Integrative mechanisms of blood pressure regulation in humans and rats: cross-species similarities

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Charkoudian N, Gusman E, Joyner MJ, Wallin BG, Osborn J. Integrative mechanisms of blood pressure regulation in humans and rats: cross-species similarities. Am J Physiol Regul Integr Comp Physiol 298: R755–R759, 2010. First published January 13, 2010; doi:10.1152/ajpregu.00607.2009.—As our understanding of the importance of individualized medicine continues to grow, the clinical relevance of interindividual variability in hemodynamic variables is receiving increasing attention. However, it is not known whether the rat, which is often used for studies of cardiovascular regulation, exhibits similar interindividual variability. In the present study, we evaluated whether the magnitude of interindividual variability in cardiac output (CO) and total peripheral resistance (TPR) was similar in humans and in rats. We assessed interindividual variability of mean arterial pressure (MAP), CO, and TPR during control conditions in normotensive humans (n = 40) and during normotension and deoxycorticosterone acetate-salt hypertension in Sprague-Dawley rats (n = 16). Humans and rats showed marked interindividual variability in CO and TPR but low variability in MAP. During deoxycorticosterone acetate-salt hypertension, CO was maintained, but TPR was elevated compared with the baseline period. We conclude that the magnitudes of interindividual variability of MAP, CO, and TPR are qualitatively similar in humans and rats, providing support for the relevance of this variability in both species and suggesting that studies in rats could be designed to address questions specific to individualized medicine in hypertension.

circulation; cardiac output; vasoconstriction; arterial pressure; sympathetic nervous system

BLOOD PRESSURE VARIABILITY has been of interest to cardiovascular scientists and physicians for decades. The variations in blood pressure within a given human or animal over time (intraindividual variability) have been extensively studied using a wide variety of approaches, ranging from frequency analysis for noninvasive measurement of autonomic function in humans (16, 19, 23), to surgical studies of the role of the baroreflex in blood pressure regulation (e.g., sinoaortic denervation studies) (6, 17), to studies of circadian and other temporal rhythms in health and disease (7, 18, 20), among others.

Less common are studies on the interindividual variability in blood pressure regulatory mechanisms: studies in which differences between and among individuals are evaluated. An illustrative example relates to measurement of sympathetic nerve activity in humans by microneurography. When the first measurements of muscle sympathetic nerve activity (MSNA) were reported, it was noted that there was a wide (7- to 10-fold) range of MSNA values among otherwise-healthy and normotensive individuals with similar arterial pressures (26). The MSNA values did not correlate with blood pressure: in individuals with reproducibly high MSNA, arterial pressure values were normal and similar to arterial pressure in individuals with reproducibly very low MSNA (26). These characteristics made it difficult for the investigators to establish a “normal range” for MSNA. The large interindividual variability was considered by some to be a limitation in terms of clinical diagnostic use and in terms of interpretation for studies of chronic blood pressure regulation in humans.

More recently, we performed a series of studies that led us to the exact opposite conclusion: we now believe that this interindividual variability has fundamental biological significance and, in fact, provides mechanistic insight into our understanding of blood pressure regulation in humans. For example, although resting activity of sympathetic vasoconstrictor nerves can vary 7- to 10-fold among healthy human subjects with similar and normal arterial pressure (4, 24, 25), we recently reported that, in young men, these interindividual differences in sympathetic nerve activity were balanced by reciprocal differences among individuals in cardiac output (CO) (4). In individuals with higher MSNA, total peripheral resistance (TPR) was also higher, but these values were balanced by lower CO, which minimized their ultimate effect on arterial pressure. We concluded from these and other studies that interindividual variability is a key factor in arterial pressure regulation and that balances between central hemodynamics (CO and stroke volume) and control of peripheral vascular resistance are important in maintaining the narrow range of arterial pressure values observed across subjects (4, 5).

Although the importance of individualized medicine has been receiving renewed attention in recent years, the clinical relevance of interindividual variability in blood pressure-regulating variables has been recognized for decades. A detailed understanding of chronic blood pressure regulatory mechanisms can potentially reveal important insight into mechanisms of human cardiovascular diseases such as hypertension. As such, it is important to investigate these mechanisms in an animal model that is suitable for detailed investigations that are not possible in humans. The rat is the species that is most commonly used to study mechanisms of cardiovascular diseases such as hypertension and heart failure. However, it is not known whether interindividual variability of hemodynamic variables occurs to the same extent in rats as in humans. Therefore, the goal of the present study was to directly compare the magnitude of interindividual variability in MAP, CO, and TPR between humans and rats.
METHODS

Human Studies

The present report represents retrospective analyses of hemodynamic data from previously published studies from our laboratory (4, 9, 10). The protocol for all human studies was approved by the Institutional Review Board of the Mayo Foundation. Forty healthy young men [25.6 ± 0.7 (SE) yr of age, 1.78 ± 0.01 m height, 78 ± 1.7 kg body wt] volunteered to participate and gave written informed consent. The subjects were nonsmokers and had no history of cardiovascular or other chronic diseases. They were asked not to consume anything except water within 2 h before the experiment and not to consume caffeine or alcohol within 24 h of the experiment.

Upon arrival to the laboratory, subjects rested quietly in the supine position during instrumentation. With use of aseptic technique after local anesthesia with 2% lidocaine, a 5-cm, 20-gauge catheter was placed in a radial or brachial artery. This catheter was connected to a pressure transducer that was placed at heart level and used for measurement of arterial pressure. A three-lead ECG was used for continuous monitoring of heart rate (HR).

CO was measured using the open-circuit acetylene uptake technique, as previously described (3, 4, 12). This technique has been validated against direct Fick measurements of CO for a range of CO values (12). The instrumentation period included a practice measurement of CO to familiarize the subject with the procedure. This practice value was not included in the CO data presented in RESULTS.

Multunit MSNA was recorded with a tungsten microelectrode in the peroneal nerve, posterior to the fibular head, as described by Sundlöf and Wallin (26). The recorded signal was amplified 80,000-fold, band-pass filtered (700–2,000 Hz), rectified, and integrated (resistance-capacitance integrator circuit, time constant 0.1 s) by a nerve-traffic analyzer.

We continuously recorded arterial pressure, ECG, and integrated MSNA during 20 min of supine rest. CO was measured in duplicate during the last 5–7 min of the recording period.

Data collection and analysis. Data were sampled at 240 Hz and stored on a personal computer for offline analysis. Mean arterial pressure (MAP) was calculated as the time integral over the pressure pulse. MSNA and MAP are reported as averages over the 4 min immediately preceding the first CO measurement. CO is reported as the average of the two measurements for each individual. TPR was calculated as MAP / CO.

Sympathetic bursts in the integrated neurogram were identified by a custom-manufactured semiautomated analysis program; burst identification was then corrected by visual inspection by a single investigator, as previously reported (3, 4, 15).

Rat Studies

Surgical procedures. Studies were conducted in 16 male Sprague-Dawley rats (250–300 g body wt; Charles River Laboratory, Wilmington, MA). The animals were housed in an animal room with controlled temperature and a 12:12-h light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota and were conducted in accordance with institutional and National Institutes of Health guidelines.

All surgical procedures were conducted using aseptic technique. Eight of the 16 rats were anesthetized with pentobarbital sodium (50 mg/kg ip), unilaterally nephrectomized as previously described (11), and allowed to recover for 1 wk. All rats were surgically instrumented for hemodynamic measurements. Subsequently, rats with intact kidneys (2-K) or uninephrectomized (1-K) rats were anesthetized in a chamber with isoflurane, intubated, and ventilated with a rodent ventilator (model 683, Harvard Apparatus, South Natick, MA). Anesthesia was maintained by administration of 2.0% isoflurane in 100% oxygen using a vaporizer (model 61020WOB, Brickford, Wales Center, NY) connected to the ventilator. A telemetry transmitter (model TP11PA-C40, Data Sciences International, St. Paul, MN) was used for continuous monitoring of MAP and HR. The body of the telemetry transmitter was placed in the lower abdomen, and the transmitter catheter was passed through the abdomen wall and advanced into the descending aorta via the femoral artery. A silicone rubber catheter was advanced into the vena cava via the femoral vein. The ascending aorta was exposed by a midline thoracotomy, and a transit-time ultrasonic flow probe (model SB2.5, Transonic Systems, Ithaca, NY) was placed around the ascending aorta for measurement of CO. The venous catheter and flow probe cable were tunneled subcutaneously to the back of the neck, externalized through a midscapular skin incision, and passed through a flexible spring attached to a custom-made rodent vest.

Upon recovery from anesthesia, rats were placed in individual cages, and the flow probe cable was connected to an electrical swivel (model SL6C, Kent Scientific, Torrington, CT) above the cage. Once they were fully awake, the rats were injected with bumetanide HCl (0.015 mg iv) for analgesia. A 0.1% NaCl diet (Research Diets, New Brunswick, NJ) and distilled water were provided ad libitum. For the first 3 days of the recovery period, ampicillin (15 mg), tobramycin (2 mg), bumetanide (Bumex, 0.015 mg), and 5% dextrose (5 ml) were intravenously injected daily. Rats were allowed ≥14 days to recover from surgery before hemodynamic measurements were obtained. Experimental protocol. Since the magnitude of doxycorticosterone acetate (DOCA)-salt hypertension is dependent on whether the animals are intact or uninephrectomized, experiments were conducted in 2-K and 1-K rats before instrumentation. This allowed us to measure hemodynamic function over a wide range of arterial pressure in DOCA-salt hypertensive rats.

Baseline MAP, HR, and CO were recorded for 7 days while the rats drank distilled water; then the rats were switched to a 0.9% NaCl-0.2% KCl drinking solution for the duration of the protocol, 50 mg of DOCA in silicone were implanted subcutaneously, and measurements were continued for an additional 21 days, as previously described (11).

Data collection and analysis. MAP and HR were measured with a telemetry transmitter and monitored by a receiver (model RPC-1, Data Sciences) that was mounted behind individual rat cages and connected to a data-exchange matrix. The CO signal collected by the flow probe around the ascending aorta was connected to a flowmeter (model T-206, Transonic Systems); the output was digitized using an analog-to-digital converter (model C111V, Data Sciences) and then sent to a data-exchange matrix. Data acquisition and analysis were performed using Dataquest ART version 2.2 software on a Dell XPS B866 computer. MAP, HR, and CO were monitored continuously at 500 Hz for the duration of the protocol. TPR was calculated by dividing MAP by CO. CO and TPR data are expressed as absolute units or normalized to body weight. CO, MAP, and TPR data are expressed as 24-h averages. Data analysis was performed for the final day of the baseline period and the last day of the DOCA-salt period, at which time MAP had reached a new steady-state level.

Statistics. Interindividual variability (CO, TPR, and MAP) was evaluated using coefficient of variation (CV = SD / mean). The relationships between CO and MSNA and between MSNA and TPR were assessed using first-order linear regression (SigmaStat and SigmaPlot, SPSS). Differences between 2-K and 1-K rats during baseline and DOCA-salt periods were compared by two-way analysis of variance for repeated measures followed by the Holm-Sidak method for all post hoc comparisons (SigmaStat version 3.5). Statistical significance was accepted for P < 0.05.

RESULTS

As shown in Table 1, there were no differences between 2-K and 1-K rats during the baseline periods for MAP, CO, TPR, or HR. Figure 1 shows interindividual variability in MAP, CO, and TPR in humans (A) and rats (B).
Table 1. 24-h values for hemodynamic variables in 2-K and 1-K rats on the final day of the baseline period and the last day of DOCA-salt treatment

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>CO, cl·min⁻¹·kg⁻¹</th>
<th>TPR, mmHg·cl⁻¹·min⁻¹·kg⁻¹</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-K</td>
<td>1-K</td>
<td>2-K</td>
<td>1-K</td>
</tr>
<tr>
<td>Baseline</td>
<td>101 ± 1</td>
<td>105 ± 3</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>120 ± 7*</td>
<td>135 ± 4*</td>
<td>21 ± 1</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). 2-K, rats with kidneys intact; 1-K, uninephrectomized rats; MAP, mean arterial pressure; CO cardiac output; TPR, total peripheral resistance; HR, heart rate; DOCA, deoxycorticosterone acetate. *P < 0.05 vs. baseline (within-group comparison).

Figure 2 shows the inverse relationship between CO and MSNA (A) for the 40 human subjects during normotension and the direct relationship between MSNA and TPR (B). Linear regression analysis indicated a statistically significant relationship for both variables with MSNA (P < 0.001).

Next, we evaluated MAP, CO, and TPR during the steady-state phase of DOCA-salt hypertension in our rat model. The DOCA-salt protocol resulted in a steady-state increase in MAP of ~20 mmHg in 2-K rats and ~30 mmHg in 1-K rats (Table 1). On the last day of DOCA-salt hypertension, mean MAP and TPR were significantly elevated in 2-K and 1-K rats, whereas mean CO was unchanged compared with the baseline period.

DISCUSSION

Our goal in these studies was to evaluate whether interindividual variability in hemodynamic control mechanisms is as important in arterial pressure regulation in rats as we previously showed it to be in humans (4, 5). We report that the magnitude of interindividual variability in hemodynamic variables was strikingly similar between species. Indeed, the CVs (among individuals) for MAP, CO, and TPR were nearly identical in rats and humans. In both species, we noted marked interindividual variability in CO and TPR, in contrast to low interindividual variability in MAP (Fig. 1). In humans, an inverse relationship between CO and MSNA appears to be an important contributor to the balance of factors that maintain normotension in healthy individuals (Fig. 2). Furthermore, in DOCA-salt hypertensive rats, the increase in MAP appears to be due primarily to an increase in TPR (Table 1). Since DOCA-salt hypertension is a sympathetic neurogenic model of hypertension, human (Fig. 2) and rat (Table 1) data support an important role for the sympathetic nervous system in an integrated balance of factors that contribute to the regulation of arterial pressure.

MAP is a function of CO and TPR (i.e., MAP = CO × TPR), and an inverse relationship between CO and MSNA in humans (Fig. 2) (4) supports the idea that the sympathetic nervous system contributes importantly to the maintenance of a hemodynamic balance and normal blood pressure in healthy young men. The mechanism whereby basal sympathetic nerve activity is regulated to maintain the balance is not known. The arterial baroreceptor reflex is one possibility. However, reports of normal levels of sympathetic activity in sinoaortic-dener-
the present study are consistent with the theory that a system for long-term control of arterial pressure exists within the central nervous system, and neurogenic hypertension could result from alterations of this system (21, 22).

The methods employed to measure hemodynamic variables and the experimental conditions under which these measurements were made were quite different between species. Whereas arterial pressure and CO were measured at a single time point in humans in a clinical setting, both variables were monitored continuously throughout the light and dark cycle in rats in their home cage. CO was measured in humans by an indirect approach, in contrast to an implanted flow probe on the ascending aorta in the rat. Despite these marked differences in methodology and experimental conditions, the intersubject variability, as measured by the CV, was qualitatively identical in rats and humans (Fig. 1). It is unlikely that the quantitative differences in hemodynamic variables are an artifact of the methods of measurement. It is important to note that the measurements involved are themselves quite reproducible in individual subjects over time. In humans, the interindividual differences in MSNA are reproducible over many years (8, 26). CO as measured in the present study is also reproducible and compares closely with direct Fick measurements over a range of CO values (12). In the rat, MAP was measured using radiotelemetry and CO was measured via a chronically implanted aortic probe, and data for both variables were calculated over a 24-h period. Again, despite these differences in the methods of measurement in rats and humans, the variability of MAP, CO, and TPR (expressed as the CV) was nearly identical in both species, as was the CO-TPR relationship. Taken together, we believe that this robust quantitative similarity between species, despite the different methodologies, provides strong support for the hypothesis that the hemodynamic mechanisms responsible for establishing the small degree of intersubject variability of MAP are similar in rats and humans.

Our data do not provide evidence regarding the origin of the interindividual variability of CO and TPR. In humans, there is some evidence that interindividual differences in sympathetic nerve activity may have a genetic basis (27). If so, genetic mechanisms may also contribute to the interindividual differences in CO and TPR. On the other hand, the Sprague-Dawley rats used in the present study would likely have less interindividual genetic variability than the human subjects, but the variability in CO, TPR, and MAP in the rats was comparable to that in the humans (Fig. 1). Potential genetic mechanisms, therefore, are beyond the scope of the present study.

**Perspectives and Significance**

Clearly, rats are not small humans. Any model has its limitations: whereas human studies are limited by the invasiveness of experimental approaches that are used in animal models, data obtained in rats are sometimes limited in terms of how much they can be “translated” into clinical relevance for the human patient. In this sense, our present analysis has been helpful, in that one would not expect an inbred strain of rats to exhibit the same extent of interindividual variability as a group of randomly chosen humans, yet the variability and hemodynamic balances were qualitatively and quantitatively very similar between species. This suggests a fundamental “conserved” importance to the variability and hemodynamic balances we
have reported in humans (4, 5) and gives another dimension of support for the use of the rat model in studies relevant to human blood pressure regulation.

A limitation of the present work is that all the data were obtained from young males of each species. We recently showed that both aging and female sex can alter the balance between central hemodynamics and sympathetic control of peripheral vascular resistance (9, 10). The present analysis does not allow us to evaluate whether the balances among variables in rats are also altered by aging or female sex. An additional limitation was that since this report represents a retrospective analysis of existing data, the data collection and timelines for human and rat protocols were quite different. Although the similarities were very robust and, therefore, clear, despite differences in experimental design, in future direct comparisons, it will be important (to the extent possible) to design comparable protocols between the two species.

In summary, we report that the magnitude of the interindividual variability in MAP, CO, and TPR was strikingly similar between humans and rats. As we previously reported, there was a significant inverse relationship between CO and MSNA in rats. Further studies involving comparisons, it will be important (to the extent possible) to design comparable protocols between the two species.

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DISCLOSURES
No conflicts of interest are declared by the author(s).

REFERENCES