Antihypertensive Effect of 0.1-Hz Blood Pressure Oscillations to the Kidney

Benno Nafz, MD; Jens Stegemann; Morton H. Bestle, MD; Nadja Richter, PhD; Erdmann Seeliger, MD; Ingrid Schimke, PhD; H. Wolfgang Reinhardt, MD; Pontus B. Persson, MD

Background—Physiological blood pressure (BP) fluctuations with frequencies >0.1 Hz can override renal blood flow autoregulation. The influence of such immediate changes in renal perfusion pressure (RPP) on daily BP regulation, eg, via shear stress–stimulated liberation of renal endothelial NO, however, is unknown. Thus, we studied the effects of such RPP oscillations on renal function and on systemic BP during the onset of renal hypertension.

Methods and Results—Seven beagles (randomly assigned to each of the following protocols) were chronically instrumented for the measurement of systemic BP, RPP, and renal excretory function. An inflatable cuff was used to reduce and to oscillate RPP over 24 hours in the freely moving dog. Reducing RPP to 87±2 mm Hg diminished excretion of sodium and water and doubled plasma renin activity (PRA, n=7, P<0.01) but had no significant effect on urinary nitrate excretion (n=6), a marker of NO generation. Superimposing 0.1-Hz oscillations (±10 mm Hg) onto the reduced RPP blunted hypertension, returned fluid excretion almost to control levels, and doubled renal sodium elimination. Nitrate excretion peaked at 8 hours, only to return to control values shortly thereafter. PRA, conversely, was significantly reduced during the last third of the experimental protocols.

Conclusions—BP fluctuations transiently stimulate NO liberation and induce a reduction in PRA, which enhances 24-hour sodium and water excretion and markedly attenuates the acute development of renovascular hypertension. (Circulation. 2000;101:553-557.)

Key Words: hemodynamics ■ hypertension ■ kidney ■ Fourier analysis ■ renin

The average level of arterial blood pressure (BP) is a major determinant of future cardiovascular complications in hypertension.1 In addition, recent investigations show that the dynamic properties of BP are also of significance for the development of hypertension-related end-organ damage in patients.2–5 Thus, changes in the dynamic properties of BP, which usually coincide with hypertension,4,6 seem to establish an independent risk factor for cardiovascular complications.

Little is known regarding the influence of such short-term changes in BP on kidney function, a crucial control element for long-term BP regulation.7,8 Conversely, the inability of the renal blood flow (RBF) autoregulation to effectively buffer fast BP fluctuations is well recognized.9,10 It seems likely, therefore, that spontaneous BP oscillations (BPOs), which are not effectively buffered by RBF autoregulation, enhance intrarenal shear stress at the level of the vascular wall. Shear stress, in turn, is known to enhance the liberation of vasoactive substances, eg, endothelium-derived NO, along the renal vascular tree in vitro. Hence, one may expect a profound impact of BPOs on medullary blood flow, on renal excretory function,11,12 and on longer-term BP regulation.13,14

There have been several efforts to determine which systems contribute to modulations in BP levels and BPOs.6,15,16 Nonetheless, the effects of BPOs on regulation of systemic BP remain unknown. We therefore investigated the impact of induced BPOs on the onset of renovascular hypertension, especially with regard to excretory function of the kidney, to urinary nitrate excretion (UNO3), and to renal renin release in the conscious, freely moving dog.

Methods

The experiments were carried out on 7 adult, chronically instrumented, purebred female beagles. The general status of the body weight–matched dogs (13.2±0.2 kg) was controlled daily (including body temperature and erythrocyte sedimentation rate). Infections were prevented by a catheter-restricted antibiotic-lock technique.17 Daily intake was controlled with respect to food composition and feeding time. At least 5 days before the first experiment, each animal was fed an individually prepared diet containing 91 mL water, 5.5 mmol Na+, and 3.5 mmol K+ per kg body wt. The last feeding was offered for 30 minutes at 10 PM, ie, 10 hours before an experiment was started the next morning. Any leftovers were fed via a gastric tube, if necessary.

During the experiments, the animals moved freely and undisturbed in their 9-m2 kennels. Meanwhile, data recording, control of renal
perfusion pressure (RPP), continuous urine sampling, and blood sampling were done via a swivel system from an adjacent room (see Reference 18 for details). For reasons of social well-being, at least 1 more dog (placed in a neighboring kennel) accompanied the dog under investigation in the air-conditioned, sound-protected animal room. A 12-hour dark/light cycle with electronically induced ½-hour dawn breaking and dusk falling was used to further standardize environmental conditions.

All experiments and the care of the dogs were supervised by an ethical committee and were approved of and performed in accordance with the German Animal Protection Law.

Surgery
All surgery was performed under aseptic conditions. General anesthesia was introduced by 8 mg/kg body wt methohexital IV and maintained under controlled ventilation with ~1% halothane in combination with a 2:1 mixture of N₂O:O₂. Then, 2 catheters were advanced via the femoral arteries into the abdominal aorta. This was done in such a way that the tips were placed distally and proximally to both renal arteries, respectively. An inflatable cuff was positioned around the aorta, above the origin of the renal arteries and between the tips of the catheters. Finally, a bladder catheter was implanted to allow continuous urine collection. All catheters and the cuff lead were tunnelled subcutaneously to the dog’s neck, where they were exteriorized. A recovery period of ≥3 weeks was allowed between the surgery and the first experiment.

Protocols and Measurements
BP was measured in the abdominal aorta, above and below the inflatable cuff, by means of Honeywell micro pressure transducers (type 136p05051) and pressure processors (Plugsys bus system, Hugo Sachs Electronic). Heart rate was derived from the BP signal by a rate meter (Gould pressure processor). The cuff and the distal catheter were connected to an electropneumatic pressure control system, a modified version of a previous device,19 which allowed us to reduce and to oscillate RPP around a preset level with high precision. After analog-to-digital conversion (DASH1602, Keithley Instruments), BP and HR were stored on line on an IBM-compatible PC (sampling rate 20 Hz).

The bladder catheter was used to collect urine continuously into a fraction sampler (1 tube every 20 minutes). Urine flow (UF) was determined by weighing. Blood samples were taken with the proximal arterial catheter into precooled tubes every 4 hours (ie, at 9 AM, 1 PM, 5 PM, 9 PM, 1 AM, and 5 AM) and placed on ice. After centrifugation at 4°C, plasma was separated and stored at −20°C.

All dogs were randomly assigned to the following protocols: protocol 1, Con: A 24-hour time control without external modulations in RPP to determine basal values of BP, HR, UF, sodium excretion (UNa V), potassium excretion (UK V), UNaO, and plasma renin activity (PRA). Protocol 2, P85: During this protocol, BP was elevated by reduction of mean RPP to 85 mm Hg over the 24-hour recording period. All other measurements were performed as during protocol 1. Protocol 3, Osc: To determine the influence of BPOs on renovascular hypertension, RPP was sinusoidally oscillated over 24 hours with a frequency of 0.1 Hz (amplitude, 10 mm Hg) around the same mean value as used during protocol 2.

Analyses
Sodium and potassium concentrations were determined by flame photometry (AFM 5052, Eppendorf). A ¹²⁵I-labeled radioimmunoassay (Du Pont New England Nuclear) was used to measure PRA. To determine nitrate, [¹⁵N]KNO₃ (Sigma) was used as internal standard. The nitrate of the samples was converted to nitrobenzene by shaking for 30 minutes with a precooled (−80°C) mixture of trifluoroacetate anhydrous sulfuric acid (120 µL) and benzene (500 µL, both from Sigma). After separation and neutralization of the benzene layer, 1 µL of the organic phase was injected into a temperature program–controlled gas chromatograph (Varian 3400) equipped with an XTI-5 capillary column (Resteck Corp.). Subsequent selective monitoring with a Varian Saturn mass spectrometer operating in the positive ion/chemical ionization mode (with methane as reactant gas) allowed the estimation of the ratio between endogenous nitrate (m/z 124) and the ¹⁵N-labeled internal standard (m/z 125). Although this method is more costly than the commonly used Griess-Ilosvay reaction, it is very specific, thereby avoiding interference with many other compounds that contain NOₓ.20

Statistics
Mean values of the hemodynamic data were obtained by beat-to-beat integration; all other data were averaged by calculation of arithmetic means. The Student-Newman-Keuls test was used for statistical comparisons. A probability level of <0.05 was taken to indicate significance. All data are presented as mean±SEM.

Results
The 24-hour mean values of HR ranged from 81±8 bpm (P85) to 91±7 bpm (Con) without any significant difference between the protocols (Osc: 86±2 bpm). Figure 1 shows original recordings of RPP (left) and 1-minute mean values of BP (right) of a single dog. During control, RPP and mean BP showed a remarkable variability within seconds (A) and hours (D), thereby ranging between 80 and 140 mm Hg. A reduction in RPP did not exert a major influence on rapid fluctuations, eg, the physiological pulsations in RPP, whereas slower changes in RPP were effectively suppressed in our experiments (B). This intervention elevated BP to 170 mm Hg at the end of the 24-hour recording period (E). The influence of BP fluctuations was assessed by superimposing 0.1-Hz oscillations onto the reduced RPP (C). As depicted in F, the pressure oscillations markedly attenuated the hypertensive effect of P85. Because the time course of BP (D through F) revealed a wide variability and major differences between the protocols, 24-hour mean values were used to
for overall comparisons. Thus, 24-hour BP was elevated from 113±3 mm Hg (Con) to 142±5 mm Hg during P85 (P<0.01 versus Con, RPP=87±2 mm Hg, n=7). Superimposing RPP oscillations (24-hour mean: 85±2 mm Hg, n=7) significantly attenuated this increase in BP. Thus, 24-hour BP increased only by 8 mm Hg, to 121±7 mm Hg (P<0.01 versus P85, P=NS versus Con).

The respective time courses of UF (same dog as in Figure 1) are shown in Figure 2 (left), together with group means of 24-hour electrolyte excretion (right). As indicated, UF varied spontaneously by a factor of ∼4 during Con (A) and Osc (C) but declined after some initial fluctuations to low levels during P85 (B). In contrast to these short-term changes in UF, which differed remarkably between the dogs, the 24-hour mean fluid excretion was stably reduced by P85 to nearly 50% of control (288±33 versus 557±49 mL/24 h, h<0.01, n=7). This effect was nearly abolished during Osc. Thus, UF increased to 484±48 mL/24 h (h<0.01 versus P85, P=NS versus Con, P=NS versus Con). The changes in UF among the 3 protocols were paralleled by modulations in UrNa and UrK: P85 induced a fall in UrNa (open bars) to ∼20% of control (h<0.01, n=7, D and E), Osc (F) doubled UrNa in comparison to P85 (7.9±1.1 versus 3.3±0.8 mmol/24 h, h<0.05, n=7) but failed to reestablish normal sodium excretion (P<0.01 versus Con: 17.3±2.1 mmol/24 h). Renal K+ handling was not so strongly affected by P85 (Con, 22.7±1.4 mmol/24 h; P85, 15.1±2.4 mmol/24 h; P<0.05, n=7) and returned to normal during Osc (23.4±2.6 mmol/24 h, P=NS versus control, P<0.05 versus P85, n=7).

In contrast to BP and fluid and electrolyte excretion, the 8-hour mean values of UrNO3 (Figure 3, A through C) showed a marked transient increase during Osc (shaded bars). Thus, after a maximum at the first 8 hours of the recording (A), NO declined quickly to control levels (B and C), indicating that NO liberation is not directly responsible for the prolonged changes in BP and renal fluid and electrolyte excretion. No significant differences were observed between Con and P85 (Figure 3, open bars versus solid bars). Osc reduced 24-hour mean values of PRA by 30% in comparison with P85 (5.0±0.6 versus 7.5±0.9 ng angiotensin I · mL⁻¹ · min⁻¹, h<0.05, P=NS versus Con, n=7), suggesting an important role of the renin system in the observed antihypertensive effect. Interestingly, however, the differences in PRA among the protocols increased slowly (D through F). Thus, no significant increase in PRA was detected within the first 8 hours of the experiments (D), and the difference between P85 and Osc gained significance only during the last 8 hours of the recordings (F). No direct coupling between the time course of NO, or PRA and electrolyte excretion, UF, or BP was observed.

### Discussion

A vast number of studies over recent years have focused on the description of rapid BP changes and the possibility of using spectral analysis of BP as a tool for quantifying sympathetic and vagal tone.21,22 The importance of BPOs as an individual parameter in BP control, however, remains to be established. The major finding of the present study is that oscillations in RPP alleviate the onset of renal hypertension in the freely moving dog. This antihypertensive effect is probably mediated by the combined action of the following mechanisms: a marked increase in renal fluid and electrolyte excretion, as perhaps achieved by the observed transient
increase in NO liberation, and the prevailing attenuation of pressure-dependent renin release.

Hypertension is a major cardiovascular risk factor, thus being in part responsible for the principal cause of death in the industrialized nations.23 It was recognized early, however, that hypertension is commonly accompanied by changes in the dynamic properties of BP.24 Indeed, it seems likely that BPO itself may be intertwined with the regulation of BP. For instance, surgical or pharmacological interruption of the baroreceptor reflex, or the NO system, elevates 24-hour BP and also enhances short-term BPOs in the conscious animal.14,25,26 Conversely, a possible interaction of BPOs with longer-term BP regulation is suggested by the results of 24-hour ambulatory BP recordings, which have shown that enhanced SD of BP coincides with renal end-organ damage in hypertensive subjects.4

Thus, it is conceivable that the kidney does not merely constitute an important control element in the BP regulation network but may also be a target organ for BPOs. The established concepts of BP regulation offer several entry points by which BPOs may interact with longer-term BP.

According to the renal/body-fluid–pressure-control concept, fluid and electrolyte excretion is crucial for long-term BP regulation (see Reference 8 for review). In line with this hypothesis, several investigators reported that an impaired capability of the kidney to form adequate amounts of urine induces hypertension.7,8,27 Conversely, BP modulates renal fluid and sodium handling, which has been reported to be closely dependent on renal medullary hemodynamics.11 Hence, a BP-dependent increase in medullary blood flow may induce a more or less pronounced washout of the osmotic gradient within the renal medulla and thereby attenuates the ability of the kidney to form concentrated urine.11,12 Therefore, it seems likely that BP-induced changes in renal hemodynamics can modulate longer-term BP via a change in renal fluid and sodium excretion. In our experiments, we used sinusoidal 0.1-Hz RPP oscillations in the conscious dog to investigate this pathway. Such BPOs are not effectively buffered by the autoregulation of RBF and may therefore impinge on renal excretory function. Indeed, the induced BPOs led to an increase in fluid and electrolyte excretion (Figure 2).

It is widely accepted that BPOs can also modulate shear stress at the vascular wall. Endothelial shear stress stimulates NO release from arteries, thereby inducing vasodilation and changes in intrarenal hemodynamics.28,29 Hence, it is not surprising that recent studies suggest a close coupling between renal NO liberation and hypertension.14 Unfortunately, the direct assessment of renal NO liberation is still impossible in the freely moving animal. Thus, we determined U\textsubscript{NO3} as a measure for NO. To obtain the highest possible accuracy, we performed all experiments during the postabsorptive state when influences of food composition on U\textsubscript{NO3} are minimized. In addition, a very specific mass spectrometric analysis, instead of the Griess-Ilosvay reaction, was used. This avoids possible interferences with most physiological NO\textsubscript{x} compounds.20 Nonetheless, the stimulation in NO liberation was detectable only during the first 8 hours of the experiments. Thus, the prolonged antihypertensive effect of BPOs during renovascular hypertension (Figure 1) cannot be explained by a direct effect of the enhanced NO release.

The activity of the renin-angiotensin system was lower during Osc than during P85 (Figure 3). In light of the well-established stimulus-response curve of pressure-dependent renin release, an attenuated renin release is a surprise. Under short-term steady-state conditions (stepwise pressure reduction), a pronounced influence of RPP on renin release has been detected only below \(\approx 90\) mm Hg.30 Thus, the minima of the RPP oscillations should lead to a pressure-dependent stimulation in renin release, whereas maxima in RPP should not reduce renin release to the same degree. Therefore, if one assumes that possible hysteresis effects can be neglected, one would expect higher PRA during Osc than during P85. In addition, the 24-hour mean value of RPP was slightly but not significantly lower during Osc than during P85 (85±2 versus 87±2 mm Hg), which may also have contributed to an elevated PRA. Thus, the influence of BPOs on a reduction in renin release is probably underestimated in our experiments. Obviously, the BPOs used exert a major influence on renin release in the freely moving dog. The renin-angiotensin system interacts with BP regulation via the direct vasoconstrictory effects of angiotensin II and via the influence on renal fluid and sodium handling. This suggests that the sustained antihypertensive effect of Osc on renovascular hypertension is mediated mainly by lowered PRA. In contrast to the prolonged effect on PRA, the stimulation of NO generation seems to be important only during the first 8 hours of our experiments.

It must be kept in mind, however, that further influences of BPOs on the intrarenal microcirculation may participate in the observed antihypertensive effect: BPO-induced changes in local blood flow probably modulate physical forces along the nephron. Likewise, it is plausible that BPOs interfere with the local release of many vasoactive substances (eg, prostaglandins and kinins), thereby changing local hemodynamics and kidney function.

With respect to the wide spectrum of spontaneous BP fluctuations that have been observed in hypertensive subjects, it is emphasized that our approach covers only a specific BPO and therefore cannot simply be extended to other BPOs that have also been observed during hypertension.

Nonetheless, the data demonstrate the importance of BP dynamics for kidney function and provide evidence that the investigated BPOs can attenuate RPP (P85)-induced changes in PRA, volume, and electrolyte homeostasis and thereby alleviate renal hypertension in our experiments. Interestingly, \(\approx 0.1\)-Hz oscillations in muscle sympathetic nerve activity and RR interval have been reported to be markedly attenuated during heart failure and blunted during severe heart failure in humans.31 Given that the patterns of muscle sympathetic nerve activity and RR interval are mirrored by corresponding BPOs, one may hypothesize that BPOs facilitate excretion of fluid and electrolytes and reduce PRA, thereby improving the prognosis of these patients.

Conclusions

Using a new approach to chronically oscillate RPP around a reduced RPP in conscious dogs, we demonstrate that BP
changes in the range of several seconds enhance daily sodium and fluid excretion and attenuate the BP elevation during the onset of renal hypertension. The RPP oscillations also induce a transient increase in NO liberation and lead to a sustained reduction in renin activity, which have been shown to be major factors in long-term BP control. These results may be a first step in understanding the importance of short-term BP fluctuations for the development of hypertension.

Acknowledgments

We thank K. Dannenberg, D. Bayerl, A. Gerhardt, and S. Molling for technical assistance and the employees of the Forschungseinrichtung Experimentelle Medizin for perfect animal caretaking.

References

Antihypertensive Effect of 0.1-Hz Blood Pressure Oscillations to the Kidney
Benno Nafz, Jens Stegemann, Morton H. Bestle, Nadja Richter, Erdmann Seeliger, Ingolf Schimke, H. Wolfgang Reinhardt and Pontus B. Persson

_Circulation_. 2000;101:553-557
doi: 10.1161/01.CIR.101.5.553

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circ.ahajournals.org/content/101/5/553

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/