Renal Sympathetic Denervation Suppresses Postapneic Blood Pressure Rises and Atrial Fibrillation in a Model for Sleep Apnea

Dominik Linz, Felix Mahfoud, Ulrich Schotten, Christian Ukena, Hans-Ruprecht Neuberger, Klaus Wirth, Michael Böhm

Abstract—The aim of this study was to identify the relative impact of adrenergic and cholinergic activity on atrial fibrillation (AF) inducibility and blood pressure (BP) in a model for obstructive sleep apnea. Obstructive sleep apnea is associated with sympathovagal disbalance, AF, and postapneic BP rises. Renal denervation (RDN) reduces renal efferent and possibly also afferent sympathetic activity and BP in resistant hypertension. The effects of RDN compared with β-blockade by atenolol on atrial electrophysiological changes, AF inducibility, and BP during obstructive events and on shortening of atrial effective refractory period (AERP) induced by high-frequency stimulation of ganglionated plexi were investigated in 20 anesthetized pigs. Tracheal occlusion with applied negative tracheal pressure (NTP; at −80 mbar) induced pronounced AERP shortening and increased AF inducibility in all of the pigs. RDN but not atenolol reduced NTP-induced AF-inducibility (20% versus 100% at baseline; \(P=0.0001\)) and attenuated NTP-induced AERP shortening more than atenolol (27±5 versus 43±3 ms after atenolol; \(P=0.0272\)). Administration of atropine after RDN or atenolol completely inhibited NTP-induced AERP shortening. AERP shortening induced by high-frequency stimulation of ganglionated plexi was not influenced by RDN, suggesting that changes in sensitivity of ganglionated plexi do not play a role in the antiarrhythmic effect of RDN. Postapneic BP rise was inhibited by RDN and not modified by atenolol. We showed that vagally mediated NTP-induced AERP shortening is modulated by RDN or atenolol, which emphasizes the importance of autonomic disbalance in obstructive sleep apnea-associated AF. Renal denervation displays antiarrhythmic effects by reducing NTP-induced AERP shortening and inhibits postapneic BP rises associated with obstructive events. (Hypertension. 2012;60:172-178.) ● Online Data Supplement

Key Words: obstructive sleep apnea ■ postapneic blood pressure rise ■ atrial fibrillation ■ renal denervation

Patients with obstructive sleep apnea (OSA) show a high prevalence of atrial fibrillation (AF),1–3 and OSA is considered an etiologic factor in the development of hypertension4 and in the progress of resistant hypertension.5 Severe bradycardia and atrioventricular conduction disturbances together with postapneic blood pressure (BP) rises during the arousal are frequently seen in OSA and suggest sympathovagal activation.6,7 Although enhanced vagal tone is known to induce shortening of the atrial effective refractory period (AERP), increased sympathetic tone may increase spontaneous triggered activity, both of which, when simultaneously occurring, could induce and maintain AF.8 In addition, repetitive postapneic BP surges may lead to atrial structural changes and, thus, an arrhythmogenic substrate for AF. Previously, we showed that negative tracheal pressure (NTP) during obstructive respiratory events leads to pronounced shortening of the AERP, thereby perpetuating AF.9 These electrophysiological changes were mainly mediated by increased vagal tone because they were completely inhibited by atropine or bilateral vagotomy. However, less is known about the relative impact of adrenergic and cholinergic activity on AF inducibility and maintenance in OSA.

We hypothesized that modulation of the sympathetic nervous system might reduce AF susceptibility and postapneic BP rises in OSA. Renal denervation is a new therapeutic approach to reduce sympathetic activity, BP, and apnea-hypopnea index (OSA severity) in resistant hypertension.10–13 However, the effect of RDN on AF inducibility and BP during and after obstructive events is unknown. We tested the effect of denervation of the afferent and efferent renal sympathetic nerves shown previously to reduce renal and whole body sympathetic activity10–12 and compared it with β-receptor blockade by atenolol on atrial electrophysiological changes and postapneic BP rises in a pig model for OSA. To check whether RDN displays its
antiarrhythmic effects by a modulation of sensitivity of the intrinsic cardiac nervous system, we investigated the influence of RDN on the sensitivity of right atria to stimulation of autonomic ganglionated plexi (GP).

Methods
All of the animal studies were performed in accordance with the guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996).

Experimental Model for OSA
In 15 chest-closed male castrated pigs (25–30 kg) of the German Landrace (anesthetized with 20% urethane, 0.8 mL/kg IV load, 0.4 mL/kg per hour of maintenance and 4% α-chloralose, 0.4 mL/kg IV load, 0.1 mL/kg per hour of maintenance), a tracheotomy was performed to place an endotracheal tube. This tube was used for tracheal occlusion and to apply different levels of NTPs by a negative pressure device.9,14 The Mueller maneuver (forced inspiration against airway obstruction after deep expiration generating a negative pressure of ~40 mm Hg) is used in the clinical setting to simulate conditions, particularly negative thoracic pressure, during OSA.6,7,15,16 As a modification of this maneuver, we applied NTP at −80 mbar during tracheal occlusion corresponding with NTPs found in patients with OSA.6,7,15,16

BP was measured by a TIP-catheter (Millar PC 350; Millar Instruments, Houston, TX) in the femoral artery. Bipolar body surface ECG was recorded using subcutaneous needle electrodes in the classic lead II arrangement. The ECGs were analyzed concerning heart rate and conduction times according to the American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society Recommendations for the Standardization and Interpretation of the ECG before and after RDN or atenolol. The QT interval was corrected using the Bazett formula (QTc = QT/√RR interval). Blood gas analyses (P02, P02, pH, and O2 saturation) were performed directly before and at 2 minutes of each tracheal occlusion with applied NTP. In 7 animals, both kidneys were approached through bilateral retroperitoneal flank incisions. Both kidneys were surgically denervated by cutting all of the visible nerves in the area of the renal hilus and by stripping ~1 cm of the adventitia from the renal artery. The area was then moistened with a 20% phenol/ethanol solution for 10 to 15 minutes. Left renal flow was measured with a Doppler flow probe (transit time flowmeter module system from Transonic Systems, Inc) positioned on the blood vessels. After RDN, the animals were allowed to re-equilibrate for 1.5 hours. Significant reduction of the reproducible postanesthetic BP-rise and the absence (<=5% change) of a decrease in renal blood flow induced by tracheal occlusion with applied NTP were taken as evidence of the completeness of RDN. Sham surgical procedure with kidney exposition without renal denervation was performed in 3 additional pigs serving as a time control. Results for vehicle time controls for atenolol and atropine have been published previously.9,14 Vehicle application did not change NTP-induced electrophysiological parameters or hemodynamics within 4 hours.

Five additional pigs (35–41 kg) were anesthetized with pentobarbital,17 and a right thoracotomy at the fourth intercostal space provided access to the right lung and trachea through a pericardiotomy. At the caudal end of the sinus node between the right superior and inferior pulmonary vein, a plaque electrode containing 2 electrodes was sutured on the fat pad (anterior right GP). For electric GP stimulation, the following stimulation parameters were used: frequency, 20 Hz; stimulus duration, 0.1 ms; and voltage, 0.1 to 5.0 volts. GP stimulation, which slowed heart rate by ~50% at baseline, was applied before and 1.5 hours after bilateral RDN.

Electrophysiological Examinations
During normal breathing, AERP was measured at a basic cycle length of 400, 300, and 240 ms before and after RDN or atenolol. In addition, AERP measurements at a representative basic cycle length of 300 ms and AF inducibility were performed before, during, and after tracheal occlusion. An extended description of electrophysiological examinations is available as an online-only Data Supplement.

Drugs
Atenolol (3 mg/kg) and atropine were dissolved in 0.5 mL of dimethyl sulfoxide and 2.5 mL of polyethylene glycol 400. All of the solutions were injected over 5 minutes. Vehicle as a control was injected 15 minutes before the second baseline NTP run in all of the animals of group 1. Atenolol was used in a concentration that resulted in a maximal heart rate reduction and AERP-prolonging effect in previous studies.9,14

Experimental Design
The electrophysiological study protocol and the experimental design are depicted in Figure 1. AERP measurements were performed and AF inducibility was investigated during normal breathing and at every 30 seconds during 2 minutes of tracheal occlusion with applied NTP at −80 mbar. Recovery of the AERP was shown by AERP measurement at 15 minutes between the different tracheal occlusion maneuvers. After the first run, both kidneys were approached through bilateral retroperitoneal flank incisions in group 1, and vehicle was given to all of the animals of group 2. Bilateral RDN was performed in group 1, and atenolol was given to all of the animals of group 2. Two additional runs of the electrophysiological study protocol were performed in exactly the same manner 15 minutes and 1.5 hours after RDN and 15 minutes after atenolol. In group 3, AERP shortening induced by high-frequency stimulation of GPs was investigated at baseline and 1.5 hours after RDN. Sham surgical procedure was performed in 3 additional pigs serving as a time control.

Statistics
Data are presented as mean±SEM. For comparisons of single repeated measures only, a paired Student t test was used. For multiple repeated-measures comparisons with the same baseline, repeated-measures 2-way ANOVA was used, followed by the Dunnet test to compare individual mean differences if ANOVA was significant. A P value <0.05 was considered significant. For all of the statistical calculations software, Eversat V5 based on SAS 8 was used.

Results
Electrophysiological Effects of RDN and Atenolol During Normal Breathing
Figure 2 shows the effects of bilateral RDN (Figure 2A) and atenolol (Figure 2B) on AERP during normal breathing. Atenolol increased the AERP by 27.2±3.2 ms at 300-ms basic cycle length (P=0.0001). RDN did not influence AERP significantly during normal breathing. During normal breathing, atrioventricular conduction, estimated as PQ interval in ECG, and heart rate were reduced by RDN and atenolol. P-wave duration, QRS duration, and cQT time were not modified by RDN or atenolol (Figure S1, please see the online-only Data Supplement).

Effect of RDN and Atenolol on NTP-Induced AERP Shortening
Tracheal occlusion without applied NTP did not change the AERP, neither before nor after RDN or atenolol. In contrast, the application of NTP during tracheal occlusion induced a pronounced AERP shortening (from 167.8±7.9 to 105.2±10.6 ms; P=0.0001). In 3 control animals, repetitive full electrophysiological study protocols, including NTP maneuvers and sham operation, were conducted over the time frame similar to those of the treated animals. NTP-induced changes in electrophysiology and recovery at each repetition over time were constant. In Figure 3, the effect of RDN and atenolol followed by atropine on NTP-induced AERP shortening during tracheal occlusion

Linz et al Renal Denervation and Atrial Fibrillation 173

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is shown. RDN reduced NTP-induced AERP shortening (AERP shortening, 27±5 ms after RDN versus 43±3 ms after atenolol; \(p=0.0272\); Figure 4A) and NTP-induced multiple action potential (MAP) shortening at 70% of repolarization (MAP shortening, 22±2.1 ms after RDN versus 47±7.2 ms after atenolol; \(p=0.0024\); Figure 4B) more than atenolol. However, atropine applied after the administration of atenolol or RDN completely inhibited NTP-induced AERP shortening and MAP shortening.

**Effect of RDN on Sensitivity of Right Atria to Stimulation of Autonomic GP**

RDN did not influence AERP shortening induced by high-frequency stimulation of ganglionated plexi (GP).

**Effect of RDN and Atenolol on NTP-Induced AF Inducibility**

AF was not inducible by a single premature stimulus during the AERP measurement procedure during normal breathing. NTP-induced AERP shortening and MAP shortening were associated with increased inducibility of AF by a single premature stimulus during the AERP measurement from 0% during normal breathing to 100% during tracheal occlusion with applied NTP (\(p=0.0001\)).

In Figure 4C, the effects of RDN and atenolol on NTP-induced AF inducibility are shown. RDN inhibited NTP-induced AF inducibility. Atenolol did not reduce NTP-induced AF inducibility significantly, but inducible AF duration was shortened (27 versus 122 seconds at baseline; \(p=0.0037\)). After administration of atropine after RDN or atenolol, no episode of AF was inducible.

**Effect of RDN on Sensitivity of Right Atria to Stimulation of Autonomic GP**

RDN did not influence AERP shortening induced by GP stimulation as a result of programmed electric stimulation (AERP shortening, 51±4 versus 56±5 ms after RDN; \(p=0.2915\)).
Effect of RDN and Atenolol on Postapneic BP Rises and Blood Gases

Postapneic changes in BP after bilateral RDN or atenolol are shown in Figure 5. RDN abolished postapneic BP surge. Suppression of the postapneic BP rise was not observed after atenolol. NTP-induced changes in blood gases were not modified by RDN or atenolol (Table S1).

Discussion

In this study we showed that postapneic BP rises were abolished by RDN but not by atenolol. Arrhythmogenic electrophysiological changes during tracheal occlusion with applied NTP, mainly characterized by a pronounced shortening of AERP, can be modified by RDN and atenolol. However, AF inducibility was inhibited by RDN only and not

Figure 3. Changes in atrial effective refractory period during tracheal occlusion. Changes in atrial effective refractory period (AERP; dotted line, left ordinate) during tracheal occlusion with applied negative tracheal pressure (NTP) at −80 mbar for 120 seconds (continuous line, right ordinate) before (A) and after renal denervation (RDN) or atenolol (B) followed by atropine (C). Representative time curves of a pig of group 1 (RDN followed by atropine, □) and a pig of group 2 (atenolol followed by atropine, ●) are shown.

Figure 4. Electrophysiological changes during tracheal occlusion. A, Effect of renal denervation (RDN) and atenolol followed by atropine on changes in atrial effective refractory period (AERP) induced by tracheal occlusion with applied negative tracheal pressure (NTP) at −80 mbar (ordinate). B, Representative atrial multiple action potential (MAP) recordings during AERP measurements before and after RDN or atenolol. C, Percentage of tracheal occlusions with inducible atrial fibrillation (AF; ordinate) and effect of RDN and atenolol followed by atropine.
Several observations suggest that the autonomic nervous system plays an important role in both the initiation and the maintenance of AF in humans. Vagal nerve stimulation and acetylcholine infusion shorten the AERP. By contrast, activation of sympathetic activity showed variable effects on AERP. Interestingly, studies in lone AF patients and in animal models of both intermittent rapid atrial pacing and congestive heart failure have indicated that AF onset is associated with simultaneous sympathovagal activation rather than with an increase in vagal or sympathetic drive alone.

Previously we have shown increased AF susceptibility and AF duration caused by pronounced AERP shortening in a pig model for OSA. NTP during breathing attempts against the obstructed upper airways was identified to be a trigger for AERP shortening. AERP shortening was mediated by pronounced vagal activation because it was completely inhibited by atropine and bilateral vagotomy. However, increased negative thoracic pressure, hypoxia, and hypercapnia during obstructive apnea are associated with severe bradycardia and atrioventricular block together with postapneic BP rises, suggesting simultaneous sympathovagal activation. Direct recordings of muscle sympathetic nerve activity showed increased sympathetic activation during apneic episodes in OSA patients or in apnea divers. Sympathetic activity may increase profoundly during the application of NTP, resulting in the pronounced postapneic BP rise.

The sympathetic nervous system can be modulated by blockade of β-receptors and RDN in humans. NTP-induced AERP and MAP shortening were significantly reduced by RDN and atenolol. This indicates that sympathetic modulation affects NTP-associated electrophysiological changes. Atropine after RDN or atenolol completely inhibited NTP-induced AERP and MAP shortening, showing that vagal activation beyond sympathetic activation is one significant determinant of NTP-induced shortening of atrial refractoriness.

The suppression of vagally mediated AERP and MAP shortening during obstructive events by RDN or atenolol indicates an interaction between the acetylcholine-induced AERP shortening and the reduced activation of the sympathetic nervous system. Consistently, Sharifov et al showed that acetylcholine-mediated AF was facilitated by isoproterenol, which decreased the threshold of acetylcholine concentration for AF induction and increased AF duration. Patterson et al showed that the synergistic action of both the sympathetic and the parasympathetic neurotransmitters is required to initiate rapid ectopic discharges in superfused pulmonary arteries.

Figure 5. Postapneic blood pressure rise. Original registration of postapneic blood pressure changes in a pig before (control) and 1.5 hours after renal denervation (RDN; A). Postapneic blood pressure changes (ordinate) before (control; B) and 1.5 hours after RDN (C) and 15 minutes after atenolol (D).
vein preparations. Indeed, we observed that AF was induced by spontaneous atrial premature beats during tracheal occlusion in some animals, which may represent the occurrence of ectopic discharges triggering AF. However, this spontaneous induction of AF was too rare for a systematic evaluation.

RDN but not atenolol reduced NTP-induced AF inducibility. Catheter-based denervation of the kidneys by ablation of the efferent sympathetic and afferent somatic fibers has been shown to be effective in reducing both renal and whole-body norepinephrine spillover and muscle sympathetic nerve activity. Altering the signals from the kidney to the hypothalamus is expected to impact downstream organ systems, including the vasculature and the heart. Catheter-based RDN in patients with resistant hypertension effectively reduced BP and improved insulin resistance and sleep apnea severity. Interestingly, NTP-induced postapneic BP surges were attenuated by RDN, suggesting a sympatho-inhibitory effect. However, similar effects have not been reported for β-blocker treatment, clearly indicating a different mechanism of action. Possibly, RDN might additionally modify α-adrenoceptor pathways, which are not influenced by atenolol. Interestingly, activation of the intrinsic cardiac nervous system results in atrial arrhythmias that involve intrinsic cardiac neuronal α-adrenoceptors.

RDN resulted in a more pronounced inhibition of NTP-induced AERP shortening compared with atenolol, which might explain the superior antiarrhythmic effect of RDN compared with atenolol. Changes in blood gases during tracheal occlusion or P-wave duration as a marker of atrial conduction were neither modified by RDN nor by atenolol, suggesting an antiarrhythmic effect independent of changes in blood gases or atrial conduction. RDN and atenolol prolonged PQ interval and reduced heart rate, which may represent the modulation of sympathetic activity. First supportive data for the potential antiarrhythmic effect of acute RDN has been published recently by Ukena et al. They reported reduced ventricular tachyarrhythmias in patients with electrical storm and congestive heart failure after catheter-based RDN. However, data from clinical studies powered to detect these effects are lacking.

Importantly, long-term OSA has been shown to be associated with significant atrial remodeling characterized by atrial enlargement, reduction in voltage, site-specific and widespread conduction abnormalities, and longer sinus node recovery in humans. Apart from the described antiarrhythmic electrophysiological effects of RDN, RDN might be able to attenuate the development of an atrial proarrhythmic substrate by inhibiting postapneic BP surges, as shown in this study. In addition, RDN has been proven to reduce OSA severity in OSA patients.

We tested, whether parts of the antiarrhythmic effects of RDN can be attributed to a modulation of the sensitivity of the intracardiac autonomic nervous system containing GPs. GPs may modulate the interactions between the extrinsic and intrinsic cardiac autonomic nervous system and contain efferent cholinergic and adrenergic neurons influencing the atrial myocardium. GP ablation combined with a pulmonary vein ablation procedure has been shown to further improve success rates of reversion in paroxysmal AF patients. In a dog model for central sleep apnea, GP ablation inhibited AF inducibility. In this study, AERP shortening induced by high-frequency stimulation of GPs was not modified by RDN. This suggests that changes in sensitivity of GPs do not play a role in the antiarrhythmic effect of RDN.

**Limitations**

Spontaneous induction of AF was too rare for a systematic evaluation. Therefore, we applied a premature beat during tracheal occlusion with applied NTP to induce AF. AERP measurement in the left atrium and the investigation of the spatial distribution of refractoriness and of inhomogeneity in conduction and conduction velocity in the atrium would have required thoracotomy. In pigs with thoracotomy, application of NTP would not be possible. In addition, catheter-based renal nerve ablation in humans is possibly not complete, as indicated by a norepinephrine spillover reduction by only ~50%. We performed complete surgical renal nerve ablation in this study. The precise measurements of sympathetic activity and GP activation during and after obstructive events deserve further studies.

**Conclusions and Perspectives**

RDN reduced postapneic BP rises, which may attenuate OSA-associated structural atrial arrhythmogenic remodeling. In addition, vagally mediated NTP-induced AERP and MAP shortening is modulated by RDN or atenolol, which emphasizes the importance of autonomic disbalance in OSA-associated AF. RDN but not atenolol reduced AF inducibility. RDN displays antiarrhythmic effects independent of a modulation of sensitivity of intracardiac GP, suggesting that GPs do not play a relevant role in the antiarrhythmic effect of RDN. Modulation of the autonomic nervous system by RDN might be useful to reduce atrial arrhythmogenesis and deserves to be tested in clinical studies and in AF models for structural and/or electric atrial remodeling.

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**Disclosures**

None.

**References**

178 Hypertension July 2012

What Is New?

- RDN displays atrial antiarrhythmic effects by modulating vagally mediated atrial electrophysiological changes and reduces postapneic BP rises in OSA, thereby attenuating the development of an atrial arrhythmogenic substrate.

What Is Relevant?

- OSA causes combined sympathovagal activation potentially leading to hypertension and increased susceptibility to AF.
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Short title: Renal Denervation and Atrial Fibrillation

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Methods

Electrophysiological examinations
During normal breathing, atrial effective refractory period (AERP) was measured at a basic cycle length (BCL) of 400, 300 and 240 ms before and after RDN as described previously. Additionally, AERP measurements at a representative basic cycle length (BCL) of 300 ms were and AF-inducibility were performed before, during, and after tracheal occlusion. Atrial responses to the pacing procedure were visualized by monophasic action potential (MAP) recordings from the endocardium of the right atrium by a MAP pacing catheter (combined MAP- & stimulation catheter, 7F, Foehr Medical Instruments GMBH, Seeheim, Germany). The catheter was inserted via a femoral vein. The tip of the catheter was advanced to the lateral right atrium to record a stable and sharp MAP-signal. Determination of the diastolic pacing threshold (0.5-1 mV) before each tracheal occlusion maneuver revealed no significant changes during the experimental period. A train of 5 basic stimuli (S1, pulse duration 1 ms) at twice diastolic pacing threshold was followed by an extra-stimulus (S2) starting about 30 ms below the expected AERP with a 5-ms increment (UHS 20, universal heart stimulator; Biotronik, Berlin, Germany). The shortest coupling interval able to elicit a propagated atrial response minus 3 ms was taken as the AERP. For analysis of right atrial MAP-signals, position of the electrode was the same as used for AERP measurements (see above). A particular effort had to be made to obtain atrial MAPs of sufficient quality. The catheter was left at one location, confirmed by a regular and stable baseline and amplitude of the MAPs during NTP-procedures. Right atrial MAP duration was evaluated from 70% repolarization during regular pacing (BCL=300ms). During the AERP-measurement procedure the shortest premature S2-extrastimulus resulting in a propagated response frequently induced episodes of AF. When atrial MAP-signals showed an irregular rapid activation (cycle length <200 ms, duration >5 seconds), AF was diagnosed. NTP-maneuver after RDN or atenolol was repeated twice in all animals. The mean percentage of NTP-maneuvers triggering AF was used to describe AF-inducibility. Inducible AF-duration was determined.

References:

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**Table S1:** Effect of RDN and atenolol on NTP-induced changes in blood gases.
Fig. S1: ECG-intervals at baseline and 15 min after bolus administration of atenolol and 1.5 hours after RDN during normal breathing. Black bars: baseline, grey bars: RDN, white bars: atenolol.