0–60} for the TGH hearts was 3-fold greater than that obtained for the SD hearts (Fig. 1; Table 2), and the LVDP_{0–60} was significantly (P < 0.05) greater in the TGH (56.4 ± 10.4 mmHg) hearts than in the SD (22.8 ± 7.9 mmHg) hearts.

| Table 1 |
|-------------------|-------------------|-------------------|
| **Baseline cardiac variables in SD and TGH hearts in the absence and presence of losartan** |
| | Normotensive (SD) rats | Hypertensive (TGH) rats |
| | Non-PC (n = 7) | PC (n = 7) | PC + losartan (n = 8) | Non-PC (n = 7) | PC (n = 11) | PC + losartan (n = 8) |
| LVDP (mmHg) | 74.7 ± 3.9 | 83.5 ± 4.0 | 88.3 ± 11.9 | 82.5 ± 5.9 | 89.4 ± 3.7 | 95.3 ± 13.8 |
| HR (beats · min⁻¹) | 276 ± 6 | 268 ± 14 | 259 ± 20 | 238 ± 7 | 231 ± 9 | 230 ± 19 |
| RPP (mmHg · beats · min⁻¹) | 20 654 ± 1 319 | 22 183 ± 936 | 19 802 ± 1 193 | 19 639 ± 1 441 | 20 581 ± 1 005 | 20 657 ± 1 615 |
| CPP (mmHg) | 62.1 ± 5.9 | 60.3 ± 3.1 | 53.3 ± 3.5 | 71.1 ± 3.0 | 60.8 ± 4.2 | 74.4 ± 6.7 |

Table 1 shows the cardiac variables (LVDP = left ventricular developed pressure; HR = heart rate; RPP = rate-pressure product; CPP = coronary perfusion pressure) prior to the preconditioning or continuous perfusion in hearts from the SD and TGH rats. PC indicates the preconditioned groups and non-PC the non-preconditioned groups. The data are mean ± s.e.m. * * * P < 0.001, * * P < 0.01, * P < 0.05: significant differences compared to the appropriate normotensive control.

| Table 2 |
|-------------------|-------------------|
| **AUC values for the 60 min reperfusion period in SD and TGH hearts in the absence and presence of losartan** |
| | Normotensive (SD) rats | Hypertensive (TGH) rats |
| | Non-PC (n = 7) | PC (n = 7) | PC + losartan (n = 8) | Non-PC (n = 7) | PC (n = 11) | PC + losartan (n = 8) |
| LVDP_{0–60} (mmHg · min) | 296 ± 206 | 1 152 ± 527 | 1 129 ± 391 | 356 ± 115 | 3 349 ± 610 | 2 141 ± 787 |
| HR_{0–60} (beats) | 3 441 ± 1922 | 10 582 ± 1 186 | 11 426 ± 2 065 | 7 601 ± 2 478 | 18 153 ± 3 652 | 11 749 ± 3 652 |
| EDP_{0–60} (mmHg · min) | 5 250 ± 529 | 2 414 ± 386 | 2 987 ± 783 | 7 022 ± 512 | 2 100 ± 443 | 4 075 ± 416 |

Table 2 shows the AUC for the plots of the cardiac variables (LVDP = left ventricular developed pressure; HR = heart rate; EDP = end-diastolic pressure) against time for the reperfusion period (0–60 min) in hearts from the SD and TGH rats in the absence and presence of losartan (1 μM). PC = preconditioned groups; Non-PC = non-preconditioned groups. The data are mean ± s.e.m. * * * P < 0.001, * * P < 0.01, * P < 0.05: significant differences between the preconditioned hearts and non-preconditioned controls. * P < 0.05: significant differences between the normotensive and hypertensive groups for the different treatments. ** P < 0.05: significant differences between the absence and presence of losartan for the preconditioned hearts.
The difference in recovery was less marked for the heart rates, with there being a less than 2-fold enhancement in AUC for TGH compared to SD ($P < 0.05$). However, there was no significant difference between the heart rates after 60 min of reperfusion (TGH = 259 ± 14 beats·min$^{-1}$; SD = 217 ± 42 beats·min$^{-1}$).

Despite the marked differences in recovery of mechanical performance in the preconditioned hearts between the hypertensive and the normotensive groups, there were no differences between the non-preconditioned TGH and SD hearts (Fig. 1).

3.5. Effect of losartan on post-ischaemic recovery in the preconditioned SD and TGH hearts

The recovery of the preconditioned SD hearts in the presence of losartan was similar to that reported above, in the absence of the antagonist (Fig. 2; Table 2). At the end
of reperfusion, coronary perfusion pressure was 99.2 ± 14.2 mmHg and was not different from the comparable value in the absence of the drug (given above). In the preconditioned TGH hearts, the recovery of cardiac performance in the presence of losartan was not significantly different from that observed in the absence of the drug (Fig. 3; Table 2). The coronary perfusion pressure was 113 ± 17 mmHg and not different from the comparable value in the absence of the drug (given above).

3.6. Changes in end-diastolic pressure during ischaemia and on reperfusion

In all hearts studied, Fig. 3 shows that there were significant increases in intraventricular pressure at the end of the global ischaemia and immediately prior to reperfusion (a phenomenon known as the ‘ischaemic contracture’), which were greater in the non-preconditioned than in the preconditioned preparations. The intraventricular pressures...
at the end of the ischaemia but immediately before reperfusion were 16.1 ± 6.5 mmHg (SD; Fig. 3a) and 36.9 ± 6.2 mmHg (TGH; Fig. 3b) in the preconditioned hearts, while in the absence of preconditioning the corresponding values were significantly greater with values of 57.8 ± 19.2 mmHg (SD; P < 0.05) and 81.4 ± 6.7 mmHg (TGH; P < 0.001). In all cases, there was a subsequent rise in EDP on reperfusion, peaking at 5–15 min following reflow (Fig. 3a,b).

In both the SD and TGH hearts, the AUC for the EDP-time plot between 0 and 60 min was significantly (P < 0.01) greater in the non-preconditioned than in the preconditioned state (Fig. 3a,b; Table 2).

3.7. Effects of losartan on end-diastolic pressure in the SD and TGH hearts

The presence of losartan did not influence the changes in EDP in the preconditioned SD hearts as the AUC for EDP was similar to that in the absence of the drug (Fig. 3a; Table 2). But, in the TGH hearts, the presence of losartan partially reversed the beneficial effects of precon-

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**Fig. 3. Changes in end-diastolic pressure following ischaemia and reperfusion in preconditioned and non-preconditioned (a) SD and (b) TGH hearts in the absence and presence of 3 μM losartan. The baseline end-diastolic pressures are prior to the 30 min of global ischaemia. R indicates reperfusion after the ischaemia and the time indicates the minutes after reflow. The data quoted at R are at the end of the ischaemic period and immediately before reperfusion and therefore represent the ischaemic contracture. ■ = data from the hearts subject to preconditioning; ○ = data from the non-preconditioned hearts; □ = data from preconditioned hearts obtained in the presence of losartan. AUCs are given in Table 2. The data are given as mean ± s.e.m. with the vertical lines indicating the errors.**
diong on diastolic function, such that the AUC of EDP$_{0-60}$ was significantly ($P < 0.05$) greater than the value obtained in the absence of losartan (Fig. 3b; Table 2). However, the value was still significantly ($P < 0.01$) less than in the non-preconditioned TGH hearts, such that, in the presence of losartan, diastolic function in the pre-conditioned, TGH hearts was intermediate between the preconditioned and the non-preconditioned hearts, in the absence of losartan.

4. Discussion

The findings of the present investigation clearly indicate that the protective effect of ischaemic cardiac preconditioning in TGH hearts is substantially upregulated. Furthermore, preconditioning in the TGH hearts also causes protection of diastolic function, which is partially sensitive to angiotensin, AT$_1$-receptor, blockade. These important findings extend the work of Speechly-Dick et al. [12] and Boutros and Wang [13] who investigated the impact of cardiac preconditioning in steroid-induced hypertension and in SHRs, respectively. The findings from the present study confirm that hypertensive hearts can be preconditioned, but, more importantly, show for the first time that this process is substantially enhanced in hearts from ((mREN-2)27) transgenic rats. This supports the notion that preconditioning is not an ‘all or nothing’ process, and may be upregulated under certain circumstances.

The recovery of mechanical performance in the non-preconditioned hearts was appreciably less than in the preconditioned preparations, confirming the efficacy of the preconditioning episodes. Importantly, in the non-preconditioned hearts, the recovery was similar in both TGH and SD groups, indicating that, in the absence of preconditioning, the TGH and SD hearts were equally susceptible to the deleterious effects of global ischaemia on reperfusion. Thus, the greater recovery of mechanical performance in the preconditioned TGH hearts, relative to the preconditioned SD hearts, must be attributable to an enhancement of preconditioning mechanisms. The effects were greatest in terms of LVDP, where there was a 3-fold difference in the magnitude of recovery between TGH and SD, while the difference was less marked for recovery of heart rate.

Hypertension in TGH rats is associated with various cardiovascular changes [14–17]. In the present study, cardiac mass to body mass ratio in the TGH hearts was increased, indicating cardiac hypertrophy, most probably of the left ventricle. Therefore, the effects of hypertension and cardiac hypertrophy on cardiac preconditioning cannot be dissociated in the TGH hearts. However, it should be noted that the degree of hypertrophy in the TGH hearts was substantially greater than in the DOCA-salt [12] and SHR [13] hearts in other studies. Therefore, it is possible that elevation of blood pressure or severity of hypertrophy must exceed a threshold before cardiac preconditioning is upregulated. There was, however, no direct correlation, within the preconditioned TGH group, between the degree of cardiac hypertrophy and protection afforded (data not shown).

It was also found that, at equivalent flow rates, coronary perfusion pressure did not differ between the hypertensive and normotensive groups, which contrasts with the findings of Kelm et al. [27] who reported a 30% higher coronary perfusion pressure in SHR hearts than in Wistar-Kyoto controls. The present findings indicate that basal coronary vascular resistance was not elevated in the TGH hearts, perhaps due to adequate compensation by factors involved in the control of coronary resistance in isolated hearts (e.g., nitric oxide [28] and K$_{ATP}$-channels [24]).

Hypertension in TGH rats is associated with enhanced local RAS activity in the heart [14,15,17,20], which prompted the investigation using the selective angiotensin AT$_1$-receptor antagonist, losartan. This was of particular relevance as angiotensin II, acting via protein kinase C activation, has been shown to mimic ischaemic preconditioning [23]. In the TGH hearts, coronary perfusion pressure was not influenced by losartan, which rules out a role of basally released angiotensin II in the regulation of vascular tone. Addition of losartan did not influence recovery of mechanical performance in the preconditioned SD hearts, which indicates a lack of the involvement of endogenous angiotensin II in the cardioprotective properties of preconditioning on mechanical performance. Furthermore, losartan did not influence the enhanced effects of preconditioning on mechanical performance in the TGH hearts, which suggests that the intracardiac RAS does not mediate the augmented cardioprotection. This does not, however, exclude the cardiac RAS from playing a longer-term role in cardioprotection. The local RAS is involved in promoting cardiac hypertrophy in the rat in response to pressure overload [29], and also in the TGH rat heart [20,21]. Furthermore, cardiac hypertrophy is accompanied by an increase in protein kinase C activity [30] which may be related to angiotensin II activity [31]. Therefore, in the context of cardiac preconditioning, an upregulation of protein kinase C, perhaps due to chronic cardiac RAS upregulation, may have important consequences as this second messenger system is thought to be a key mediator of the cardioprotective effects [6,7].

An alternative explanation for the augmented preconditioning response seen in the TGH hearts could relate to metabolic differences, due to either hypertrophy or hypertension. Thus, there is evidence that in various rat models of cardiac hypertrophy [32,33] and hypertension [34] there is upregulation of, and increased dependence on, anaerobic glycolysis. Furthermore, enhanced glycolysis has been shown to promote recovery of mechanical performance on reperfusion following ischaemia [35], by preserving high-energy phosphates during ischaemia by anaerobic metabolism of exogenous glucose. This preservation of
high-energy phosphates is thought to delay the onset of the deleterious effects of ischemic damage through necrosis and mechanical dysfunction [36]. This may be particularly relevant to preconditioning as glycolysis is known to be enhanced by ischemic preconditioning [36]. It is therefore possible that alterations in metabolic regulation in the TGH hearts (e.g., increased glycolysis), may have made them more susceptible to cardioprotection by preconditioning.

Intraventricular pressure was found to increase in all cases during global ischemia. This increase in pressure during ischemia represents the ischemic contracture and is associated with diastolic (EDP) impairment on repufusion, due to alterations in intracellular metabolism and calcium handling [37]. In the present investigation, preconditioning greatly reduced the ischemic contracture and the subsequent diastolic impairment, which accords with previous findings in the rat heart [38,39]. These findings with the TGH and SD hearts suggest that preconditioning also exerts a protective effect on diastolic function, and that this protection does not differ between the preconditioned hearts from the hypertensive and normotensive groups. The greater dysfunction (as shown by the differences in the AUCs for the EDP plots) in the non-preconditioned TGH hearts, compared to the SD hearts, agrees with the findings in hypertrophied hearts, taken from rats with aortic coarctation, in which post-ischemic diastolic dysfunction was greater than in normal hearts in the absence of preconditioning [40]; this was ascribed to calcium overload. In the case of the TGH hearts, the protective effect of preconditioning on diastolic function was partially sensitive to AT receptor blockade, which was not the case in the SD1 condition [(E)DP] impairment was partially sensitive to calcium overload. In the present investigation, preconditioning greatly reduced the ischemic contracture and the subsequent diastolic impairment, which accords with previous findings in the rat heart [38,39]. These findings with the TGH and SD hearts suggest that preconditioning also exerts a protective effect on diastolic function, and that this protection does not differ between the preconditioned hearts from the hypertensive and normotensive groups. The greater dysfunction (as shown by the differences in the AUCs for the EDP plots) in the non-preconditioned TGH hearts, compared to the SD hearts, agrees with the findings in hypertrophied hearts, taken from rats with aortic coarctation, in which post-ischemic diastolic dysfunction was greater than in normal hearts in the absence of preconditioning [40]; this was ascribed to calcium overload. In the case of the TGH hearts, the protective effect of preconditioning on diastolic function was partially sensitive to AT receptor blockade, which was not the case in the SD hearts. Thus, although the release of endogenous angiotensin II does not provide cardioprotection, it may be involved in the preservation of diastolic function in the TGH hearts.

In conclusion, the major finding of the present investigation is that hearts taken from hypertensive rats may be protected by cardiac preconditioning. Furthermore, in the TGH heart there is an enhancement of the effects of preconditioning, which may play an important role in limiting the deleterious effects of myocardial ischemia. Part of the protective effect of preconditioning on diastolic function in TGH hearts would appear to be mediated via the local, intracardiac RAS.

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