Sympathetic and angiotensin-dependent hypertension during cage-switch stress in mice

Dexter L. Lee, R. Clinton Webb, and Michael W. Brands

Department of Physiology, Medical College of Georgia, Augusta, Georgia 30912-3000

Submitted 7 May 2004; accepted in final form 9 August 2004

Lee, Dexter L., R. Clinton Webb, and Michael W. Brands. Sympathetic and angiotensin-dependent hypertension during cage-switch stress in mice. Am J Physiol Regul Integr Comp Physiol 287: R1394–R1398, 2004.—The goal of this study was to determine the dependence of the acute hypertensive response to a novel model of acute psychosocial stress on the sympathetic and renin-angiotensin systems. Baseline mean arterial pressure (MAP), heart rate (HR), and locomotor activity were measured with telemetry in mice for a 1-h period and averaged 98 ± 1 mmHg, 305 ± 3 beats/min, and 5 ± 1 counts, respectively. Stress was induced by placing a mouse into a cage previously occupied by a different male mouse, and this increased MAP, HR, and activity in the control group by 40 ± 2 mmHg, 204 ± 25 beats/min, and 68 ± 6 counts, respectively. Each variable gradually returned to baseline levels by 90 min after beginning cage switch. Pretreatment with terazosin (10 mg/kg ip) significantly reduced the initial increase in MAP to 12 ± 6 mmHg, whereas MAP for the last 45 min was superimposable on control values. Atenolol (10 mg/ml drinking water) had no effect to blunt the initial increase in MAP but had a growing effect from 10 min onward, decreasing MAP all the way to baseline by 60 min after starting cage switch. Captopril (2 mg/ml drinking water) treatment caused a very similar response. All three treatments significantly decreased the area under the blood pressure curve, and the blood pressure effect could not be attributed uniformly to effects on HR or activity. These data suggest that our novel model of psychosocial stress causes an initial α1-receptor-dependent increase in MAP. The later phase of the pressor response is blocked similarly by a β1-receptor antagonist and an ACE inhibitor, independent of HR, suggesting that the β1-dependent blood pressure effect is due, in large part, to the renin-angiotensin system.

Mean arterial pressure; sympathetic nervous system; renin-angiotensin system

Psychosocial stress increases arterial pressure and also has been linked to chronic renal and cardiovascular disease, including hypertension (1, 9, 17, 19). Stress causes activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenocortical axis, with responses that include increases in body temperature, blood pressure, heart rate (HR), and plasma glucocorticoid concentration. Interference with either system compromises the body’s ability to respond to the stressors (2, 16, 20). The well-described effects of the SNS are mediated through α- and β-adrenergic receptors located on the heart and throughout the vascular system, which includes the stimulation of renin secretion and subsequent increase in ANG II production (3, 4, 7, 8, 10, 18).

We have a new experimental model of psychosocial stress that is caused by placing a male mouse in a cage previously occupied by another male mouse. Termed cage switch (CS), this is a modification of earlier models of stress, in which rats or mice are switched to a clean cage or a cage with a layer of water at the bottom (12–15, 21). The hypertension in those models is ~20 mmHg, but reproducible, and the unique feature of our CS model is that it capitalizes on the reproducibility and combines the more robust hypertension characteristic of psychosocial stress (~40 mmHg) but without complex caging paradigms or fighting. The pressor response is reproducible, takes over 90 min to subside, and is accompanied by significant increases in HR and motor activity. The goal of this study was to determine the roles of α- and β-adrenergic receptors in mediating these responses to stress in this new model. In addition, the effects of α- and β-blockade led us to test the hypothesis that ANG II also played a significant role in the rapid hypertensive response to stress in this model.

Methods

Animals. Procedures involving animals were approved by the Animal Care and Use Committee of the Medical College of Georgia and complied fully with those approved by the American Veterinary Medical Association Panel on Euthanasia. Surgery was performed on male C57BL/6J (Jackson Laboratories C57BL/6J 000664) mice (6 to 8 wk of age) to implant a blood pressure transmitter (Data Science, PA-C20). Briefly, the mice were anesthetized with isoflurane at 2–3% in a stream of 100% oxygen mixed with room air. A Data Sciences transmitter catheter was inserted into the left carotid artery via a 10-mm incision on the ventral neck region over the trachea, and the transmitter body was routed to a subcutaneous pocket in the midscapular region. The incision was closed with Ethicon Ophthalmic suture. After recovery, mice were housed in individual cages in the laboratory animal facilities with a 12:12-h dark-light cycle and provided with standard laboratory chow and water ad libitum. Because CS experiments were conducted from 1000 to 1500 h, baseline mean arterial pressure (MAP) was measured 19 h/day, from 1500 to 1000 h the next day. CS experiments were not begun until normal circadian rhythm was reestablished (~4–7 days postsurgery).

Stress. All mice were placed in a clean cage for 3 days before CS stress. On the day of the study, the mice were observed for 120 min in their home cages, undisturbed from the room in which they were housed before testing. These were the last 2 h of the 19-h measurement period (i.e., from 0800 to 1000 h). The CS then was begun at 1000 h by removing mice from their original cage and placing them randomly into a cage previously occupied by a different male mouse for a period of 5 h. Because we could not measure consistent effects of any treatment on the blood pressure response to stress after ~90 min, only the first 90 min following CS were analyzed for peak and area under the curve (AUC) responses.

Sham CS. As a control for handling, mice in a separate group (n = 5) were picked up and placed back in their own cage.

Address for reprint requests and other correspondence: M. W. Brands, Dept. of Physiology, Medical College of Georgia, Augusta, GA 30912-3000 (E-mail: mbrands@mec.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Experimental groups. Terazosin (α1-receptor antagonist) group (n = 5): terazosin (10 mg/kg) was administered via an intraperitoneal injection 3 h before CS stress. Atenolol (β1-receptor antagonist) group (n = 6): atenolol (10 mg/ml) was administered in the drinking water beginning 7 days before CS stress. Captopril (angiotensin-converting enzyme inhibitor) group (n = 5): captopril (2 mg/ml) was administered in the drinking water beginning 7 days before CS stress. Mice that were treated with terazosin were given a period of 2 wk for a washout period before being treated with atenolol. Likewise, mice that were treated with atenolol were given a period of 2 wk before being treated with captopril. In each case, MAP, HR, and locomotor activity returned to pretreatment levels before starting atenolol or captopril. In each case, MAP, HR, and locomotor activity returned to pretreatment levels before starting atenolol or captopril. The control group (n = 9) involved no drug treatment. Control animals were subjected to CS stress with each drug treatment group and then grouped together for analysis and plotting.

Data analysis. Data are expressed as change from average baseline values with means ± SE. The average baseline value was taken from the last 60 min before beginning CS. The first 60 min were not analyzed because the effect of intraperitoneal terazosin injection was evident there. This handling effect was confirmed in pilot studies using intraperitoneal saline injection. MAP, HR, and motor activity data were collected at 500 Hz for 5 s/min throughout the baseline and stress periods and then were averaged in 5-min intervals. MAP, HR, and motor activity are expressed as millimeters of Hg, beats per minute, and counts, respectively. Each point on the graphs represents an average of five 1-min data points for MAP, HR, and activity. The AUC was calculated with the trapezoid method over the first 45 min after starting CS. Comparisons were made with one-way ANOVA and Dunnett’s post hoc test. Means were considered significantly different if P < 0.05.

RESULTS

The baseline values for MAP, HR, and motor activity were 98 ± 1 mmHg, 505 ± 3 beats/min, and 5 ± 1 counts, respectively, during the 2 h before CS. CS caused increases in MAP, HR, and motor activity in control mice that peaked at averages of 39 ± 2 mmHg, 223 ± 13 beats/min, and 68 ± 6 counts, respectively (Fig. 1). The AUCs for MAP, HR, and locomotor activity were 1,612 ± 107, 6,211 ± 968, and 2,418 ± 218 mmHg/min, respectively. These responses occurred within 10 min after the initiation of CS stress and returned to baseline values ~2 h later (Fig. 1).

Terazosin significantly reduced MAP and locomotor activity, while increasing baseline HR. Baseline MAP, HR, and motor activity averaged 89 ± 1 mmHg, 592 ± 3 beats/min, and 1 count, respectively. Terazosin significantly reduced the peak change in MAP (22 ± 3 mmHg), HR (119 ± 47 beats/min), and motor activity (43 ± 7 counts) compared with control responses during CS stress (Fig. 1). The AUCs for MAP, HR, and locomotor activity during terazosin treatment were not
significantly different from control: 1,097 ± 115, 4,725 ± 1,293, and 1,538 ± 436 mmHg/min, respectively.

In the atenolol-treated groups, after 7 days of atenolol in the drinking water, baseline MAP, HR, and motor activity before CS stress were 98 ± 1 mmHg, 417 ± 3 beats/min, and 8 ± 1 counts, respectively, and the HR was significantly less than that of control animals. The peak change in MAP during CS stress was 37 ± 3 mmHg. The peak changes in HR and motor activity were 148 ± 20 beats/min and 43 ± 4 counts, respectively, and were significantly different from control (Fig. 2). The AUC for the MAP was significantly decreased in animals treated with atenolol (796 ± 163 mmHg/min) compared with control (1,612 ± 107 mmHg/min); however, the AUCs for HR and locomotor activity were not significantly different from control: 5,301 ± 745 and 1,643 ± 330 (mmHg/min), respectively (see Fig. 4).

Captopril treatment significantly decreased baseline MAP and HR, but not motor activity, with average values over the 1-h period of 73 ± 1 mmHg, 479 ± 3 beats/min, and 3 ± 1 counts, respectively. The peak changes in MAP, HR, and motor activity during CS stress averaged 40 ± 5 mmHg, 240 ± 18 beats/min, and 53 ± 5 counts, respectively, and were not different from control (Fig. 3). Similar to the atenolol response, the AUC for the MAP was attenuated significantly in captopril-treated animals compared with controls (914 ± 141 mmHg/min). The AUCs for HR and locomotor activity were not significantly different from control: 7,863 ± 1,351 and 2,178 ± 346 mmHg/min, respectively (Fig. 4).

When the sham switch mice were picked up and returned to their own cage, the average peak changes for MAP, HR, and motor activity were 22 ± 5 mmHg, 122 ± 9 beats/min, and 31 ± 5 counts, respectively. These all were significantly less than the control group CS response, and the time for return to control also was less. The AUC is shown in Fig. 4.

**DISCUSSION**

This study provided evidence that the rapid, peak increase in MAP following onset of psychosocial stress is due, to a significant extent, to α-adrenergic mechanisms, whereas β-adrenergic mechanisms played a quantitatively greater role be-

![Fig. 3. Comparisons of the MAP, HR, and activity responses between control and captopril-treated mice. Mice were given captopril (2 mg/ml) in the drinking water for 7 days before psychosocial stress. Captopril significantly attenuated the area under the curve for the pressor response (A) to stress but did not cause significant changes in HR (B) or activity (C). *Significant difference in the peak response (P < 0.05).](http://ajpregu.physiology.org/)

![Fig. 4. Comparisons of the area under the curve (AUC) for MAP, HR, and activity. The AUC is calculated by using the change from baseline of the MAP (A), HR (B), and activity (C) during cage-switch stress. *Significant difference compared with control (P < 0.05, ANOVA).](http://ajpregu.physiology.org/)
At the beginning ~20 min after the stress began. Moreover, the important role of β-adrenergic receptor activation in the blood pressure response was linked more closely with the renin-angiotensin system than to changes in HR.

α-Adrenergic receptor activation is a well-established mechanism for vasoconstriction and is an important mechanism for rapid increases in sympathetic-mediated increases in blood pressure or vascular tone. The SNS is also a well-known participant in the acute response to stress (4, 7, 8). It was important, however, to quantify its role in this new acute stress model that combines psychosocial stress and open-field type, i.e., CS, stress (12–15). Our terazosin data show that the rapid, peak rise in MAP to this level is due almost entirely to α1-adrenergic receptor activation, because peak MAP during terazosin treatment was only 20 mmHg and it occurred 10–20 min after starting CS, compared with 40 mmHg at 1–3 min in the control group. It also was interesting that locomotor activity was attenuated significantly. This effect of α1-receptor blockade has been described previously (11), but the cause-and-effect relationship between the blood pressure and activity responses is not known.

The effect of atenolol on the integrated response to the stress maneuver was remarkable for several reasons. First, the complete lack of effect of atenolol on the rapid upswing in MAP to ~40 mmHg during the first 1–3 min after initiating CS lends further credence to the role of α1-receptors in mediating that response. Atenolol did have a significant effect on MAP, however, but that did not start to become evident until ~15–20 min after CS. The area under the MAP curve for the atenolol group was significantly less than the control group, and that was due, therefore, to effects in the latter stages of the response.

It is important to note that this effect of atenolol was not due to suppression of the HR response to stress. During the period in which the increase in MAP in the atenolol group was becoming more and more attenuated compared with the control group response, the increase in HR from baseline was superimposable on the HR response in the control group. Atenolol did decrease baseline HR significantly, and it also blunted the initial increase in HR during the first 10–15 min after CS. However, thereafter the change in HR tracked right along with the control group response, and that was precisely the period in which the blood pressure deviation was greatest between the groups.

This raises two questions. 1) Why did HR increase at all during stress if there was significant β-receptor blockade? 2) What was the mechanism for the blood pressure effect of atenolol if it was not through an effect on HR? Regarding the increase in HR during stress, we did not quantify the degree of blockade by administering a β1-agonist, so we cannot rule out a role for transient "escape" from receptor blockade during stress. However, the nearly 100-beats/min decrease in baseline HR provides good evidence of blockade. Moreover, if the competitive antagonist were hypothesized to be overwhelmed during a stress-induced surge in sympathetic activity, then one might predict this would be most evident during the peak HR response, yet the peak increase was attenuated by ~34% and it was the sustained HR response that was not affected. Although we still cannot rule out a role for incomplete receptor blockade, another possibility is that the increase in HR during stress was due to withdrawal of parasympathetic tone rather than increases in cardiac sympathetic activity. The effects of atropine in conscious mice reported by Just and Ehmke (6) do not support a strong parasympathetic influence on baseline HR in mice, which is consistent with data from conscious rats showing that baroreflex-mediated tachycardia is due mainly to sympathetic activation (5). But it is possible that both escape from our β-receptor blockade regimen as well as parasympathetic control of HR contributed to the stress-induced tachycardia we measured in the atenolol-treated and control mice.

Regarding the mechanism for the blood pressure response during atenolol, it is important to note that despite the fact that the increase in HR from baseline was not different between groups during stress, the increase in blood pressure was markedly attenuated by atenolol. Thus there was a significant HR-independent effect of atenolol on the hypertensive response to stress. Because β1-receptor activation is an important mechanism for stimulation of renin secretion by the SNS, this suggested that renal β1-receptors may have been the most important target of atenolol in this blood pressure response. The captopril data supported this hypothesis by showing an attenuated increase in MAP during stress that was almost an identical response to that in the atenolol-treated group. Captopril treatment had no effect on the HR or blood pressure responses. Thus, even though we did not measure renin secretion, these effects of β1-receptor blockade and inhibition of angiotensin-converting enzyme provide strong evidence that ANG II played an important role in the acute hypertensive response. Previous studies suggested a role for ANG II in acute stress-induced increases in blood pressure (3, 10, 18); however, the findings are not universal. Moreover, it is not known to what extent the blood pressure response is due to peripheral actions or to central actions to modulate SNS activity. Our data do not provide insight into this aspect of the response, but they do provide strong evidence that in this unique model of psychosocial stress, ANG II plays a major role in the blood pressure response.

This study, therefore, describes a new model of acute psychosocial stress caused by placing male mice into cages that have been occupied previously by different male mice. The increase in telemetry-measured MAP followed by CS stress is ~40 mmHg and it is accompanied by significant increases in HR and motor activity. This robust hypertensive response can be broken into a rapid α1-receptor-dependent phase and a sustained β1-receptor-dependent phase, and the β1-effect on blood pressure appears to be independent of increased HR and due almost exclusively to control of the renin-angiotensin system. Further studies that quantify renin secretion, during control and atenolol treatment conditions, and the role of central vs. peripheral actions of ANG II will be needed to better delineate these relationships.

GRANTS
This study was supported by National Institutes of Health Grants HL-74167, HL-56259, HL-75625, and T32-HL-66993.

REFERENCES


