Smoking accelerates the progression of hypertension-induced myocardial hypertrophy to heart failure in spontaneously hypertensive rats☆

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Abstract

Objective: Myocardial hypertrophy often develops in response to hypertension, and it is causal to and an independent predictor of heart failure. Several risk factors modify the progression of hypertrophy, the associated progressive impairment of myocardial function, and eventually the transition to overt congestive heart failure. The aim of the present study was to investigate the effects of smoking on the progression of pressure-dependent myocardial hypertrophy.

Methods: Spontaneously hypertensive rats (SHR) were used as a model for pressure-dependent hypertrophy. SHR were exposed to mainstream smoke from the Kentucky reference cigarette 2R4F (450 μg total particulate matter/l) or to fresh air (control), 5 days a week, twice for 1 h per day with a 30-minute fresh air exposure break for 30, 60, or 90 days. Endpoints for hypertrophy-associated changes were heart weight to body weight ratio, ventricular expression of hypertrophy-associated genes, ischemic tolerance, and inotropic responsiveness to isoprenaline in post-ischemic hearts.

Results: Smoke-exposed SHR showed a significant elevation in heart weight to body weight ratio, increased mRNA expression of atrial natriuretic factor (ANF), transforming growth factor (TGF)-β1, ornithine decarboxylase (ODC), and parathyroid hormone-related protein in both ventricles compared to controls. Hearts from smoke-exposed SHR showed a reduced recovery after 30 min global ischemia during the first 5 min of reperfusion and loss of inotropic stimulation after 30 min reperfusion. Smoke cessation was sufficient to reverse most of these alterations. WKY exposed to smoke did not develop similar changes.

Conclusion: Our data indicate that several aspects of myocardial hypertrophy are accelerated by smoking.

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Keywords: Ischemia; Reperfusion; ODC; PTHrP; TGF-β1

1. Introduction

Myocardial hypertrophy is causal to and an independent predictor of heart failure [1]. Among the variety of potential risk factors, hypertension is one of the most well-known factors that induce myocardial hypertrophy [2]. The common understanding of pressure-dependent myocardial hypertrophy is that hypertrophic growth of cardiomyocytes compensates for the increased workload generated by hypertension. The molecular and cellular mechanisms converting load into growth are still not completely understood; however, it is well established that compensatory hypertrophy, irrespective of the initial responses of the heart to pressure overload, progresses to the non-compensated form with clinically severe symptoms of cardiac dysfunction (reviewed in Ref. [3]).
Spontaneously hypertensive rats (SHR) have been used as an experimental model to study the transition of hypertension-induced myocardial hypertrophy to heart failure, because they exhibit several characteristics that reflect those in the human heart under conditions of hypertension [4–6]. SHR of advanced age exhibit depressed myocardial contractile function and ventricular fibrosis, characterized by impaired left ventricular (LV) function, ventricular dilatation, and reduced ejection fraction [7]. In SHR, hypertension is accompanied by activation of the renin–angiotensin–aldosterone system (RAS). Angiotensin II, a highly active vasoconstrictor, induces the ventricular expression of transforming growth factor (TGF-β) component of this system, induces the ventricular expression of transforming growth factor (TGF-β1) in vitro and in vivo [8,9]. TGF-β1 was found to be elevated when SHR start to develop heart failure [7]. One mechanism by which TGF-β1 modifies heart function and regulation is an altered responsiveness of β2-adrenoceptors [10,11]. Once exposed to TGF-β1, activation of these receptors transcriptionally induces ornithine decarboxylase (ODC) [12]. ODC is the rate-limiting enzyme of polyamine metabolism. Because polyamines stabilize RNA, their up-regulation supports the development of cardiac hypertrophy. Hypertrophy-related changes are further supported by increased atrial natriuretic factor (ANF) mRNA levels, a widely accepted molecular marker of myocardial hypertrophy [13].

Parathyroid hormone-related protein (PTHrP) is another protein that is regulated by TGF-β1. The cytokine down-regulates cardiac PTHrP [13]. PTHrP exerts various cardiac effects via binding to cardiac specific receptors and also exerts intracrine effects on endothelial and smooth muscle cells (reviewed in Refs. [14] and [15]). Altered PTHrP responsiveness, altered β-adrenoceptor responsiveness, and increased ODC expression contribute to the progression of myocardial hypertrophy to heart failure in SHR. The loss of ischemic tolerance is another early feature indicating progression of heart failure in this model [16].

Although SHR have been widely used to investigate the basic mechanisms by which hypertension contributes to the transition of hypertrophy to heart failure, limited data are available on the combined effects of various risk factors in the presence of hypertension. The main risk factors contributing to myocardial hypertrophy and subsequent heart failure in the presence of hypertension are age, gender, diabetes, and obesity. Smoking is another risk factor of great clinical relevance, but data on the effects of smoking on the progression of hypertrophy to heart failure are lacking. In this study we used SHR to test our hypothesis that smoking modifies the progression of hypertrophy by altering the expression of the aforementioned hypertrophy-associated genes and impairs ischemic tolerance. The effects of hypertension on myocardial hypertrophy, expression of hypertrophy-associated genes, ischemic tolerance, and β-adrenoceptor responsiveness were compared to the combined effects of smoking and hypertension in SHR.

2. Materials and methods

2.1. Experimental design

The study was conducted in two parts. In the first part, SHR (n = 10 each group) were exposed to fresh air (sham) or to cigarette mainstream smoke for 30, 60, or 90 days. In the second part, SHR and Wistar–Kyoto rats (WKY), serving as normotensive controls, (n = 8 each group) were exposed to fresh air (sham) or to cigarette mainstream smoke for 90 days while another group of SHR was exposed to cigarette mainstream smoke for 30 days followed by 60 days of exposure to fresh air (recovery). Male SHR (both series; Charles River Laboratories Italia, Calco, Italy) and WKY (second series; Charles River Deutschland, Sulzfeld, Germany) were 8 weeks old at the start of the study. In the first series rats were exposed to mainstream smoke in flow-past exposure chambers without plungers. In order to better define smoke uptake, in the second series exposure tubes with plungers were used.

2.2. Animals and exposure

Male SHR (Charles River Laboratories Italia, Calco, Italy) and WKY (Charles River Deutschland, Sulzfeld, Germany) were 8 weeks old at the start of the study. Male rats were chosen for this study due to the estrogen-dependent effect on PTHrP expression and the less severe development of reduced ischemic tolerance [17]. Exposure was nose-only, 5 days per week, 2 times 1 h per day with a 30-minute fresh air break between the 2 h. Mainstream smoke from the University of Kentucky Reference Cigarette 2R4F was diluted to 450 µg total particulate matter (TPM) per liter. TPM exposure of rats was calculated on the basis of daily exposure normalized to body surface and corresponds to 1.5-fold of a dosage that can be calculated for humans. All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health. The animal experiments were previously approved by the Belgian Institutional Animal Care and Use Committee of PHILIP MORRIS Research Laboratories. None of the animals used in this study died during the exposure period. The development of hypertension was monitored in a small subgroup of animals in parallel by telemetry (n = 3). SHR exposed to smoke developed blood pressure values of 203 ± 8 mm Hg, comparable to our earlier observation in non-smoking SHR using the tail-cuff method (Ref. [19]; 201 ± 7 mm Hg). Moreover, smoking alone did not increase blood pressure in normotensive rats (121 ± 9 mm Hg).

2.3. Langendorff perfusion

Functional analysis was performed on isolated hearts. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal®, CEVA, Brussels, Belgium) at a dose of 75 mg/kg body weight and the hearts were rapidly removed.
The aorta was cannulated for retrograde perfusion with a 16-gauge needle connected to a Langendorff perfusion system. A polyvinyl chloride balloon was inserted into the left ventricle through the mitral valve and held in place by a suture tied around the left atrium. The other end of the tubing was connected to a pressure transducer for continuous measurement of left ventricular pressure. The balloon was filled to give an end-diastolic pressure of 10 mm Hg and balloon volume was held constant thereafter. A second transducer connected to the perfusion line just before the heart was used to measure coronary perfusion pressure. The perfusion system consisted of a warmed storage container for perfusate solutions, a rotary pump, and a pressure control chamber in which the hearts were kept at a temperature of 37°C.

After Langendorff perfusion, the right ventricle of the heart was separated from the left ventricle and septum. Samples of both ventricles were snap frozen in liquid nitrogen and homogenized. In samples from 30 and 90 days, total RNA from ventricular tissue was extracted with RNA-Clean (AGS; Heidelberg, Germany) according to the manufacturer’s protocol. Reverse transcription reactions were performed as previously described [12]. Aliquots (1 μg) of the synthesized cDNA were used for real-time reverse transcriptase polymerase chain reactions (RT-PCR) using I-CYCLER (Biorad, Germany) and SYBR-green as the fluorescence signal. The expression of TGF-β1, ODC, ANF, and PTHrP was normalized to the housekeeping gene hypoxanthine phosphoribosyl transferase (HPRT). Ratios were adjusted to a relative expression of 1 for the right ventricle of sham SHR at 30 days. The sequences of the primers used in this study are given in Table 1.

2.6. Immunoblotting

To determine protein expression of ANF, TGF-β1, and β-actin, ventricles (60 days) were homogenized and transferred to a lysis buffer (composition in mmol/l: cacodylate 10, NaCl 150, CaCl2 20, sodium acetate 1.5, ZnCl2 0.001, Triton X100 (0.01% vol/vol), pH 5.0). After centrifugation the supernatant was diluted with Laemmli buffer. Sodium dodecyl sulphate (SDS)-gel electrophoresis was performed as described before [18]. Proteins were separated by a 10% (w/v) SDS-polyacrylamide gel electrophoresis (acrylamide:bisacrylamide 30:1). After SDS-gel electrophoresis, proteins were transferred onto reinforced nitrocellulose sheets by semi-dry blotting. The sheets were saturated with 2% (wt/vol) bovine serum albumine (BSA) and incubated for 2 h with 0.2 μg/ml primary antibody (polyclonal rabbit-anti-ANF (RBI/Biotrend, Cologne, Germany), polyclonal rabbit-anti-TGF-β1 (RBI/Biotrend, Cologne, Germany) and polyclonal rabbit-anti-β-actin (Sigma-Aldrich, Germany)). After the sheets were washed alkaline phosphatase-labeled goat anti-rabbit-

Table 1
Sequences of primers used for real-time RT-PCR (see Refs. [13,28])

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<td>CCA GCG TCG TGA TTA GTG AT</td>
<td>CAA GTC TTT CAG TCA GCC GTT CC</td>
</tr>
<tr>
<td>ANF</td>
<td>ATG GCC TCC TTC TCC ATC AC</td>
<td>TCT TGC TTC CAG GAA GCT G</td>
</tr>
<tr>
<td>TGF-β1</td>
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<td>GTG GAG TAC ATT TTC TTT GCT</td>
</tr>
<tr>
<td>ODC</td>
<td>GAA GAT GAG TCA AAC GAG CA</td>
<td>AGT AGA TGT TTG GCC TCT GG</td>
</tr>
<tr>
<td>PTHrP</td>
<td>CGG TGT TCC TGC TGA GCT A</td>
<td>TGC GAT CAG ATG GTG AAG GA</td>
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2.4. Heart weight to body weight ratios

The progression of myocardial hypertrophy was determined by heart weight to body weight ratio. External fluid was completely removed from the excised heart before weighing.

2.5. Quantification of mRNA expression

After Langendorff perfusion, the right ventricle of the hearts was separated from the left ventricle and septum. Samples of both ventricles were snap frozen in liquid nitrogen and homogenized. In samples from 30 and 90 days, total RNA from ventricular tissue was extracted with RNA-Clean (AGS; Heidelberg, Germany) according to the manufacturer’s protocol. Reverse transcription reactions were performed as previously described [12]. Aliquots (1 μg) of the synthesized cDNA were used for real-time reverse transcriptase polymerase chain reactions (RT-PCR) using I-CYCLER (Biorad, Germany) and SYBR-green as the fluorescence signal. The expression of TGF-β1, ODC, ANF, and PTHrP was normalized to the housekeeping gene hypoxanthine phosphoribosyl transferase (HPRT). Ratios were adjusted to a relative expression of 1 for the right ventricle of sham SHR at 30 days. The sequences of the primers used in this study are given in Table 1.

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IgG antibodies were added for another 2 h. Bands were visualized by alkaline phosphatase activity using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. For quantification, density of the protein bands for ANF, TGF-β1 and β-actin was determined by ImageQuaNT (Molecular Dynamics Krefeld, Germany) and normalized to β-actin.

2.7. Statistical analysis

Results are expressed as means±SE. Differences were analyzed by one-way ANOVA followed by Student–Neumann–Keuls post-hoc analysis. Results were considered statistically significant at *p*<0.05.

3. Results

3.1. The effects of cigarette mainstream smoke exposure on the progression of myocardial hypertrophy

SHR exposed to smoke for 30, 60, or 90 days showed retarded body weight development compared to sham animals (Table 2). However, despite the difference in body weight, there were no differences in heart weight between smoke-exposed and sham animals at any time point, resulting in an increased heart weight to body weight ratio at 30, 60, and 90 days. The heart weight to body weight ratio in smoke-exposed SHR was 17.8±5.8% higher at day 30 and remained elevated throughout the experiment (18.1±6.9% at day 90), without further increase (Table 2). In SHR that were allowed to recover from smoking after the initial 30 days of smoke
Fig. 3. Time course of the left ventricular developed pressure (LVDP) during ischemia and reperfusion in hearts of smoke-exposed SHR and WKY. Data are expressed in mm Hg (left) or % of basal (right). Recovery = 30 days smoke exposure followed by 60 days sham exposure. Data are means±SE. n = 10 (A–C) or 8 (D–E). *, p<0.05 vs. sham.
Fig. 4. Time course of the left ventricular end-diastolic pressure (LVEDP) during ischemia and reperfusion in hearts of smoke-exposed SHR and WKY. Data are expressed in mm Hg (left) or % of basal (right). Recovery = 30 days smoke exposure followed by 60 days sham exposure. Data are means±SE. n = 10 (A–C) or 8 (D–E). *, p<0.05 vs. sham.
Fig. 5. Time course of the perfusion pressure during ischemia and reperfusion in hearts of smoke-exposed SHR and WKY. Data are expressed in mm Hg (left) or % of basal (right). Recovery = 30 days smoke exposure followed by 60 days sham exposure. Data are means ± SE. n = 10 (A–C) or 8 (D–E).
exposure, heart weights were still elevated at day 90; however, because these animals showed a significant increase in body weight, the heart to body weight ratios were again normal at the end of the recovery period. In WKY, smoking increased mean heart to body weight ratios, but this increase did not reach the level of statistical significance (11.7±3.3%; Table 2). The heart to body weight ratio was lower in WKY compared to SHR in all groups.

Expression of hypertrophy-associated genes was analyzed by real-time RT-PCR. Ratios were adjusted to a relative expression of 1 for the right ventricle of sham SHR at 30 days. ANF expression was significantly higher in the left ventricle than in the right ventricle of SHR sham groups at 90 days (Fig. 1A). In smoke-exposed SHR, ANF expression in both ventricles was higher at day 30 compared to sham, with a further increase with continued smoke exposure seen in the left ventricle only (Fig. 1A). Ventricular TGF-β1 expression was not elevated in the left ventricle compared to the right ventricle at day 30 or 90. However, at day 30, it was elevated in the smoke-exposed group in both ventricles (Fig. 1B). Although no differences were seen among the groups in ventricular ODC expression at day 30, ODC expression did increase with continued smoke exposure and was statistically significant at day 90 (Fig. 1C). This was similar for the ventricular expression of PTHrP (Fig. 1D).

The smoke-dependent increase in ventricular RNA expression of ANF and TGF-β1 was confirmed by protein levels in the 60-day samples. Representative immunoblots are shown in Fig. 2A. The quantitative analysis revealed ANF protein expression to be significantly higher (1.33±0.07-fold) in the left ventricle compared to the right ventricle, with a further increase in the smoke-exposed group reaching a level of 1.67±0.09-fold relative to the control right ventricle (Fig. 2B). The expression of TGF-β1 was also 1.22±0.13-fold higher in the left ventricle compared to the right ventricle of sham rats with a trend toward higher values in the smoke-exposed group, reaching a level of 1.29±0.21-fold (not statistically significant; Fig. 2C).

3.2. Post-ischemic recovery of the hearts

All hearts were analyzed ex vivo for heart function in a Langendorff perfused heart model. At baseline LVDP was higher in all smoke-exposed SHR groups compared to sham (103±14 mm Hg vs. 87±12 mm Hg at day 30, 104.7±13.5 mm Hg vs. 85.7±14.7 mm Hg at day 60, and 103.2±9.8 mm Hg vs. 76.4±12.9 mm Hg at day 90 (p<0.05; Fig. 3A–C). In the second series of experiments, baseline LVDP was again higher in smoke-exposed SHR at day 90: 120.0±16.7 mm Hg vs. 71.8±18.7 mm Hg (p<0.05; Fig. 3D). In the SHR recovery group (30 days smoke exposure followed by 60 days sham exposure) it reached 91.5±20.3 mm Hg and was no longer statistically significant compared to sham (Fig. 3D). No differences were seen between sham and smoke-exposed WKY rats: 68.8±12.4 mm Hg vs. 71.1±11.0 mm Hg; Fig. 3E).

After characterization of baseline parameters, hearts were exposed to 30 min of zero-flow ischemia, followed by reperfusion for 30 min. Time course of these experiments are given in Figs. 3–5, indicating the development of LVDP, LVeDP and perfusion pressure (i.e., coronary resistance) during ischemia and reperfusion. As indicated by the pronounce peak in LVDP after reperfusion, hearts from sham-exposed SHR recovered rapidly from zero-flow ischemia during the first 5 min. Thereafter, LVDP decreased again but recovered during the rest of the 30-minute period. The main difference in all smoke-exposed SHR groups is the progressive development of reduced recovery during the initial 5 min with increasing exposure duration (Fig. 3A–C). There was also a tendency to a diminished late recovery in the smoke-exposed SHR groups compared to sham during the 30-minute reperfusion period. In the SHR recovery group there was no difference in the early phase of reperfusion compared to sham (Fig. 3D). No loss of early reperfusion recovery was seen in hearts from normotensive WKY (Fig. 3E).

For LVeDP, there was a clear trend towards a higher LVeDP after reperfusion in the SHR smoke-exposed groups (Fig. 4A–C). However, in contrast to LVDP recovery, the difference between smoke-exposed and sham rats was still present in the recovery group (Fig. 4D). LVeDP levels were lower in smoke-exposed normotensive WKY compared to sham (Fig. 4E).
Coronary resistance was elevated in all SHR groups during the reperfusion period compared to the pre-ischemic values without any differences between smoke-exposed and sham groups (Fig. 5A–D). No differences were seen in coronary resistance for normotensive WKY (Fig. 5E).

3.3. β-Adrenergic responsiveness of post-ischemic hearts

The β-adrenergic responsiveness of the hearts was investigated 30 min after reperfusion. The addition of isoprenaline at a final concentration of 100 nmol/l produced a comparable positive chronotropic effect in all groups (Fig. 6A). However, the effect of isoprenaline on LVDP disappeared in SHR after 30 days of smoke exposure and was negative at days 60 and 90 (Fig. 6B). This paradoxical loss of function was not seen in the SHR recovery group. No paradoxical effect was seen in hearts from normotensive WKY (Fig. 6B).

4. Discussion

4.1. Main findings

The aim of this study was to investigate whether smoking directly modifies the progression of myocardial hypertrophy in the spontaneously hypertensive rats (SHR), a well-established model for pressure-induced hypertrophy in the literature. The relatively young animals used in this study developed myocardial hypertrophy but no obvious signs of heart failure over the 90 days. Smoking was shown to accelerate the progression of myocardial hypertrophy, favoring the progression to heart failure as described for this animal model [7]. This was demonstrated by the heart weight to body weight ratio, which was already increased after 30 days of smoking; the expression of the hypertrophy-associated genes, i.e., ANF, TGF-β1, and ODC; a reduced ischemic tolerance, as indicated by a reduced early recovery period; and finally by a loss of positive inotropic stimulation in post-ischemic hearts. These same changes have also been seen in older SHR that were not exposed to smoke [16,19].

4.2. Smoking and cardiac hypertrophy

Smoking and cardiac hypertrophy are known clinical risk factors for cardiovascular disease [20]. To identify potential interactions between them, we needed to be able to control other risk factors, such as gender, hyperglycemia, hypercholesteremia, and obesity; therefore we used only male rats fed a standard diet. On the basis of this model of hypertension-dependent hypertrophy, we observed an acceleration of the hypertrophic process in SHR exposed to smoke. This conclusion is based on the increased heart weight to body weight ratio found in the smoke-exposed groups compared to the sham-exposed groups and weakens when we consider that smoke-exposed animals had a slower body weight development. The lower body weight in age-matched animals accounted for the increased heart weight to body weight ratio. Nevertheless, when comparing body weight-matched animals, heart weights alone are indeed significantly higher. This holds true, for instance, for smoke-exposed rats at 60 days compared to sham rats at 30 days (see Table 2). Another way to evaluate cardiac hypertrophy is to use data on hypertrophy-related gene expression, i.e., mRNA and protein levels. ANF was used as an established marker of myocardial hypertrophy. The data clearly show an increase in ANF expression in the smoke-exposed SHR group at all time points, both on the mRNA and the protein level. The simultaneous increase in ANF expression and heart weight to body weight ratio highlights the prevalence of cardiac hypertrophy already after 30 days of smoke exposure. The SHR model mimics a pathophysiological development of heart disease induced by hypertension quite well, due to the time-dependent progression of the heart specific adaptation. Nevertheless, future studies must confirm these results in other hypertension-dependent models as well.

4.3. Smoking, heart failure, and ischemic tolerance

The data from our experiments do not provide direct evidence of smoke-induced heart failure or severe heart disease within the time span under investigation. The rats tolerated the smoke exposure quite well and basal heart function ex vivo was not impaired. In contrast, basal LVDP was even more pronounced in the smoke-exposed groups compared to the sham groups, particularly after 90 days. The data on the basal ex vivo function of the hearts cannot be superimposed on in vivo heart function because this is a Langendorff preparation. Therefore, due to the lack of afterload, the mechanical work performed by the hearts is low and this is different from the in vivo situation. For this reason we decided to expose the hearts to an established stress protocol. Impaired ischemic recovery is an established complication of myocardial hypertrophy, which develops progressively in this model [16,19]. Based on our experience with this model, we exposed the hearts to 30 min of global ischemia and investigated the recovery during the subsequent 30-minute reperfusion period [17,19]. The data showed improved early recovery from ischemia within the first minutes of reperfusion in hearts from smoke-exposed animals. This impairment progresses during the duration of smoke exposure. It was highly reproducible in both parts of the study, and did not develop further in the recovery group after smoking cessation. Reduced post-ischemic recovery did not develop in normotensive WKY exposed to smoke. Our study indicates no impairment in recovery in normotensive WKY, but this may be due to the endpoints we used. An increased sensitivity to ischemia/reperfusion was also shown before in normotensive rats after smoke exposure in another studies [21,22]. A similar impairment of the recovery during reperfusion was previously shown in SHR during ageing [19], where the changes in post-ischemic recovery seen in 12-month-old SHR are similar to those seen in 5-month-old rats in our study after 90 days of smoke exposure. Thus, smoking seems to accelerate the process of hypertrophy to heart failure in the presence of hypertension in
this model. The most remarkable finding is the lack of sufficient recovery in the first minutes after reperfusion, when activation of adrenoceptors by catecholamines released from the transmural nerve endings may play a major role. Following this line of argument, we exposed the hearts post-ischemically after 30 min to isoprenaline and determined the chronotropic and inotropic responsiveness to \( \beta \)-adrenoceptor stimulation. As expected, the addition of isoprenaline to the perfusion system significantly increased heart rates and LVDP. However, in hearts from smoke-exposed SHR, the ability of isoprenaline to increase LVDP progressively abated and was finally converted into a small negative effect after 90 days of smoke exposure. No such loss of effect or even paradoxical effect occurred in respect to the chronotropic response. This led us to speculate that the \( \beta \)-adrenoceptor coupling per se is not modified in the smoke-exposed SHR. Instead, another likely reason why isoprenaline does not increase LVDP in smoke-exposed SHR after 60 and 90 days might be the induction of a negative force–frequency-relationship. In control experiments on one-year-old male SHR, which had already developed an impaired post-ischemic recovery, a negative force–frequency-relationship was found between 240 and 300 beats per minute, which did not occur in hearts from age-matched normotensive WKY (unpublished data). Future studies are required to investigate whether smoke-exposed hearts indeed develop a negative force–frequency-relationship. However, irrespective of the outcome of such future studies, the loss of early post-ischemic recovery in combination with the impaired \( \beta \)-adrenoceptor-dependent inotropic responsiveness may be taken as an early indicator for the development of heart failure.

4.4. Smoking and expression of TGF-\( \beta \)-1 and TGF-\( \beta \)-dependent regulated genes

In addition to the functional data described above, the changes in the ventricular expression of TGF-\( \beta \)-1 may also be taken into consideration when evaluating the impact of smoking on the progression of hypertension-induced hypertrophy and the transition to heart failure. In this model, ventricular expression of TGF-\( \beta \)-1 increases within months to values exceeding that of normotensive WKY. The mechanism by which ventricular expression of TGF-\( \beta \)-1 is increased has been evaluated before and depends on angiotensin II. Moreover, inhibition of either angiotensin II receptors or angiotensin converting enzymes reduces the ventricular expression of TGF-\( \beta \)-1 and prolongs the life span of these animals [23]. For these reasons, ventricular expression of TGF-\( \beta \)-1 may be considered a predictor of the transition from hypertrophy to heart failure. In this context one of the most remarkable results we observed is the early increase in TGF-\( \beta \)-1 mRNA expression in hearts from smoke-exposed SHR. There is a general increase in ventricular TGF-\( \beta \)-1 expression in all SHR, in line with the well-known progression of this cytokine in SHR. Again, the main effect of smoking is an acceleration of already ongoing processes in this model. As TGF-\( \beta \)-1 mRNA expression could not be confirmed by a significant increase in TGF-\( \beta \)-1 protein expression at day 60, we further focussed on TGF-\( \beta \)-1-dependent regulated genes, to prove the significance of this observation, i.e. ODC and PTHrP. ODC is an indicator for the activation of the \( \beta \)_2-adrenoceptor system. The \( \beta \)_2-adrenoceptors couple to hypertrophy only under conditions in which a TGF-\( \beta \)-1-dependent activation occurs first [10,11]. It is in line with these suggestions, that ODC expression was already elevated in SHR after 90 days of smoke exposure following an increased ventricular TGF-\( \beta \)-1 expression. Again, similar findings have been demonstrated before in older hypertensive rats [13]. PTHrP in the ventricle seems to be part of the endothelium-dependent control of ventricular function [13]. The observation that the ventricular expression of PTHrP was also increased in smoke-exposed SHR was unexpected. Previously we demonstrated that TGF-\( \beta \)-1 down-regulates vascular expression of PTHrP in stroke-prone SHR, in transgenic mice over-expressing TGF-\( \beta \)-1, and in vitro [13]. Thus, we expected a down-regulation of this factor as well. However, in the smoke-exposed SHR, ventricular expression of PTHrP is elevated. This does not contradict our earlier conclusion that TGF-\( \beta \)-1 is activated in ventricles from smoke-exposed SHR because we were recently able to show that factors increasing the expression of PTHrP (i.e., estrogens) are sufficient to counterbalance the depressive effect of TGF-\( \beta \)-1 [17,24]. The question remains as to why PTHrP expression is elevated in this model. Preliminary experiments performed on isolated microvascular endothelial cells support the idea that nicotine increases endothelial PTHrP expression (data not shown). The remaining question is whether increased PTHrP expression is good or bad for the hearts of SHR. In general, vascular effects of PTHrP can be cardioprotective. On the other hand, PTHrP is sufficient to induce paradoxical effects on cardiovascular target cells of SHR [25]. The fact that PTHrP is released from endothelial cells during ischemia leads to the conclusion that local PTHrP concentrations are high at the beginning of reperfusion. While PTHrP was sufficient to improve post-ischemic recovery in normotensive WKY it worsens the situation in older SHR [19]. Thus, keeping in mind that smoking seems to accelerate the ongoing processes in these hypertensive hearts, one may speculate that PTHrP may also at least contribute to the insufficient early phase of reperfusion.

We observed changes in the expression of hypertrophy-associated mRNA levels that were quite comparable to those found in hearts from SHR and normotensive rats without a subsequent ischemia/reperfusion protocol as used in this study [19]. Therefore, we do not believe that this may influence the results presented here. ANF has nevertheless a hypoxia-regulated element. However, control experiments performed in our laboratory at the same model revealed that it required at least 45 min ischemia and 120 min reperfusion before ANF mRNA rises.

5. Conclusion

Smoking and hypertension are main risk factors for cardiovascular disease in man. Our study provides mechanic
evidence that these two factors interact with each other in the SHR. In this established model of hypertension-induced myocardial hypertrophy, smoking accelerates the progress of ventricular changes in regard to the expression of hypertrophy-associated genes. As a consequence, hearts will develop heart failure earlier. This was observed in our study mainly by the impaired early post-ischemic recovery, an established complication of myocardial hypertrophy which develops progressively in the SHR model. In the advanced stages of myocardial hypertrophy, both experimental and clinical studies have shown that when the high workload is maintained, hypertrophy progresses to contractile dysfunction, ventricular dilatation, and decreased tolerance to ischemia/reperfusion [26]. However, interventions reported to be cardioprotective in experimental models of ischemia/reperfusion have not been seen to improve clinical outcome in patients. One of the main reasons for this failure, as identified by a working group of the National Heart, Lung, and Blood Institute of the US, is the use of animal models that do not adequately approximate the clinical setting. In the experimental setting, healthy, juvenile animals are used, whereas the human situation is characterized by comorbidity (e.g., hypertension), usually in the elderly, and other variables (e.g., nutritional status or smoking) [27]. Our study was conducted in accordance with the recommendations that this working group gave to overcome these obstacles. The data clearly indicate that the combined effect of smoking and hypertension accelerates the progression of myocardial hypertrophy compared to either of these individual risk factors. As parameters under investigation we analyzed cytokine expression, reduced ischemic tolerance and different β-adrenoceptor responsiveness. All these parameters are well known to contribute to heart failure on the basis of hypertension. Nevertheless, future studies are needed to investigate the impact of smoking on established hypertensive heart disease in elder animals and on further functional parameters and survival. Our study also shows the benefits of smoking cessation, irrespective of other risk factors, such as hypertension.

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