Rapid Eye Movement Sleep Deprivation and Hypertension

Genetic Influence

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We studied the importance of genetic predisposition in the development of stress-induced hypertension in the spontaneously hypertensive rat (SHR), Wistar-Kyoto (WKY) rat, and borderline hypertensive rat (BHR; first-generation offspring of SHR and WKY). Rats were submitted to seven 72-hour sessions of rapid eye movement sleep deprivation (REM-sd) every other week during 13 weeks. Tail arterial pressure was determined throughout the experiment. At the end of the study, mean arterial pressure (direct measurement), sympathetic activity (acute blockade with propranolol and phentolamine), and ventricular weight were determined. Results showed that REM-sd induced sustained hypertension only in rats with a partial predisposition to developing hypertension (BHRs). Values of tail arterial pressure at the end of the study were BHR REM-sd, 175±1.6 mm Hg and control BHR, 155.9±0.9 mm Hg, p<0.05; SHR REM-sd, 219±2.6 mm Hg and control SHR, 211.9±3.4 mm Hg, NS; WKY REM-sd, 123.9±2 mm Hg and control WKY, 125.4±2.2 mm Hg, NS. Stressed groups showed higher reduction of mean arterial pressure than their controls when submitted to sympathetic blockade (SHR REM-sd, -75.7±13.2 mm Hg and control SHR, -60±4.5 mm Hg, p<0.05; BHR REM-sd, -38.4±3.6 mm Hg and control BHR, -24.3±2.1 mm Hg, NS; WKY REM-sd, -34.4±2.5 mm Hg and control WKY, -25.6±3.3 mm Hg, NS). REM-sd increased ventricular weight in all strains. These increments showed no correlation with blood pressure. In conclusion, chronic and intermittent REM-sd induced sustained hypertension only in rats with a partial predisposition to developing hypertension (BHRs) and increased ventricular weight in all strains. (Hypertension 1992;19[suppl II]:II-202-II-206)

A clear relation between stress and hypertension has proved difficult to show. In animal species, acute stress almost always induces blood pressure (BP) elevations. However, in the majority of studies, chronic stress has failed to induce sustained hypertension. The models of stress used and the genetic susceptibility to the development of hypertension have been suggested to be responsible, at least in part, for the difficulty in causing this form of hypertension. Food-shock conflict can increase BP only in the Dahl strain of salt-sensitive rat. Immobilization, sensorial stimuli, or exposure to a cold environment causes chronic BP elevation in spontaneously hypertensive rats (SHRs) but not in genetically normotensive Wistar-Kyoto (WKY) rats. Furthermore, Lawler et al. have shown that shock-shock conflict paradigm induced sustained hypertension only in rats with a partial predisposition to developing hypertpension (borderline hypertensive rat, BHR). In these animals, BP remains elevated even after discontinuation of stress and is associated with cardiac hypertrophy. Thus, these data suggested that genetic inheritance may facilitate stress-induced chronic hypertension.

To further analyze the role of genetic influence on stress-induced hypertension, we submitted three strains of rats (SHR, maximal genetic background to developing hypertension; WKY, minimal genetic background; and BHR, partial genetic background) to several stress sessions induced by rapid eye movement sleep deprivation (REM-sd). We used REM-sd because we previously observed that this model of stress can cause significant BP increases in normotensive rats. The influence of REM-sd on sympathetic activity and cardiac ventricular weight was also determined.
Methods

Fifty animals (18 WKY, 14 SHR, 18 BHR) aged 11 weeks and weighing 170–290 g were studied. BHRs were the F1 offspring of male SHRs and female WKY rats. Rats were generously provided by the animal facilities of the Escola Paulista de Medicina and Faculdade de Medicina de Botucatu, São Paulo, Brazil. They were kept at room temperature with free access to tap water and solid diet (Agrovita, São Paulo, Brazil).

Experimental Protocol

Experimental groups of each strain were WKY REM-sd (n=9), BHR REM-sd (n=8), and SHR REM-sd (n=7). These animals were submitted to seven 72-hour stress sessions at 2-week intervals. The total study period was 13 weeks, and the evaluation of sympathetic activity was carried out (see below) during the last week. During REM-sd sessions, rats were placed on a small platform whose top just protruded above the surface of surrounding water. The platform was so small that, with the loss of postural muscle tone at the onset of each REM period, the animal awakened as it began to fall toward the water. Thus, this platform procedure deprived the rat of REM sleep. Control groups (C) were WKY-C (n=9), SHR-C (n=7), and BHR-C (n=10). All control groups were followed for the same period and had BP determined as in the experimental groups, but they were not submitted to REM-sd stress and were kept in their regular plastic cages.

Chronic BP changes were assessed by tail arterial pressure (TAP) using a tail microphonic method in an unanesthetized animal. TAP was determined at the beginning and end of the weeks in which animals were not submitted to stress. Results are presented as the mean of these two measurements.

Pharmacological Blockade of the Sympathetic Nervous System

At the end of the experiment, an acute pharmacological blockade of the sympathetic nervous system (SNS) was performed. For this purpose, catheters (PE-10) were placed into the femoral artery and vein of all experimental and control rats with rats under ether anesthesia. Twenty-four hours later, with the unrestrained animals placed in individual cages and with free access to water and food, mean arterial pressure (MAP) was recorded with a physiograph (model 900, Gould Instruments, Cleveland, Ohio) with a Statham P23AA pressure transducer connected to the arterial catheter. After a 1-hour control period, SNS blockade was induced by intravenous administration of propranolol (2 mg/kg, Ayerst Laboratories, Ft. Dodge, Iowa) and phentolamine (10 mg/kg, CIBA-GEIGY Laboratories, Summit, N.J.). Both drugs were administered with a 30-minute interval. MAP was recorded for 90 minutes after the initial injection. Results are expressed as differences in MAP values before propranolol and 60 minutes after phentolamine administration.

After sympathetic blockade was completed, animals were anesthetized, and the heart was removed and rinsed with saline. Ventricular weight (left and right) was determined after atria and vessels were removed. The right adrenal gland and testis were also removed and weighed. The stomach was incised, and the presence or absence of gastric ulcers was analyzed in a fresh tissue preparation.

Results are expressed as mean±SEM. TAP variations of each group throughout the experiment were analyzed by repeated-measures analysis of variance. One-way analysis of variance was used at the end of the study to compare differences in MAP, sympathetic activity, and ventricular, testicular, and adrenal weights among all groups. Multiple comparisons were made with Duncan’s multiple range test. Student’s t test for unpaired observations was used to compare TAP values of experimental versus control groups for each strain. Pearson’s correlation test was used to investigate the correlation between ventricular weight and BP or decreases in MAP induced by SNS blockade. Differences between means were considered significant at a value of \( p < 0.05 \).

Results

TAP values of all groups studied during the 12-week period are depicted in Figure 1. At the 13th week, TAP was not determined because an acute SNS blockade was performed. TAP values during the weeks when REM-sd was induced (weeks 1, 3, 5, 7, 9, and 11) are not shown because acute effects of stress were not the aim of the study. As can be seen in Figure 1 (bottom tracings), WKY groups were normotensive at the beginning of the study and exhibited only small fluctuations of TAP throughout the 12-week period. In the SHR-C group (top tracing), TAP increased progressively during the study. In stressed SHRs, a similar rise in TAP was observed. REM-sd did not induce an additional increment in TAP and thus did not change the expected BP curve. On only one occasion (10th week), TAP of the stressed SHR group was significantly higher than that of the control. On the other hand, in the BHR strain (middle tracings), chronic and intermittent REM-sd caused significant increases in TAP when compared with TAP values of BHR-C or with baseline. Important and progressive increments in TAP were observed by the fourth week. MAP values (direct measurement) obtained at the end of the study confirm these results. MAP values in stressed BHRs were higher than those of the BHR-C group (155.9±3.7 and 139.3±2.8 mm Hg, respectively; \( p < 0.05 \)). No significant difference in MAP values of experimental and control WKY groups was observed (114.7±2.1 and 107.2±3.1 mm Hg, respectively). MAP values in the stressed SHR group were even lower than those of the BHR-C group (155.9±3.7 and 139.3±2.8 mm Hg, respectively; \( p < 0.05 \)). Figure 2 shows values of MAP drops induced by SNS blockade. As can be seen,
these drops were more intense in all stressed groups. However, statistical significance was obtained only in SHR groups (SHR REM-sd, $-75.7 \pm 13.2$ mm Hg and SHR-C, $-60 \pm 4.5$ mm Hg; $p<0.05$).

Table 1 contains values of ventricular, testicular, and adrenal weights for all groups. It can be seen that chronic and intermittent REM-sd caused statistically significant increases in ventricular weight in all groups. Ventricular weight showed no correlation with MAP values. Only in the WKY strain was an inverse correlation between ventricular weight and MAP fall during SNS blockade found ($r=0.58$). Adrenal hypertrophy occurred in all stressed groups, but this increment in adrenal weight was significant only in the SHR group. Testis atrophy was observed only in stressed WKY rats. All stressed groups had a higher incidence of gastric ulcer when compared with controls (WKY REM-sd, five of nine; WKY-C, two of nine; BHR REM-sd, three of eight; BHR-C, none of ten; SHR REM-sd, two of seven; SHR-C, none of seven).

Discussion

Our results show that chronic and intermittent stress induces significant and sustained BP elevation in rats with a partial predisposition to developing hypertension (BHRs). On the other hand, rats with a maximal (SHRs) or minimal (WKY rats) genetic inheritance to becoming hypertensive showed no changes in their BP curve patterns. Our findings agree with those obtained in BHRs submitted to shock-shock conflict.8-9 In these animals, a chronic and sustained hypertension was observed even after several weeks of stress interruption. Thus, BHRs appear to be a model suitable for the study of the chronic effects of stress on BP. According to our data, an increase in sympathetic activity cannot explain the additional increment in BP observed in stressed BHRs, because WKY REM-sd and SHR REM-sd also exhibited increases in sympathetic activity with no modification in their BP curve profiles. An increase in vascular peripheral resistance with no modification in cardiac output and increase in sympathetic activity of renal nerves has been described in stress-induced hypertension in BHRs exposed to a shock-shock conflict,12-13 but we cannot analyze the importance of these mechanisms because we did not measure these parameters.

Data from the WKY group reinforce the hypothesis that animals with no genetic background to developing hypertension are more resistant to increased BP after being stressed. Accordingly, no significant changes in BP were observed after 12 weeks of REM-sd. Similar results were obtained in normotensive rats exposed to shock-shock conflict,8 cold environment,7 or immobilization.5-7 In the WKY group, testis atrophy, which did not occur in stressed BHRs or SHRs, was observed. Reduction in testosterone levels, which is associated with lower BP values,14 may have played a role in the failure of
REM-sd to induce BP elevation in WKY rats, because they developed testis atrophy.

The SHR group also did not show changes in their natural BP curve profile. Thus, REM-sd does not cause further BP increases in SHRs. Our results agree with those obtained in SHRs submitted to psychosocial stress or immobilization stress. Previous work showed that several models of stress (visual, auditory, or electric shock) cause additional BP increments in SHRs. We do not have a clear explanation for this discrepancy with our results. Differences in BP levels before the stress sessions could have influenced the different outcomes. In our study, animals with an initial BP of 188 mm Hg were used, whereas animals with lower initial values (166 mm Hg) were used in the other study. It is possible that the model of stress we used was not capable of causing additional increments if BP levels have naturally reached values equal to or higher than 188 mm Hg. However, in young (5 weeks old) SHRs submitted to REM-sd, no anticipation of BP elevation or further increments in BP were observed (unpublished observations). Thus, the different method of stress we used (REM-sd) may explain the absence of BP elevation in SHRs.

At the end of the study, we observed that all stressed groups developed ventricular hypertrophy. The increase in ventricular weight did not correlate with BP levels. This result coincides with previous reports that showed no relation between ventricular weight and BP. The role of the SNS as a factor in the development of ventricular hypertrophy is not clear. In our study, a good correlation between sympathetic activity and ventricular weight was observed only in the WKY strain. Thus, our data suggest that REM-sd may activate other mechanisms that may cause ventricular hypertrophy.

In conclusion, chronic and intermittent REM-sd causes sustained and progressive increments in BP only in animals with a partial predisposition to developing hypertension (BHRs). Furthermore, REM-sd also induces ventricular hypertrophy even when no increases in BP are observed.

**References**


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