THE IMPACT OF RENAL DENERVATION ON THE BAROREFLEX CONTROL OF HEART RATE IN AN EXPERIMENTAL MODEL OF REDUCED RENAL MASS, SALT SENSITIVE HYPERTENSION

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THE IMPACT OF RENAL DENERVATION ON THE BAROREFLEX CONTROL OF HEART RATE IN AN EXPERIMENTAL MODEL OF REDUCED RENAL MASS, SALT SENSITIVE VOLUME-OVERLOAD HYPERTENSION (Abstract): **Background**. Beyond lowering arterial blood pressure in hypertensive patients, renal denervation (RDNx) has also been shown to improve the cardiac autonomic nervous system, particularly in sympathetically mediated cardiovascular disorders. Nevertheless, little is known about the efficiency of renal nerve ablation in non-mediated sympathetically diseases. Therefore, our aim was to investigate the influence of renal denervation on the cardiac autonomic nervous system in an experimental rat model of salt sensitive, volume-overload hypertension developed after surgical reduction of renal mass (RRM) by 75-80% and salt loading. **Material and methods:** The dynamic time-dependent cardiac autonomic system analyses such as the baroreflex sensitivity (BRS), the power of the fluctuations of the heart rate in high frequency range (0.75-3Hz, HF power) and both short and long-term beat-to-beat variability were calculated from continuously recorded cardiac cycles before and after surgical RRM by 80%, including here RDNx and central sympathoinhibition with clonidine. **Results:** The BRS decreased significantly throughout the entire 2 weeks of the development of salt-sensitive, volume overload hypertension induced after surgical RRM and high salt diet. After RDNx, the HF power decreased during the first 7 days, paralleling the alterations of both long and short-term heart rate variability. Global sympathoinhibition after clonidine administration significantly improved the cardiac autonomic nervous system as reflected by the substantial enhancement of the BRS, the HF power and both short and long-term heart rate variability. **Conclusions:** The present results indicate that in salt-sensitive, volume overload hypertension, the cardiac autonomic nervous system is impaired, and these alterations are not improved over the long term by RDNx. On contrary, central sympathoinhibition after clonidine administration following RDNx significantly improved BRS, the HF power and both short- and long-term beat-to-beat variability. **Keywords**: HYPERTENSION, RENAL DENERVATION, CARDIAC AUTONOMIC NERVOUS SYSTEM, BAROREFLEX, HEART RATE VARIABILITY.

The arterial baroreflex plays a crucial role in maintaining the homeostasis of the cardiovascular system by reflexively modulating the balance between sympathetic and parasympathetic activity (1-3). Baroreflex dysfunction has been associated to many cardiovascular diseases that lead to autonomic imbalance and thus likely contribute to the progression of disease. Impairment of the baroreflex control of the heart rate has been found in patients with heart failure, myocardial infarction, coronary artery disease and essential hypertension (2, 4-7). While baroreflex dysfunction *per se* is correlated with a high mortality risk (8-10), reductions in heart rate variability (HRV), likely due to impaired baroreflex control of the cardiac autonomic outflow, has also been recognized as a predictor and a risk factor for developing life-threating atrial and ventricular tachyarrhythmia (11-16). Furthermore, since dysfunction of the baroreflex system has also been described in patients with treatment resistant hypertension (RHT) (17) these patients are likely exposed to arrhythmic risk (18).

Recent clinical and experimental studies indicate that ablation of the renal nerves may improve both baroreflex sensitivity (BRS) and HRV in patients with RHT (19, 20). Importantly, these effects occur in parallel to the lowering of blood pressure, thus indicating a putative beneficial cardioprotective effect. Overactivation of sympathetic efferent renal nerves increases blood pressure by stimulating renin secretion and renal tubular sodium reabsorption and interruption of these pathways by renal denervation (RDNx) is considered to underlie most of the antihypertensive effects of this non-pharmacological approach (21). However, RDNx also interrupts sympathoexcitatory afferent signaling from the kidneys, which may contribute to global sympathoinhibition, in addition to the renal-specific effects. An improvement in the baroreflex control of sympathetic outflow or suppression of the central sympathetic outflow via hypothalamic pathways has been suggested to mediate these latter effects (22, 23). Although the lowering of blood pressure and the improvement of autonomic function following RDNx appear to be efficient in conditions associated with sympathetic overactivation, it remains unclear whether these beneficial effects are also manifest in the absence of an increased sympathetic outflow.

The remnant kidney model is mainly used to investigate the pathophysiology of the RHT related to non-ischemic nephron loss and has the major advantage to closely replicate many of the abnormalities of this clinical condition (24). In this model of salt-sensitive, volume overload hypertension following surgical reduction of 75-80% of the renal mass, we have previously shown that the global sympathetic nervous system activity is actually reduced and RDNx does not further lower sympathetic activity, thus lacking sustained antihypertensive effects (25). However, it is unclear whether baroreflex function and HRV are altered during the development of salt-sensitive hypertension and whether RDNx may have beneficial effects on the baroreflex control of heart rate in the absence of a sustained antihypertensive and global sympatholytic effect. Considering the recent observations regarding the anti-arrhythmic effects in patients undergoing RDNx (19, 26-29), we hypothesized that renal nerve ablation may improve cardiac BRS and HRV in this clinically relevant model of human RHT. This evaluation in this experimental model which shares many of char-

acteristics of RHT may be important clinically because many patients with RHT are at high risk for detrimental cardiac arrhythmias (17, 18).

To test this hypothesis, spontaneous oscillations of blood pressure and heart rate were continuously analyzed in the time and frequency domains, to determine the daily time course of cardiovascular dynamics under different salt-loading conditions during normal renal function and throughout the progression of salt-sensitive hypertension following reductions in renal function. Furthermore, the ability of the renal denervation to improve cardiac baroreflex, and ultimately the HRV was evaluated. Finally, the response of the cardiovascular dynamics to global suppression of sympathetic outflow with clonidine was assessed.

MATERIAL AND METHODS

All experiments were performed according to the European Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes and were approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași. As previously describe (25), eight adult male Sprague Dawley rats (28-32 weeks of age) were used in this study. The animals were obtained from the National Research Institute "*Cantacuzino*" (Bucharest, Romania). Rats were housed in a temperature (21-23°C) and humidity-controlled environment with a 12 hours light/dark cycle, with *ad libitum* access to food and water. Animals were fed a standard rodent diet containing either 0.8 % NaCl (Normal salt, NS), 0.1 % NaCl (Low Salt, LS) or 4 % NaCl (High Salt, HS) (National Research Institute "Cantacuzino" Bucharest, Romania). The long-term cardiovascular responses (24 hours averages) data from this study have been presented in a previous publication (25). The present study was designed to investigate the detailed time-dependent dynamic changes of baroreflex control of heart rate, as well as HRV.

Animal preparation

All surgical procedures aimed for continuous acquisition of blood pressure, surgical reduction of renal mass and renal denervation of the remnant kidney have been previously described (25).

Data acquisition and analysis

Arterial blood pressure was continuously recorded using implantable radio telemetry system. For signal acquisition and wireless charging of the implanted telemeters rats were placed on TR181 smart pads (*Millar, Inc., Houston, TX*)(25). The individual 24-h BP waveforms were acquired continuously at a sampling frequency of 2000 Hz using a PowerLab 16/35 data acquisition system (*ADInstruments, New South Wales, Australia*). All individual cardiac cycles were recorded continuously (on every day between 7.00 AM to 6.00 AM recordings, excluding the one hour necessary for daily animal care). The dynamic time-dependent cardiac autonomic system analyses were performed under both normal and reduced renal mass conditions, with the associated phases of the developing and established volume-overload hypertension, including here the renal denervation and the central sympathoinhibition with clonidine (in doses of 300 and 150 μg/kg/day in the drinking water with daily adjustment of the concentration per fluid intake to attain desired drug intakes). For subsequent time- and frequency-domain analyses, daily time series of beat-to-beat

systolic blood pressure (SBP) and pulse intervals (PI) were generated from artifact-free 23 hours long blood pressure waveforms with LabChart Pro v8.1.8 (*ADInstruments, New South Wales, Australia*) and peak detection algorithms.

Estimation of baroreflex control of heart rate

BRS was computed using frequency domain analyses as previously described. Briefly, a spectral analysis of the daily beat-to-beat data based on the Fast Fourier Transformation (FFT) was performed using MATLAB routines (*R14; MathWorks, Natick, MA*) using algorithms we have previously described (16, 30). The BRS was estimated as the magnitude of the transfer function between SBP and PI within 0.05 - 0.5 Hz frequency range.

Heart rate variability measurements

HRV measurements were performed using both time and frequency domain analyses. Long-term heart rate variability was obtained from time domain analyses entailing in determination of the standard deviation (SD) of all pulse intervals during a 23-hours. period. Short-term variability was computed as the means of the standard deviation calculated for all 5-min segments for the entire 23 hours period (SDNN) (31). The power of the heart rate oscillations within 0.75-3 Hz band (HF power) from the daily R-R interval variations, reflecting the parasympathetic control of the heart rate, was computed using the non-parametric lomb periodogram algorithm implemented into LabChart module (*LabChart Pro v8.1.8*).

Experimental design

Normal renal function and associated salt resistant hypertension

1. After implantation of the telemeters, the rats were allowed to recover for 2 weeks until the circadian rhythmicity was fully restored. Cardiac autonomic nervous system responses from control period were recorded while the animals were fed with normal salt (NS) diet. To assess the cardiac autonomic nervous system regulation during normal renal function and associated salt resistant hypertension, the salt intake varied as follows: for 7 days the rats were fed with LS, followed by another 7 days of HS diet. At the end of the second week, the LS diet was initiated and maintained until the mean arterial blood pressure and heart rate values plateaued around the levels from control period.

Reduced renal mass and associated salt sensitive hypertension

Cardiac autonomic nervous system responses were assessed continuously throughout the entire experimental periods as described below. Surgical RRM was performed as previously (25). Following the post-surgical recovery period, the HS diet was initiated and maintained for the next 6 weeks. After two weeks of HS diet, when arterial blood pressure significantly increased and plateaued at hypertensive levels, RDNx of the remnant left kidney was performed as previously described (25). Two weeks after RDNx, the cardiac autonomic nervous system responses to centrally sympatholytic clonidine were assessed. Finally, in the last week of the experiment, the rats were subjected again on LS diet.

Statistical analysis

Results are expressed as Mean ± SE. One-way, repeated measures ANOVA followed by Dunnett's multiple comparison test were used (*Prism 6.01, GraphPad Software*) to compare the experimental

periods mentioned here below and the differences were considered statistically significant for $p<0.05$.

Normal renal function

→ daily cardiac autonomic nervous system regulation during:

1) LS (days 5-11; 19-21) to NS control period (days 2-3);

2) HS (days 12-18) to NS control period (days 2-3);

Reduced renal mass and associated salt sensitive hypertension

→ daily cardiac autonomic nervous system regulation during:

1) HS (days 40-53) to LS (days 38-39);

2) HS + RDNx (days 54-67) to HS (days 52-53);

3) HS + RDNx + clonidine 300 μg/kg/day (days 68-73); + clonidine 150 μg/kg/day (days 74-76); + clonidine washout (days 77-81) to HS + RDNx (days 66-67);

4) LS (days 82-87) to HS + RDNx (days 80-81).

RESULTS

Responses during normal renal function and associated salt resistant hypertension.

As shown in the figure 1A, the estimated BRS as the magnitude of the transfer function between systolic blood pressure and pulse interval did not change during both LS and HS. While the HF power (fig. 1B) increased in the last day of the LS, it decreased significantly throughout the entire HS period. Although long term beat-to-beat variability did not suffer any alterations (fig. 2A), short term beat-to-beat variability decreases during both LS and HS periods (fig. 2B), paralleling the HF power.

Figure 1

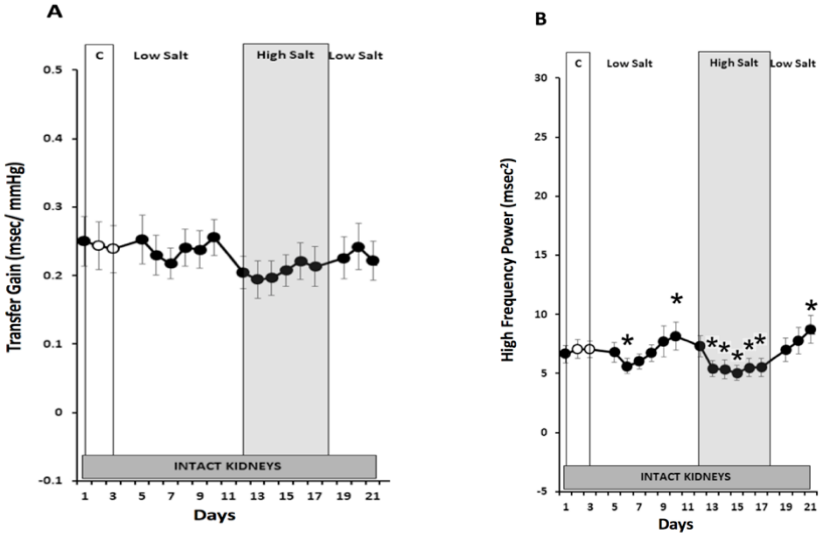

Fig. 1. Baroreflex sensitivity (**Panel A**) and the power of the fluctuations of the heart rate in high frequency range - HF power - **(Panel B)** responses to varying salt intake during normal renal function. Values are men ± SEM. Normal renal function: * $p < 0.05$ vs. NS Control (days 2-3). Respective baseline days are depicted as (○).

Figure 2

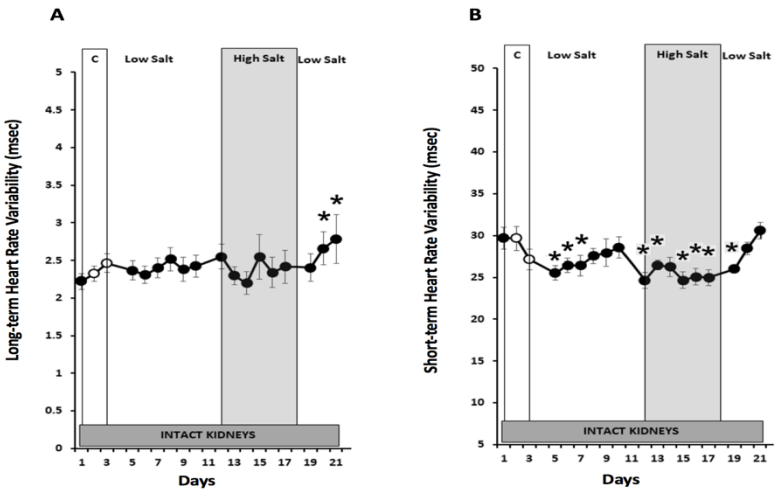

Fig. 2. Long (**Panel A**) and short-term (**Panel B**) beat-to-beat heart rate variability responses to varying salt intake during normal renal function. Values are men ± SEM. *Normal renal function*: * $p < 0.05$ vs. NS Control (days 2-3). Respective baseline days are depicted as (○).

Responses to renal denervation during established salt-sensitive, volume overload hypertension

Compared to the last 2 days of previous period of HS loading, the estimated BRS slightly increased at the end of the 2 weeks after renal denervation (fig. 3). As shown in figure 4, the HF power decreased during the first week after renal denervation, paralleling the alterations of the long- and short-term beat-to-beat variability (fig.5 and fig. 6). These changes induced by renal denervation were only transient, as all variables gradually returned towards pre-denervation levels within a week and were stable subsequently, except estimated baroreflex sensitivity which remained at higher levels than control period.

Responses to central sympathoinhibition during established salt-sensitive, volume overload hypertension

As depicted in figure 3, global sympathoinhibition induced by clonidine administration in doses of 300μg/ kg significantly increased the BRS. Moreover, the HF power significantly increased (fig. 4) and all these changes were accompanied by a marked increase of both long- and short-term beat-to-beat variability (fig. 5 and fig. 6). Then, during the clonidine dose tapering (150 μg/ kg) and the following washout period, all the variables gradually returned to post-denervation values.

Responses to salt restriction after reduced renal mass

No further changes of the BRS occurred along the last week of the experiment when the animals were on LS diet. The HF power slightly increased at the end of the last week (fig. 4), accompanied by the same variation of the short-term heart rate variability (fig. 6).

Figure 3

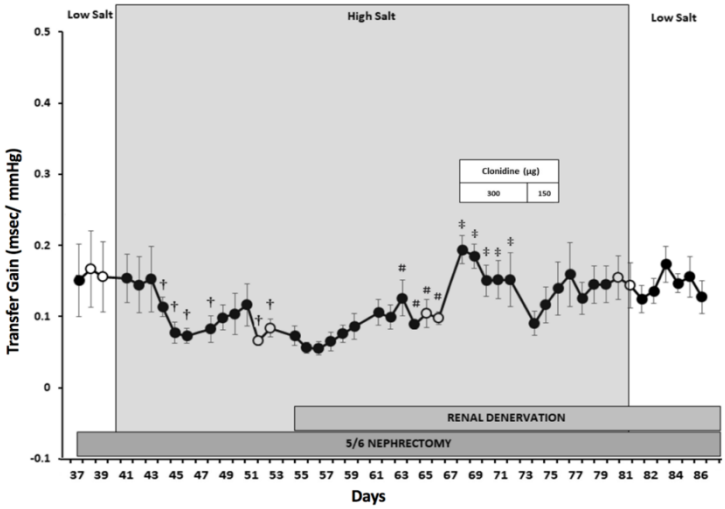

Fig. 3. Baroreflex sensitivity responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); Respective baseline days are depicted as (○).

Figure 4

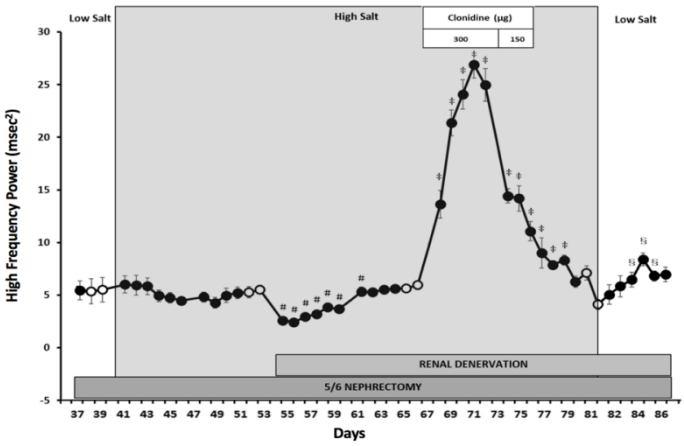

Fig. 4. Responses of the power of the heart rate fluctuations within high frequency range to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); § $p < 0.05$ vs. baseline Clonidine washout (days 79-80). Respective baseline days are depicted as (○).

Figure 5

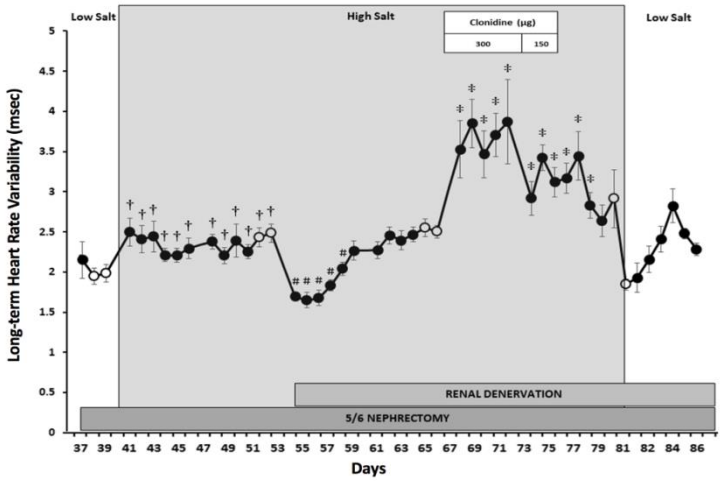

Fig. 5. Long term beat-to-beat heart rate variability responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); Respective baseline days are depicted as (○).

Figure 6

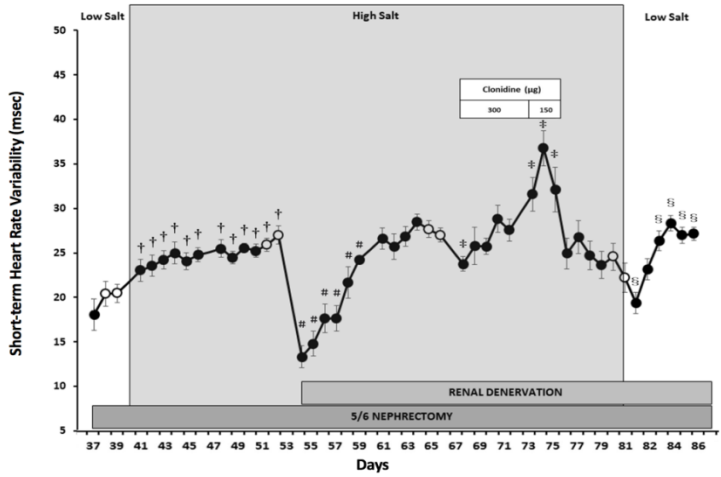

Fig. 6. Short term beat-to-beat heart rate variability responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); § $p < 0.05$ vs. baseline Clonidine washout (days 79-80); Respective baseline days are depicted as (○).

DISCUSSION

A significant reduction of the estimated BRS during the development of salt-sensitive, volume overload hypertension following non-ischemic reduction of the renal mass and HS diet was found in this experimental study. Our results are in line with previously published data from experimental and clinical studies that show a blunted baroreflex sensitivity in both non-ischemic (32) and ischemic renovascular hypertension (33, 34). Although in the ischemic forms of the renovascular hypertension the impairment of the BRS is associated with the activation of the sympathetic nervous system (34, 35), little is known about the mechanisms responsible for the cardiac autonomic nervous system dysregulation in the non-ischemic renovascular arterial hypertension. While BRS decreased during high salt diet and associated progression to salt-sensitive, volume overload hypertension, both long and short-term heart rate variability did not paralleled the baroreflex evolution, possible due to involvement of the low-pressure cardiopulmonary mechanoreceptors in the heart rate control triggered by fluid overload (36). Moreover, we also found that the estimated BRS tended to be decreased during salt loading with normal kidney function although this effect did not reach statistical significance. During this time, both time- and frequency domain measures of heart rate variability were lower, as compared to normal salt intake. These findings are consistent with clinical (37) and experimental studies (38, 39) indicating that the sensitivity of the baroreflex control of heart rate is depressed during high salt diet in normotensives as a consequence to reduced sensitivity of the mechanoreceptors, possibly due to local alterations in the ionic milieu in the carotid sinus region (40-42). Tonic increases in central sympathetic outflow may also be responsible for the reduced variability of heart rate during exposure to high salt levels. Indeed, we have previously shown that during salt loading on normal kidney function, the sympathetic activity estimated from the power of the blood pressure oscillations in the low frequency range was increased (25). Furthermore, in normotensive rats, the magnitude of the sympathetic nervous system activation is directly correlated with the level of dietary sodium intake because of a NaCl-induced sensitization of the central autonomic pathways (43), likely involving neurons within rostral ventrolateral medulla (44-46). Although the activation of the sympathetic nervous system mediated through central mechanisms in response to salt loading could underlie the impairment of the cardiac autonomic modulation during salt-resistant hypertension, other mechanisms are likely responsible for the impairment of the BRS during salt-sensitive hypertension since the global sympathetic activity is actually decreased as we have previously described (25).

Recent experimental and clinical investigations suggest that beyond antihypertensive effects, renal denervation is also effective in reducing the incidence of arrhythmias particularly in cardiovascular diseases associating high levels of sympathetic activation such as chronic heart failure (47-51). In contrast, in this model of salt sensitive hypertension, in the absence of overt sympathoexcitation, we found that renal denervation did not improve baroreflex sensitivity, cardiac parasympathetic tone or short- and long-term heart rate variability. Furthermore, during the first week after renal nerve ablation these parameters decreased significantly and returned to pre-denervation levels only during the second week after denervation. Together with our previous findings indicating the lack of

sustained antihypertensive effects of renal denervation (25), our current data suggest that the failure of renal denervation to lower global sympathetic activity in this model of salt sensitive hypertension and non-ischemic reduction of renal function may also be responsible for its inability to improve the cardiac autonomic regulation.

The above contention is further supported by the responses to central sympathoinhibition with clonidine. In parallel with lowering sympathetic activity (25), Clonidine also significantly improved the sensitivity of the baroreflex system, the parasympathetic tone to the heart and both short and long term beat-to-beat heart rate variability. The improvement of the baroreflex sensitivity reported in our study is in line with the other results showing that by exerting a direct effect on baroreceptors themselves (52) or by stimulating the alpha-2 adrenoceptors from the *vagus nuclei* (53), clonidine is able to increase the cardiac parasympathetic tone and therefore the baroreflex sensitivity and heart rate variability.

CONCLUSIONS

The major findings of this study are: 1) during salt-sensitive, volume overload hypertension developed after non-ischemic reduction of renal mass, the BRS is reduced, 2) in this phenotype of non-sympathetically mediated arterial hypertension RDNx does not improve over the long term the BRS and 3) central sympathoinhibition after clonidine administration following RDNx led to a significant improvement of the BRS, parasympathetic modulation of the heart rate and both short and long term HRV.

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