Augmentation of Spontaneous Hypertension by Chronic Stress in Rats

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Although genetic elements are involved in essential hypertension, it is very likely that many environmental factors influence the development of the genetic factors and determine the course of this disease. Among various environmental factors recent interests have been directed toward the correlation between emotional stress or mental factors and hypertension from the view point of psychosomatic medicine.

On the other hand in the field of experimental pathology on hypertension successful induction of hypertension by stress such as auditory stimuli have been reported. These successes, however, are only restricted to some special animals. These facts indicate that both genetic and environmental factors are responsible for the development of hypertension.

In order to clarify the relationship between genetic and environmental factors, we have applied several kinds of chronic stresses to Spontaneously Hypertensive Rats (OKAMOTO and Aoki), which develop hypertension without any treatment in 100 per cent. The purpose of this article is to report the effects of chronic stress on the existing spontaneous hypertension or its development, and on hypertensive pathological lesions in these rats.

Materials and Methods

Spontaneously hypertensive rats (hereafter abbreviated as SHR) used in these experiments were 94 males and 33 females. Most of them were F₁₁ and F₁₈ rats, the offspring obtained by the repetitional matings of a male (T10202) and a female (T10204) of F₁₀ rats in the pedigree previously reported. The other SHR were F₁₆, 160112-115, 160142-145, 160159-163, 160223-225, 160260-263, 160303-306, supplied by Okamoto's lineage in our department. As the control normotensive rats (hereafter abbreviated as NR) 99 male and 10 female age-matched rats of Wistar strain were used. They were supplied by the Animal Center Laboratory of Kyoto University, from the strain of which SHR had been separated. SHR and NR were fed with stock chow diet CA-1 (Japan CLEA Co.) and tap water for drinking. Both SHR and NR except for cold exposed groups were housed in the same animal house under the stable conditions of temperature (22-25°C) and humidity (50-60%).

1) Immobilization (restraint)

In SHR and NR, the siblings were divided into stress and nonstress groups, respectively from 70 to 90 days after birth. In total of 9 series of experiments stress group consisted of 54 SHR (38 males and 16 females) and 38 NR (33 males and 5 females), while nonstress group included 44 SHR (27 males and 17 females) and 28 NR (23 males and 5 females). SHR and NR of the stress group were restrained supinely on boards for 2-10 hours daily, 100-130 hours in total during 20-30 days. The restraint boards were devised to support the rats in a supine position by their paws with strings and to fix their neck with collars which prevented them from biting their paws.

2) Combined visual, auditory and electric stimuli

The siblings of 40-day old SHR and NR were divided respectively into a stress group (4 male SHR and 5 male NR) and a nonstress group (4 male SHR and 10 male NR). The stress groups were put in

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"stress" cages and exposed to randomly combined stress of a high-pitched buzzer (duration 3–7 sec, 5–6 times per min.), flickering of incandescent lamps of 100 V, 100 W in the cages (40–60 times per min.) and electric shock from the electrified floors of the cages (20–40 V alternating current of 60 Hz, duration 0.5–1 sec, 5–6 times per min.) for 1–4 hours daily from the 40th day up to the 18th week after birth. The stimuli were randomly imposed by our modified shocking unit, the circuit diagram of which is shown in Fig. 1.

3) Chronic cold exposure

One or two rats of stress groups (10 male SHR and 15 male NR) were housed in each cage kept in a cold environment (2–10°C) from 3 months after birth for 7 weeks. The nonstress group consisted of 11 male SHR and 13 male NR from the same siblings as the stress groups. They were kept at room temperature in the above-mentioned animal house.

Blood pressure was measured indirectly at tail once weekly from at least 3 weeks before stress-loading till 1 to 10 months after the experiment. During the experiment stress was not loaded on the day when blood pressure was measured.

The organs of the sacrificed or autopsy cases were histologically examined (only in the immobilization experiment, but not in other stress groups).

RESULTS

1) Immobilization (Fig. 2)

During chronic stress-loading SHR showed a decrease in body weight, while their blood pressure was prominently increased over 210 mm Hg.

Fig. 2. Effect of chronic stress (immobilization) on blood pressure in spontaneously hypertensive rats.
Notes: Statistically significant difference
from the value of the stress nonloaded SHR (#; p<0.001, ++; 0.001<p<0.01),
from the value of the stress loaded NR (•; P<0.001, ••; 0.001<p<0.01),
from the value of the stress nonloaded NR (+; p<0.02<p<0.05).
For the details of loaded stress, see text.

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Hg in 8 out of 38 rats and sometimes reached 260 mmHg. One case with such severe hypertension presented an abnormal posture like decortication rigidity due to apoplexy on the 20th day of stress-loading (Fig. 5). In SHR which did not show a marked increase in blood pressure during chronic stress-loading, blood pressure was prone to increase after the final stress when their body weight recovered. In NR only some of the stress group showed a slight increase in blood pressure during or after chronic stress-loadings. Their blood pressure one month after stress loading, however, was not different from that of the nonstress group. The data were summarized in Fig. 2 (the data are expressed in M±SE. SE is used only in this figure because of enough number of the cases observed.) and the detailed data were as follows.

In all 9 series of experiments the stress and nonstress groups of male SHR had no difference in blood pressure before stress-loadings (167±10, 166±10 mmHg, M±SD) and the former showed a significantly (p<0.001) higher blood pressure (196±19 mmHg) than the nonstress group (178±14 mmHg) 2 or 7 days after stress-loadings. The increments during this period in the former and the latter group were 29±16 and 12±10 mmHg and there was a significant (p<0.001) difference between them. Still one month after stress-loadings the increments were significantly (p<0.001) different between them (30±16, 14±12 mmHg) and their blood pressures were 198±20 and 181±13 mmHg, respectively. On the other hand, the blood pressure of the stress and nonstress groups in male NR (122±6, 119±6 mmHg) were not different before stress-loadings and the former group had higher (p<0.05) blood pressure (132±10 mmHg) than the latter (127±5 mmHg) after the chronic stress, but the increments during this period (11±10, 8±6 mmHg) were not significantly different.

In female SHR also, the stress group showed a significant (p<0.001) increment (29±11 mmHg) and a significantly (p<0.001) higher blood pressure (187±14 mmHg) in comparison with the nonstress group (10±8, 170±12 mmHg) after the chronic stress and there were still significant (p<0.01) differences in the blood pressure and the increment between them. In female NR, however, there were no significant

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difference in blood pressure after the chronic stress between the stress and nonstress groups. These results showed that the effect of chronic stress on blood pressure was significantly greater and was maintained longer in SHR than in NR.

2) Combined visual, auditory and electric stimuli (Fig. 3)

Blood pressure in the stress and nonstress groups of SHR (127±1, 129±13 mmHg) was not different before stress-loadings and became significantly different (p<0.01) between them (181±8, 164±6 mmHg) in the 5th week of stress-loadings. This difference, 17-31 mmHg in the mean, was maintained thereafter and finally the blood pressure in the former (204±8 mmHg) was significantly (p<0.01) higher by 29 mmHg in the mean than the latter (175±2 mmHg).

NR showed no difference before the stress in the stress and nonstress groups (113±8, 109±7 mmHg) and in the 6th week of stress-loadings the difference between them (138±6, 127±5 mmHg) was 11 mmHg in the mean and significant (p<0.05), and ranged from 11 to 15 mm Hg thereafter. After the final stress the blood pressure in the stress group (141±7 mmHg) was significantly (p<0.05) higher by 15 mm Hg in the mean than that in the nonstress group (126±6 mmHg).

These results revealed that the chronic stress of this kind affected blood pressure in both SHR and NR, and that the increment was greater in SHR than in NR.

3) Chronic cold exposure (Fig. 4)

Blood pressure in the exposed and nonexposed groups was not different before cold exposure in SHR (172±16, 173±17 mmHg) and NR (126±9, 125±6 mmHg). In the 3rd week of cold exposure the exposed SHR showed a significantly (p<0.05) higher blood pressure (186±16 mmHg) than the nonexposed group (171±13 mmHg), while there was no significant difference between two group in NR (128±9, 127±4 mmHg). In the 7th week, the difference was clear (p<0.01) between the exposed and nonexposed groups in SHR (207±15, 182±21 mmHg). However, no difference was noted in two NR groups (132±10, 128±6 mmHg) again.

Fig. 4. Effect of chronic cold exposure on blood pressure in spontaneously hypertensive rats.
Notes: SHR: spontaneously hypertensive rats, NR: normotensive control rats. Statistically significant difference from the value of non-exposed groups: ††; p<0.01, +; 0.01<p<0.05.
For the details of experimental conditions, see text.

This experiment provided an evidence that chronic cold exposure slightly raised blood pressure in SHR, while it exerted no remarkable influence in NR.

4) Hypertensive vascular lesions in stress (immobilization)-loaded SHR

Stress-loaded SHR developed severe hypertension (over 210 mmHg) during or after the immobilization stress and maintained it even 1 month after in 8 out of 38 males (21%), while only one out of 24 nonstress SHR (4%) devel-

Fig. 5. Cerebral hemorrhage in two stress loaded SHR. 11902: A male SHR developed severe hypertension (blood pressure was 260 mmHg and frequently scaled over), suddenly showed abnormal posture like decortication rigidity on the 20th day (95 hours) of stress-loading (immobilization) and was sacrificed. 11910: A male SHR maintained severe hypertension around 240-250 mmHg for 2 months after stress-loading (immobilization) and died from extensive cerebral hemorrhage.

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opposed such hypertension. Chronic stress augmented spontaneous hypertension or accelerated the development of severe hypertension in younger age and in higher incidence, and continued to do so.

Pathological studies revealed hypertensive cardiovascular lesions in almost all of SHR with such severe hypertension. The incidence of hypertensive lesions in histologically examined 31 cases (19 males and 12 females, all SHR of the earlier 6 series of immobilization experiments) was as follows: Two cases of gross cerebral hemorrhage (Fig. 5), 2 cases died of abdominal hemorrhage from mesenteric arteries (Fig. 7), 1 case of retinal bleeding, hypophyseal bleeding, and hemorrhagic infarction of adrenal glands (Fig. 11), respectively. Totally, macroscopical bleeding lesions were noted in 5 cases (16%). Other lesions were cerebral softening (1 case) (Fig. 6) microscopical but rather extensive myocardial scar or fibrosis (2 cases) (Fig. 10), periarteritis nodosa and/or sclerotic arterial lesions of mesenteric arteries and other visceral vessels (8 cases, 26%) (Fig. 8), and nephrosclerosis (7 cases, 23%) which consisted of malignant nephrosclerosis (4 cases) (Fig. 9) with angionecrosis and benign nephrosclerosis (2 cases). Angionecrosis in the arteries of various organs such as testicular and thymic arteries was also noted in the cases with malignant hypertension.

On the other hand, the nonstress group of SHR, 3 or 4 months after birth, that is, 1 or 2 months after the development of hypertension, showed only mild hypertensive lesions. Although the incidence of vascular lesions was very high in older SHR as reported in another article, only 2 cases of periarteritis nodosa

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and 1 case of benign nephrosclerosis were noted in 27 nonstress SHR examined. There was no hemorrhagic lesions in these SHR. No hypertensive lesions were noted in stress and nonstress groups of NR.

These observations provided an evidence that chronic stress not only augmented the spontaneous hypertension but also aggravated it in younger age or accelerated the malignant change of it.

5) Organ weight and endocrine organs in stress (immobilization)-loaded SHR

As for the organ weight, detailed data of which were reported previously¹, the stress group of SHR showed a rather marked increase in adrenal weight and a decrease in thymus

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weight in comparison with the nonstress group of SHR or stress-loaded NR.

Hypertrophied adrenals in stress-loaded SHR appeared likely to show an exaggerated histological response to stress compared with stress-loaded NR. As shown in Fig. 11, typical hyperplasia of the fascicular zone consisting of clear cells, hyperemia and even hemorrhagic infarction were noted in them.

In the pituitaries of stress-loaded SHR, hyperplasia, degranulation, vacuolation or hyalinization of basophils were more frequently noted than in those of stress-loaded NR or nonstress group of SHR. One case of stress-loaded SHR which died 9 months after the chronic stress showed an adenomatous hyperplasia of chromophobe cells accompanied with marked bilateral adrenal hypertrophy.

Fig. 11. Adrenal hypertrophy in stress (immobilization) loaded rats (HE × 20).
A. A nonstressed male control (BP 124 mm Hg, weight of adrenals 37 mg).
B. Adrenal hyperplasia in a male control rat sacrificed on the 20th day of stress-loading (BP 123 mmHg, weight of adrenals 60 mg).
C. "Hemorrhagic infarction" of the markedly hypertrophic adrenal in a male SHR sacrificed on the 20th day of stress-loading (BP 260 mmHg, weight of adrenals 158 mg).
D. Prominent adrenal hypertrophy with hyperemia in a stress-loaded female SHR (BP 200 mmHg, weight of adrenals 142 mg).
DISCUSSION

This experiment has proved that such chronic stress as immobilization, combined visual, auditory and electric stimuli and cold exposure augment spontaneous hypertension which develops without any treatment and aggravates hypertensive lesions. On the other hand, the same chronic stress induced only a slight and transient elevation of blood pressure in normotensive Wistar rats and failed to develop sustained hypertension.

FARRIS et al. succeeded in inducing so-called audiogenic hypertension only in emotional gray Norway rats. HALL and HALL observed that chronic stress by electric shock slightly increased blood pressure only in a few or some animals and augmented the vascular lesions in hormone-induced hypertension. DAHL et al. reported that various chronic stresses failed to enhance the existing hypertension in salt-sensitive rats. When we consider the present results together with these other authors' reports, we come to a conclusion that genetic elements, diathesis or other intrinsic factors of the animals employed in the experiment are rather important for stress-induced augmentation of hypertension. It is further speculated that genetic factors or diathesis, in cooperation with environmental factors, contribute to the induction of hypertension in experimental neurosis in animals or to the induction or aggravation of human hypertension by stress.

OKAMOTO and his coworkers' extensive studies on the mechanism of development of hypertension in SHR have provided some evidences suggestive of dysregulation of autonomic nervous system, i.e., sympathetic-vasomotor and -adrenomedullary systems, and of slight overactivity of endocrine systems, including hypophyseo-adrenocortical and -thyroidal axes. The involvement of both endocrine and autonomic nervous systems in the mechanism of spontaneous hypertension indicates that the hypothalamo-medullary portion, i.e., central nervous system concerning the regulation of autonomic nervous and endocrine systems, might possibly be responsible for the development of this hypertension.

On the other hand, as cardiovascular responses elicited by electric stimulation of hypothalamic defense area were similar to alerting response under stress conditions, FOLKOW and RUBINSTEIN applied chronic intermittent electric stimulation of the hypothalamic defense area instead of chronic stress-loading and observed a slight elevation of blood pressure. This defense area has a close anatomical connection with the vasomotor center. Extensive hypothalamic destruction including this area decreased blood pressure slightly in normotensive Wistar rats. In SHR, enzyme histochemical studies revealed intense activity of some enzymes in the several nuclei of the postero-medial part of the hypothalamus and brain stem compared with the findings on control rats. When the fiber connection between posterior hypothalamus and the lower part of the brain was partly cut at the posterior hypothalamo-mesencephalic level in an acute experiment, slight but abrupt fall of blood pressure was observed and this decrease was greater in SHR than in NR. These evidences support the idea that intrinsic disorder of the center of autonomic nervous system participates in the development and maintenance of hypertension and that this center is prone to be activated by stress and augments or aggravates hypertension through sympatho-vasomotor or -adrenomedullary system. On the contrary, the results of the present experiment consist with hypothetically

![Diagram of stress and hypertension](image-url)

Fig. 12. Stress and hypertension (revised from Mills, L. C.)

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proposed dysregulation of this part of the central nervous system.

The systems involved in the response to stress are summarized in relation to hypertension as shown in Fig. 12. Immobilization and visual, auditory and electric stimuli are suitable stressors to give emotional stress and to activate these systems. Not only sympathovasomotor or sympatho-adrenomedullary systems are activated by stress in SHR as mentioned above, but also hypophyseo-adrenocortical system is mobilized and is supposed to be involved in the augmentation of hypertension in SHR, because histological response to stress such as adrenocortical hypertrophy, hyperemia and hemorrhagic infarction appeared more intense in SHR than in NR.

By cold exposure thyroid function is activated and catecholamine is mobilized. In SHR, thyroidal release rate was increased greater than in NR by cold exposure and this difference was not diminished after adrenalectomy or pharmacological sympathectomy. Consequently, thyroidal function may also participate in augmentation of existing hypertension in SHR.

Aggravation of cardiovascular lesions is probably induced by cooperative effects of vascular spasm of sympathetic origin and hormonal environment proper to SHR or hormone induced metabolic changes, because stress-induced vascular lesions are reported to be severer in hormone-induced hypertension.

Although hypothalamo-sympatho-vasomotor and/or -adrenomedullary systems and hypothalamo-hypophyseo-adrenocortical and/or thyroidal axes are speculated to be involved in the stress-induced augmentation or aggravation of spontaneous hypertension, other possibilities cannot be eliminated and quantitative evaluations of the above factors are to be analyzed in the future.

**Summary**

The alteration of blood pressure in Spontaneously Hypertensive Rats (Okamoto and Aoki, hereafter abbreviated as SHR) was observed under various kinds of stresses and their hypertensive vascular lesions were histologically examined.

1) **Effect of immobilization** Seventy to 90 day-old SHR (male 38, female 16) andagematched normotensive rats of Wistar strain (male 33, female 5, hereafter abbreviated as NR) were restrained supinely on a board for 2-10 hours daily, 100-130 hours in total during 20-30 days. a) Stress-loaded SHR showed a highly significant increase ($p < 0.001$) in blood pressure (male $29 \pm 16$, female $29 \pm 11$ mmHg) at the end of a series of stress-loadings in comparison with either control SHR (male $11 \pm 8$, female $10 \pm 8$ mmHg) or control NR (male $8 \pm 6$, female $5 \pm 3$ mmHg), and maintained it even one month after the final stress, while a slight elevation of blood pressure ($11 \pm 8$ mmHg) was observed only at the end of the stress-loadings in male NR. b) Stress-loaded SHR developed severe hypertension (over 210 mmHg) during or after the stress-loadings in 8 out of 38 males, and pathological studies made on them revealed cerebral hemorrhage, abdominal hemorrhage, retinal bleeding, malignant and benign nephrosclerosis, periarteritis nodosa and angioneerosis in the various organs, some of which corresponded to the lesions in malignant hypertension.

2) **Effect of combined visual, auditory and electric stimuli** SHR and NR were loaded with a combined stress of high pitched buzzer (duration 3-7 sec., 5-6 times per min.), flickering of incandescent lamp (100 V, 100 W, 40-60 times per min.) and electric shock (20-40 V Alternating current of 60 Hz, 0.5-1 sec., 5-6 times per min.) for 1-4 hours daily from the 40th day up to the 18th week after birth. a) Stress-loaded NR (male 5, $141 \pm 7$ mmHg) showed a significantly ($p < 0.05$) higher blood pressure (by $11-15$ mmHg) than control NR (male 10, $126 \pm 7$ mmHg) after the 6th week following the onset of the stress-loadings. b) Rise in blood pressure in stress-loaded SHR (male 4, $205 \pm 8$ mmHg) was significantly ($p < 0.01$) greater than that in control SHR (male 4, $175 \pm 5$ mmHg), and the increment of blood pressure ($17-31$ mmHg) in the former compared with the latter was evidently larger in comparison with that ($11-15$ mmHg) of stress-loaded NR compared with control NR.

3) **Effect of chronic cold exposure** Ten SHR and 15 NR were exposed to cold tempera-
ture (2–10°C) from 3 to 4 months after birth for 7 weeks. In the 3rd week and 7th week of cold exposure the exposed SHR showed significantly (p<0.05, p<0.01) higher blood pressure (186±16, 207±15 mmHg) than nonexposed SHR (171±13, 182±21 mmHg), respectively, while no difference was noted between the exposed NR and nonexposed 13 NR.

4) These results provided an evidence that some kinds of chronic stress-loadings augment hypertension and aggravated hypertensive lesions in SHR and it is speculated that stress might activate the causative mechanisms of the spontaneous hypertension hypothetically supposed in the hypothalamo-medullary-autonomic nervous system and the hypothalmo-adenohypophyseo-adrenocortical and -thyroidal systems.

REFERENCES


