Baroreflex control of renal sympathetic nerve activity during air-jet stress in rats

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Kanbar R, Oréa V, Barrès C, Julien C. Baroreflex control of renal sympathetic nerve activity during air-jet stress in rats. Am J Physiol Regul Integr Comp Physiol 292: R362–R367, 2007. First published September 14, 2006; doi:10.1152/ajpregu.00413.2006.—The effects of acute emotional stress on the sympathetic component of the arterial baroreceptor reflex have not yet been described in conscious animals and humans. Arterial pressure (AP) and renal sympathetic nerve activity (RSNA) were simultaneously recorded in 11 conscious rats before and during exposure to a mild environmental stressor (jet of air). Baroreflex function curves relating AP and RSNA were constructed by fitting a sigmoid function to RSNA and AP measured during sequential nitroprusside and phencyclidine administrations. Stress increased mean AP from 112 ± 2 to 124 ± 2 mmHg, heart rate from 381 ± 10 to 438 ± 18 beats/min, and RSNA from 0.80 ± 0.14 to 1.49 ± 0.23 μV. The RSNA-AP relationship was shifted toward higher AP values, and its maximum gain was significantly (P = 0.01) increased from 9.0 ± 1.3 to 16.2 ± 2.1 normalized units (NU)/mmHg. The latter effect was secondary to an increase (P < 0.01) in the range of the RSNA variation from 285 ± 33 to 619 ± 59 NU. In addition, the operating range of the reflex was increased (P < 0.01) from 34 ± 2 to 41 ± 3 mmHg. The present study indicates that in rats, the baroreflex control of RSNA is sensitized and operates over a larger range during emotional stress, which suggests that renal vascular tone, and possibly AP, are very efficiently controlled by the sympathetic nervous system under this condition.

arterial pressure; baroreceptor reflex; gain; sympathetic nervous system

IN LABORATORY ANIMALS AND human subjects, emotional stress evokes parallel increases in arterial pressure (AP), heart rate (HR), and sympathetic nerve activity (SNA) (1, 2, 17). In sleeping or quiet resting subjects, pharmacologically induced increases in AP result in reflex decreases in HR and SNA. From this simple observation, it is logical to hypothesize that emotional stress inhibits or even suppresses the arterial baroreceptor reflex. Support to this hypothesis came initially from studies performed on anesthetized animals. Specifically, electrical stimulation of the hypothalamic defense area of cats, which mimics the cardiovascular and autonomic effects of emotional stress (8), was shown to inhibit baroreceptive neurons in the nucleus tractus solitarius (22). In the latter study, however, it could not be excluded that electrical stimulation had activated fibers of passage. Therefore, the question was recently revisited by using a pharmacological approach. It was found that in urethane-anesthetized rats, disinhibition of neurons of the defense area with a GABA_A receptor antagonist induced a rightward shift of the renal SNA (RSNA) baroreflex function curve and increased its sensitivity (20).

It is amazing that the effects of emotional stress on the characteristics of the sympathetic baroreceptor reflex have not yet been reported. However, indirect evidence has been obtained in conscious rats that the sympathetic vascular component of the baroreceptor reflex is still operating during stress because pressor and regional vasoconstrictor responses to air-jet stress are enhanced in sinoaortic baroreceptor denervated rats (29). This would imply that the amplitude of the pressor response accompanying exposure to emotional and psychological stressors is, at least partly, determined by the sensitivity of the sympathetic baroreceptor reflex. This question is of importance from a (patho)physiological point of view simply because stress-induced pressor episodes probably contribute to the spontaneous variability of AP (14), and because AP variability is increasingly recognized as a cardiovascular risk factor, especially in hypertensive patients (18, 21, 25).

The aim of the present study was, therefore, to examine the effects of emotional stress on the sympathetic baroreflex function curve. For this purpose, RSNA and AP were simultaneously recorded in conscious rats under resting conditions and during acute exposure to a mild environmental stressor (jet of air).

METHODS

Animals and surgery. Experiments were performed on 11 male Sprague-Dawley rats aged 9 to 10 wk (Charles River Laboratories, L’Arbresle, France). Animals were housed individually with free access to food and water and were maintained on a 12:12-h light-dark cycle. All experiments were approved by the local Animal Ethics Committee.

The day before the study, rats were anesthetized with halothane (2% in oxygen) and received a single prophylactic injection of penicillin G (50,000 IU sc). One femoral arterial and two femoral venous catheters were inserted into the lower abdominal aorta and the inferior vena cava for AP measurement and drug administration, respectively. Catheters were routed subcutaneously and exteriorized between the scapulae. Then, 4 to 6 h later, rats were reanesthetized with pentobarbital sodium (60 mg/kg ip), and the left renal nerve was exposed via a flank incision. After careful isolation, a major branch of the renal nerve was placed on a bipolar platinum-iridium electrode and insulated with a silicone gel (604A and B; Wacker Chemie, Munich, Germany). The electrode cable was secured to back muscles and exteriorized at the same site as the catheters. The plug was protected in a small cap sewn to the skin. Rats received an injection of ketoprofen (2 mg/kg ip) and were allowed 16–18 h to recover from anesthesia (28).

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Data acquisition and experimental protocol. AP was measured by connecting the arterial catheter to a precalibrated pressure transducer (TNF-R; Ohmeda, Bilthoven, The Netherlands) coupled to an amplifier (model 13–4615-52; Gould, Cleveland, OH). RSNA was amplified (×50,000), band-pass filtered (300–3,000 Hz; model P-5111; Grass, Quincy, MA), and rectified by an analog homemade rectifier including a low-pass filter with a cut-off frequency of 150 Hz. Using a computer equipped with an analog-to-digital converter (model AT-MIO-16; National Instruments, Austin, TX), and LabVIEW 5.1 software (National Instruments), we sampled the AP and RSNA signals at 500 and 5,000 Hz, respectively.

AP and RSNA were simultaneously and continuously recorded before (baseline) and during application of a mild emotional stress elicited by means of a jet of air blown into the cage (2, 14, 29) for 20 min. The baroreflex control of RSNA was assessed under baseline conditions in the awake resting state (twice at a 60-min interval) and 10 min after starting the air-jet stress. Baroreflex testing consisted of first decreasing mean AP (MAP) to ~50 mmHg with a bolus injection of sodium nitroprusside (100 μg/kg iv) and then increasing MAP from that level to ~160 mmHg with an infusion of phenylephrine (50 μg·kg⁻¹·min⁻¹ iv for 30 to 40 s) (Ref. 7; see Fig. 3). At the end of the recording session, the long-acting ganglionic blocker chlorisondamine was administered (2.5 mg/kg iv). On completion of the experiment, the rats were euthanized with an intravenous overdose of pentobarbital sodium, and RSNA was recorded for an additional 25- to 30-min period.

Data analysis. AP and RSNA time series were resampled at 1 Hz by averaging data over consecutive 1-s periods. Average values of 1 s of AP are referred to as MAP values in the following of the text. Within each 1-s segment, HR was estimated as the mean interbeat interval. Since there was no significant difference between postmortem RSNA values and those measured after chlorisondamine administration, the background noise level was taken as the mean value calculated over a 2-min period under ganglionic blockade. This value was then subtracted from all RSNA data for subsequent analyses. A reference RSNA value was obtained by calculating the mean of the two 2-min average values measured just before each baroreflex testing under baseline conditions. RSNA was then expressed as a percentage of this reference value, which was taken to equal 100 normalized units (NU).

The RSNA-MAP baroreflex relationship was assessed by analyzing data collected from the maximum nitroprusside-induced fall in AP up to the maximum phenylephrine-induced rise in AP. The duration of the whole AP variation was ~60 s. A sigmoid function was then fitted to the MAP-RSNA data pairs (see Fig. 4) by an iterative least-squares procedure (SigmaPlot 2000; SPSS, Chicago, IL): RSNA = P1/[1 + exp(P2(MAP-P3))] + P4, where P1 is the full range of RSNA changes, i.e., the difference between the upper (calculated maximum RSNA activation) and lower (calculated maximum RSNA inhibition) plateaus; P2 is a slope coefficient; P3 is MAP at half the RSNA range (which is also the inflection point of the sigmoid curve where the slope is maximum); and P4 is the lower plateau. From these parameters, the upper plateau (P4 + P3), the threshold pressure (Pthr = P3 – 2P2), the saturation pressure (Psat = P3 + 2P2) and the operating range (Pout – Pin) were derived. Pthr and Psat are MAP values at the points where the tangent at inflection point of the sigmoid curve intersects with the upper and lower plateaus, respectively. At these points, RSNA lies 11.9% (of the RSNA range) below and above the upper and lower plateaus, respectively (6, 19). The operating range indicates the range of MAP over which RSNA is responsive. The first derivative of the sigmoid function was computed to determine the baroreflex gain across the full range of MAP variations, including the maximum gain (Gmax), which can also be calculated as P1/P2.

Statistics. Values are means ± SE. Paired comparisons were performed using the nonparametric Wilcoxon signed rank test.

RESULTS

Effects of emotional stress on cardiovascular variables. Behavioral responses to stress were consistent between animals. When the jet of air was suddenly blown into the cage, there was a startle response and AP rose to a maximum (Fig. 1). Thereafter, rats usually moved until they found a place in the cage where they stayed immobile for the rest of the test. AP and RSNA stabilized 7 to 8 min after the onset of the stress trial, thus allowing the assessment of the baroreflex control of RSNA under stable conditions (Fig. 2A and C). By contrast, HR increased progressively and did not reach a stable level after 10 min of exposure to the air jet (Fig. 2B). When considering the 2-min average values recorded immediately before baroreflex testing (i.e., 2 h and 1 h before, and at 8–10 min after the onset of the stress), it was observed that MAP increased from 112 ± 2 to 124 ± 2 mmHg, HR from 381 ± 10 to 438 ± 18 beats/min and RSNA from 0.80 ± 0.14 to 1.49 ± 0.23 μV. Differences are significant (P = 0.003 in all cases).

Effects of emotional stress on the sympathetic component of the baroreceptor reflex. Under both baseline and stress conditions, RSNA showed clear changes in response to drug-induced alterations of AP (Fig. 3). A four-parameter sigmoid equation could be satisfactorily fitted to all MAP-RSNA baroreflex relationships (Fig. 4), as demonstrated by high coefficients of determination (Table 1). The two baroreflex curves obtained under baseline conditions at a 1-h interval were very similar (Fig. 5), and their describing parameters did...
not differ significantly. These parameters were therefore averaged (Table 1).

Baroreflex curves obtained during stress were shifted toward higher MAP levels (Fig. 5A and Table 1). The increases in midrange MAP (P₃) and reference MAP did not differ significantly (9.5 ± 2.6 mmHg vs. 12.3 ± 1.8 mmHg, respectively; P = 0.155) and were positively and linearly related (R = 0.68, P = 0.022). In addition, the upper plateau was increased and the lower plateau was decreased so that the range of RSNA variations (P₁) was more than doubled (Fig. 5A and Table 1). As a consequence, the maximum gain of the MAP-RSNA baroreflex curve was increased by ∼80% during stress (Fig. 5B and Table 1). The operating range of the sympathetic baroreflex was also significantly increased due to an increase in the saturation MAP (Table 1). Finally, it was observed that the stress-induced increase in MAP tended to be inversely related to the maximum gain measured under baseline conditions (R = −0.58, P = 0.063).

The effects of emotional stress on the cardiac component of the baroreceptor reflex could not be assessed with the method used in this study. After sodium nitroprusside administration, HR remained elevated when AP had returned to, or even was higher than, baseline levels during phenylephrine infusion (Fig. 3A and B). This resulted in a marked hysteresis in the HR response to AP changes, which precluded a proper analysis of baroreflex curves.

DISCUSSION

Emotional stress in rats resets the AP-RSNA baroreflex function curve to higher AP levels and increases its maximum gain and its operating range. Taken together, these observations suggest that renal vascular tone, and possibly AP, are very efficiently controlled by the sympathetic component of the arterial baroreceptor reflex during acute exposure to an emotional stressor.

Methodological aspects. In the present study, baseline baroreflex testing was performed in awake, quiet rats. Under this condition, the characteristics of the AP-RSNA baroreflex function curves showed a good reproducibility at a 1-h interval, thus allowing safe comparisons with curves obtained under stressful conditions after a similar time interval. During air-jet stress, rats stayed motionless so that changes in baroreflex function curves most probably reflected the purely “emotional” effects of stress. One limitation of the method, however, is that it was not appropriate for the study of the cardiac component of the baroreceptor reflex, due to a marked hysteresis in the HR response to the sequence of AP changes. The reasons for this hysteresis are unclear. It is possible that the profound fall in AP induced by sodium nitroprusside evoked a reflex release of adrenomedullary catecholamines. Circulating catecholamines have longer half-lives than neurally released catecholamines and thus might have opposed and retarded the subsequent vagally mediated slowing of HR induced by phenylephrine infusion. Experiments performed on adrenal demedullated rats would be necessary to clarify this point. In practice, it might be advisable to allow a recovery period between the administrations of vasoactive drugs to assess the baroreflex control of HR (11, 23, 24).

Effects of emotional stress on the sympathetic baroreflex. Air-jet stress increased both MAP and RSNA. This was accompanied by a resetting of the AP-RSNA baroreflex function curve toward higher AP levels. This result accords with previous studies in conscious rats reporting significant rightward shifts of the AP-RSNA curve during treadmill exercise (23).
and grooming (24). A significant resetting of the AP-HR baroreflex function curve has also been reported during air-jet stress in borderline hypertensive rats (13) and in rabbits (26). Another major effect of air-jet stress was a range-dependent increase in the maximum baroreflex gain. Again, this was consistent with a previous report in exercising rats (23). Stress-induced alterations in the AP-RSNA baroreflex function curve are probably of central origin as very similar changes in the characteristics of the reflex have been reported in anesthetized rats during pharmacological disinhibition of neurons of the defense area (20).

The marked stress-induced increase in RSNA observed in this and previous (2, 17) studies probably resulted in renal vasoconstriction. An increase in renal and mesenteric vascular resistances has been observed during air-jet stress in rats (16, 29). Despite concomitant vasodilatation in skeletal muscles (16, 29), the net hemodynamic effect of air-jet stress is an increase in vascular resistances in the lower part of the body, as could be demonstrated by Doppler blood flow measurements at the level of the subdiaphragmatic aorta (29). This hemodynamic effect is probably responsible for a large part of the stress-induced increase in AP. The present finding that the sympathetic component of the baroreceptor reflex still operates during emotional stress is consistent with our previous observations in sinoaortic baroreceptor denervated rats that led us to propose that the baroreflex system limits renal and splanchnic vasoconstriction while enhancing vasodilatation in the skeletal muscles, hence favoring the hyperemic response in the latter circulation while reducing its cost in terms of pressor effect and thus, workload on the heart (29). However, this hypothesis does not require that the gain of the reflex should be increased and its operating range enlarged. We are therefore left with speculations about the necessity for the cardiovascular system to be prepared to face stronger challenges during “fight or

Fig. 3. Time course of changes in AP, HR, and RSNA induced by the sequential administration of sodium nitroprusside (SNP) and phenylephrine (PHE) in 1 conscious rat, under baseline conditions (A) and during air-jet stress (B).
flight” behavior. An enhanced baroreflex function would possibly be beneficial to regional and systemic hemodynamic adjustments accompanying such behavior. This hypothesis is supported by a study in rabbits demonstrating that air-jet stress increases the blood loss necessary to produce hypotension (27).

Limitations of the study. The absence of data on the cardiac component of the baroreceptor reflex might be disappointing to the reader. However, such data have already been reported in the literature (13, 26). Furthermore, in conscious rats, HR variations play little role in controlling spontaneous AP fluctuations, which is demonstrated by the lack of effect of acute cardiac autonomic blockade on AP variability (3). It is of note, however, that a buffering effect of HR variability on AP variability can be unmasked in rats with chronic sympathectomy (10).

We did not analyze the effects of air-jet stress on AP variability. The reason is twofold. First, effects of air-jet stress on fast rhythms of AP have been thoroughly described in rats (2, 4, 12). Secondly, an increased sympathetic baroreflex gain is expected to attenuate low and very low frequency components of AP variability (5, 15). The experimental protocol used in the present study did not allow analysis of AP fluctuations with periods longer than 3–4 min. More importantly, the behavioral effects of air-jet stress eliminated the movement-related component of AP variability, which is a major source of slow AP perturbations in rats (10, 30). The latter effect of air-jet stress would thus have biased any comparison with AP variability observed under baseline conditions in freely behaving rats.

Table 1. Effects of emotional stress on the characteristics of the MAP-RSNA baroreflex function curve

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<th>Baseline</th>
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<td>$P_4$, NU</td>
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<th>Derived Model Parameters</th>
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<td>$P_{\text{thr}}$, mmHg</td>
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<tr>
<td>Operating range, mmHg</td>
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<tr>
<td>$G_{\text{max}}$, mmHg/NU</td>
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Values are means ± SE ($n = 11$ rats). Baseline parameters were averaged from the 2 baroreflex curves. $R^2$, coefficient of determination (observed vs. predicted values); $P_1$, RSNA range; $P_2$, slope coefficient; $P_3$, mean arterial pressure (MAP) at midrange; $P_4$, lower plateau; $P_{\text{sat}}$, threshold MAP; $P_{\text{thr}}$, saturation MAP; $G_{\text{max}}$, maximum gain; NU, normalized units. P values refer to comparisons between baseline and stress conditions.

Fig. 4. Example of baroreflex function curves constructed by fitting a sigmoid function to RSNA and MAP measured during sequential SNP and PHE administrations, before (○) and during (●) air-jet stress. Data are from the same rat as in Fig. 3. $R^2$, coefficient of determination (observed vs. predicted values).

Fig. 5. Baroreflex relationships between MAP and RSNA determined under baseline conditions (base 1 and base 2) and during air-jet stress. Group average ($n = 11$ rats) parameters were used to generate baroreflex function curves (A) and their first derivative (B). In B, white and black circles show the gain observed at the reference MAP value under baseline and stress conditions, respectively.
Acknowledgments

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References


Perspectives

The present study provides the first demonstration that the sympathetic baroreceptor reflex is reset and sensitized during emotional stress. This result, taken together with previous reports in rats during exercise (23) and grooming (24), raises the possibility that the characteristics of the sympathetic baroreceptor reflex might vary spontaneously during daily life, e.g., with changes in behavioral and emotional states. A proper evaluation of this hypothesis would require developing a continuous index of sympathetic baroreflex sensitivity. Such indices are available for the cardiac component of the baroreceptor reflex, and it has indeed been shown recently that a spontaneous index of vagal baroreflex gain exhibits large, slow fluctuations in humans (9).

From a clinical perspective, it is worth noting that baseline baroreflex gain tended to be inversely related to the amplitude of the pressor response to stress. This observation needs to be confirmed in a larger series of animals, and could also be investigated in humans.