Plasma Cyclic Nucleotides in Spontaneously Hypertensive Rats: Hyperresponse to Acute Hot Stress

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Abstract—An acute hot stress caused a sharp increase in plasma cyclic AMP and cyclic GMP in both spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). This hot stress-induced increase in plasma cyclic AMP was observed even after chemical sympathectomy elicited by 6-hydroxydopamine or depleting the catecholamine stores in adrenergic neurons by tyramine or reserpine, but was no longer observable after β-adrenergic blockade by propranolol, blockade of autonomic ganglia by hexamethonium, adrenomedullation or anesthesia by pentobarbital. These results indicate that the initial stimulation of the central nervous system evoked the release of catecholamines from the adrenal medulla which could activate adenylate cyclase via the stimulation of β-adrenoceptors on the cell surface. The increment of plasma cyclic GMP was not influenced by prior blockade of the peripheral autonomic nervous system, but was totally abolished by pentobarbital, indicating that cyclic GMP generated within the central nervous system in response to the hot stress would be directly related to its increase in the peripheral blood stream. The plasma cyclic AMP and cyclic GMP responses were greater in adult SHR than in young SHR and young and matured WKY. The predominant response of plasma cyclic AMP might be due to a greater release of catecholamine from the adrenal medulla in matured SHR. The hyperresponse of plasma cyclic GMP in adult SHR remains to be fully elucidated. The increased cyclic nucleotide responses in SHR might be an important factor in the maintenance of hypertension.

Spontaneously hypertensive rats (SHR) have been used for the investigation of human essential hypertension as a suitable model by many workers (1, 2), especially to study the possibility that initiation and/or maintenance of the hypertension in SHR is associated with aberrations in the central and peripheral nervous systems (3–11). The plasma concentration of cyclic nucleotides was recently reported to reflect the activity of the autonomic nervous system: the plasma cyclic AMP increased when endogenous catecholamines were released from sympathetic neuronal terminals by a s.c. injection of tyramine or from the adrenal medulla upon insulin-induced hypoglycemia (12–14), whereas the plasma cyclic GMP increased when cholinergic agents such as acetylcholine and carbamylcholine were s.c. injected and decreased when atropine was s.c. injected as a muscarinic inhibitor (14, 15). Accordingly, anomalous plasma cyclic nucleotide responses not only to pharmacological stimuli but also to emotional or physical stresses would possibly be observed in SHR at the stage of pre- and/or fixed-hypertension.

In the present study, we show rapid elevation of the plasma concentration of cyclic nucleotides in rats exposed to high temperature. This cyclic nucleotide response was larger in matured SHR than in young SHR and in normotensive Wistar-Kyoto rats (WKY).
Materials and Methods

Animals: Spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) from which SHR had been derived were obtained from the Department of Pathology, Kinki University School of Medicine and The Animal Center Laboratory of Kyoto University, respectively. The rats were made specific-pathogen-free by means of the Caesarean technique for the mother rats and maintained as such by Mr. H. Ishige in the Research Laboratories, Yamasa Shoyu Company Ltd. They were bred by sib-matings and kept in air-conditioned rooms (22±2°C with humidity of 55±5 per cent) on a 12 hr light-dark cycle with food pellets and water ad libitum. In the present study, male rats older than 5 weeks of age were used for the experiment after a 20 hr fast. Theophylline (10 mg/kg), a phosphodiesterase inhibitor, was injected into rats 30 min before the hot stress (13).

Measurement of blood pressure: Systolic blood pressure was non-hemorrhagically and indirectly measured the day before the experiment by using a rat tail manometer (Natsume Seisakusho Company Ltd., Tokyo, Japan) without anesthesia. The average of 3 recordings of the pressure taken at approx. 15 sec intervals was used for the blood pressure value.

Exposure of rats to high temperature: In order to load a hot stress on animals, rats were individually placed for 2 min in a warming box (20.5 x 21.5 x 27.5 cm, Natsume Seisakusho Company Ltd., Tokyo, Japan) at varying high temperatures. Blood samples were taken immediately before and after the hot stress.

Determination of plasma cyclic AMP and cyclic GMP concentration: Whole blood specimens (0.05 ml) withdrawn from the tail vein were rapidly mixed with 0.15 ml of saline containing 10 mM EDTA-4Na. After centrifugation (2540xg, 5 min), the supernatant (0.1 ml), as diluted plasma, was directly submitted to a sensitive radioimmunoassay of cyclic AMP and cyclic GMP as described in the previous paper (16). Yamasa cyclic AMP and cyclic GMP assay kits were used for these analyses.

Reagents: Sources of reagents were as follows: hexamethonium chloride and tyramine hydrochloride, Nakarai Chemicals Ltd., Kyoto, Japan; 6-hydroxydopamine, Sigma Chemical Company, St. Louis, U.S.A.; reserpine (Apoplon®), Daiichi Seiyaku Company Ltd., Tokyo, Japan; propranolol (Inderal®), Sumitomo Kagaku Kogyo, Osaka, Japan; phentolamine (Regitin®), Takeda Chemical Industry Ltd., Osaka, Japan; atropine sulfate, E. Merck A.G., Darmstadt, Germany; pentobarbital (Nembutal®), Dai-nippon Pharmaceutical Company Ltd., Osaka, Japan. Other reagents were of analytical grade from commercial sources.

Results

Blood pressure and the base-line level of plasma cyclic nucleotides in SHR and WKY of different ages: Development of hypertension was followed by measuring the blood pressure of rats at frequent intervals. As shown in Table 1, the blood pressure of WKY was 121 ±2 mmHg at 5 weeks of age and gradually rose to 132±1 mmHg by 10 weeks of age. Thereafter it was kept at this level. On the other hand, the blood pressure of SHR was significantly higher than that of WKY after 6 weeks of age and reached the hypertension level at 10 weeks, with further gradual increases thereafter. The plasma cyclic nucleotide levels at a resting state were determined. Apart from the blood pressure, the plasma concentrations of cyclic AMP and cyclic GMP were stable and did not show any fluctuation during this period.

Increases in plasma cyclic nucleotides after hot stress: The exposure of rats to high temperature caused an increase in plasma cyclic AMP in a temperature-dependent manner as shown in Fig. 1A. All rats exposed to the highest temperature, 80°C, displayed an acute symptom of weakness which was maintained for 20–30 min after the cessation of the stress. The elevation of plasma cyclic AMP at 70°C was rapid in onset and of short duration; the rise reached a peak immediately after the hot stress and returned to the initial level in 30 min at the latest in adult rats (Fig. 2A). The elevation of plasma cyclic AMP by the stress was always significantly higher in matured SHR which were already hyper-
tensive than in normotensive WKY of comparable age at each temperature. There was no significant difference in the increment in plasma cyclic AMP between young SHR which were at a pre-hypertensive or early-hypertensive stage and young WKY when they were exposed to 70°C (Fig. 1A). The plasma concentration of cyclic GMP also

Table 1. Blood pressure and plasma cyclic nucleotide levels in male rats of different ages

<table>
<thead>
<tr>
<th>Age (week)</th>
<th>Rat strain</th>
<th>Blood pressure (mm Hg)</th>
<th>Plasma concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cyclic AMP (pmol/ml)</td>
</tr>
<tr>
<td>5</td>
<td>SHR</td>
<td>124±4</td>
<td>62.0±7.2</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>121±2</td>
<td>65.4±8.2</td>
</tr>
<tr>
<td>6</td>
<td>SHR</td>
<td>149±2</td>
<td>66.1±2.0</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>123±3*</td>
<td>65.2±3.6</td>
</tr>
<tr>
<td>10</td>
<td>SHR</td>
<td>181±2</td>
<td>72.2±4.9</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>132±1*</td>
<td>70.2±5.5</td>
</tr>
<tr>
<td>15</td>
<td>SHR</td>
<td>190±4</td>
<td>65.4±3.5</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>132±1*</td>
<td>61.6±3.5</td>
</tr>
<tr>
<td>20</td>
<td>SHR</td>
<td>198±3</td>
<td>63.7±2.9</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>134±2*</td>
<td>63.2±1.8</td>
</tr>
</tbody>
</table>

Values represent means±S.E.M. The number of animals used was 10 for each group. * Difference between SHR and WKY is statistically significant (P<0.05). See "Materials and Methods" for details.

Fig. 1. Increases in plasma cyclic nucleotides in response to hot stress with varying temperatures. Adult and young rats were 16 and 6 weeks old, respectively. Results are means±S.E.M. from 5 animals. Figures under columns represent the temperature (°C). *(P<0.05) and **(P<0.01) show significant difference as compared with the control group at 22°C. See "Materials and Methods" for details.
Fig. 2. Time-course of hot stress-induced increases in plasma cyclic nucleotides. Adult rats at 16 weeks of age were exposed to 70°C for 2 min as indicated by solid squares. Blood specimens were withdrawn at intervals before and after the hot stress. Mean±S.E.M. (n=5) is plotted as a symbol with an attached vertical line. ●, SHR. ○, WKY. *(P<0.05), **(P<0.01): Significantly higher than the value before the hot stress.

Fig. 3. Effect of hexamethonium on hot stress-induced increases in plasma cyclic nucleotides. Rats (14 weeks old) were subcutaneously injected with 50 mg/kg of hexamethonium (C6) 15 min before hot stress. Results are expressed as means±S.E.M. The number of observations is 5 for each group. In this and following figures, open and stippled (or striped) columns mean the plasma concentration of cyclic nucleotides before and after the hot stress, respectively. *(P<0.05), **(P<0.01): Difference is statistically significant between “before” and “after”.
significantly increased upon the hot stress, and the increment became greater as the temperature rose (Fig. 1B). As shown in Fig. 2B, the rise of plasma cyclic GMP at 70°C was as abrupt and temporary as that of cyclic AMP. The stress of 70°C or 80°C caused a significantly larger increase in plasma cyclic GMP in matured SHR than in WKY of the same age. In the young rats of both strains, there was little difference in the increment of plasma cyclic GMP caused by the 70°C exposure, just as was the case with plasma cyclic AMP. The largest elevation of plasma cyclic AMP and cyclic GMP after the hot stress was observed in matured SHR.

Response of rats with restricted autonomic nervous activity to hot stress: Possible involvement of the autonomic nervous system in the hot stress-induced increase in plasma cyclic AMP and cyclic GMP was studied by using matured rats which showed a high response to the hot stress. Autonomic ganglion blockade by hexamethonium prevented the plasma cyclic AMP in rats from elevation in response to the hot stress (Fig. 3A), while hexamethonium did not affect the increase in plasma cyclic GMP, though it was slightly smaller than in rats without the blockade (Fig. 3B). Hexamethonium itself elevated the plasma cyclic AMP level in WKY as shown in Fig. 3A. The destruction of sympathetic nerve terminals by 6-hydroxydopamine did not affect the hot stress-induced increase in plasma cyclic nucleotides (data not shown).

A typical phenomenon of tachyphylaxis is observed when rats were twice challenged by tyramine, a catecholamine releaser from the presynaptic vesicle of postganglionic fibers of adrenergic nerves. The tachyphylaxis was reproduced as shown in Fig. 4; the first injection of tyramine into rats caused a sharp increase in plasma cyclic AMP and cyclic GMP, but the second injection (1 hr later) was without effect in this regard, reflecting that the presynaptic vesicles were depleted of

![Fig. 4](image-url)
catecholamines by the first injection. When the rats thus preinjected once with tyramine were next subjected to hot stress instead of the second challenge of tyramine, the levels of plasma cyclic AMP and cyclic GMP sharply rose by just the same degree as those observed in noninjected rats (Fig. 4A and B). Thus, the increase in plasma cyclic AMP and cyclic GMP was not accounted for by the action of catecholamines that could be liberated in response to tyramine.

One shot of reserpine 24 hr before the hot stress did not interfere with the action of hot stress to increase the plasma concentration of cyclic nucleotides in both SHR and WKY (Fig. 5A and B). However, 5-day reserpinitization completely suppressed the hot stress-induced increase in plasma cyclic AMP and cyclic GMP in WKY, though it was without effect in SHR (Fig. 5A and B). Adrenomedullation of rats totally abolished the increase in plasma cyclic AMP after hot stress, but had no effect on the response of plasma cyclic GMP as shown in Fig. 6A and B.

Effect of adrenergic and cholinergic blocking agents on hot stress-induced increase in plasma cyclic nucleotides: The increase in plasma cyclic AMP caused by the hot stress was totally blocked by pretreatment with propranolol, a β-adrenoceptor antagonist (Fig. 7A), which was without effect on the increase in plasma cyclic GMP under the same condition (Fig. 7B). Phenotolamine, an α-adrenergic antagonist, was very effective in potentiating the plasma cyclic AMP response to the hot stress (Fig. 7A), while it was without effect on the hot stress-induced increase in plasma cyclic GMP (Fig. 7B). Atropine, a muscarinic cholinergic blocking agent at doses of 0.1 mg (Fig. 7B) or 10 mg/kg (data not shown) did

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**Fig. 5.** Effect of reserpinitization on hot stress-induced increase in plasma cyclic nucleotides. Reserpine (5 mg/kg) was subcutaneously administered 24 hr before the hot stress (Res x1) or once a day for 5 days (Res x5). Results are shown as the mean±S.E.M. The number of rats used (15 weeks old) was 5 for each group. Asterisks indicate the significant difference between “before” and “after” the hot stress in this and following figures (*, P<0.05; **, P<0.01).
Fig. 6. Abolition of hot stress-induced increase in plasma cyclic AMP by adrenomedullation. Rats (16 weeks old) had been adrenomedullated 6 days before the experiment. Results are shown as means±S.E.M. (n=6).

Fig. 7. Effect of phentolamine, propranolol or atropine on hot stress-induced increase in plasma cyclic nucleotides. Phentolamine (10 mg/kg), propranolol (1 mg/kg) or atropine (0.1 mg/kg) was subcutaneously injected into rats (18 weeks old) 15 min before the hot stress. The number of rats used was 5 for each group.
not suppress the increase in the stress-induced plasma cyclic GMP level, nor was the increase in plasma cyclic AMP suppressed (Fig. 7A).

Abolition by pentobarbital of hot stress-induced increase in plasma cyclic AMP and cyclic GMP: Neither plasma cyclic AMP nor cyclic GMP increased after hot stress when rats were anesthetized by an i.p. injection of pentobarbital (Table 2). Pentobarbital anesthesia by itself did not elevate the plasma cyclic nucleotide levels; it is distinct from diethylether anesthesia which causes significant increases in the plasma concentration of both nucleotides (13).

Discussion

The cellular cyclic nucleotides which are generated in response to stimulation of receptors of certain hormones and neurotransmitters are generally known to be released from the cell (17, 18) and to serve as a source of plasma cyclic nucleotides. Changes in the plasma concentration of cyclic nucleotides are hence useful as indices of the activity of the autonomic nervous system (13-15) and possibly the central nervous system. The present study has shown that the plasma concentration of cyclic AMP and cyclic GMP in rats exhibited significant increases in response to the neuronal tension caused by exposure of rats to high temperature for a short period (2 min), though the origin of plasma cyclic nucleotides remained to be determined. Moreover, we have found that the increase in plasma cyclic AMP and cyclic GMP after the hot stress was much greater in adult SHR than in young SHR and WKY at all ages examined. It is very likely that the increase in plasma cyclic nucleotides by the stress was dependent on the activity of endogenous substances such as hormones and neurotransmitters which are capable of increasing the concentration of cyclic nucleotides within the cell as a result of activation of the receptor-coupled enzyme, adenylate or guanylate cyclase.

It is very likely that hot stress directly gives rise to stimulation of the central nervous system. This causes release of catecholamine from the adrenal medulla, but not from the nerve endings of adrenergic neurons, via the effluent autonomic nervous system, eventually leading to the increase in plasma cyclic AMP as a result of $\beta$-adrenoceptor stimulation by released catecholamines. The experimental evidence in support of this conclusion is summarized as follows: (1) Hot stress failed to raise the plasma cyclic AMP level, (i) when rats had been adrenomedullated (Fig. 6A), (ii) when the transmission via the autonomic ganglia had been blocked by hexamethonium (Fig. 3A), (iii) when the central nervous system had been depressed by pentobarbital (Table 2). (2) The plasma cyclic AMP response to the hot stress was abolished by a $\beta$-adrenolytic agent, propranolol (Fig. 7A). The results that a single injection of reserpine (Fig. 5A), 6-hydroxydopamine or tyramine (Fig. 4A) did not suppress the hot stress-induced increase in plasma cyclic AMP implies that the catecholamines released after the hot stress did not originate from the sympathetic neuronal terminals since the principal site of action of these agents was the sympathetic neuronal terminals (19).
The increment of plasma cyclic GMP after the hot stress was not blocked by hexamethonium, atropine and phentolamine, in contrast to the finding that the increase in plasma cyclic GMP caused by a s.c. injection of acetylcholine combined with physostigmine was completely suppressed by hexamethonium or atropine (15), and the increase in cyclic GMP caused by an infusion of noradrenaline was inhibited by phentolamine (20). Only pentobarbital was effective in abolition of the hot stress-induced increment in plasma cyclic GMP (Table 2). Though conclusive evidence is lacking, it seems likely that excitement in the central nervous system induced by the stress may result in stimulation of receptors, other than cholinergic muscarine or α-adrenergic ones, which, activating guanylate cyclase, eventually lead to increases in plasma cyclic GMP.

The hyperresponse of plasma cyclic AMP in adult SHR to hot stress is supposed to be due to a greater content of catecholamines in adrenals (21) and a more sensitive releasing mechanism of epinephrine in a stressful state (10, 22) because the increase in plasma cyclic AMP caused by a s.c. injection of epinephrine or norepinephrine was the same for both strains (data not shown). However, the detailed explanation is not available for the hyperresponses of plasma cyclic AMP and cyclic GMP in matured SHR.

The 5-day reserpinization elicited a refractoriness of plasma cyclic AMP to the hot stress in WKY, but not in SHR (Fig. 5A). Since the action of reserpine to deplete stores of catecholamines is slower and less complete in the adrenal medulla than in other tissues (23), it is very likely that the adrenal medulla in WKY was depleted of catecholamines only after 5-day reserpinization. Probably, the adrenal medulla of SHR would be so resistant to reserpine that catecholamine was still present in the secretory granules even after 5-day reserpinization. The hot stress-induced increase in plasma cyclic GMP was also unobservable in WKY after repeated reserpinization (Fig. 5B), indicating that a certain substance responsible for increases in cyclic GMP was lacking in WKY under these conditions. Adult SHR seem to be resistant to the action of reserpine in the adrenal medulla and in the central nervous system.

Since plasma cyclic nucleotides in adult SHR with fixed-hypertension, not in young SHR, was more responsive to the hot stress than those in WKY, it is reasonable to suppose that the hyperresponse of adult SHR is related to the maintenance of the hypertensive state rather than the initiation of the syndrome.

References
10 Nakamura, K. and Nakamura, K.: Enhanced sympathetic activity in young spontaneously hypertensive rats is not the trigger mechanism