Restraint Stress
Differential Cardiovascular Responses in Wistar-Kyoto and Spontaneously Hypertensive Rats

Stuart J. McDougall, Jeremy R.A. Paull, Robert E. Widdop, Andrew J. Lawrence

Abstract—With the use of a restraint stress paradigm, both normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) underwent acute (1-hour restraint in a Perspex tube), chronic (1-hour restraint for 10 consecutive days), or no-restraint (control) stress. Rats experiencing chronic restraint were previously implanted with telemetric probes to measure heart rate and blood pressure. Basal (prestress session) cardiovascular variables did not change during the course of the study (SHR: mean arterial pressure 129±1 mm Hg, heart rate 288±4 bpm; WKY rats: mean arterial pressure 103±1 mm Hg, heart rate 285±3 bpm). Restraint caused tachycardia and pressor responses in the WKY rats and SHR, but these effects were greater in the hypertensive strain. The duration of restraint-induced tachycardia did not change in the WKY rats between acute and chronic stress; however, a graded reduction in the duration of restraint-induced tachycardia occurred in the SHR, decreasing to WKY rat levels by day 7 of the 10-day regimen. These data indicate that although the WKY rats can effectively “cope” within a single period of restraint, the coping mechanism is apparently impaired in the SHR compared with the WKY rats. A reduced capacity to cope with processive stressors may thus have an affect on cardiovascular regulation and represent an additional risk factor in hypertension. (Hypertension. 2000;35:126-129.)

Key Words: stress ■ hypertension ■ tachycardia

Although the effects of various stressors on animals are largely similar, due to hypothalamo-pituitary-adrenal and sympathoadrenal system activation, it seems that the central processes by which the response is generated, at any time for any given stressor, are highly specific. For example, as one encounters particular stressors regularly (eg, restraint/immobilization stress), some aspects of the resulting stress response (eg, adrenocorticotrophic hormone secretion) may occur to a lesser degree.1,2 This is known as coping (adaptation or habituation) and presumably occurs due to changes within the central nervous system nuclei that regulate the stress response.3

Central control of the cardiovascular system is achieved via a complex network of interconnected nuclei. Although the basic control is primarily mediated at the level of the medulla oblongata, significant supramedullary modulation also occurs.4 For example, the locus ceruleus makes up a large portion of the central noradrenergic system, which is thought to play an important role in the initiation of the stress response5 and can regulate sympathetic outflow.6

The involvement of stress in the pathophysiology of hypertension has always been suspected, although there is a lack of clear evidence documenting a direct link between stress and hypertension. Nevertheless, the hypertensive state seems to result in differential responses compared with normotensive counterparts; spontaneously hypertensive rats (SHR) show a greater increase in heart rate (HR) and blood pressure (BP) after air jet stress than do Wistar-Kyoto (WKY) rats.6 More recently, an experimental paradigm was described in which normotensive rats that had been implanted with radiotelemetric probes to monitor HR and body temperature were exposed to restraint stress.7 This type of study offers the advantage of removing additional stresses, such as the necessity to tether animals to obtain functional correlates of the stress response.

In the present study, we used the same experimental paradigm to investigate the cardiovascular effects of restraint stress in the SHR and WKY rats. The aims of the present study were to delineate any change in HR and BP in the SHR and WKY rats during acute and chronic restraint stress and to thereby determine whether the ability to cope differs between the strains.

Methods
Experimental procedures were approved by the Monash University Animal Ethics Committee and performed according to the guidelines of the National Health and Medical Research Council of Australia for animal experimentation. All rats were obtained from the Austin Hospital (Heidelberg, Victoria) and maintained on a 12-hour light/
dark cycle with standard laboratory rat chow and water available ad libitum.

**Experimental Design**

Age-matched (15 to 16 weeks old) male WKY rats (n=15) and SHR (n=15) were divided into 3 groups (n=5 per group): (1) control (no stress), (2) acute stress (60 minutes), and (3) chronic stress (60 minutes daily for 10 days). Rats subjected to restraint were housed singularly, whereas control rats maintained social contact. Previous studies have demonstrated that isolation housing of adult rats per se is not stressful; indeed, rats need to be isolated from weaning to show signs of stress/anxiety.\(^8\) The stress imposed involved confining rats inside aerated Perspex tubes (6 cm in diameter; Plastic Labs) for 60 minutes between 9AM and noon. This procedure of restraint stress was performed once for the acute study and for 10 consecutive days for the chronic study, as previously described by Sweerts et al.\(^9\)

In the group of rats undergoing chronic restraint stress, radiotelemetric probes (TA11-PAC40; Data Sciences International) were implanted into the abdominal aorta, with the animals under methohexitone sodium (60 mg/kg IP) anesthesia, 10 days before the stress regimen. Rats were housed separately, with the home cage placed on a receiver (RLA-1020; Data Sciences International), and each rat was observed and handled daily during the 10-day recovery period. On the next day, a 2-hour baseline measurement of HR, systolic BP (SBP), diastolic BP (DBP), and locomotor activity was recorded between 9AM and noon with use of the telemetry system. For the next 10 consecutive days, rats experienced restraint for 60 minutes (at the same time for each rat between 9 AM and noon), during which cardiovascular measurements were taken for 10 seconds every minute (at 500 Hz) beginning 30 minutes before and ending 30 minutes after the restraint period. Mean arterial pressure (MAP) was calculated as \[(SBP-2DBP)/3\]+DBP. Behavior was observed and noted throughout the 2-hour period.

**Statistical Analysis**

All data are represented as mean±SEM. Telemetry data were collected every minute, and changes in HR and MAP (both maximum and duration) were determined for each restraint period, with the average of the initial 30-minute prerestraint period used as baseline. The area under the curve (AUC) was calculated to provide an indication of the duration of the response. AUC data were compared with the use of 1-way ANOVA with repeated measures for analysis between the restraint stress days in each strain and 2-way ANOVA with repeated measures for analysis between the strains. Post hoc testing with a Dunnett or Newman-Keuls test was performed as appropriate. Changes in maximum responses were analyzed in a similar fashion. In all cases, \(P<0.05\) was considered significant.

**Results**

**Cardiovascular Effects**

In the radiotelemetry (chronic) group, resting MAP, which was averaged during the 30-minute control (prestress) period during 11 days, was significantly elevated in the SHR (129±1 mm Hg) compared with the WKY rats (103±1 mm Hg, \(P<0.01\)), whereas resting HR did not vary between strains (SHR 288±4 bpm; WKY rats 285±3 bpm). Consecutive periods of restraint had no effect on resting prestress HR or MAP on each day (data not shown).

On day 1 (acute stress), in both strains of rat, there were immediate increases in HR and MAP on the commencement of restraint stress (Figure 1). There were no significant differences between the strains with respect to maximal tachycardic and pressor responses on day 1 (Figure 2). In the WKY rats, at the end of the restraint period, HR and MAP values returned toward resting values but remained above baseline until the end of the first restraint session. However, in the SHR, HR was labile during acute stress, and MAP plateaued and remained near maximum during the entire restraint period (Figure 1). Thus, the duration of HR and MAP changes was significantly increased in the SHR compared with in the WKY rats (Figure 3).

On repeated periods of restraint, there were no changes during days 1 to 10 in maximum tachycardia or pressor effects within strains, although the increase in MAP in the SHR was significantly greater than that of the WKY rats when analyzed during the 10-day period (Figure 2). In the WKY rats, with multiple experiences of restraint stress, there

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**Figure 1.** Radiotelemetry recordings of HR (A and C) and MAP (B and D) for both WKY rats (A and B) and SHR (C and D) on day 0 (baseline) (♦), day 1 (●), and day 10 (◇) of restraint regimen (n=5 per strain). Dashed lines at 30 and 90 minutes indicate period of restraint. All other restraint days have been omitted for clarity.
was no significant change in the duration (as indicated by the AUC) of stress-induced tachycardia or pressor responses (Figure 3). In the SHR group, the duration of stress-induced pressor response did not vary with repeated stress but was significantly greater than that seen in the WKY rats. Interestingly, there was a significant decrease in the duration of tachycardia as stress sessions progressed in the SHR (Figure 1), such that the duration of tachycardia in the SHR gradually decreased to levels similar to those of the WKY rats by day 7 (Figure 3). Hence, there was a significant difference between duration (AUC) for HR between the strains that was most evident in the earlier days of the regimen (days 1 to 6).

Behavioral Effects
The behavior exhibited by rats during restraint stress included piloerection, pricked ears, tail stiffening, urination, defecation, increased respiration, and vocalization. On release from restraint, there was substantial activity (general exploration, rearing, grooming) during the next 30 minutes. However, the amount of activity (movement across a horizontal plane) from day 1 to 10 was not significantly different within or between strains (data not shown).

Discussion
The present study represents the first systematic comparison, with the use of radiotelemetry, of the cardiovascular responses of the WKY rats and SHR to repeated restraint stress. There are a vast number of components to any stress response. For example, increases in plasma adrenocorticotropic hormone and corticosterone levels due to restraint\textsuperscript{10,11} and in plasma norepinephrine and epinephrine levels due to immobilization stress\textsuperscript{12} have been reported. Restraint stress has also been reported to increase HR and BP,\textsuperscript{7,13} largely due to sympathoadrenal system activation. In the present study, we used HR and MAP as functional indicators of restraint-induced stress to compare the WKY rats and SHR with respect to their ability to cope with chronic restraint stress. Indeed, a differential ability to cope with restraint stress between the WKY rats and SHR was manifested in a slowly adapting reduction in tachycardia in the hypertensive group. The current data indicate that the WKY rats apparently coped more effectively with restraint stress than did the SHR. This interpretation is based on the findings that the duration of tachycardia did not change significantly as stress proceeded from acute to chronic in the WKY rats, whereas the duration of tachycardia was significantly longer in the SHR in the earlier stages of the stress regimen but gradually decreased to the levels of the WKY rats during 7 days. Similarly, the time courses of pressor responses exhibited by the WKY rats and SHR were markedly different during restraint stress, with MAP tending toward baseline during the restraint period only in the WKY rats. However, unlike HR alterations, the duration of restraint-induced hypertension within each strain did not vary from day 1 to 10. Interestingly, Chen and Herbert,\textsuperscript{7} using a similar form of restraint as that used in the present study, also reported that the duration of
tachycardia decreased during chronic stress, but they used a different normotensive rat strain (Lister Hooded rats). However, the maximum changes in HR were markedly less than those in the present study, and no BP data were presented in that previous study.

Collectively, these observations demonstrate plasticity in HR during restraint stress and that hypertension per se may impair the ability to cope with progressive stress sessions, as demonstrated by the slow adaptation of stress-induced tachycardia in the SHR. However, there was no modulation of stress-induced pressor activity, which may reflect the fact that a number of factors are likely to regulate BP (e.g., sympathetic activation, cardiac output, total peripheral resistance, structural remodeling) as opposed to the predominant neural influences that determine HR.

As noted earlier, HR is primarily under neural control, and as such, the present findings are clearly suggestive of a process of neuronal adaptation within brain nuclei that is associated with cardiovascular regulation. It would therefore be of interest to perform molecular/biochemical studies within rat brains after such stress paradigms in an attempt to determine neurochemical correlates for the observed physiological responses. Such a strategy clearly may be indicative of potential therapeutic targets for specific cardiovascular disorders characterized by dramatic changes in HR (e.g., panic disorder, acute anxiety, sympathetic activation associated with depression).

In conclusion, it was found that the WKY rats are able to cope with restraint stress to the extent that HR and MAP almost normalize within the first encounter, whereas the SHR require multiple experiences before stress-induced tachycardia adapts to the levels of the WKY rats. Moreover, the mechanisms regulating such responses are likely to be highly specific. Therefore, although the observed cardiovascular changes occurring subsequent to restraint stress indicate the possibility of impaired coping mechanisms in the hypertensive state, it remains to be determined which neural circuits and transmitter systems are implicated in this process. Such information will clearly be of value in drug discovery programs.

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References
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