Hypoxic pressor response, cardiac size, and natriuretic peptides are modified by long-term intermittent hypoxia

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1Department of Clinical Pharmacology, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden; and 2Department of Pharmacology and Toxicology, University of Oulu, FIN-90020 Oulu, Finland

Kraicz, Holger, J arkko Magga, Xiang Ying Sun, Heikki Ruskao, Xioahe Zhao, and J an Hedner. Hypoxic pressor response, cardiac size, and natriuretic peptides are modified by long-term intermittent hypoxia. J. Appl. Physiol. 87(6): 2025–2031, 1999.—We investigated whether the effect of long-term intermittent hypoxia (LTIH) on cardiovascular function may be modified by preexisting genetic traits. To induce LTIH experimentally, cycles of 90-s hypoxia (nadir 6%) followed by 90-s normoxia were applied to six Wistar-Kyto and six spontaneously hypertensive rats during 8 h daily. Comparison with the same number of control animals after 70 days revealed no alteration of intra-arterial blood pressure or heart rate. Blood pressure responsiveness to a brief hypoxic stimulus was enhanced in the LTIH animals, regardless of strain, whereas the hypoxia-induced increase in heart rate was abolished. In the spontaneously hypertensive but not the Wistar-Kyto rats, LTIH increased left ventricular weight-to-body weight ratio and content of atrial natriuretic peptide mRNA. Expression of B-type natriuretic peptide was unchanged (Northern blot). Slightly increased right ventricular weight-to-body weight ratios in the LTIH animals were associated with higher right ventricular atrial natriuretic peptide and B-type natriuretic peptide mRNA amounts. Consequently, the effects of LTIH on different components of cardiovascular function appear incompletely related to each other and differentially influenced by constitutional traits.

INTERMITTENT SYSTEMIC hypoxia due to respiratory pauses in patients with obstructive sleep apnea (OSA) is closely accompanied by recurrent increases in sympathetic activity, blood pressure (BP), and heart rate (HR) during sleep (21). Moreover, this repeated hemodynamic activation has been used to explain the increased nocturnal release of natriuretic peptides into the circulation, a foremost hormonal characteristic of OSA (28). Because this process is altered during LTIH. Moreover, atrial and B-type natriuretic peptide (ANP and BNP, respectively) act as growth inhibitors in several models of cardiac hypertrophy (6, 7), not least of which, hypoxic pulmonary hypertension (27). Potential adaptations of ANP and BNP turnover might therefore have direct implications for the development of long-term cardiovascular sequelae of LTIH.

Prospective studies to verify causality between LTIH and cardiovascular disease are difficult to perform in humans. Apart from ethical problems, repeated hypoxia cannot be isolated from other physiological phenomena associated with obstructive apneas in long-term human experiments. Moreover, long-term sequelae of LTIH might be modified by preexisting, e.g., genetic, characteristics, which may be difficult to identify and control in humans. Experimental induction of long-term intermittent hypoxia over 35 days in Sprague-Dawley and Wistar-Thomae rats has previously been shown to increase resting BP and to accelerate LV growth (13, 15). However, it is not known whether the potential impact of LTIH on BP and heart weight interacts with constitutional traits determining the development of hypertension and cardiac hypertrophy. To address this question, the present study included both normotensive Wistar-Kyto rats (WKY) and spontaneously hypertensive rats (SHR).

Short-term hypoxic stimuli result in transient tachycardia as well as splanchic vasoconstriction associated with a brief BP increase in rats (2, 32). On the basis of previous findings in patients with OSA (23), we hypothesized that this pressor response to short-term systemic hypoxia might be sensitized by prior LTIH exposure, genetic factors, or an interaction of both. Therefore, we measured the BP and HR changes during short hypoxic challenges in conscious WKY and SHR after LTIH.

Finally, we hypothesized that LTIH induces an increase in LV and RV synthesis of natriuretic peptides, consistent with the observation that hypoxia and increased cardiac workload upregulate the expression of ANP and BNP in the ventricular myocardium (38). It is unknown, however, whether this increased expression is maintained during LTIH and, if so, whether the cardiacANP and BNP mRNA contents parallel hemodynamic changes. Because this adaptation might be modi-

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Adaptations to Long-Term Intermittent Hypoxia

MATERIALS AND METHODS

Animals. Adolescent WKY and SHR in the 200-g range were purchased from Dr. Møllegaard (Copenhagen, Denmark). Body weights were determined at baseline and at the end of the study. Food and water were given ad libitum at all times except during hypoxic exposure. Lights were on between 0700 and 1900. All experimental procedures were approved by the Animals Ethics Committee of Göteborg University.

LTIH exposure. Repeated reductions in inspiratory oxygen fraction (FIO2; Oxygenanalyzer 5595, BOC Ohmeda) from 21 to 6% (Fig. 1) were induced in a transparent plastic chamber holding all test animals. The hypoxic episodes were initiated by discharging pure nitrogen into the chamber and terminated after 90 s by evacuation of the hypoxic gas mixture and replacement with room air. Normoxic periods lasted for a further 90 s before the onset of a new cycle. A damping device at the air-nitrogen supply dissipated the gas stream and prevented direct jets into the chamber. Intermittent hypoxia was applied daily from 0900 to 1700, i.e., during the animals’ normal sleeping period. This procedure induced no apparent alteration of the behavioral sleep-wake cycle. Control animals were handled according to the same routines but were allowed to continuously breath room air. The control chamber was placed adjacent to the test chamber to achieve a similar noise exposure to closure of gas valves.

Intra-arterial measurement of BP. Rats were instrumented with catheters (PE 50) placed in the left femoral artery under methoxital (25 mg/kg ip) anesthesia 3 days before BP measurements. Cannulas were filled with heparin in saline (100 U/ml), sealed, and exteriorized at the nape of the neck. Catheters were connected to a Grass model P 23 DC pressure transducer (Gould, Oxnard, CA). HR was derived from the systolic upstroke of the BP curve and plotted via calculation of the area under the BP and HR curves. MAP and HR during the 6-s interval with the highest readings within these two 30-s intervals are referred to as maximal MAP and HR. Values recorded immediately before the respective hypoxic stimulation were subtracted from each measurement value obtained during hypoxia, yielding hypoxia-induced changes in MAP (mmHg) and HR (beats/min), which were then averaged over the three hypoxic cycles.

Heart weight and ventricular expression of ANP and BNP mRNA. At the end of the experiments, the animals were decapitated. Hearts were dissected, cleaned from adhesions, divided into LV (containing septum) and right ventricle (RV; free wall portion), weighed, and immediately frozen at 70°C for storage until analysis. For isolation of RNA, the tissue was homogenized in guanidine-isothiocyanate solution (Ultraturrax, J anke & Kunkel). Samples of 20 and 15 µg RNA for Northern and dot blot analyses were obtained by ultracentrifugation through a CsCl cushion (41) and transferred to a nitrocellulose membrane (Schleicher and Schull BAS 85). Full-length probes of rat ANP cDNA (a gift from Dr. Peter L. Davies, Kingston, ON) (41) and BNP cDNA (a gift from Dr. Kazuwa Nakao, Kyoto, Japan) (37) as well as a full-length cDNA probe complementary to glyceraldehyde-3-phosphate dehydrogenase (17) were labeled with [32P]dCTP (Quick Prime Kit, Pharmacia) before overnight hybridization of the membranes at +42°C in 5 x saline sodium citrate (SSC; 1 x SSC being 0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0), 0.5% SDS, 5 x Denhardt’s solution, 50% formamide, and 100 µg/ml sheared herring sperm DNA. The membranes were subsequently washed three times in 0.1 x SSC with 0.1% SDS (20 min each, 50°C) and exposed to X-ray film with Cronex Lightning Plus intensifying screens (DuPont, Wilmington, DE) at -70°C. Autoradiograms generated by dot blots were scanned with a densitometer (Millipore Imaging Systems, Ann Arbor, MI). Amounts of LV and RV ANP and BNP mRNA were calculated from dot blot analysis normalizing individual hybridization signals to corresponding glyceraldehyde-3-phosphate dehydrogenase mRNA signals (41).

Ventricular ANP- and BNP-IR. For radioimmunoassay (RIA) analysis of tissue ANP-1R, guanidine thiocyanate extracts were diluted 1:400 in RIA buffer (43) and assayed without further extraction. Duplicates of 100 µl were incubated with 100 µl rabbit anti-rat ANP antiserum (final dilution 1:200,000) (43) at +4°C for 48 h. After a second incubation with 100 µl of 125I-ANP99–126 at +4°C for 24 h, γ-globulin in 500 µl of 8% polyethylene glycol 6000 was added and antibody-bound radioactivity was determined after separation by centrifugation at 3,000 g for 40 min. Synthetic rat ANP99–126 was used for the standard curve, which was tested for parallelism with extract dilution curves.

Extract samples for RIA analysis of BNP-IR were diluted 1:300 in RIA buffer (43), and duplicates of 100 µl were performed in the test chamber during the morning hours after a further day of rest and 2 days of intermittent hypoxia. Resting systolic and diastolic BPs as well as HR were recorded in each animal no earlier than 12 h after the last hypoxic exposure. One-minute averages of visually stable and artifact-free recordings were determined after at least 30 min of undisturbed conditions in the test chamber and immediately before the initiation of hypoxic challenges. Mean arterial pressure (MAP) was calculated as the arithmetic mean of systolic and diastolic values. With ongoing BP and HR recording, rats were subsequently exposed to three consecutive cycles of short-term hypoxia (FIO2 = 6%) by using the same time pattern as for LTIH (see above and Fig. 1). During each cycle, average MAP and HR were determined separately over two consecutive 30-s intervals after the onset of hypoxia via calculation of the area under the BP and HR curves. MAP and HR during the 6-s interval with the highest readings within these two 30-s intervals are referred to as maximal MAP and HR. Values recorded immediately before the respective hypoxic stimulation were subtracted from each measurement value obtained during hypoxia, yielding hypoxia-induced changes in MAP (mmHg) and HR (beats/min), which were then averaged over the three hypoxic cycles.

Heart weight and ventricular expression of ANP and BNP mRNA...
incubated at +4°C for 40 h with an equal volume of anti-rat BNP antiserum (a gift from Dr. Kazuwa Nakao) (37) by using a final dilution of 1:50,000. The samples were thereafter incubated at +4°C for 40 h with an equal volume of anti-rat BNP antiserum (a gift from Dr. Kazuwa Nakao) (37) by using a final dilution of 1:50,000. The samples were thereafter incubated at 4°C for 40 h with an equal volume of anti-rat BNP antiserum (a gift from Dr. Kazuwa Nakao) (37) by using a final dilution of 1:50,000. The samples were thereafter incubated at 4°C for 40 h with an equal volume of anti-rat BNP antiserum (a gift from Dr. Kazuwa Nakao) (37) by using a final dilution of 1:50,000. The samples were thereafter incubated at +4°C for 40 h, and antibody-bound radioactivity was determined as nanograms per milligram wet weight.

Statistics. The influence of the two factors, strain (WKY vs. SHR) and LTIH (control vs. LTIH), on resting MAP and HR, hypoxia-induced MAP and HR changes, LV and RV weight-to-body weight ratios, and ventricular contents of mRNA and IR of natriuretic peptides were estimated by between-group ANOVA. Planned between-group comparisons (Fig. 2) were tested by using the least significant difference test, and differences between groups with P values < 0.05 were considered statistically significant.

RESULTS

Body and heart weights. At the beginning of the experiment, SHR weighed slightly more than WKY but within either strain there was no weight difference between control and test animals. At the end of LTIH exposure, body weight was lower in the SHR compared with the WKY (P < 0.0001, Table 1) but was primarily reduced in the LTIH compared with control animals (P < 0.0001). This negative effect of LTIH on body weight gain was more pronounced in the SHR than in the WKY (P = 0.003 for the interaction between strain and LTIH).

Ventricular weights. After LTIH, LV weight-to-body weight ratio (Table 1) was higher in the SHR compared with WKY (P < 0.0001). This contrast was accentuated by LTIH exposure (P = 0.0001). Compared with the control animals of the same strain, LV weight-to-body weight ratio after LTIH was increased by 19.3% in the SHR but was unchanged in the WKY. RV weight-to-body weight ratio was higher in the SHR compared with the WKY (P = 0.001) and appeared to be increased after LTIH (P = 0.058) in both strains (+ 20% in SHR vs. WKY and + 40% in SHR vs. SHR-CON). This compared with the WKY (P = 0.001) and appeared to be increased after LTIH (P = 0.058) in both strains (+ 20% in SHR vs. WKY and + 40% in SHR vs. SHR-CON).

Table 1. Structural and hemodynamic variables after long-term intermittent hypoxia

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY-CON (n = 6)</th>
<th>WKY-LTIH (n = 6)</th>
<th>SHR-CON (n = 6)</th>
<th>SHR-LTIH (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Weight at end of experiment, g</td>
<td>362 ± 18</td>
<td>316 ± 13</td>
<td>348 ± 17</td>
<td>263 ± 6</td>
</tr>
<tr>
<td>LV weight/body weight, g/kg</td>
<td>2.19 ± 0.07</td>
<td>2.16 ± 0.11</td>
<td>2.69 ± 0.20</td>
<td>3.21 ± 0.14</td>
</tr>
<tr>
<td>RV weight/body weight, g/kg</td>
<td>0.42 ± 0.05</td>
<td>0.48 ± 0.07</td>
<td>0.56 ± 0.15</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Resting</td>
<td>120.1 ± 11.1</td>
<td>125.2 ± 12.4</td>
<td>178.3 ± 16.5</td>
<td>171.3 ± 21</td>
</tr>
<tr>
<td>Maximal change during hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>282 ± 29</td>
<td>292 ± 30</td>
<td>395 ± 35</td>
<td>390 ± 23</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of animals; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive; CON, control; LTIH, long-term intermittent hypoxia; LV, left ventricle; RV, right ventricle; MAP, mean arterial pressure; HR, heart rate. Structural and hemodynamic variables at end of experiment in WKY and SHR without (WKY-CON, SHR-CON) and with (WKY-LTIH, SHR-LTIH) prior LTIH exposure are shown. MAP and HR changes are based on measurements made during the first 60 s of hypoxia of 3 separate consecutive cycles and refer to resting conditions immediately before hypoxic exposure.blast was accentuated by LTIH exposure (P = 0.0001). Compared with the control animals of the same strain, LV weight-to-body weight ratio after LTIH was increased by 19.3% in the SHR but was unchanged in the WKY. RV weight-to-body weight ratio was higher in the SHR compared with the WKY (P = 0.001) and appeared to be increased after LTIH (P = 0.058) in both strains (+ 20% in SHR vs. WKY and + 40% in SHR vs. SHR-CON).

Fig. 2. Means and SE of arterial blood pressure (MAP; A) and heart rate (B) in Wistar-Kyoto (WKY; filled symbols) and spontaneously hypertensive rats (SHR; open symbols) after long-term intermittent hypoxia (circles) or control conditions (squares). Measurement points refer to normoxic conditions (unstimulated) and average values derived from 3 consecutive hypoxic cycles (FIO2 5 6%). Shown are significant within-group changes from values measured in unstimulated state. *P < 0.05; **P < 0.01; ***P < 0.001 (least significant difference test).
Hemodynamic measurements. Unstimulated MAP at the end of the LTIH-exposure period (Table 1, Fig. 2) was higher in the SHR than in the WKY (P < 0.0001). LTIH exposure did not affect resting MAP (P = 0.89) in either strain (P = 0.37 for strain × LTIH interaction). In contrast to the BP results under resting conditions, however, the increase in MAP during the first minute of hypoxia was not predicted by the strain of the animals (P = 0.62 and P = 0.74 for mean and maximal change in MAP, respectively) but was significantly enhanced by prior LTIH exposure (P = 0.038 and P = 0.021 for mean and maximal change, respectively). Compared with the respective control groups, mean hypoxia-induced change in MAP was nearly doubled in the SHR and more than tripled in the WKY after LTIH (Table 1). No significant interaction between strain and LTIH was found (P = 0.42 and P = 0.34 for mean and maximal MAP increase, respectively).

Unstimulated HR was higher in the SHR compared with the WKY (P < 0.0001) but was not influenced by prior LTIH (P = 0.78; interaction: P = 0.68). Short-term hypoxic exposure raised HR in the SHR and WKY control animals by an average of 14 and 22%, respectively (Table 1, Fig. 2). In the LTIH-exposed animals, by contrast, this HR response was practically abolished (P = 0.003 and P = 0.0004 for differences in mean and maximal HR between control and LTIH animals, respectively). This effect of LTIH on HR acceleration did not significantly differ between SHR and WKY (P = 0.85 and P = 0.77 for mean and maximal HR change, respectively). When change in HR during short-term hypoxia was controlled for the mean change in MAP, essentially the same ANOVA results were obtained for both mean and maximal hypoxia-induced HR responses (data not shown).

Natriuretic peptides. The amount of ANP mRNA in the LV of the SHR (Fig. 3) was approximately twice that in the WKY (P < 0.0001). LTIH further increased the LV expression of ANP mRNA by ~40% in the SHR but had no effect on the content of LV ANP mRNA in the WKY (P = 0.025 for strain × LTIH). The LV content of BNP mRNA was 32% higher in the SHR compared with the WKY (P = 0.015) but was uninfluenced by LTIH exposure (P = 0.80).

In the RV, only LTIH exposure (ANP: P = 0.033; BNP: P < 0.0001), but not the difference in rat strain (ANP: P = 0.84; BNP: P = 0.57), contributed significantly to the variability in ANP and BNP mRNA content (Fig. 3). In a comparison of all LTIH with all control animals, RV ANP and BNP mRNA were both increased by ~40% after LTIH. In contrast to the cardiac mRNA levels, neither ANP- nor BNP-IR was explainable by rat strain, LTIH exposure, or interaction of these factors (Fig. 3; P values not shown).

Fig. 3. Means and SE of atrial natriuretic (ANP) and B-type natriuretic peptide (BNP) mRNA amounts (A and B, respectively) as well as ANP and BNP immunoreactivity (IR; C and D, respectively) in left and right ventricles of WKY and SHR after LTIH (WKY-LTIH and SHR-LTIH, respectively) or control conditions (WKY-CON and SHR-CON, respectively). Shown are significant differences between LTIH and control groups of same strain or between animals of different strains but with same treatment. *P < 0.05; ***P < 0.001 (least significant difference test).
DISCUSSION

Our results indicate that the inbred differences between WKY and SHR predominate over the effect of LTIH on unstimulated MAP and HR. In contrast, the responsiveness of MAP and HR to short-term hypoxia appears substantially influenced by exposure to LTIH but independent of the strain investigated. In the SHR but not in the WKY, LTIH exposure may be associated with increased LV weight-to-body weight ratio and upregulated expression of ANP mRNA. In the right hearts of both WKY and SHR, LTIH may increase the ventricular expression of both natriuretic peptides without being a potent stimulus for ventricular growth.

The failure of LTIH to alter basal MAP in this study contrasts with results reported by Fletcher and co-workers (15), who demonstrated an increase in MAP of ~14 mmHg after 35 days of intermittent hypoxia in Wistar (Thomae) rats. Differences in the intermittent hypoxia protocol used in the present study might explain this discrepancy. First, Fletcher et al. selected a comparably low $\mathrm{FIO}_2$ level for their hypoxic stimuli (3–5%). In rats, $\mathrm{FIO}_2$ levels above 5%, as chosen in our study, may cause less prominent increases in MAP (2, 33). Consequently, the hypoxic challenges in our protocol may result in less pronounced autonomic and hemodynamic fluctuations during LTIH. During extended exposure, this difference may account for a weaker modulatory influence on cardiovascular homeostasis. Second, the hypoxic challenges in previous LTIH studies were considerably shorter and repeated with a higher frequency (120/h compared with 20/h). Apart from the lower total number of hypoxic challenges per time unit thereby achieved with our experimental setup, the extended hypoxic challenges may have allowed slower acting vasodilatory mechanisms to counteract and to finally attenuate the initial BP rise before the onset of the next normoxic period (24, 33). Moreover, the dampening effect of hypocapnia on hypoxia-induced BP rise because of hyperventilation (3) may have been more pronounced during the longer hypoxic periods in our model. On the whole, the balance between vasoconstrictive and vasodilatory influences at the end of each hypoxic cycle may have been closer to equilibrium in our setting than with short hypoxic challenges. A third difference from the previously described model was the longer overall duration of LTIH in the present study (70 vs. 35 days). However, if LTIH had resulted in sustained BP elevation at an earlier time point during our experiment, hypertension would have been expected rather to become structurally established (16, 29). Long-term adaptive mechanisms countering a potential hypertension-promoting effect of intermittent hypoxia have, to our knowledge, not been demonstrated. Finally, Fletcher et al. (15), using the lowest stable BP value recorded for >10 min as an outcome variable, might have estimated unstimulated BP with higher precision, thus increasing statistical power in their studies.

Increased MAP after LTIH has recently been reported by another group (20). However, compared with our study, these authors used a different species (Sprague-Dawley rats), a different pattern of intermittent hypoxia (with shorter cycles and a hypoxic plateau lasting no longer than 5–7 s), as well as a shorter exposure period (30 days). Moreover, BP was measured in anesthetized and instrumented animals, and the study report does not specify whether recovery after the termination of intermittent hypoxia was allowed. Thus the ensuing lack of comparability is based on a variety of experimental factors, the systematic study of which might explain why our study failed to demonstrate a sustained increase in unstimulated MAP during LTIH.

Responsiveness of the cardiovascular chemoreceptor reflex was studied by stimulating arterial chemoreceptors with brief hypoxic challenges. In rats, increased chemoreceptor discharge activates the sympathetic nervous system (4, 18), resulting in splanchnic vasoconstriction and tachycardia (32). The ensuing BP rise may secondarily be attenuated or terminated by superimposition of this response by local vasodilatory effects of hypoxia (24, 33). Activation of vagal afferents as a result of hypoxemia-induced hyperventilation, by contrast, does not affect the hemodynamic response to hypoxia in rats (32). In the present study, responsiveness of MAP to short-term hypoxic challenges was increased after LTIH, regardless of strain. Because this effect of LTIH was not caused by a facilitation of hypoxia-induced tachycardia (Table 1, Fig. 2), it remains that LTIH exposure facilitated an increase in either stroke volume or total peripheral resistance (or both) during short-term hypoxia. A previous study using borderline hypertensive rats and shorter hypoxic cycles (2/min) with more severe hypoxia ($\mathrm{FIO}_2$, nadirs of 2–4% during both LTIH and acute hypoxic challenges) could not find a significantly enhanced hypoxic pressor response after LTIH (12). The discrepancies between these findings remain unexplained but may be attributable to the methodological differences.

Hypoxia-induced tachycardia as seen in the control rats was strongly inhibited by prior LTIH in both strains studied. At first sight, this observation might be explained by baroreceptor stimulation as a result of the hypoxia-induced increase in MAP. However, variability in hypoxia-induced HR change was only in part explainable by the change in MAP ($r^2 = 49\%$ in the WKY and 3% in the SHR). Moreover, the effect of LTIH in suppressing tachycardia during short-term hypoxia was still significant after statistical allowance for the change in MAP. This finding is in accordance with data showing that prevention of BP increase during hypoxia with a barostat does not influence the balance between cardiac vagal and sympathetic activity, the net activation of which determines the direction and level of the HR change during hypoxia (18). Moreover, repetitive pressor stimuli during LTIH may have been expected to result in baroreflex adaptation (10), allowing tachycardia to arise despite the increase in MAP. As a consequence, increase in MAP during hypoxia seems insufficient to explain the dampened hypoxia-induced HR response in the LTIH animals. Instead, strong coactivation of the vagal in relation to the sympathetic cardiac
discharge during short-term hypoxia after LTIH (42) may have occurred without involvement of baroreflex mechanisms. Alternatively, attenuation of tachycardia during short-term hypoxia might have been the result of disturbed cardiac pacemaker function after LTIH, because hypoxia can modify sympathetic (30) as well as vagal (39) synaptic transmission at the sinus node and also alter the structure and function of the sinus node itself (19). Finally, as circulating epinephrine might contribute to the normal HR increase during short-term hypoxia (40), reduced secretory capacity of the adrenal medulla or increased cardiac metabolism (31) of catecholamines after LTIH is a conceivable explanation for our observation.

Regardless of the rat strain, LTIH caused a significant, although less pronounced, increase in the RV expression of ANP and BNP mRNA than previously studied biological stimuli (1, 36, 38). This finding, together with the modest effect of LTIH on RV weight-to-body weight ratio, suggests that LTIH provides a rather mild pathological stimulus to the RV. In the LV, ANP mRNA levels paralleled the distribution of ventricular weight-to-body weight ratios over the four groups studied. The highest values in the SHR LTIH group are likely to reflect an adaptation to repeated increases in workload during LTIH on the basis of inbred hypertension and LV hypertrophy (5, 11, 26, 41). Alternatively, LTIH may have resulted in a further enhancement of sympathetic outflow (14), a potent stimulus of ANP secretion (25, 44). This mechanism, which may also account for the moderate increase in RV natriuretic peptide mRNA in our study, is compatible with previous results from Fletcher et al. (14), who found enhanced epinephrine and norepinephrine concentrations in the RV of LTIH-exposed rats. However, increased sympathetic tone is less likely to explain higher LV in the LTIH-exposed SHR, because previous results in Wistar rats showed no effect of chemical sympathectomy on LTIH-induced increase in LV weight-to-body weight ratio (14).

LTIH exposure modified neither LV weight-to-body weight ratio nor LV expression of ANP mRNA in the WKY rats. Therefore, the effects of LTIH on cardiac growth and ANP mRNA expression appear to depend on preexisting hypertension, LV hypertrophy, or some other constitutional trait present in the SHR, although specification of this interaction requires further study.

The failure of LTIH to further elevate LV amounts of BNP mRNA in SHR is not explainable by our study but may be due to differences in the regulation of the transcriptional activity for ANP and BNP (34, 35). Because the transcription of BNP mRNA has been reported to respond faster but more briefly to some biological stimuli (34, 35), we cannot exclude that the LV expression of BNP mRNA was increased at an earlier stage during our experiment (38). Finally, our failure to demonstrate changes in the ventricular levels of ANP- or BNP-IR despite increases in mRNA levels after LTIH may be explained by a rapid release of peptides into the circulation without prior storage (constitutive mode of secretion), resulting in unchanged myocardial peptide concentrations despite increased synthesis (41).

Our finding that increased LV weight-to-body weight ratio and enhanced LV expression of ANP mRNA after LTIH were observable in the SHR but not in the WKY illustrates that at least some of the long-term consequences of LTIH may be modified by preexisting constitutional traits. However, increased hemodynamic responsiveness after LTIH was found in both strains, which is in accordance with data from human studies suggesting that OSA may lead to independent forms of cardiovascular or autonomic dysfunction without necessarily causing arterial hypertension (9, 22, 23). Future investigations will show whether these mechanistically separate alterations of cardiovascular homeostasis are clinically relevant pathogenic links between OSA and manifest cardiovascular disease.

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