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Relationship between the Regulation of Blood Pressure and *in vivo* Noradrenergic Neural Activities in the Locus Coeruleus of Young Spontaneously Hypertensive Rats

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Abstract – The purpose of the present study was to address whether the *in vivo* noradrenergic neural activities in the locus coeruleus are related to the development of hypertension. Two groups of the animals were prepared, 1) young SHR and 2) age-matched normotensive control, WKY. At the age of 6 weeks, blood pressure and the releases of NE and DOPEG from the locus coeruleus in young SHR and WKY were measured by *in vivo* microdialysis at two different conditions; 1) normal and 2) elevated state of blood pressure by systemically injected phenylephrine. Basal releases of NE and DOPEG from the locus coeruleus were 0.415±0.089 pg/20 min and 1.311±0.293 pg/20 min in SHR and 0.204±0.078 pg/20 min and 1.492± 0.365 pg/20 min in WKY. The basal release of NE of SHR was significantly greater than that of WKY. Phenylephrine treatment caused elevation of blood pressure in both SHR and WKY in dose-dependent manner. Following phenylephrine injection, the releases of NE and DOPEG from the locus coeruleus of SHR were significantly decreased, whereas there was no significant changes of NE in WKY. The results from the present study suggests that the noradrenergic nervous system in the locus coeruleus may contribute as one of the triggering factors for the expression of hypertension in young SHR.

Keywords □ Spontaneously hypertensive rats, blood pressure, norepinephrine, 3,4-dihydroxyphenylethylene glycol, locus coeruleus, *in vivo* microdialysis, hypertension

The importance of central noradrenergic system has been implicated in the development of hypertension in spontaneously hypertensive rats (SHR) (Mannelli *et al.*, 1990; Ciriello, 1987; Winternitz *et al.*, 1984). The locus coeruleus is a major site of the origin of noradrenergic neurons in the central nervous system (Dahlstrom and Fuxe, 1965). It is interconnected with other regions of the central nervous system involved in the cardiovascular regulation such as the nucleus tractus solitarius, hypothalamus and rostral ventrolateral medulla (Pieribone *et al.*, 1991; Foote *et al.*, 1983; Cedarbaum and Aghjanian, 1978). Electric stimulation on the locus coeruleus elicited an increase in blood pressure and in heart rate (Sved, 1986; Drolet and Gauthier, 1985; Gurtu *et al.*, 1984). Injection of L-glutamate into the locus coeruleus elicited a prolonged elevation of blood pressure more greater in SHR than in normotensive Wistar Kyoto rats (WKY) (Kawasaki *et al.*, 1991).

Several studies reported that the locus coeruleus of young SHR in the state of development of hypertension has abnormal neural activity (Luque *et al.*, 1991; Koulu *et al.*, 1986; Pullen *et al.*, 1985; Wintemitz *et al.*, 1984; Saavedra *et al.*, 1978, 1975). For example, the locus coeruleus of young SHR has been found to have increased NE content (Winternitz *et al.*, 1984), turn over rate (Koulu *et al.*, 1986) and the number of α_1-adrenoceptor (Pullen *et al.*, 1985) compared with age-matched WKY. But there are some reports which are not consistant with the above results (Yao *et al.*, 1989; Koulu *et al.*, 1986; Fujino, 1984; Nagatsu *et al.*, 1976). This may be partly due to the fact that these studies were performed *in vitro*. It seems very important to investigate the function of the noradrenergic nervous system in the locus coeruleus at *in vivo* status using the

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technique to monitor the neural activities which affects the neurons particularly in the locus coeruleus area. *In vivo* microdialysis techniques satisfy this request. *In vivo* microdialysis is a powerful new technique for monitering of events occuring in the extracellular fluid including neurotransmisslon and cell metabolism as well as the application of drugs directly into the brain (Ungerstedt, 1984).

The purpose of the present study was to address whether the noradrenergic neural activities in the locus coeruleus of the young SHR may be involved in the development of hypertension. We have measured the release of NE and 3,4-dihydroxyphenyethylene glycol (DOPEG), its intraneuronal metabolite, as the index of noradrenergic neural activities by using *in vivo* microdialysis and monitored blood pressure. In the present study, two experiments were performed. First, the basal release of NE and DOPEG from the locus coeruleus of SHR and normotensive WKY was examined. Second, the changes in the release of NE from the locus coeruleus of both SHR and WKY were observed when blood pressure was increased.

MATERIALS AND METHODS

Animals

Six week old male SHR and age-matched normotensive control, WKY were used. Male and female SHR and WKY were purchased from the Charles River Japan Co. (Japan) and inbred in a good laboratory practice room in the Seoul National University (Seoul, Korea). Animal were housed in groups of four or five and maintained in air-conditioned room (23±1℃) under controlled light (12 h light and 12 h dark), with free access to the rat chow and water.

Measurement of Blood Pressure

Systolic blood pressure was measured in conscious, prewarmed and restrained rat using a tail-cuff plethysmography (Narcotrace TM-80, Houston, Tex., U.S.A.) according to the procedure described by Pfeffer *et al.* (1971).

The carotid artery of the rat was cannulated under urethane anesthesia (1.25 g/kg, i.p.). The cannula was connected to a strain gauge coupler (Type 7179, Narco Biosystem, Houston, Tex., U.S.A.). Mean arterial pressure was monitored using a physiograph (Physiograph MK-III-P, Narco Bio-system, Houston, Tex., U.S.A.).

Surgery

The rat was anesthetized with urethane (1.25 g/kg, i.p.). The carotid artery and the jugular vein of the rat were cannulated for blood pressure monitoring and phenylephrine administration, respectively. The rat was placed in a stereotaxic apparatus (Stoelting, Chicago, Ill., U.S.A.) (tooth bar at -3.3±0.4 mm). The skull was exposed and a small hole was drilled to allow implantation of a dialysis probe into the locus coeruleus at the following coordinates: AP, -1.1 mm; L, +1.1 mm from the lambda and V, -6.7 mm from the skull surface. A guide cannula was secured to the skull by using two anchor screws and dental cement. An U-shaped microdialysis probe (membrane length, 0.7 mm) was made from hollow dialysis fiber (molecular weight cut-off; 6,000 daltons, Medical Industries, Inc., Los Angeles, Calif., U.S.A.).

Microdialysis

Ringer's solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$, pH 6.0) was perfused through the dialysis probe at a rate of 1.5 μl/min during the probe implantation and subsequent experimental procedure. Perfusates were collected into an ice-cold eppendorf microtube for 20 min and assayed for NE and one of its metabolite, DOPEG by HPLC with electrochemical detector system (ECD) as descirbed below. The first 60 min-perfusate after the probe implantation was discarded. Before initiating any experimental manipulation, at least four 20 min-perfusates were collected for measurement of the basal release. Perfusates were collected for 180 min according to experimental schedule.

Histology

At the end of the experiment, the rat was transcardially perfused with 200 ml of 0.1 M phosphates buffer (pH 7.4), followed by the same amount of a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer).

On the completion of the perfusion, the brain was removed and post-fixed in a fixing solution (4% paraformaldehyde in 30% sucrose solution), at least for 1 week at 4℃. Fifty μm sections from the fixed brain were obtained by a vibratome (Vibratome 100, Technical Products International, Inc., Mo., U.S.A.). The hlstological sections were stained with neutral red to identify the placement of the microdialysis probe using a light microscope (Olympus, BH-2, Japan) with reference to the rat stereotaxic atlas of Paxinos and Watson (1986). If the probe was misplaced, neurochemical data obtained from the experiment were excluded from the analysis.

In vitro Recovery Test

In order to estimate the recovery of the compounds

across the dialysis membrane, dialysis probes were immersed in Ringer's solution containing 0.5 μM NE and 0.5 μM DOPEG and perfused with Ringer's solution at a rate of 1.5 μl/min at room temperature.

Perfusates were collected every 20 min for 80 min and the first was discarded. The amounts of NE and DOPEG in the perfusate were compared with those in the bathing solution and expressed as a percent recovery.

Mesurement of NE and DOPEG

The perfusates were assayed for NE and one of its metabolites, DOPEG by HPLC-ECD. Five μl of each perfusates was injected directly into a analytical column for chromatographic separation of catecholamines without any pretreatment through a Rheodyne injection valve equipped with a 5 μl-sample injection loop.

The HPLC system consisted of a pump (BAS PM-60, Bioanalytical Systems Inc., Lafayette, Ind., U.S.A.), a temperature controller (BAS LC-22A, Bioanalytical Systems Inc., Lafayette, Ind., U.S.A.), an electrochemical detector (BAS LC-4B, Bioanalytical Systems Inc., Lafayette, Ind., U.S.A.) and an injection valve (Rheodyne 7125, Cotati, Calif., U.S.A.). A Biophase cartridge column (Phase II ODS; C18, particle size; 3 μm, 3.2 mm ID $\times$ 100 mm length; Bioanalytical Systems Inc., Lafayette, Ind., U.S.A.) was used for the analysis. The mobile phase consisted of 0.1 M citric acid, 0.225 mM octyl sodium sulfate, 0.06% triethylamme, 0.05 mM Na_2EDTA and 9% acetonitrile (volume bases) and was adjusted to pH 2.55 with solid NaOH. Prior to use, the mobile phase was filtered and degassed by ultrasonification at least for 1 h. Working potential of electrochemical detector was set at +0.7 V relative to the Ag/AgCl reference electrode, and the detector sensitivity was set at 1 nA/V. The flow rate was 0.5 ml/min. A standard solution of NE and DOPEG was freshly prepared and analyzed on the day of experiment. The amount of NE and DOPEG in the perfusates was determined by the comparison of peak heights with those of standards.

Data obtained from 5 μl perfusates were converted to the NE or DOPEG release for 20 min of experiment period (pg/20 min). The % release was calculated by dividing the release by mean of the basal release.

Statistical Analysis

Data were analyzed by Student's t-test for unpaired data, ANOVA test and Newman-Keuls test. Each value was expressed as mean$\pm$S.D. and statistical significance was accepted for $p<0.05$.

RESULTS

Blood Pressure

The systolic blood pressure of young SHR was significantly higher than that of young WKY (114.9$\pm$1.0 mmHg, SHR vs 95.0$\pm$1.1 mmHg, WKY, n=20 $p<0.01$). The mean arterial pressure was decreased following anesthesia with urethane administration (1.25 g/kg, i.p.) in both animals, but it was significantly higher in SHR than in WKY (98.2$\pm$6.3 mmHg vs 80.3$\pm$7.3 mmHg, n=20 $p<0.01$).

***In vitro* Recovery of NE and DOPEG**

The relative recovery of probe was estimated *in vitro*. The recoveries across the dialysis membrane, estimated from a Ringer's solution containing 0.5 μM NE and 0.5 μM DOPEG, were 12.0$\pm$2.5% for NE and 14.1$\pm$1.6% for DOPEG (n=20),

Effects of High K^+ stimulation and NE reuptake Inhibition on NE Release

In order to confirm that NE in perfusates was originated from the neuronal tissue, whether high K^+ stimulation and reuptake blocker of NE, desipramine, affect the amount of NE in perfusates was tested. Ringer's solution was perfused at a rate of 1.5 μl/min throughout the experiment except at 80 min when high K^+ (120 mM) or desipramine (1 mM) was added to Ringer's solution for 10 min and 30 min, respectively. As shown in Fig. 1, high K^+ stimulation significantly increased the NE release to about 250% over the basal release from the locus coeruleus. Local perfusion of desipramine into the locus coeruleus significantly increased the release of NE in the locus coeruleus.

Basal Releases of NE and DOPEG

The mean basal releases of NE and DOPEG in the locus coeruleus of SHR were 0.415$\pm$0.089 pg/20 min and 1.311$\pm$0.293 pg/20 min, respectively. The mean basal releases of NE and DOPEG in WKY were 0.204$\pm$0.078 pg/20 min and 1.429$\pm$0.365 pg/20 min, respectively. The basal NE release was significantly greater in SHR compared with that of WKY ($p<0.01$).

Effects of Pressor Response on Releases of NE and DOPEG

In order to address whether the enhanced neural activities of the locus coeruleus in young SHR contribute to the rise of blood pressure, we monitored the changes of the NE release from the locus coeruleus when blood pressure was increased. Fig. 2 shows that phenylephrine

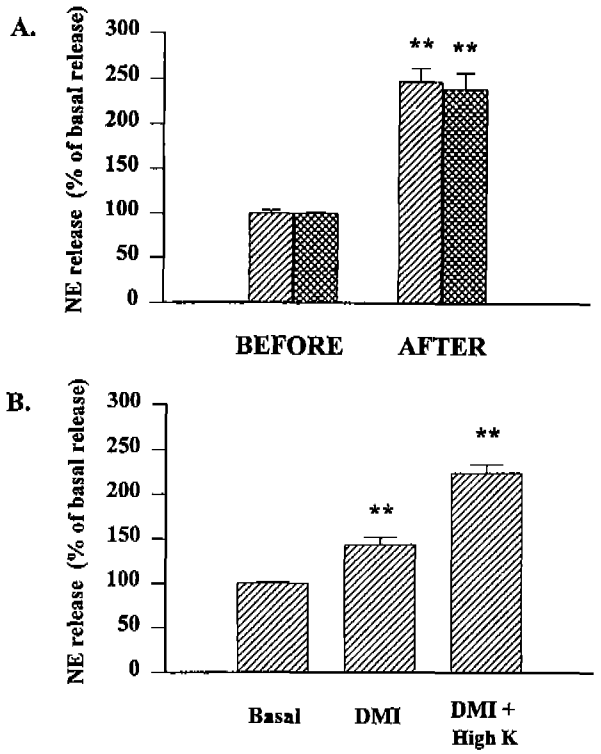

Fig. 1. A) Effect of high K^+ stimulation on the release of NE from the locus coeruleus of SHR and WKY. Ringer's solution was perfused (1.5 μl/min) throughout the experiment except at 80 min when high K^+ (120 mM) was added to Ringer's solution for 10 min. Each bar represents the mean±S.D. of the data from at least 5 animals. Asterisk indicates a significant difference from the level before high K^+ stimulation (*p<0.05, **p<0.01). ▨, SHR; ▩, WKY. BEFORE: basal release of NE (considered as 100%), AFTER: high K^+ evoked release of NE B) Effect of reuptake blockade on the release of NE from the locus coeruleus of 6-week-old SHR. Desipramine (1 mM) was perfused into locus coeruleus via microdialysis probe for 30 min at 100 min and desipramine with high K^+ was perfused *via* microdialysis probe for 30 min at 200 min. The mean of NE release (basal release), obtained from the perfusates samples until 100 min was considered as 100%. Each bar represents the mean±S.D. of the data from at least 5 animals. Asterisk indicates a significant difference from the basal release (**p<0.01).

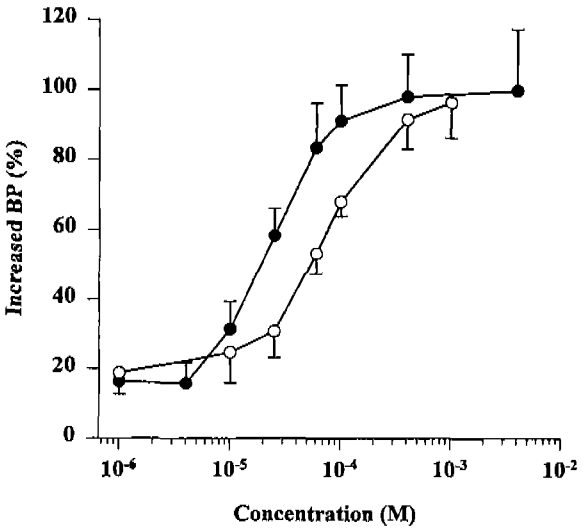

Fig. 2. Dose-response curve of phenylephrine on pressor response of SHR and WKY. Each concentration of phenylephrine solution (0.5 ml/350 g of body weight) was administered to the animals through the jugular vein. Blood pressure before phenylephrine administration was considered as control and the increment of blood pressure was expressed as percentage. Each symbol represents the mean±S.D. of the data from at least fifteen animals. EC_{50} values are 2×10^{-5} M and 5.78×10^{-5} M for SHR (●) and WKY (○), respectively.

treatment caused elevation of blood pressure in SHR and WKY in dose-dependent manner. EC_{50} Of phenylephrine was 2.0×10^{-5} M in SHR and 5.78×10^{-5} M in WKY.

The releases of NE and DOPEG were measured following the administration of 10^{-5} M, 2.0×10^{-5} M and 10^{-3} M of phenylephrine to the SHRs. The releases of NE and DOPEG in the WKY rats were measured following the administration of 10^{-5} M, 5.78×10^{-5} M and 10^{-3} M of phenylephrine. The releases of NE and DOPEG from the locus coeruleus of SHR were decreased when blood pressure was increased by phenylephrine administration (Fig. 3 and 4). NE release but not DOPEG release was significantly reduced after 10^{-5} M phenylephrine administration and both NE and DOPEG releases were significantly reduced after 2×10^{-5} M and 10^{-3} M phenylephrine administration to SHR.

In WKY, there was no significant change in the release of NE following increase in blood pressure. DOPEG release was significantly decreased by 5.78×10^{-5} M and 10^{-3} M phenylephrine administration.

DISCUSSION

The purpose of the present study was to address whether the noradrenergic neural activity in the locus coeruleus of the young SHR is involved in the development of hypertension. Six week old SHR were chosen for the present study, because young SHR have been known to be in the developmental stage of hypertension (Okamoto and Aoki, 1963): WKY, from which SHR were developed by selective inbreeding, were used as their normotensive control.

The systolic blood pressure of 6 week old SHR was significantly higher than that of age-matched normotensive

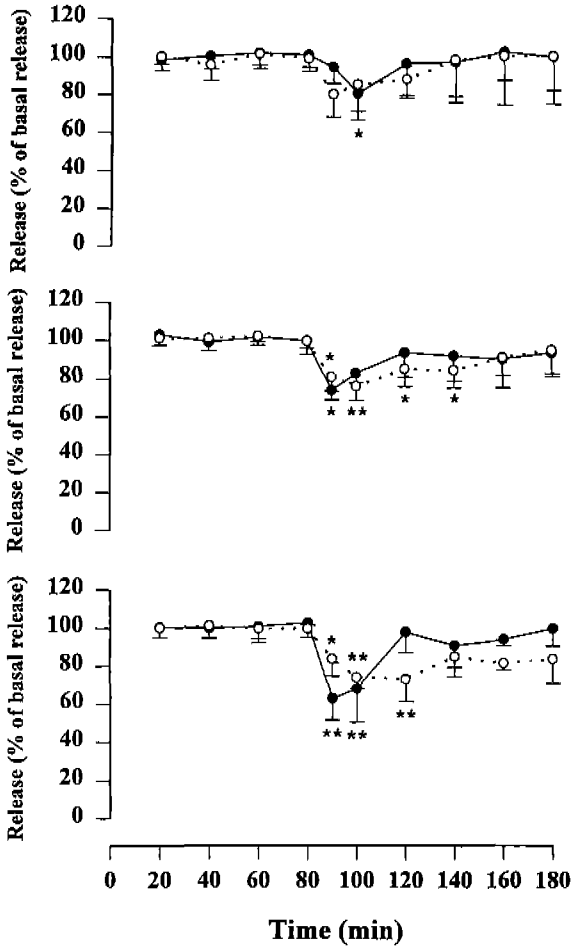

Fig. 3. Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE and DOPEG from the locus coeruleus of 6-week-old SHR. Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein at 80 min. The concentration of phenylephrine was 10^{-5} M (upper panel), 2×10^{-5} M (middle panel) and 10^{-3} M (lower panel). The mean of the release, obtained from the perfusates samples until 80 min was considered as 100% (basal release). Each symbol represents the mean±S.D. of the data from 7 animals. ● norepinephrine; ○, 3,4-dihydroxyphenylethylene glycol (DOPEG). Asterisk indicates a significant difference from the basal release (*p<0.05, **p<0.01).

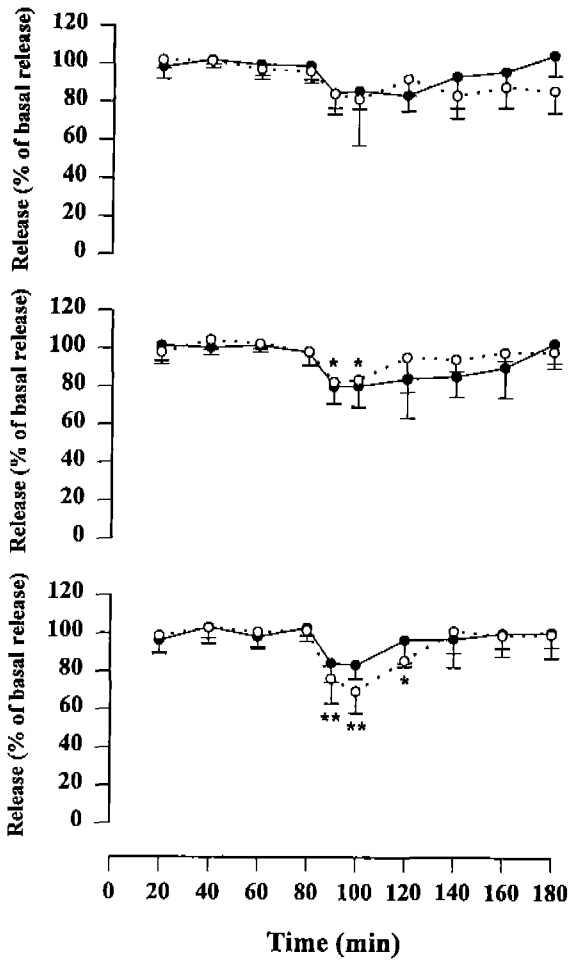

Fig. 4. Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE and DOPEG from the locus coeruleus of 6-week-old WKY. Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein at 80 min. The concentration of phenylephrine was 10^{-5} M (upper panel), 5.78×10^{-5} M (middle panel) and 10^{-3} M (lower panel). The mean of the release, obtained from the perfusates samples until 80 min was considered as 100% (basal release). Each symbol represents the mean±S.D. of the data from 7 animals. ● norepinephrine; ○, 3,4-dihydroxyphenylethylene glycol (DOPEG). Asterisk indicates a significant difference from the basal release (*p<0.05, **p<0.01).

WKY. The mean arterial pressure was decreased after urethane anesthesia in both animals but the mean arterial pressure of SHR was significantly higher than that of WKY. The results demonstrated that 6 week old SHR used in the present study was in the development of hypertension as described by others. SHR are remarkably susceptible to environmental stress and the central nervous system and peripheral organs of SHR respond strongly to environmental condition (Nomura and Okamura, 1989; Okamoto *et al.*, 1972). For this reason, the present experiment was performed under urethane anesthesia. Urethane is known to cause minimal cardiovascular and respiratory system depression (Flecknell, 1987).

High K^+ and reuptake inhibitor of NE, desipramine treatments increased the level of extracellular NE in the locus coeruleus, which proved that NE was originated from the neural tissue.

The basal release of NE from the locus coeruleus of the young SHR was significantly greater than that of the young WKY. This suggests that the basal noradrenergic

neural activities of the locus coeruleus in young SHR are enhanced. Previous studies reported some conflicting results with regard to the NE content, turnover and enzyme activities. Wintemitz *et al.* (1984) reported that 5 week old SHR showed significantly increased NE content in the locus coeruleus compared with that of WKY. Turnover (Koulu *et al.*, 1986) of NE in the locus coeruleus of young SHR was increased compared with that of young WKY and activities of tyrosine hydroxylase and dopamine β-hydroxylase (Luque *et al.*, 1991; Saavedra *et al.*, 1978, 1975) in the locus coeruleus of young SHR were decreased compared with those of WKY. On the contrary, Koulu *et al.* (1986) reported that NE content in the locus coeruleus of young SHR was not significantly different from that of young WKY. This may be partly due to the fact these studies were performed *in vitro* using postmortem tissues. Also it is difficult to infer the noradrenergic neural activity from the NE content because the level changes could reflect alterations in the rate of synthesis, degradation and/ or release. Thus further studies using *in vivo* sampling techniques are necessary to obtain more reliable measurements of biochemical parameters. *In vivo* microdialysis is to moniter the release of neurotransmitters in the discret brain *in vivo*. *In vivo* microdialysis have characteristic of closed system and minimal tissue damage. In the present study *in vivo* microdialysis performed.

In the present study, the greater basal release of NE from the locus coeruleus of young SHR suggests that the basal noradrenergic neural activities of the locus coeruleus in young SHR are enhanced. The observation could be interpreted as follows; 1) the enhanced neural activities of the locus coeruleus in young SHR may be involved in the development of hypertension. Alternatively, 2) the enhanced neural activities of the locus coeruleus in young SHR may be secondary effect of hypertension.

In order to address whether the enhanced neural activities of the locus coeruleus in young SHR contribute to the rise of blood pressure, we monitered the changes of the NE release from the locus coeruleus when blood pressure was increased. To elevate blood pressure, phenylephrine was injected. EC_{50} of phenylephrine in young SHR, 2×10^{-5} M was lower than that of young WKY, 5.78×10^{-5} M. The vascular bed of young SHR seemed to have greater sensitivity to phenylephrine. This higher sensitivity of vascular bed may be involved in the development of hypertension in young SHR in some part. It was reported that the vascular bed of SHR had enhanced responsiveness to NE or 5-hydroxytryptamine which was administered systemically (Ceng and Shibata, 1980; Dietz *et al.*, 1978). These were consistant with our results.

Phenylephrine caused increase in blood pressure followed by decrease in the releases of NE and DOPEG from the locus coeruleus. The more presser response was, the greater the NE release was decreased. These results implicated that the enhanced neural activities of the locus coeruleus in young SHR is not secondary effect of hypertension.

In cats, the presser response elicited by vascular constriction or hypervolemia reduced the release of NE in the locus coeruleus. Conversely, the fall in blood pressure caused by a controlled haemorrhage enhanced the release of NE (Singewald and Philippu, 1993; Singewald *et al.*, 1993). In the rats, hypotension elicited by haemorrage or drugs has been reported to increase neuronal activity in the locus coeruleus (Valentino, 1989; Quintin *et al.*, 1986; Elam *et al.*, 1985). The decrease in the locus coeruelus discharge rate in response to hypertension induced by increased blood volume and NE has been observed (Olpe *et al.*, 1985; Elam *et al.*, 1984). Haemorrage also increases spontaneous firing in the locus coeruleus of the cat and volume load was found to decrease noradrenergic neuronal unit activity (Morilak *et al.*, 1987; Ward *et al.*, 1980).

The locus coeruleus innervates brain areas involved in cardiovascular control, such as hypothalamus, nucleus tractus solitarius and rostral ventrolateral medulla (Pieribone and Aston-Jones, 1991; Foote *et al.*, 1983). In the posterior hypothalamus or nucleus solitarius tractus (area A2) the changes of NE release in response to altered blood pressure are similar to those found in the locus coeruleus (Guyenet, 1984; Moore and Guyenet, 1983; Philippu *et al.*, 1981).

These findings suggest that the noradrenergic nervous system in the locus coeruleus is involved in the regulation of blood pressure.

The changes of NE release were significant only in the young SHR not in the WKY. It seems that the noradrenergic nervous system of the locus coeruleus in young SHR has hypersensitivity to increase in blood pressure. However, in young WKY just a decreased tendency of changes could be observed. It may be explained by the relatively long collection time. In the present study the pressor response was relatively short, while in

other studies pressor response was prolonged. It can mask rapid changes of NE release in WKY

Microinjection of L-glutamate into the locus coeruleus of SHR elicits the greater pressor response than that in WKY (Kawasaki *et al.*, 1991). The pressor responses to the electrical stimulation of the locus coeruleus are greater in SHR (Kawamura *et al.*, 1978). These findings imply that the noradrenergic neural activities of the locus coeruleus in SHR is facilitated compared to WKY.

The changes of DOPEG were significantly decreased in both the young SHR and WKY by increase of blood pressure. These results implicated that DOPEG was changed faster and more pronounced in response to the changes of blood pressure than NE release. Also, basal DOPEG in the locus coeruleus of the young SHR was not different from that of the young WKY. These suggest that DOPEG level do not reflect noradrenergic neural acivity.

In summary, the basal noradrenergic neural activities of the locus coeruleus in young SHR were greater compared with in WKY and the releases of NE and DOPEG from the locus coeruleus following the increase in blood pressure were significantly decreased only in young SHR. These results suggest that increased basal activities and hypersensitivity of the noradrenergic nervous system of the locus coeruleus in young SHR might be one of the underlying factors for the development of hypertension in this animal model.

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