Blood pressure variability and urine flow in the conscious dog

BENNO NAFZ, HEIMO EHMKE, CLAUS D. WAGNER, HARTMUT R. KIRCHHEIM, AND PONTUS B. PERSSON

Institut für Physiologie der Charité, Humboldt Universität zu Berlin, D-10117 Berlin; and Physiologisches Institut der Universität Heidelberg, D-6900 Heidelberg, Germany

METHODS

All experiments were performed on 13 female, chronically instrumented, conscious, adult foxhounds, weighing 17.5–25 kg, that had free access to water and received a standard dog diet (Alma H 5003, 4 g/kg NaCl). The daily sodium intake was 110 mmol. Experiments were approved and performed according to the German Animal Protection Law.

Implantation Surgery

All operations were done under aseptic conditions in an operating room. General anesthesia was induced by pentobarbital, and the animal was intubated and ventilated. General anesthesia was maintained with isoflurane in oxygen. The animal was placed in a supine position with the head and neck excised and wrapped with gauze. A single incision was made along the dorsolateral aspect of the neck and carried through the platysma and the muscles to the carotid artery. The carotid artery and the jugular vein were dissected in the cranial direction and tied with bulldog clamps. The vessels were incised and cannulated using 10 Fr catheters and connected to pressure transducers (Statham, P231B). Both catheters were connected to saline-lubricated pressure transducers. The left carotid artery was cannulated using a 10 Fr catheter. After the carotid artery was clamped, a short longitudinal incision was made to access the internal carotid artery. The internal carotid artery was cannulated using a 10 Fr catheter, and the cannula was filled with a 50% saline solution. The left jugular vein was cannulated using a 10 Fr catheter, and a short longitudinal incision was made to access the internal carotid vein. The internal carotid vein was cannulated using a 10 Fr catheter, and the cannula was filled with a 50% saline solution. The cannulas were connected to saline-lubricated pressure transducers. The pressure transducers were calibrated against the mercury manometer. The animal was positioned with its head in a stereotaxic device, and the stereotaxic device was connected to a computerized data acquisition system. The computerized data acquisition system was connected to a computer, and the data were recorded using Chart software. The data were recorded continuously for the duration of the experiment. The data were analyzed using the appropriate statistical methods. The results were considered statistically significant at P < 0.05. The data are represented as mean ± SEM. The results are representative of at least three experiments. The number of animals is indicated in parentheses. The Student t-test was used to compare the means of the groups. The results are expressed as mean ± SEM. The results are representative of at least three experiments. The number of animals is indicated in parentheses. The Student t-test was used to compare the means of the groups.

A second mechanism often suggested to influence renal hemodynamics and UV is the so-called tubuloglomerular feedback mechanism (TGF) (21, 29). Several studies have revealed that dynamic alterations of TGF over 20–30 s are closely intertwined with fluctuations of not only arterial blood pressure (AP), but also renal blood flow (RBF) (2, 10). Consequently, physiological variations of blood flow, in addition to, or even independent of, changes in blood pressure, may modulate intrarenal mechanisms such as the TGF. In light of these studies, it appears likely that physiologically occurring short-term blood pressure oscillations (BPO), which can bypass the renal autoregulation, or spontaneous variations in RBF may have a profound impact on short-term UV.

It was the goal of the present study, therefore, to clarify the influence of short-term variations in RPP and RBF on UV in 13 resting conscious foxhounds on a normal-sodium diet. This was achieved by the following protocols. 1) A single-step reduction in RPP was performed to determine the response times of RBF and UV in the conscious dog. 2) RPP, RBF, and UV were measured under control conditions over a period of 4 h. After correction of the RBF and UV values for the response times, we investigated the extent to which spontaneous short-term variations in UV were coupled with fluctuations in RPP or in RBF. 3) To clarify the individual effects of RPP and RBF on UV at a reduced RPP, we superimposed sinusoidal pressure oscillations with a frequency of 0.9 mHz on RPP. This induces a phase shift of ~180° between RPP and RBF in the conscious dog; thus RPP and RBF become uncoupled (30).
bital sodium (20 mg/kg body wt iv; Nembutal, Abbott) and maintained, after endotracheal intubation, by spontaneous ventilation with halothane and N\textsubscript{2}O. Two polyurethane catheters were implanted into the abdominal aorta, such that the tips were placed distally or proximally to both renal arteries, respectively. An inflatable cuff was placed around the aorta, above the origin of the renal arteries and between the tips of the arterial catheters. An ultrasound flow probe (type 6S5, Transonic Systems, Ithaca, NY) was positioned around the left renal artery. In addition, a Silastic catheter was inserted into the ureter and advanced into the left renal pelvis. The cuff and the distal catheter were connected to an extracorporeal electropneumatic pressure control system, which allowed us to reduce and maintain the RPP on a preset level with high precision (22). All catheters, cables, and the cuff lead were tunneled subcutaneously to the dog's neck, where they were exteriorized. To prevent catheter-related infections, the ureteral catheter was flushed daily with 10% sulfamethoxazole (Bactrim, Hoffmann-La Roche). The arterial catheters were filled daily with a diluted heparin solution (Braun, Melsungen, Germany).

Measurements

Blood pressure was measured in the abdominal aorta, above and below the constricting cuff, by means of pressure transducers (type P23Db, Statham) and Gould pressure processors (Gould, Cleveland, OH). Heart rate (HR) was recorded instantaneously with a rate meter (model 4600 pressure processor, Gould). RBF was measured continuously via the ultrasound transit time flow probe, placed around the renal artery (6 mm ID). To determine UV, the ureteral catheter (0.5 mm ID, 0.9 mm OD) was connected to a closed collecting system: a fraction sampler located in a Perspex chamber, a drop counter, and a servo system, which was used to stabilize the pressure in the chamber to \(-30\) cmH\textsubscript{2}O. The suction necessary for complete collection of urine was determined before the experiments by measuring UV at different suction pressures; UV became independent of suction at a pressure of \(-20\) cmH\textsubscript{2}O. The drop counter (precision of the timer = \(3.6 \times 10^{-5}\) s) was used to determine the time between two subsequent urine drops. In addition, the tubes of the fraction sampler were used to determine the average UV of two subsequent urine drops. In the equation: UV = volume per drop/time between two drops. The accuracy of this system is independent of catheter dead space, since the catheter is full of urine during the experiments and fluid is incompressible. The precision is, however, limited by the drop volume and the time between two drops. During the experiments, drop volume was \(-5.6\) µl \((178 \pm 1\) drops/ml). Thus a UV of 0.3 ml/min (Fig. 1) was determined with a resolution of \(-5.6\) µl/s \(-1\) drop/s (0.3 ml/min \times 178 drops/ml = 53.4 drops/min or \(-1\) drop/s).

During the experiments, AP, RPP, HR, RBF, and UV (analogue output of the drop counter) were recorded directly with an analog recorder (model 2600, Gould). After analog-to-digital conversion, all data were stored on-line by a computer system (Labtech Notebook, IBM-compatible personal computer). HR and UV were sampled every 5th s. Direct signals of the AP, RPP, and RBF were concomitantly stored with a sampling rate of 10/s.

Experimental Protocols

All experiments were performed between the 13th day and the 8th wk after the implantation surgery. The dogs were trained to rest in a recumbent position on a padded bench during the experiments, and the laboratory was strictly isolated from disturbances. Between 7:00 and 8:00 AM, the dogs were brought to the padded bench and connected to the recording instruments via extension cables. The last meal was provided 15–18 h before the experiment; water was available ad libitum. Urine was collected as described above. After 1–1.5 h of stabilization, the experiments were started. Only one experiment on one dog was conducted per experimental day.

Protocol 1: estimation of transition times between RPP, RBF, and UV. At resting blood pressure, AP, RPP, and RBF were continuously recorded with a sampling rate of 10/s (HR and UV with a sampling rate of 0.2/s). After stabilization, the extracorporeal control system was adjusted to reduce mean RPP to 80 mmHg. The pneumatic cuff was deflated, and \(-10–20\) min were allowed for recovery. Thereafter, the cuff was inflated briskly using the electrical values from the memory of the control system, which were obtained during the first short period of pressure reduction. Thus RPP was reduced in a single step to 80 mmHg. The data obtained by the pressure reduction in a single step were used to calculate response times between RPP and UV as well as between RBF and UV. These values were then used to correct for the time lag of the RBF and UV response.

Protocol 2: influence of spontaneous variability of RPP and RBF on UV. After stabilization, RBF, RPP, HR, and UV were recorded for 4 h without intervention. Throughout the experiments the dogs lay quietly on their right side. In addition to the electrical data obtained via the pressure processors and
the flowmeter, the analog output of the drop counter was used to determine the UV with high precision.

Protocol 3: dissociation between RPP and RBF. RPP was adjusted via the extracorporeal control system to 80 mmHg. Then the control system was used to superimpose sinusoidal blood pressure waves with an amplitude of about ±10 mmHg and a frequency of 0.9 mHz. Thus RPP varied continuously between ~70 and ~90 mmHg. A period of 30 min was allowed for stabilization before the data collection began. Thereafter, RBF, RPP, and UV were determined as described for protocol 2.

Data Analysis

Protocol 1. The last 10 min before the pressure step were used to calculate mean ± SD from the baseline data. A linear interpolation was used to connect data points.

Protocols 2 and 3. Mean values were derived from the original data by averaging within a 60-s window. Thus every 4-h recording resulted in 4 × 60 = 240 data points. Interindividual means were obtained by averaging the data triplets (RPP, RBF, UV) of every dog with the data triplets of the other dogs using RPP as a matching criterion. The interdependence among RPP, RBF, and UV was determined by a subsequent correlation analysis. The two-tailed Student's t-test was used for comparisons. P < 0.05 was taken to indicate significance. Values are means ± SE unless stated otherwise.

RESULTS

No significant changes in HR (101 ± 4, 97 ± 4, and 94 ± 6 beats/min for control, step function, and induced BPO, respectively) or in AP (105 ± 3, 104 ± 3, and 119 ± 4 mmHg for control, step function, and induced BPO, respectively) were observed between the protocols.

The response time of RBF and UV vs. RPP was determined in seven dogs by single-step pressure reduction. Figure 1 shows original recordings from a single experiment. Inflation of the aortic cuff (dashed vertical line) led to a precipitous fall in RPP from 104 ± 3 to 80 mmHg (Fig. 1, top). The sudden change in RPP caused RBF to decrease within a few seconds from 332 ± 38 to 100 ml/min. After this transient fall, RBF increased slowly but did not reach the baseline level within the following 50 s. UV, as measured via the drop counter at the end of the ureteral catheter, followed the precipitous reduction in RPP (Fig. 1, bottom) with a response time of ~10 s. The response time of the experiments was determined as indicated in Fig. 2: a ≥2 SD reduction from the baseline was compared with the corresponding points of RBF and UV. The resulting response time was ~1 s between RPP and RBF (0.4 ± 0.1 s; Fig. 2, middle). In contrast to this short latency, the response of UV to the sudden fall in RPP was calculated to be 8.1 ± 0.8 s (n = 7, P < 0.01 vs. RBF; Fig. 2, bottom). This sluggish response is not due to dead space, since the ureteral catheter was always filled and maintained on a constant suction via the extracorporeal pump.

The 60-s mean values of the 13 dogs of the control protocol are shown in Fig. 3. To investigate the influence of spontaneous changes in RPP and RBF on UV, the UV data were plotted against RPP (x-axis) and RBF (y-axis). During the recording period, mean RPP varied spontaneously from ~95 to 115 mmHg, without any systematic change (“trend”). As indicated by the course of the surface grid, spontaneous changes in UV were more or less randomly related to increases or decreases in RPP. In contrast, the steep slope of the surface grid with respect to RBF revealed a close coupling of RBF on
UV under spontaneous conditions. Mean RBF was $334 \pm 36$ ml/min during this control period (Fig. 4). As shown in Fig. 4, top, the physiological short-term variability of RBF was well correlated to the observed changes in UV ($r = 0.94$, $P < 0.001$). Thus a spontaneous, short-term increase in RBF by 10% of the baseline level induced an increase of ~23% in UV. Surprisingly, we observed no significant correlation of RPP with UV, as indicated in Fig. 4, bottom ($r = 0.09$, $P = NS$).

During protocol 3, RPP was forced with a frequency of 0.9 mHz around a mean value of 80 mmHg (Fig. 5, RPP). As indicated by the pulsatile signals of RPP and RBF, this dissociated the maxima in RPP and the maxima in RBF, in contrast to the maxima in UV (Fig. 5, RBF and UV), which coincided with the maxima in RPP.

Figure 6 shows mean values of the eight dogs from protocol 3, in which pressure oscillations were superimposed on RPP (81 ± 2 mmHg). As indicated in Fig. 6, top, RPP was forced with a period length of ~1,100 s (1,112 ± 4 s) and an amplitude of nearly ±10 mmHg. Because of the time constants of the systems involved in renal autoregulation, the minimum in RBF (Fig. 6, middle) was reached when the maximum in RPP was observed. In contrast to this dissociation between RPP and RBF, UV (Fig. 6, bottom) reached its maximum with a phase shift of only a few seconds to RPP (7 ± 3 s). The interdependence of RPP, RBF, and UV is demonstrated by a three-dimensional surface grid (Fig. 7). An overall steep slope of the surface with respect to RPP as well as with respect to RBF was observed. Thus the minimum in UV coincided with low RPP and high RBF, whereas the maximum in UV was observed at high RPP and low RBF. Figure 8 shows the correlation analysis of the 240 mean values of these experiments. The slope of the RPP-UV regression line (Fig. 8, top) was $6.38 \mu l \text{ UV} \cdot \text{mmHg}^{-1} \cdot \text{min}^{-1}$. Thus a 10% increase in RPP led to an ~15% increase in UV ($r = 0.95$, $P < 0.001$). Because of the induced phase shift, changes in RBF were inversely correlated to the oscillations in UV ($r = -0.85$, $P < 0.001$, slope = $-3.45 \mu l \text{ UV/ml}$).

**DISCUSSION**

The data of the present study reveal a close relationship between spontaneous oscillations in RBF, i.e., changes around a normal baseline level of minutes to several hours, and UV. Surprisingly, we observed no coupling between the spontaneous variability of RPP and UV during these control experiments (Fig. 4). At first sight, this observation seems to be in contrast to the well-established phenomenon of pressure diuresis (3, 8, 9). However, as indicated by protocol 3, if RPP was manipulated, we observed a marked pressure dependency of UV.
These data describe only the short-term behavior of urine production. Changes in, e.g., vasopressin levels and alterations in RPP will most likely compensate for longer periods of inadequate urine formation. Several points must be kept in mind when the results of our experiments are compared with other studies that focus on pressure diuresis. In the present study we induced sinusoidal RPP oscillations to phase shift RBF from RPP. It is inherent to this approach that we control RPP and hence eliminate some of the physiologically occurring RPP variations at other frequencies. However, this protocol did not depulsate the signal, as seen in Fig. 5.

A problem of earlier approaches was the need for averaging. Rapid changes in urine formation are not readily measured in the awake animal. The determination of short-term UV suffers from biologic (bladder, renal pelvis) or experimental (catheter) dead spaces. A simple way to partly overcome these effects is the use of longer sampling periods.

In our experiments we used a ureteral catheter, which was advanced into the left renal pelvis together with a suction pump and a drop counter, to determine UV with the highest possible resolution and with minimum dead space. Thus we were able to investigate the interdependence between physiological oscillations in RPP, RBF, and UV, even in the range of several seconds. The data of the present study reveal very short response times of only several seconds between changes in RPP and subsequent modulations in RBF or UV (protocol 1, Fig. 1). This agrees with the work of Steele and co-workers (27), who used an anesthetized rat model to determine stimulus response times between RPP and UV by the use of pharmacologically or mechanically (aortic occlusion) induced changes in RPP. Under these conditions, the group observed a response time of $\sim 5$ s between induced changes in RPP and UV. The importance of RBF on UV, however, remains unclear.

A second issue that is important in the interpretation of this study is the potential masking of pressure diuresis by the sympathetic nervous system. Short-term variability in resting AP is, to a great extent, controlled by the action of the autonomic nervous system.
system (26, 28, 32). An increase in sympathetic tone not only elevates AP but also enhances renin release and reduces UV, RBF, and renal sodium excretion (1, 5, 6, 13, 15, 16).

Despite these reservations, several studies support a role for RBF fluctuations in urine formation, and it is intriguing that spontaneously occurring RPP oscillations of minutes to several hours are poorly coupled with changes in UV (protocol 2, Fig. 4). In halothane-anesthetized rats, Yip and co-workers (31) observed spontaneous oscillations in single nephron blood flow as well as in tubular pressure. The spontaneous short-term variability in renal vascular resistance may change peritubular pressures and medullary blood flow or modulate the intrarenal transport of sodium and water (4) and, therefore, alter UV without any influence of the autonomic nervous system. In addition, changes in shear stress, as induced by changes in blood flow, increase NO production, which alters the plasma levels of renin in the conscious animal (24). This may also contribute to the observed modulations in UV (11, 23).

To clarify the observed lack of pressure diuresis and the short-term coupling of RBF on UV under baseline conditions, we used a common feature of feedback-controlled systems, the settling time. Every feedback-controlled system requires a certain period of time to compensate for initial changes in the controlled parameters. With respect to renal autoregulation of blood flow, the fastest feedback mechanism known so far is the myogenic response. Changes in RPP that are faster than the settling time of the vascular smooth muscle cells cannot, or at least not fully, be compensated for. Thus, in protocol 1, RBF and UV declined rapidly in response to the induced fall in RPP. On the other hand, very slow fluctuations in RPP, well within the range of the settling time of the RBF autoregulation, are effectively buffered (30). Between these very slow and the fast oscillations in RPP, there is a frequency range in which the settling time of the renal RBF autoregulation is similar to the half-period length of the oscillations in RPP. In this case, a decrease in RPP leads to an overshooting vasodilation. Thus minimum RPP coincides with maximum RBF, and, in turn, maximum RPP is observed at minimum RBF. In other words, there is a phase shift between RPP and RBF of 180°. In the conscious resting dog, BPO with a frequency of 0.9 mHz, corresponding to a wavelength of –1,100 s (Fig. 6), led to this phase shift in RBF autoregulation (30). During these oscillations around a reduced RPP, an increase in RPP is extremely well coupled with an increase in UV (Fig. 7). In contrast to this observation, the effect of RBF on UV was totally overridden (Fig. 8). Hence, once blood pressure is perturbed, pressure diuresis is reestablished. It must be emphasized, however, that these data do not provide insight into the long-term importance of RBF variability for urine formation. Further studies using longer observation periods may help clarify the overall importance of “blood-flow dependent diuresis.” Additionally, it must be kept in mind that the RPP occasionally exceeded the lower limit of RBF (~75 mmHg) and glomerular filtration rate (~80 mmHg) autoregulation (12) during protocol 3. This may have contributed in part to the observed pressure dependence of UV. We assume that the phase shift between RPP and RBF is not dependent on the lower limit of autoregulation; otherwise this phase shift should disappear at RPP above ~80 mmHg.

Our results reveal that when RPP fluctuates spontaneously around a normal baseline level, changes in RBF rather than in RPP coincide with changes in urine production. Conversely, experimentally induced 0.9-mHz oscillations in RPP of ~80 mmHg lead to a profound pressure-dependent response of UV, irrespective of RBF fluctuations.

Perspectives

It is widely accepted that long-term RPP is closely intertwined with urine formation and blood pressure regulation. Although it is clear that, in hypertension, short-term oscillations in AP are affected as well as renal hemodynamics, very little is known about the influence of such fluctuations on UV. In the present study, spontaneous variations of RBF had a marked effect on UV, in contrast to changes in RPP. However, a phase shift between RPP and RBF showed that as soon as RPP is not maintained at its normal level, short-term oscillations in UV become entirely pressure dependent. Thus spontaneous short-term BPO plays no major role in the control of UV. However, such BPO may be of particular importance when RPP fluctuates around pathological levels, e.g., in hypertension.

We thank I. Keller, A. Klein, L. Mahl, and E. Röbel for expert technical assistance and skillful animal care.

This study was supported by the German Research Foundation.

Address for reprint requests: B. Nafz, Institut für Physiologie der Charité, Humboldt-Universität Berlin, Tucholskystrasse 2, D-10117 Berlin, Germany.

Received 4 August 1997; accepted in final form 2 January 1998.

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