A Possible Role of Gonad and Renal Prostaglandin E2 on the Development of Hypertension in Spontaneously Hypertensive Rats*

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Accepted September 25, 1984

Abstract—A possible role of prostaglandin E2 (PGE2) in the regulation of blood pressure in spontaneously hypertensive rats (SHR) was investigated. The inhibition of PG synthesis by chronic indomethacin treatment accelerated the elevation of blood pressure with the tendency to decrease renal PGE2. We, therefore, confirmed that PGE2 in SHR may play a role in the antihypertensive mechanism. In this connection, the participation of renal PGE2 in the retardation of the development of hypertension in male SHR induced by orchiectomy was examined. Urinary PGE2 which reflects the renal PGE2 level tended to keep a higher level in the castrated group. Urinary electrolytes excretion also inclined to augment in the castrated group throughout the experiment. These results indicate that renal PGE2 may participate in the gonads-mediated blood pressure regulation system, although the mechanism of the retardation of spontaneous hypertension induced by orchiectomy remains obscure.

Spontaneously hypertensive rats (SHR), which were originally developed by Okamoto and Aoki (1), are normotensive at birth, although their blood pressure rises as they mature. Although SHR have been recognized as a model which is adequate for human essential hypertension, the pathogenesis of hypertension in SHR is still obscure. There are many factors in the regulation of blood pressure; for example, the central nervous system, cardio-vascular system, adrenal hormones, sympathetic nervous system, renin-angiotensin-aldosterone system, kal-likratin-kinin system, prostaglandins (PGs), water-electrolyte metabolism, and so on.

Iams and Wexler reported that early gonadectomy retarded the development of hypertension in SHR (2, 3). Since pituitary ACTH as well as circulating adrenal steroids increased following ovariectomy, these workers suggested that the hypertension inhibiting effect of early gonadectomy was due to an interruption of the genetically mediated setting of the hypothalamic-pituitary-adrenal-gonadal axis and that some gonadal steroid or some adrenal steroid, alone or combined, was responsible for the expression of the spontaneous hypertension. Moreover, Masubuchi et al. (4) demonstrated that late gonadectomy also interfered with the elevation of blood pressure in SHR. These reports indicate that gonads and sex hormones may play important roles in the regulation of blood pressure in SHR.

On the other hand, it was reported that PGE2 level in the kidney, which was reflected in urinary PGE2 content (5), was increased with the elevation of blood pressure in SHR and that this alteration was associated with the enhanced PG synthetase activity in renal medulla accompanied by the reduced 15-OH PG dehydrogenase activity in the renal cortex (6, 7). This enzyme is known to

* This study was presented in a preliminary form at the 69th Regional Meeting (Kanto Area) of the Japanese Pharmacological Society, Tokyo, October, 1983.
catalyze the initial step in PG metabolism. These papers dealt with the speculation that an abnormality of renal PGE$_2$ might be involved in the pathogenesis of hypertension in SHR. Therefore, the role of renal PGE$_2$ in the regulation of blood pressure in SHR has been widely discussed. The effect of acute or chronic treatment with indomethacin, a potent PG synthesis inhibitor, on blood pressure in SHR and other hypertensive rats has been investigated in several papers; however, their results are not always in agreement (8-11).

The present study was, therefore, designed to confirm the effect of the PG synthesis inhibition by indomethacin on blood pressure and to examine whether or not the retardation of the development of hypertension following castration in male SHR would be related to renal PGE$_2$ level.

Materials and Methods

Indomethacin (2.5 mg/kg/day) suspended in sesame oil or the vehicle alone was injected subcutaneously to male SHR (bred in Hoshino Lab.) from 5 to 13 weeks of age. In the other experiments, male SHR were castrated at 4 weeks of age, and the control group was sham operated at the same age. In all the groups of rats, urine was collected for 6 hr once a week. All the animals were housed in metabolic cages on a cycle of 12 hr lights on and off. Temperature and humidity were controlled at 23±1°C and 55±2%, respectively. Animals were fed on a commercial Rat Chow (Oriental Yeast Co.) containing 11.3 mEq per 100 g of sodium and 19.2 mEq per 100 g of potassium. Food and tap water were provided ad libitum.

The urine was acidified to pH 3 with 1 N hydrochloric acid, and PGs in the urine were extracted with 3 volumes of ether. The ether layer was evaporated at 37°C to dryness under an atmosphere of N$_2$ gas. The extracted PGs were dissolved in 0.5 ml of benzene-ethyl acetate-methanol (60:40:8.5) and applied to a silica gel (Wakogel C-100) column for separation of PGE$_2$ (12). Determination of PGE$_2$ was carried out according to the method in the previous report (13).

Systolic blood pressure was measured by the tail-cuff method once or twice a week. Animals were warmed for 3 min at an ambient temperature of 50°C just prior to the measurement.

Urinary sodium and potassium excretion was determined by flame photometry.

All the statistical analyses were performed with Student's $t$-test.

Results

The effect of indomethacin treatment on systolic blood pressure in SHR is shown in Fig. 1. The indomethacin-treated group
inclined to show an elevation of blood pressure during the experiment; moreover, it showed a significantly higher blood pressure than the control group during the blood pressure rising period, 9 to 12 weeks of age. Figure 2 shows the effect of indomethacin on urinary PGE$_2$ and urine volume in the development of hypertension in male SHR. As expected, urinary PGE$_2$ level was decreased in the indomethacin-treated group, although significant differences between the indomethacin-treated and the control groups was not confirmed at all weeks of age. Urine volume also showed the tendency to decrease at all stages in the indomethacin treated group; however, urinary sodium and potassium excretion was not influenced by indomethacin-treatment (Fig. 3).

![Figure 2: Effect of indomethacin on urinary PGE$_2$ excretion and urine volume in male SHR.](image)

![Figure 3: Effect of indomethacin on urinary electrolytes excretion in male SHR.](image)
The changes in systolic blood pressure following castration at 4 weeks of age is shown in Fig. 4. Blood pressure in sham operated male SHR progressively increased until about 10 weeks of age; thereafter, high blood pressure (181±5 mmHg) was maintained. Orchiectomy had remarkably suppressed the development of hypertension compared with the sham operation since 7 weeks of age. Besides, urinary PGE₂ clearly tended to increase in the castrated group and significant differences were observed between the castrated and the sham operated group at 10 and 12 weeks of age (Fig. 5). Urine volume did not show a remarkable change by castration as shown in Fig. 5. Similarly, urinary sodium and potassium excretion inclined to augment slightly in the castrated group throughout the experimental period (Fig. 6).

Fig. 4. Changes in systolic blood pressure following castration in male SHR. Each point shows the mean±S.E. of 4 rats. *P<0.05, **P<0.01: Significantly different from the sham operated group.

Fig. 5. Changes in urinary PGE₂ excretion and urine volume (UV) following castration in male SHR; □: sham operated, ●: castrated. Each column shows the mean±S.E. of 4 rats. *P<0.05: Significantly different from the sham operated group.
Discussion

First, we investigated the effects of indomethacin in order to determine the relationship between blood pressure and PGs in male SHR. Higher blood pressure was found by indomethacin treatment during the experiment. Our observation coincided with the report by Levy (8) that acute indomethacin treatment showed a prohypertensive action in 10-14 and 23-38 weeks old SHR and that other PG synthesis inhibitors also caused a significant elevation of blood pressure in mature SHR. Chronic administration of indomethacin (2 mg/kg/day) was shown to aggravate renal hypertension in rats (9). Similar results were found by Schölkens and Steinbach (10) using 5 mg/kg/day of indomethacin.

On the other hand, Quirion et al. (11) reported the absence of the chronic effect of indomethacin administered 3 times a week at a dose of 3.3 mg/kg on the development of DOCA/salt hypertension in rats, and that chronic administration of indomethacin (5 mg/kg twice a week) to SHR showed no significant effect on the development of hypertension. Although the discrepancy between their results and ours can not be explained completely, the weekly dose of indomethacin in our study was higher than that in the study reported by Quirion et al. Besides, they have not actually confirmed the extent of the inhibition of PGs by indomethacin. However, we measured urinary PGE₂ as an indicator of renal PGE₂ level and examined how much PGs were suppressed by indomethacin. It was observed that this dose of indomethacin (2.5 mg/kg/day), which was enough to inhibit urinary PGE₂ completely in Wistar normotensive rats (data not shown), could show only about 57% inhibition in SHR. Therefore, it was likely that the effect of indomethacin on PG synthetic activity was less in SHR than in Wistar rats.

Some workers proposed that PGE₂ revealed a vasoconstrictor effect in rats unlike in other species (14-17). At present, this problem still remains unsolved (18), but recently, Haylor and Towers reported that the intra-aortic administration of PGE₂ to rats increased renal blood flow and renal vasodilator activity (19). Our results agree with this observation, and there are some reports which suggest that PGE₂ acts as a vasodilator even in rats (20, 21). The discrepancy in the effect of PGE₂ in rats, as Haylor and Towers suggested, might depend on (i) the stimulation of renin release caused by exogenous PGE₂, (ii) the difference in the sensitivity to some vasoconstrictors, for example, angiotensin II and (iii) the experimental conditions, that is, the isolated kidney or the whole animal perfusion.
In view of this respect, we investigated by means of determining urinary PGE$_2$ as an index of renal PGE$_2$ level (5) whether renal PGE$_2$ could be associated with the retardation of development of hypertension induced by orchectomy. As shown in Figs. 4 and 5, blood pressure in castrated SHR was significantly lower than in sham operated SHR, and urinary PGE$_2$ inclined to be higher in castrated SHR. It is possible to speculate that renal PGE$_2$, at least in part, participates in the retardation of the elevation of blood pressure in castrated SHR.

Berg et al. (22) reported that early gonadectomy did not influence the elevation of blood pressure in SHR, and they suggested that the inhibitory effect of castration observed by Iams and Wexler (2, 3) contributed to anesthesia during the determination of blood pressure. The environmental conditions prior to the determination of blood pressure in our study were different from those in the report by Berg et al.; that is, our studies were performed at 50°C for 30 min, while 32–33°C for 30 min was used in theirs. The warming temperature and time prior to the determination remain to be discussed.

The adrenal system in the retardation of the development of hypertension induced by castration in SHR has been investigated (3, 4). The results failed to explain clearly the relationship between the effect of castration and the circulation level of adrenal steroids. However, they did not exclude the possibility that the interaction of adrenal and gonadal steroids might be involved in the condition of arterial wall affecting blood pressure. In addition to this hypothesis, it is likely that the renal PG system may be related to the hypothalamic-pituitary-adrenal-gonadal axis, although it is unclear whether or not renal PGE$_2$ shows vasodepressor action only within the kidney.

Urinary electrolytes showed a slight increase in the castrated group. Electrolyte metabolism may also participate in the retardation of the development of hypertension induced by castration in male SHR.

Chang et al. demonstrated that estrogen stimulated the prostacyclin synthetic activity in smooth muscle cells from rat aorta (23), but that androgen suppressed this synthesis (24, 25). It is very interesting to note that these observations including our results may indicate a close interaction between sex hormones and blood pressure.

In conclusion, our results indicated the possibility that renal PGE$_2$ is closely related to the control of blood pressure in SHR, and it also participates in the retardation of the development of hypertension induced by castration in male SHR. Further investigations on this mechanism are needed.

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