Hemodynamic response pattern predicts susceptibility to stress-induced elevation in arterial pressure in the rat

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Muller, Jay R., Khoi M. Le, William R. Haines, Qi Gan, and Mark M. Knuepfer. Hemodynamic response pattern predicts susceptibility to stress-induced elevation in arterial pressure in the rat. Am J Physiol Regulatory Integrative Comp Physiol 281: R31–R37, 2001.—Cocaine or air jet stress evokes pressor responses due to either a large increase in systemic vascular resistance (vascular responders) or small increases in both cardiac output and vascular resistance (mixed responders) in conscious rats. Repeated cocaine administration results in elevated arterial pressure in vascular responders but not in mixed responders. The present study examined the hypothesis that the pattern of cardiovascular responses to an unconditioned stimulus (UCS; air jet) is related to responses to a conditioned stimulus (CS; tone followed by brief foot shock) in individual rats. Our data demonstrate that presentation of the UCS produced variable cardiac output responses that correlated with responses to the CS (n = 60). We also determined whether individual cardiovascular response patterns to acute stress correlated with predisposition to a sustained stress-induced elevation in arterial pressure. Rats were exposed to three different stressors presented one per day successively for 4 wk and during a poststress period of 3 wk while arterial pressure was recorded periodically. Mean arterial pressure was elevated in all rats during chronic stress but, during the poststress period, remained at significantly higher levels in vascular responders but not mixed responders. Therefore, we conclude that acute behavioral stress to a conditioned stimulus elicits variable hemodynamic responses that predict the predisposition to a sustained stress-induced elevation in arterial pressure.

behavioral stress; cardiac output; systemic vascular resistance

The acute pressor response to mental arithmetic stress in humans is mediated either by an increase in cardiac output (CO) or an increase in systemic vascular resistance in most humans (6, 19, 22). Because of the response characteristics, these individuals have been referred to as cardiac and vascular responders, respectively (11, 25, 36, 47, 48). Several authors proposed that autonomic responsiveness to behavioral stress in individuals is determined primarily by genetic factors (9, 24). Others have suggested that hemodynamic responsivity to stress is predictive of which individuals will develop hypertension (19, 32, 47, 48). As yet, no longitudinal studies have linked greater vascular responsiveness in humans with the development of hypertension. However, many investigators have reported that this variable is associated with a predisposition to developing hypertension or with a family history of hypertension (9, 10, 35–37). The origins of variable hemodynamic responsiveness and its relationship to the development of hypertension are poorly understood, in part due to the lack of a good experimental model. Although several investigators have described models of stress-induced hypertension in animals (20, 23, 31, 45), these models typically require hyperresponsive strains or prolonged exposure to stress. No studies to date, however, have explored vascular reactivity to stress as a predictor for susceptibility to stress-induced elevations in arterial pressure in an animal model.

Branch and Knuepfer (4) noted hemodynamic responses in rats after administration of cocaine that were similar to those seen in humans exposed to stress. They demonstrated that cocaine elicited an acute increase in arterial pressure, which, in a subset of rats termed vascular responders, was due to a large increase in systemic vascular resistance. A second group, named mixed responders, had identical pressor responses that were mediated by an increase in both CO and a smaller increase in systemic vascular resistance. Further studies revealed that air jet stress [unconditioned stimulus (UCS)] also caused variable hemodynamic responsiveness related to those evoked by cocaine in rats (27). The pressor response to air jet was similar in vascular and mixed responders, whereas CO responses varied greatly. Vascular responders but not mixed responders developed elevations in arterial pressure with repeated administration of cocaine (5). It is not known whether the factors that determine acute response characteristics to stress or cocaine are related and correlate with the susceptibility to develop sustained stress-induced hypertension (30).

The current study was designed to examine two hypotheses. First, we predicted that variable hemodynamic responsiveness to an UCS (air jet) correlates with the...
hemodynamic responses to a conditioned stimulus (CS; hemodynamic response to a 15-s tone preceding a brief foot shock). Second, we expected that hemodynamic response variability would be related to the predisposition to develop stress-induced sustained elevations in arterial pressure such that repeated stress would elicit greater increases in arterial pressure in vascular responders compared with mixed responders.

**METHODS**

*Instrumentation.* All surgical and experimental procedures were approved by the St. Louis University Institutional Animal Care and Use Committee and followed guidelines described in the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. With an aseptic technique, male Sprague-Dawley rats (250–400 g, Harlan Sprague Dawley, Indianapolis, IN) were instrumented with a pulsed Doppler flow probe around the ascending aorta to measure ascending aortic blood flow and a femoral catheter to determine arterial pressure and heart rate as described previously (4). Briefly, in rats anesthetized with pentobarbital sodium (45 mg/kg ip), a miniaturized pulsed Doppler probe was placed snugly around the ascending aorta via a midline thoracotomy using aseptic technique. The 36-gauge insulated leads were tunneled subcutaneously to the skull, where a miniature electrical socket was mounted using dental adhesive.

After 7 to 10 days, rats were reanesthetized (using methoxyflurane) and a sterile catheter was implanted into the femoral artery for recording arterial pressure and heart rate. The CO was estimated using a directional pulsed Doppler flowmeter (Department of Bioengineering, University of Iowa). The flowmeter employed a 20-MHz incident signal with a 100-kHz sampling rate and antialiasing and auto-tracking circuits to reduce the incidence of unstable flow signals. In addition, all pulsatile flow signals were monitored continuously on an oscilloscope to verify the absence of changes in waveform. If abrupt changes were noted, the data were discarded.

Arterial pressure, heart rate, and ascending aortic blood flow in freely moving rats were recorded on a chart recorder. Changes in ascending aortic blood flow were used to estimate changes in CO. The change in systemic vascular resistance was calculated by dividing arterial pressure by the CO while the change in stroke volume was determined by dividing the CO by the heart rate. Rate pressure product was calculated by multiplying heart rate by arterial pressure. These calculations have been used to estimate hemodynamic parameters in previous studies (4, 5, 27).

*Acute stress.* Reactivity in individual rats was tested with an UCS (air jet) and a CS (tone followed by a brief foot shock). One day after arterial catheters were implanted, rats were acclimated to a Plexiglas and stainless steel test cage (25 × 30 × 30-cm, BRS/LVE, Laurel, MD) for 6 h. On the following day, rats were acclimated to the same cage for 1–2 h after a small diameter (1.77 mm ID) polyethylene tube (PE-260, Clay Adams, Parsippany, NJ) was attached to the cable connected to the socket on the skull to deliver air puffs at a distance of 10–12 mm to the face of the rat. After acclimation, six trials of a brief (1–2 s) puff of air (1.4 kg/cm²) were administered at ~10-min intervals. After the six air jet trials, the animal was subjected to 12 trials of a 15-s tone (85–90 dB) followed by a half-second shock via the test cage floor grid. The shock intensity was adjusted for each rat to elicit a flinch but not vocalization. This paradigm is similar to that described by Randall and coworkers (43). All measurements were taken within 5 s after the initiation of the tone before the ensuing foot shock. The initial response to the CS, described as the C1 response by Randall et al. (43), was recorded. Three to five days later, rats were exposed to a chronic stress procedure.

*Chronic stress.* To identify individual rats sensitive to sustained stress-induced increases in arterial pressure, we used three different stressors presented on successive days such that the animal was exposed to stressors six days a week for 4 wk. The stressors included cold water stress in which the animal was placed in a plastic cage before adding 1 cm of cold (4–6°C) water. The water was kept at this temperature for the entire 1-h cold stress period. On the following day, restraint stress was used in which the animal was immobilized in a plastic tube for a period of 1 h. Finally, on the third day the rat received 36 paired tone-shock stimuli at 5-min intervals as previously described. This series of daily stressors was repeated seven more times such that each rat received three different stressers eight times over a 4-wk period. Arterial pressure was measured immediately before each stress period whenever possible. After the chronic stress period, we recorded mean arterial pressure for 3 wk, taking six measurements over a 1-h period after a 1-h acclimation period 3–6 days/wk. Due to the prolonged experimental period, all animals were recannulated (using the right femoral artery) 2–4 days before the end of the 4-wk stress period to ensure accurate arterial pressure recordings.

*Control animals.* Eight rats were used as control subjects. Although all were cannulated for arterial pressure measurement, only five of eight were instrumented for CO determination. All animals were exposed to the acute stress paradigm (6 air jets and 12 tone-shock combinations) initially, then brought from the animal housing area to the lab daily for the 7 wk but not exposed to the stressors described above.

*Euthanasia and histologic techniques.* Animals were euthanized with pentobarbital sodium (70 mg/kg ip) after completion of the chronic stress paradigm. The hearts were fixed in formalin, sectioned, and stained with hematoxylin and eosin using standard techniques (26). An individual unfamiliar with the treatment and response characteristics of each rat examined the tissue via light microscopy.

*Data analysis.* The hemodynamic responses to the first three tone presentations were analyzed but not used to calculate the mean responses, because animals had to learn that the tone preceded the foot shock. Therefore, the responses to the six UCS and fourth through twelfth CS presentations during the acute stress period were summarized and averaged for each individual rat. With the use of CO responses, the rats were then classified as vascular or mixed responders, in a manner similar to that previously described in our laboratory (27), to facilitate the analysis of possible differences between animals at either end of the population distribution. Vascular responders had a consistent change in CO of ≤5% increase after exposure to the CS. Alternatively, rats with CO responses >5% to CS were considered mixed responders. The CO responses to acute air jet were used in a similar manner with a cutoff of 2% change to define vascular and mixed responders to unconditioned stress for Table 1 and Fig. 1. The cutoff values were derived by examining the response distribution depicted in RESULTS (Fig. 1). The cutoff values represented convenient points for separation of a primary peak from a skewed tail. The responses to the conditioned stress were less likely to change with repeated exposure and were, therefore, used to define vascular and mixed responders.
To characterize rats, hemodynamic responses at the time of peak pressure response to acute stress were analyzed using an unpaired Student’s t-test (separate variances). Arterial pressure only was measured during the chronic stress period and analyzed using an analysis of variance with repeated measures and Bonferroni’s post hoc comparison of differences at individual time points. In addition, simple regression analysis was performed to compare CO responses and changes in arterial pressure. All statistical analyses were performed using GB-STAT (Dynamic Microsystems, Silver Spring, MD). The data are expressed as the means ± SE, and differences were considered significant if $P < 0.05$.

RESULTS

Comparison of responses to UCS and CS. Rats ($n = 60$) were tested to compare hemodynamic responses to an UCS with those to a CS. Resting values for hemodynamic parameters are shown in Table 1. Within 5 s, the CS evoked a substantial increase in arterial pressure, heart rate, and variable increases in CO, systemic vascular resistance, and stroke volume (Table 1). The UCS elicited similar responses to the CS, including increases in arterial pressure, heart rate, CO, and systemic vascular resistance and a variable change in stroke volume that occurred within 1–2 s (Table 1).

Rats were divided into two groups according to the CO responses to the UCS and CS as previously described for responses to cocaine and to air jet (5, 4, 27). The distributions of the CO responses to air jet (UCS) and to tone preceding shock (CS) are shown in Fig. 1. The distributions resembled a modal peak with a skewed tail. We arbitrarily separated the peak from the skewed tail to analyze possible differences in susceptibility to sustained stress-induced increases in arterial pressure.

There was a direct correlation (as determined by linear regression) between the magnitude of the CO responses to the UCS and to the CS ($r = 0.67$, $P < 0.0001$, Fig. 2). There was no correlation for arterial pressure or heart rate responses to the CS and UCS (data not shown). There were significant differences in the CO, systemic vascular resistance, and stroke volume responses to the UCS and CS but not to the arterial pressure or heart rate changes (Table 1). Vascular responders had an elevated arterial pressure compared with mixed responders before the initial UCS testing period. There were no differences in the resting heart rate or CO in these groups.

Repeated administration of the UCS produced progressively smaller pressor responses, although the profile of the responses were not different in mixed responders compared with vascular responders (Fig. 3).

Table 1. Cardiovascular Responses to UCS and CS

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RPP, Change, %</th>
<th>CO, Change, %</th>
<th>SVR, Change, %</th>
<th>SV, Change, %</th>
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<tr>
<td></td>
<td>n</td>
<td>Resting</td>
<td>Change</td>
<td>Resting</td>
<td>Change</td>
<td></td>
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<tr>
<td>CS</td>
<td>Mixed responders</td>
<td>17</td>
<td>117.8 (2.3)</td>
<td>21.8 (2.2)</td>
<td>408 (8.8)</td>
<td>17.9 (5.1)</td>
</tr>
<tr>
<td></td>
<td>Vascular responders</td>
<td>43</td>
<td>117.9 (1.8)</td>
<td>19.9 (1.5)</td>
<td>403 (5.8)</td>
<td>9.7 (1.7)</td>
</tr>
<tr>
<td>UCS</td>
<td>Mixed responders</td>
<td>20</td>
<td>110.2 (2.4)</td>
<td>18.0 (1.7)</td>
<td>388 (8)</td>
<td>23.7 (5.8)</td>
</tr>
<tr>
<td></td>
<td>Vascular responders</td>
<td>40</td>
<td>117.1* (1.8)</td>
<td>16.8 (1.0)</td>
<td>406 (6.6)</td>
<td>13.5 (5.8)</td>
</tr>
</tbody>
</table>

Data are means ± SE (in parentheses). MAP, mean arterial pressure; HR, heart rate; RPP, rate pressure product; CO, cardiac output; SVR, systemic vascular resistance; SV, stroke volume; CS, conditioned stimulus; UCS, unconditioned stimulus. *$P < 0.05$, †$P < 0.005$ using a Student’s t-test.
In contrast, the pressor and cardiac output responses to the CS were relatively consistent over repeated trials after the first two to three trials (Fig. 3). We ascribed this to the training required for conditioning and omitted the first three trials from the calculations used to classify rats as vascular or mixed responders to a conditioned stressor. Data obtained for the CS were virtually identical on the second day and are not depicted in Fig. 3. Because of the reduced variability of the hemodynamic responses to the CS compared with the UCS, responses to the CS were used to classify rats as vascular or mixed responders during chronic stress. Rats were allowed to recover for 3–5 days before beginning the chronic stress paradigm.

Effects of chronic stress. Although most of the rats tested for acute hemodynamic responsiveness to stress were exposed to chronic stress, data from only 29 rats were compared during the 4-wk stress period and the 3-wk recovery period. The remaining rats were omitted because they had <3 wk total of arterial pressure data (pre- and poststress periods) or had no poststress data due to obstructed cannulas. The distribution of CO responses from rats used for the chronic study are depicted in Fig. 1, inset, to provide a comparison to the larger population used to compare responsiveness. Again, data at some time points (primarily week 3 of the stress period and the recovery or poststress period) were not available from all rats due to blocked arterial cannulas. The hemodynamic responses from the stress period were not different from those data obtained in the initial comparison of responses to the UCS and CS. For all subsequent analyses, animals were characterized as vascular and mixed responders according to their response to the CS.

Although the hemodynamic responses to repeated stress were not recorded during the 4-wk stress paradigm and the subsequent 3-wk recovery period, arterial pressure was recorded several times a week before exposure to the daily stressor after a 1- to 2-h acclimation period whenever arterial catheters remained patent. The changes in arterial pressures were averaged weekly in vascular and mixed responders and are depicted in Fig. 4. Repeated stress elicited a significant increase in arterial pressure in all animals over the 4-wk stress period \( P < 0.0004 \), although there were no differences between vascular and mixed responders (\( P = 0.42 \)). In contrast, the change in arterial pressure remained elevated only in vascular responders during the 3-wk poststress period (\( P < 0.0001 \)) compared with baseline (\( P = 0.0001 \)). Vascular responders had significantly greater increases in arterial pressure at 2 and 3 wk after stress compared with mixed responders using Bonferroni’s post hoc procedure. The values in parentheses represent the \( n \) value at each data point. \( n \) values varied because of occasional loss of the arterial pressure signal. *Significant differences between vascular and mixed responders (\( P < 0.05 \)).
and mixed responders during stress (P = 0.42, F = 0.68, df = 1,87). In contrast, during the entire 7-wk study, vascular responders had significantly greater increases in arterial pressure compared with mixed responders (P = 0.025, F = 5.6, df = 1,174). The difference in arterial pressure was dependent on the changes during the 3 wk after the stress presentation, which were significantly greater in vascular responders (P < 0.0001, F = 22.3, df = 1,58). During this same period, there was no significant change in arterial pressure in eight control animals (Fig. 4).

Histologic analysis of the hearts from individual rats revealed few abnormalities in either of the groups. In seven cases, a mild to moderate epicarditis was noted. This change has been noted by our laboratory in previous studies (26) and is likely to be due to alterations induced by damage to the epicardial sac during surgery to implant the aortic flow probe.

**DISCUSSION**

The present results suggest that chronic exposure to stressors causes sustained elevation in arterial pressure in a subset of rats. Susceptibility to sustained stress-induced hypertension is directly correlated with the hemodynamic response profile, particularly the relative contribution of CO and systemic vascular resistance to the pressor response. Variable CO responsiveness to stress has also been noted in humans (19, 32, 47, 48). Recent evidence has suggested that those individuals whose response to stress is primarily due to an increase in total peripheral resistance (similar to our subgroup of vascular responders) have a greater predisposition to developing hypertension or have a family history of hypertension (9, 10, 35–37) and are at greater risk of heart disease (12). The use of vascular reactivity as a risk factor for determining the development of hypertension has not been used in any longitudinal human studies. It has been suggested, however, that this may be a risk factor in the African-American population, who have both a greater propensity to develop hypertension and greater vascular reactivity to cold stress (14).

We previously demonstrated that cocaine elicited similar hemodynamic responsiveness to air jet stress in rats (27). Branch and Knupeper (5) reported that cocaine administration twice daily for 5 days caused a sustained increase (measured on the following day) in arterial pressure in vascular responders but not in mixed responders. Therefore, we hypothesized that behavioral stress would produce a greater increase in resting arterial pressure in vascular responders compared with mixed responders after exposure to chronic stress. The present results support this hypothesis. Therefore, it is possible that the rat may provide a model to understand the causes of variable responsiveness and susceptibility to sustained stress-induced hypertension.

Several models have been developed to study the causes of stress-induced hypertension. These models have typically been limited to special strains of rats (1, 31), to surgical interventions (3), or to prolonged exposure to behavioral stress (7, 15, 20, 23). The present model uses outbred Sprague-Dawley rats, a strain not known for its sensitivity to stress-induced hypertension. In previous studies from our laboratory, highly variable hemodynamic responsiveness to cocaine and to air jet stress were also observed in inbred Brown Norway and Fisher strains (unpublished data). Therefore, the varying responsiveness is not likely to be unique to this strain or this species. We propose that this may be a viable model to study the causes of sustained stress-induced increases in arterial pressure that does not require a hypersensitive strain of rat or prolonged exposure to behavioral stress (29, 30). Although our model did not produce severe hypertension, it was relatively limited in the extent of exposure to behavioral stress. Nonetheless, the data suggest a sustained effect on arterial pressure regulation in a subset of rats.

It is likely that previous studies required more prolonged stress because they were using a population of rats both susceptible and resistant to stress-induced hypertension. Indeed, several investigators describe the development of hypertension in some but not all subjects exposed to stress using rats (1, 15, 38) or monkeys (16, 21). We suggest that susceptible individuals may have characteristic hemodynamic responses to stress, although resting values may not be predictive of the propensity to develop hypertension (42).

The overall increase in arterial pressure elicited by the chronic stress paradigm was modest compared with the levels of hypertension noted in hypertensive humans or rats (3, 34, 40, 44). Most studies examining stress-induced hypertension have noted increases of pressure of 10–20 mmHg, similar to the current study (1, 15, 16, 21, 38). It is likely that the greater increases in arterial pressure noted in hypertensive humans is due to the longer duration of its development (e.g., 10–20 yr). This is more difficult to simulate feasibly in animal studies without selecting specific genetic factors that predispose individuals to develop hypertension.

Several studies proposed that hypertensive patients or those likely to develop hypertension (due to family history) had greater increases in blood pressure than normotensive patients (14, 17, 22, 36, 46). In the present study, there was no correlation between blood pressure or heart rate responsiveness to acute stress and predisposition to chronic stress-induced increases in arterial pressure. Therefore, it is likely that this strain of rat is not a useful model for studying these phenomena.

The variable responsiveness noted actually represents a continuum of hemodynamic responses. As noted in Fig. 1, the animals are arbitrarily grouped into mixed and vascular responders to create two groups and facilitate statistical comparisons of animals at either end of a wide distribution. Further examination, particularly of the extreme examples in this population may provide additional information regarding the causes of predisposition to hypertension.
Our results also suggest that the acute hemodynamic responses evoked by an UCS are directly related to responses elicited by a CS. Therefore, the type of stress may be less important than the individual responsiveness to the stressors. Indeed, our laboratory previously reported that cocaine evoked variable CO responses that were similar to those elicited by air jet (27). In fact, we noted variable CO responsiveness to a number of psychoactive agents, including amphetamine, ethanol, bromocriptine, and desipramine (5, 28, 39). These data underscore the “universal” nature of the hemodynamic response pattern evoked by a number of disparate stimuli. This is not to say that all stressors evoke similar responses, because the pattern of hormonal responses to various stressors does not support a consistent pattern (41).

The pressor response to an UCS diminishes with repeated exposure presumably due to acclimation. For this reason, we suggest that the CS elicits a more consistent response pattern in individual rats and used this response as a more reliable indication of the individual response pattern.

The histologic changes noted using light microscopy were minimal and likely to be a result of surgical implantation of the Doppler flow probe. Our laboratory has noted accentuated development of myocardial injury in vascular responders compared with mixed responders when exposed to cocaine using electron microscopy but not light microscopy (26). Therefore, we suggest that higher magnification and more detailed analysis may be required to ascertain whether myocardial injury is induced during the chronic stress paradigm.

In summary, acute pressor responses to a variety of stimuli are mediated by at least two mechanisms both in human and rat populations. Furthermore, this variable responsiveness can be used to predict sensitivity to cocaine-induced increases in arterial pressure in rats (5). An UCS can produce a similar variable acute increase; however, the pressor response diminishes with repeated exposure. The results of these experiments indicate that a CS such as the tone-shock paradigm can produce a variable acute increase similar to that seen with the UCS (air jet), yet the pressor response does not diminish over time. Finally, the hemodynamic response pattern to behavioral stress (CS) can be used to predict the predisposition to the development of sustained stress-induced elevation in arterial pressure.

**Perspectives**

At present, it is unclear whether the differences in responsiveness and predisposition to elevated arterial pressure are due to underlying genetic differences, although we recently suggested this hypothesis (30). In humans, differences in sensitivity to hypertension are likely to be determined by genetic factors, because individuals with a vascular response are more predominant in African-American men compared with Caucasian men (8, 33, 44) and in men compared with women (2, 18). Vascular responsiveness has been associated with a predisposition toward the development of hypertension, because both men and African-Americans are at higher risk for developing hypertension. Therefore, we propose that the model of response variability may allow us to examine the causes of differential responsiveness and predisposition to cardiovascular disease in an animal model. This may not only lead to new treatments but may be helpful in defining new risk factors for cardiovascular disease. Any additional information to improve clinical identification of susceptibility to cardiovascular disease would be expected to significantly improve diagnostic capability and improve health care.

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