Central noradrenergic mechanism in the regulation of blood pressure in SHR

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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 involved in the regulation of blood pressure.

Two groups of the animals were prepared, 1) SHR and 2) age-matched normotensive control, WKY. At the age of 6 and 16 weeks, blood pressure and the releases of NE from the locus coeruleus in SHR and WKY were measured by *in vivo* microdialysis at three different conditions; 1) normal, 2) elevated state of blood pressure by systemic injected phenylephrine and 3) increased state of neural activity by perfused phenylephrine into the locus coeruleus.

The basal release of NE of SHR were significantly higher 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 from the locus coeruleus of SHR were significantly decreased, whereas the significant change of NE in WKY was observed in the highest dose of phenylephrine. Phenylephrine perfusion into the locus coeruleus through microdialysis probe caused pressor responses and the pressor response in SHR was greater compared with that in WKY. The results from the present study suggests that the noradrenergic nervous system in the locus coeruleus may contribute as one of the development and maintenance factors for hypertension in SHR.

Introduction

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). Electrical stimulation of the locus coeruleus elicited an increase in blood pressure and in heart rate (Sved, 1986; Drolet and Gauthier, 1985; Gurtu *et al.*, 1984). Moreover, chemical stimulation of the locus coeruleus by microinjection of excitatory amino acid lowered the arterial pressure and heart rate (Sved, 1987).

The importance of central noradrenergic system has been implicated in the development of hypertension in spontaneously hypertensive rats (SHR) (Mannelli *et al.*, 1990). 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). But the results from the biochemical studies were not consistant (Luque *et al.*, 1991). 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 technique to monitor the neural activities which affects the neurons particularly in local area. *In vivo* microdialysis is satisfied this request. *In vivo* microdialysis is a powerful new technique for monitering of events occuring in the extracellular fluid including neurotransmission 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 SHR may be involved in the reglulation of blood pressure. We have measured the release of NE, as the index of noradrenergic neural activity by using *in vivo* microdialysis and monitored blood pressure. In the present study, three experiments were performed. First, the basal release of NE from the locus coeruleus of SHR and normotensive WKY were examined. Second, the change in the release of NE from the locus coeruleus of both SHR and WKY was observed when blood pressure was increased. Third, the change of blood pressure was observed when neural activity of the locus coeruleus was increased.

Methods

Animals

Six and sixteen 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).

Measurement of Blood Pressure

Systolic blood pressure was measured 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 Bio-system, 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 ; V, -6.7 mm from the lambda for 6 week old rats and AP, -9.8 mm ; L, +1.25 mm ; V, -6.7 mm from the bregma for 16 week old rats. 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 cutoff ; 6,000 daltons, Medical Industries, Inc., Los Angeles, Calif., U.S.A.).

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 perfused with Ringer's solution at a rate of 1.5 or 2 μl/min at room temperature. Perfusates were collected every 20 min for 80 min and the first was discarded. The amounts of NE in the perfusate was compared with that in the bathing solution and expressed as a percent recovery.

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 or 2 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 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 three 20 min-perfusates were collected for measurement of the basal release. Perfusates were collected for 180 min according to experimental schedule. The perfusates was assayed for NE by HPLC-ECD. Five μl of each perfusates was injected directly into a Biophase cartridge column (Phase II ODS; C18, particle size; 3 μm, 3.2 mm x 100 mm; Bioanalytical Systems Inc., Lafayette, Ind., U.S.A.) for chromatographic separation of catecholamines. The mobile phase consisted of 0.1 M citric acid, 0.225 mM octyl sodium sulfate, 0.06% triethylamine, 0.05 mM Na_2EDTA and 9% acetonitrile (volume bases) and was adjusted to pH 2.55 with solid NaOH. 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.

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

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 fixed solution (4% paraformaldehyde in 30% sucrose solution), at least for 1 week at 4°C. Fifty μm sections from the fixed brain were obtained by a vibratome (Vibratome 100, Technical Products International, Inc., St. Louis, Mo., U.S.A.). The histological 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).

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 ± S.D. and statistical significance was accepted for $p<0.05$.

Results

In vitro Recovery of NE

The relative recovery of probe was estimated *in vitro*. The recoveries across the dialysis membrane for NE, estimated from a Ringer's solution containing 0.5 μM NE was 12.0 ± 2.5% at rate of 1,5 ul/min and 10.4 ± 1.9% at 2.0 ul/min (n=20).

Effects of High K^+ stimulation

In order to confirm that NE in perfusates was originated from the neuronal tissue, whether high K^+ stimulation affect the amount of NE in perfusates was tested. Ringer's solution was perfused at a rate of 1.5 or 2.0 μl/min throughout the experiment except at 80 min when high K^+ (120 mM) was added to Ringer's solution for 10 min, respectively. High K^+ stimulation significantly increased the NE release to about 250% over the basal release from the locus coeruleus.

1) 6 week old rats

Blood Pressure

The systolic blood pressure of young SHR was significantly higher than that of young WKY

(114.9 ± 1.0 mmHg, SHR vs 95.0 ± 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 ± 6.3 mmHg vs 80.3 ± 7.3 mmHg, n=20, $p<0.01$).

Basal Release of NE

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

Effects of Pressor Response on Release of NE

In order to address wether 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. Phenylephrine treatment caused elevation of blood pressure in SHR and WKY in dose-dependent. EC_{50} of phenylephrine was 2.0×10^{-5} M in SHR and 5.78×10^{-5} M in WKY.

The releases of NE was measured following 10^{-5} M, EC_{50} and 10^{-3} M of phenylephrine administration to the rats. The release of NE from the locus coeruleus of SHR were decreased when blood pressure was increased by phenylephrine administration (Fig. 1). NE release was significantly reduced after 10^{-5} M, 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 (Fig. 2).

Effects of Phenylephrine Perfusion into the locus coeruleus on Blood Pressure

In order to confirm that the enhanced neural activities of the locus coeruleus in SHR contribute to the maintenance of hypertension, we monitored the change of blood pressure when neural activities of the locus coeruleus was increased. To incereased neural activities, phenylephrine was perfused into the locus coeruleus through microdialysis probe for 30 min. Phenylephrine perfusion into the locus coeruleus elicited mean arterial pressure in both SHR and WKY. Pressor response in SHR was greater compared with that of WKY (Fig. 3). And NE release from the locus coeruleus was inceased by phenylephrine perfusion (Fig. 4).

2) 16 week old rats

Blood Pressure

The systolic blood pressure of SHR was significantly higher than that of WKY (183.2 ± 2.2 mmHg, SHR vs 106.5 ± 3.2 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 (150.8 ± 9.5 mmHg vs 95.4 ± 3.2 mmHg, n=20, $p<0.01$).

Basal Releases of NE

The mean basal releases of NE from the locus coeruleus were 0.528 ± 0.048 pg/20 min in SHR and 0.348 ± 0.052 pg/20 min in WKY, respectively. The basal release of NE was significantly greater in SHR compared with that of WKY ($p<0.01$).

Effects of Pressor Response on Releases of NE

In order to address wether the enhanced neural activities of the locus coeruleus in SHR is secondary effect of hypertension, we monitered the changes of the NE release from the locus coeruleus when blood pressure was increased. Phenylephrine treatment caused elevation of blood

pressure in SHR and WKY in dose-dependent. EC_{50} of phenylephrine was 2.58 x 10^{-5} M in SHR and 2.78 x 10^{-5} M in WKY.

The release of NE was measured following 10^{-5} M, 2.5 x 10^{-5} M and 10^{-3} M of phenylephrine administration to the rats. The release of NE from the locus coeruleus of SHR was decreased when blood pressure was increased by phenylephrine administration (Fig. 5). NE release was not significantly reduced after 10^{-6} M phenylephrine administration. And NE release was significantly reduced after 2.5 x 10^{-5} M and 10^{-3} M phenylephrine administration to SHR. In WKY, there was no significant change in the NE release following increase in blood pressure by 10^{-5} M and 2.5 x 10^{-5} M phenylephrine. NE release was significantly decreased by 10^{-3} M phenylephrine administration (Fig. 6).

Effects of Phenylephrine Perfusion into the locus coeruleus on Blood Pressure

In order to confirm that the enhanced neural activities of the locus coeruleus in SHR contribute to the maintenance of hypertension, we monitored the change of blood pressure when neural activities of the locus coeruleus was increased. To incereased neural activities, phenylephrine was perfused into the locus coeruleus through microdialysis probe for 30 min. Phenylephrine perfusion into the locus coeruleus elicited mean arterial pressure in both SHR and WKY. Pressor response in SHR was greater compared with that of WKY (Fig. 7). And NE release from the locus coeruleus was inceased by phenylephrine perfusion (Fig. 8).

Discussion

The purpose of the present study was to address whether the noradrenergic neural activity in the locus coeruleus of the SHR is related to the development and maintenance of hypertension. Six week old SHR in the development stage of hypertension and sixteen week old SHR in the maintenance of hypertension were chosen for the present study. 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^+ 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 SHR was significantly higher than that of the WKY. This suggests that the basal noradrenergic neural activities of the locus coeruleus in SHR are enhanced. However, previous studies reported some contrary results with regard to the NE content, turnover, enzyme activities and adrenoceptor binding density. Wintemitz *et al.* (1984) reported that 16 week old SHR and age-matched WKY showed similar NE content in the locus coeruleus. Turnover (Koulu *et al.*, 1986) of NE and activities of tyrosine hydroxylase and dopamine β-hydroxylase (Luque *et al.*, 1991; Saavedra *et al.*, 1978) in the locus coeruleus of SHR were not different from those of WKY. On the contrary, Luque *et al.* (1986) reported that α_2-adrenoceptor binding density in the locus coeruleus of SHR was significantly increased compared with that of 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 present study *in vivo* microdialysis performed.

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

In order to address whether the enhanced neural activities of the locus coeruleus in SHR is secondary effect of hypertension, we monitered the changes of the NE release from the locus coeruleus when blood pressure was increased. To elevate blood pressure, phenylephrine was injected. Phenylephrine caused increase in blood pressure followed by decrease in the release of NE from the locus coeruleus. These results implicated that the enhanced neural activities of the locus coeruleus in SHR is not secondary effect of hypertension.

Concerning the observed to experimentally induced cardiovascular alteration, a close relation exists between the release of NE and the activity of the locus coeruleus neuron. In the cats, the pressor 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). The decrease in the locus coeruelus discharge rate in response to hypertension induced 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).

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 sytem in the locus coeruleus is involved in the regulation of blood pressure.

The changes of NE release were significant in the SHR, whereas NE release in WKY was significantly decreased by only the highest dose phenylephrine. It seems that the noradrenergic nervous system of the locus coeruleus in SHR has hypersensitivity to increase in blood pressure.

In order to confirm that the enhanced neural activities of the locus coeruleus in SHR contribute to the maintenance of hypertension, we monitored the change of arterial pressure when neural activities of the locus coeruleus was increased. By phenylephrine perfusion into the locus coeruleus mean arterial pressure was increased in both SHR and WKY. These results implicated that the enhanced neural activities of the locus coeruleus in SHR is involved in the maintenance of hypertension. Electrical stimulation of the locus coeruleus evoked a rise in arterial blood pressure (Sved, 1986; Drolet and Gauthier, 1985, 1984; Gurtu *et al.*, 1984). Microinjection of L-glutamate into the locus coeruleus elicits pressor responses (Kawasaki *et al.*, 1991). Thses

previous studies and our results suggest that the locus coeruleus play a role in the regulation of arterial pressure.

The magnitude of pressor response in SHR was greater compared with 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 finding imply that the noradrenergic neural activities of the locus coeruleus in SHR is facilitated compared to WKY.

In this study, Dialysis efficiency of phenylephrine was not estimated. Instead, we measured NE release in prefusate in order to confirm that noradrenergic neural system was stimulated sufficiently by phenylephrine perfusion. The NE release was increased by phenylephrine perfusion into the locus coeruleus. Existence of α_1- and α_2-adrenoceptor was reported. Also, it has been shown that α_1-adrenoceptor agonists facilitate the firing of the neurons in the locus coeruleus, while α_2-agonists seem to inhibition (Sakaguchi *et al.*, 1986). When α_1-adrenoceptor in the locus coeruleus was stimulated by phenylephrine perfusion, the NE release was increased. Therfore, these results demonstrated that neurons in the locus coeruleus was stimulated by phenylephrine perfusion through microdialysis probe.

In summary, the basal noradrenergic neural activities of the locus coeruleus in SHR were greater compared with in WKY and the release of NE from the locus coeruleus following the increase in blood pressure were significantly decreased only in SHR. And the pressor response elicited by phenylephrine perfusion into the locus coeruleus of SHR was greater compared with in WKY. These results suggest that increased basal activities and hypersensitivity of the noradrenergic nervous system of the locus coeruleus in SHR might be one of the underlying factors for the development and maintenance of hypertension in this animal model.

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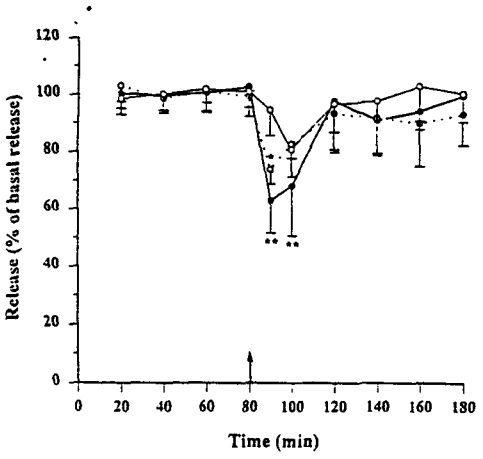

Fig. 1 Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE from the locus coeruleus of 6-week-old SHR. ↑: Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein. The mean of the release, obtained from the perfusate samples until 80 min was considered as 100% (basal release). Each symbol represents the mean ± S.D. of the data from at least 7 animals. ●, 10^{-3} M; ○, EC_{50} 2 x 10^{-5} M; ⊃, 10^{-5} M. * indicates a significant difference from the basal release (*, $p<0.05$; **, $p<0.01$)

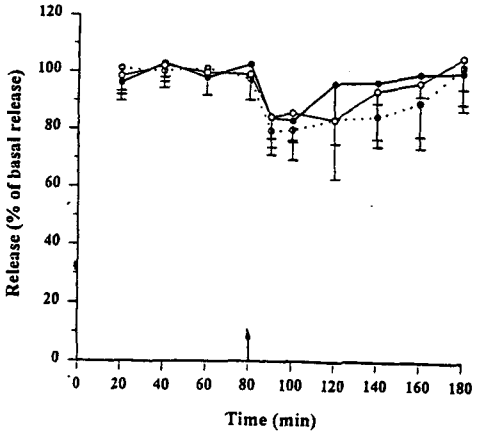

Fig. 2 Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE from the locus coeruleus of 6-week-old WKY. ↑: Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein. The mean of the release, obtained from the perfusate samples until 80 min was considered as 100% (basal release). Each symbol represents the mean ± S.D. of the data from at least 5 animals. ●, 10^{-3} M; ○, EC_{50} 5.78 x 10^{-5} M; ⊃, 10^{-5} M

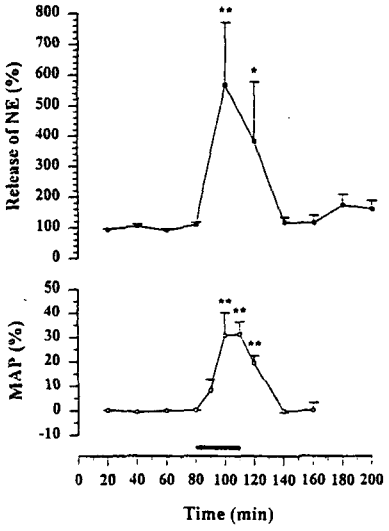

Fig. 3 Effect of phenylephrine (50 mg/ml) perfused into locus coeruleus on MAP and release of NE in locus coeruleus of 6-week-old SHR. Phenylephrine was perfused via microdialysis probe for 30 min at 80 min (→). The mean of the release, obtained from the perfusate samples until 80 min was considered as 100% (basal release). MAP before phenylephrine administration was considered as control and the increment of pressure was expressed as percentage. Each symbol represents the mean ± S.D. of the data from 10 animals. MAP (○), mean arterial pressure: NE (●), norepinephrine * indicates a significant difference from the basal release (**, $p<0.01$)

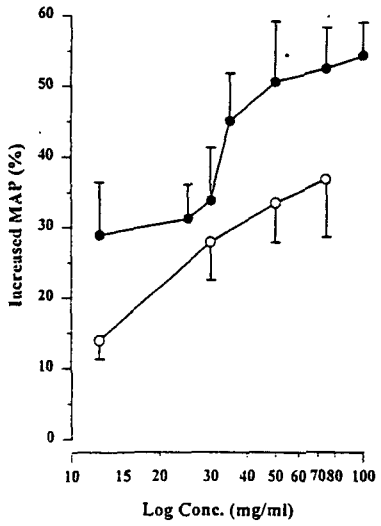

Fig. 4 Dose-response curve of phenylephrine perfused into locus coeruleus on maximal pressor response of 6-week-old SHR and WKY. Each concentration of phenylephrine solution was perfused via microdialysis probe for 30 min. Mean arterial pressure before phenylephrine administration was considered as control and the increment of pressure was expressed as percentage. Each symbol represents the mean ± S.D. of the data from at least 7 animals. ●, SHR; ⊃, WKY

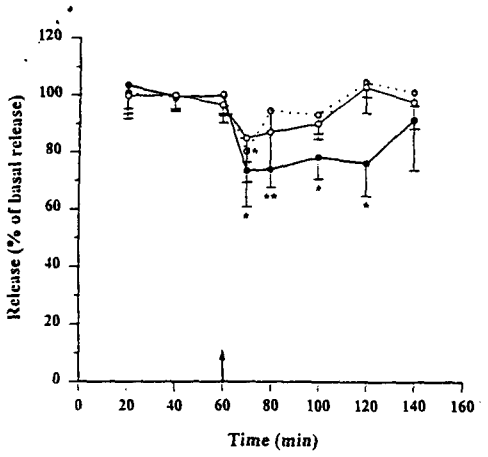

Fig. 5 Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE from the locus coeruleus of 16-week-old SHR. ↑: Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein. The mean of the release, obtained from the perfusate samples until 60 min was considered as 100% (basal release). Each symbol represents the mean ± S.D. of the data from at least 7 animals. ●, 10^{-3} M; ○, EC_{50} 2.5 x 10^{-5} M; ⊃, 10^{-3} M * indicates a significant difference from the basal release (*, p<0.05; **, p<0.01)

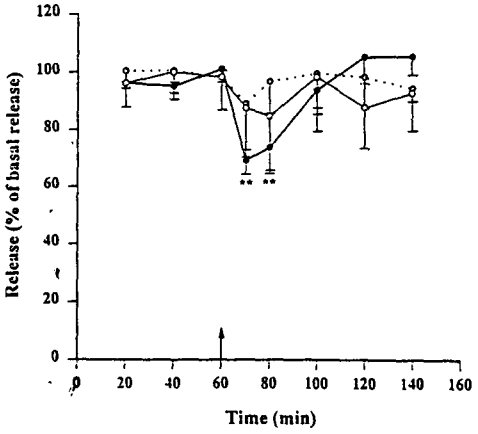

Fig. 6 Effect of systemic α_1-adrenoceptor stimulation with phenylephrine on the release of NE from the locus coeruleus of 16-week-old WKY. ↑: Phenylephrine in saline (0.5 ml/350 g of body weight) was administered through the jugular vein. The mean of the release, obtained from the perfusate samples until 60 min was considered as 100% (basal release). Each symbol represents the mean ± S.D. of the data from at least 7 animals. ●, 10^{-3} M; ○, EC_{50} 2.5 x 10^{-5} M; ⊃, 10^{-3} M * indicates a significant difference from the basal release (**, p<0.01)

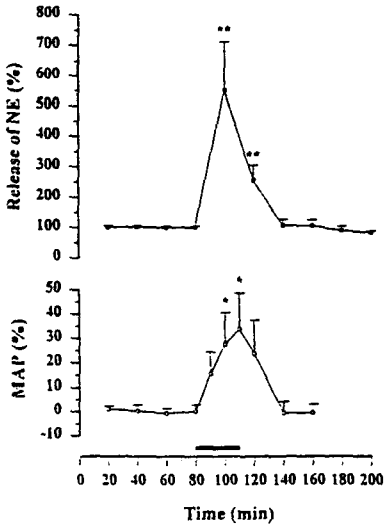

Fig. 7 Effect of phenylephrine (50 mg/ml) perfused into locus coeruleus on MAP and release of NE in locus coeruleus of 16-week-old SHR. Phenylephrine perfused via microdialysis probe for 30 min at 30 min (→). The mean of the release, obtained from the perfusate samples until 30 min was considered as 100% (basal release). MAP before phenylephrine administration was considered as control and the increment of pressure was expressed as percentage. Each symbol represents the mean ± S.D. of the data from 7 animals. MAP (⊃), mean arterial pressure; NE (●), norepinephrine * indicates a significant difference from the basal value (*, p<0.05; **, p<0.01)

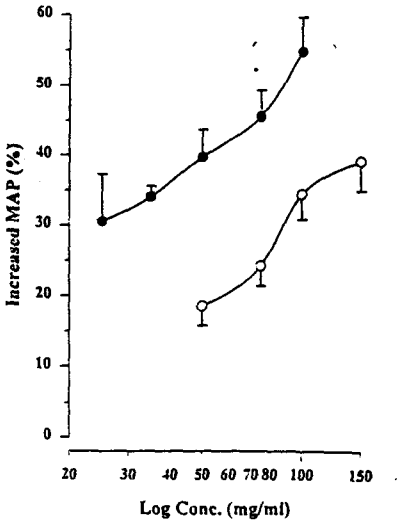

Fig. 8 Dose-response curve of phenylephrine perfused into locus coeruleus on maximal pressor response of 16-week-old SHR and WKY. Each concentration of phenylephrine solution was perfused via microdialysis probe for 30 min. Mean arterial pressure before phenylephrine administration was considered as control and the increment of pressure was expressed as percentage. Each symbol represents the mean ± S.D. of the data from at least 7 animals. ●, SHR; ⊃, WKY