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Involvement of Serotonergic Mechanism in the Nucleus Tractus Solitarius for the Regulation of Blood Pressure and Heart Rate of Rats

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ABSTRACT

In this study, it was aimed to investigate the role of serotonergic neurotransmission in nucleus tractus solitarius (NTS) for the central regulation of blood pressure and heart rate and its involvement in baroreceptor reflex activation in rats. A microinjection of 5-hydroxytryptamine (5-HT) into the NTS produced decreases in blood pressure and heart rate. Maximal decreases were 34.4±1.6 mmHg and 41.7±10.2 beats per min by 300 pmol of 5-HT. Microinjections of α-methylnoradrenaline (α-MNE) and clonidine manifested similar decreases in blood pressure and heart rate.

The hypotensive and bradycardial effects of 5-HT were blocked by previous applications of 5-HT antagonists, ritanserin, methysergide and ketanserin into the NTS, respectively. By pretreatment with reserpine and 6-hydroxydopamine (6-OHDA, i.c.v.), both hypotensive and bradycardial effects of 5-HT were significantly attenuated. Pretreatment with 5, 7-dihydroxytryptamine (5, 7-DHT, i.c.v.) enhanced the hypotensive and bradycardial effects of 5-HT. Similarly, following pretreatment with 6-OHDA, the effects of clonidine were increased. Pretreatment either with 5, 7-DHT or 6-OHDA significantly attenuated the sensitivity of baroreflex produced either by phenylephrine or by sodium nitroprusside. When either 5, 7-DHT or 6-OHDA was injected into the NTS (5, 7-DHT; 8 μg 6-OHDA; 10 μg), both of the baroreflex sensitivities were impaired. In the immunohistochemical study, the injection of 6-OHDA into the the NTS led to reduction of axon terminal varicosity, however, the injection did not reduce the numbers of catecholaminergic cell bodies. Likewise, when 5, 7-DHT was injected into the NTS, the varicosity of serotonergic axon terminals was markedly reduced.

Based on these results, it is suggested that (1) stimulation of serotonergic receptors in the NTS leads to decreases in blood pressure and heart rate as observed with the stimulation of catecholaminergic system, (2) both serotonergic and catecholaminergic receptors may be located postsynaptically, and (3) the serotonergic neurons as well as catecholaminergic neurons may have a close relevance for the activation of baroreflex.

Key Words: Nucleus tractus solitarius, Cardiovascular regulation, Serotonergic receptor, Baroreflex

INTRODUCTION

Recently, many studies have focused on the role of the nucleus tractus solitarius in the central regulation of cardiovascular function (De Jong and Nijkamp, 1976; Healy *et al.*, 1981; Laguzzi *et al.*, 1984; Palkovits and Zaborszky, 1977; Reis *et al.*, 1981). The NTS is known to have the role for relaying the information arising from peripheral baroreceptors and cardiopulmonary afferents to the vasomotor center (Kalia and Welles, 1980; Miura and Reis, 1972; Palkovits and Zaborszky, 1977). The NTS is heavily innervated by catecholaminergic neuron (Armstrong *et al.*, 1981; Koda and Bloom, 1983; Palkovits and Jakobowitz, 1974). Microinjection of noradrenaline or α-MNE produce a fall in blood pressure in association with the decrease of heart rate. Effects produced by both of these drugs are blocked by phentolamine (Kubo and Misu, 1981;

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Zandberg *et al.*, 1979). The effect of α_2 adrenoceptor agonists in the NTS was emphasized by the report of Kubo and Misu (1981), in which microinjection of α-MNE and clonidine caused a fall in blood pressure and heart rate while both variables were increased by phenylephrine and St 587.

Additionally, serotonergic system has been demonstrated to exert a modulating role for the cardiovascular system controlled by central nervous system (Kuhn *et al.*, 1980; Palkovits *et al.*, 1974; Wing and Chalmer, 1974). In the NTS, high level of 5-HT has been demonstrated in nerve terminals as well as in fibers (Gaudin-Chazal *et al.*, 1982; Pickel *et al.*, 1984; Steinbusch, 1984).

Recently, after microinjection of 5-HT into the NTS, Laguzzi and his collegues (1982; Schvaloff and Laguzzi, 1986) observed a transient fall in blood pressure and heart rate, both being blocked by pretreatment with 5-HT antagonists, metergoline or ketanserin. However, the role and importance of serotonergic neurotransmission in NTS are unclarified.

Thus, the present study was aimed to investigate the role of central serotonergic neurotransmission in the regulation of blood pressure and heart rate following microinjection of 5-HT into the NTS. The second aim is to elucidate the involvement of the serotonergic mechanism in the regulation of baroreflex sensitivity. These results are being discussed with references to those of the catecholaminergic mechanism.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250–280 g were used. Rats were housed in isolation units under constant temperature and humidity on a 12 hr light and dark cycle (6:00 a.m. −6:00 p.m.). They were given chip food and water ad libitum. The animals were anesthetized with urethane (1 g/kg) and pretreated with atropine (1 mg/kg) unless otherwise stated. Under anesthesia, the left carotid artery was cannulated with polyethylene catheter (PE 50) which contained heparinized saline (500 U/ml of 0.9% NaCl). After trachea was cannulated, bilateral vagotomy was done.

Stereotaxic procedures

Rats were prepared for direct brainstem microinjection under stereotaxic control according to the method of Kubo *et al.* (1981). Rats were mounted in a small animal stereotaxic instrument (David Kopf) with the cranium at an angle of 45° below the horizontal. Incision was made above cranial neck muscles to expose the foramen magnum. The muscles were then dissected away and atlantooccipital membrane was cut out to expose the dorsal surface of the brainstem at the level of the area postrema.

Microinjections were carried out in a volume of 100 nl over a period of 30 s via a glass cannula (outer diameter 100 μm), connected to a Hamilton microsyringe. All microinjections were made unilaterally into the NTS (0.6 mm rostral and 0.6 mm lateral to the obex, 0.6 mm below the ependymal surface). Control rats received 100 nl of saline instead. Different substance was not injected consecutively into the same brain area. The accuracy of NTS injection was confirmed by the fall in blood pressure and heart rate upon microinjection of L-glutamate (300 ng/100 nl) into the supposed area of NTS (Reis *et al.*, 1981).

The left carotid artery was cannulated for measuring the blood pressure by using Statham pressure transducer (Model P450). Heart rate was monitored by using Biotachometer (Type 7302) triggered by arterial pulses, and recorded on Physiograph (MK-IV, Narco-Biosystems Inc.)

Measurement of baroreflex sensitivity

Following anesthesia with secobarbital (35 mg/kg, i.p.), polyethylene cannulas (PE 50) were inserted into the left jugular vein and right carotid artery to facilitate i.v. injections and to record blood pressure. The graded increase or decrease in blood pressure was evoked by injecting serial bolus i.v. doses of phenylephrine and sodium nitroprusside, respectively. The slope of the linear regression line relating blood pressure and heart period represents the gain in baroreflex sensitivity expressed as milliseconds per millimeters of mercury (Goldstein, 1980).

Histological verification and immunohistochemical determinations

After cessation of experiment, the animals were perfused transcardially with zamboni fixative (Zamboni and Martino, 1967) through the left atrium. A portion of the medulla was removed and postfixed in zamboni fixative. Later, the brain tissues were cut at 25 μm sections by cryostat and

stained with cresyl violet and thereby, stereotaxic coordinates of the microinjection cannula were verified.

For monitoring of the neurotoxin lesions into the NTS, the indirect immunofluorescence procedure of Coons (1958) and Hökfelt *et al.* (1978) was used with minor modification. Briefly, the sections were incubated overnight at 4℃ in phosphate buffered saline (PBS) containing antibodies raised in rabbit against dopamine-β-hydroxylase (dilution 1:1000, personally received from Department of Pharmacology, Tokushima Medical School Japan) or in rabbit against 5-HT (dilution 1:200, BioGenex Laboratories).

The sections were then rinsed with PBS and incubated with a mixture containing either fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit IgG (dilution 1:1000, BioGenex Laboratories).

The sections were rinsed, coverslipped with a solution of glycerol and PBS (1:1) and examined with a fluorescence microscope (Zeiss). Distribution of catecholaminergic and serotonergic neurons and axon terminals were also observed in the normal rat using indirect immunofluorescence procedure.

Drugs

Serotonin creatinine sulfate (Sigma), clonidine HCl (Boehringer Ingelheim), α-methylnoradrenaline HCl (Sterling-Winthrop), methysergide maleate (Sandoz), ketanserin tartrate (Janssen), 5-hydroxy-dl-tryptophan (5-HTP, Sigma), α-methyl-*p*-tyrosine methylester (α-MPT, Sigma), phenylephrine HCl (Sigma) and sodium nitroprusside (Sigma) were diluted in saline. Ritanserin (Janssen) was diluted in dimethyl sulfoxide. 6-Hydroxydopamine hydrobromide (Sigma) and 5, 7-dihydroxytryptamine creatinine sulfate (Sigma) were diluted in 0.2% ascorbic acid solution. Reserpine (Sigma) and dl-parachlorophenylalanine (PCPA, Sigma) were suspended in 1% Tween 80.

Reserpine, α-MPT and PCPA were administered i.p. 48, 24 and 72 h prior to experiment, respectively. 6-OHDA (250 μg) was injected i.c.v two times, 3 and 7 days before and 5, 7-DHT (200 μg) was injected i.c.v. 7 days before experiment.

Statistical analysis

The results are expressed as mean±S.E.M. The statistical significance of the difference was examined by Student's t-test and the results indicating p values less than 0.05 were estimated as significant.

RESULTS

Effct of 5-HT

A microinjection of 5-HT into the NTS produced a decrease in blood pressure and heart rate (Fig. 1). Mean basal blood pressure was 108.6± 5.6 mmHg and mean basal heart rate was 395±14 beats per min (bpm). It showed biphasic patterns; one was a fall in 22.1±2.0 mmHg of blood pressure in association with decrease in 20.0±5.2 bpm of heart rate by 300 fmol of 5-HT and the other was decrease in 34.4±1.6 mmHg of blood pressure and 41.7±10.2 bpm of heart rate by 300 pmol of 5-HT. The depressor response to 5-HT was rapid and transient. It recovered to preinjection level within 1~3 min. A microinjection of saline with the same volume showed no significant effect.

Effect of 5-HTP

A microinjection of 5-HTP (100 nl in volume),

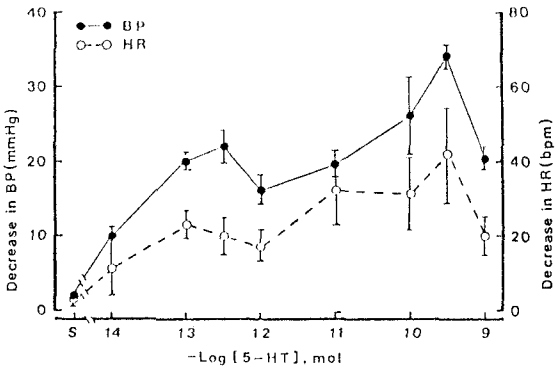

Fig. 1. Dose-response curves of serotonin (5-HT) microinjected into the nucleus tractus solitarius. Each value represents mean decreases in blood pressure (BP) and heart rat (HR) of 6 experiments. S; saline.

the precursor of 5-HT, produced dose-dependent decreases in blood pressure and heart rate. Maximum decreases were 27.5±1.4 mmHg of blood pressure and 25.0±9.7 bpm of heart rate by 3 nmol of 5-HTP (Fig. 2).

Effect of pretreatment with PCPA

Following pretreatment with an inhibitor of tryptophan hydroxylase, PCPA, the hypotensive and bradycardial responses to 5-HT and 5-HTP were observed. As shown in Table 1, there was no difference between the two groups.

Effects of 5-HT antagonists

For the purpose of identifying the significance of the biphasic patterns of 5-HT as shown above, some 5-HT antagonists were previously injected. Regardless of pretreatment with methyserside, a 5-HT_1 antagonist, or ketanserine, a 5-HT_2 antagonist, biphasic patterns of inhibition by 5-HT of blood pressure and heart rate were consistently blocked. These antagonists could not discriminate one from the other. Previous injection of ritanserin (100 pmol) elicited complete inhibition as shown in Fig. 3. A microinjection of dimethyl sulfoxide, a vehicle of ritanserin, into the NTS did not produce a significant effect.

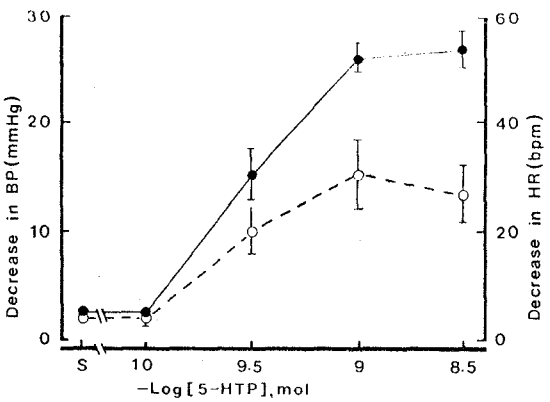

Fig. 2. Dose-response curves of 5-hydroxytryptophan (5-HTP) microinjected into the nucleus tractus solitarius. Others are the same as Fig. 1.

Responses to α_2-adrenoceptor agonists

As shown in Fig. 4, when α_2-adrenoceptor agonists, either α-MNE or clonidine was injected into the NTS, dose-dependent fall in blood pres-

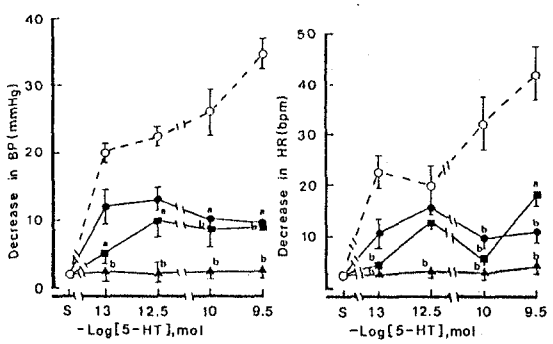

Fig. 3. Dose-response curves of 5-HT which were antagonized by 5-HT antagonists, ritanserin (▲—▲, 100 pmol), mehtysergide (■—■, 100 pmol) and ketanserin (●—●, 100 pmol). Each antagonist was microinjected into the nucleus tractus solitarius. Each value represents mean decreases in blood pressure (BP) and heart rate (HR) of 6 experiments. a, $p<0.05$; b, $p<0.01$, significantly different from control (○—○). S; saline.

Table. 1. Effect of pretreatment with PCPA (400 mg/kg, i.p.) on the cardiovascular responses to 5-HTP and 5-HT which were microinjected into the nucleus tractus solitarius

Drugs	Decrease in BP (mmHg)		Decrease in HR (beats per min)	
	Control	PCPA	Control	PCPA
5-HTP (1 nmol)	25.5±1.0	26.2±1.9	30.8±9.2	20.8±5.2
5-HT (100 pmol)	26.2±5.4	25.0±6.1	31.7±12.2	24.8±4.6

PCPA; *p*-chlorophenylalanine, 5-HT; 5-hydroxytryptamine, 5-HTP; 5-hydroxytryptophan.
Results are mean±S.E.M. of 6 experiments.

sure and heart rate were observed; maximum decrease was 38.7±2.9 mmHg of blood pressure with 52.0±6.3 bpm of heart rate by 1 nmol of α-MNE and 34.8±3.7 mmHg of blood pressure with 30.2±10.2 bpm of heart rate by 1 nmol of clonidine (Fig. 4).

Effects of pretreatment with adrenolytic drugs

Cardiovascular responses to 5-HT after pretreatment with adrenolytic drugs were observed. When pretreated with the adrenolytic drugs, reserpine (2 mg/kg, i.p.), 6-OHDA (250 μg×2, i.c.v.) and α-MPT (2 mg/kg, i.p.), respectively, the responses to 5-HT (100 pmol) were not significantly different from control (data not shown). However, the effect of 300 pmol of 5-HT on blood pressure and heart rate was significantly attenuated by pretreatment with reserpine and 6-OHDA, but not with α-MPT (Table 2). The hypotensive effect of clonidine 300 and 1000 pmol after 6-OHDA

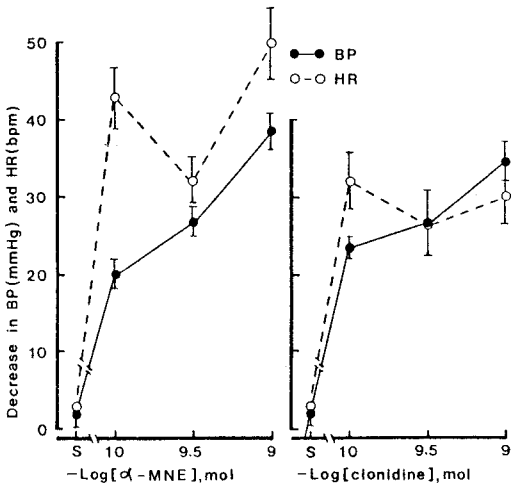

Fig. 4. Dose-response curves of α-methylnoradrenaline (α-MNE) and clonidine microinjected into the nucleus tractus solitarius. Others are the same as Fig. 3.

Table 2. Effects of pretreatment with adrenolytic drugs on cardiovascular responses to 5-HT which was microinjected into the nucleus tractus solitarius

	5-HT (300 pmol)	
	Decrease in BP (mmHg)	Decrease in HR (beats per min)
Control	34.4±1.6	41.7±10.2
Reserpine (2 mg/kg)	21.3±2.7*	13.3±3.3**
6-OHDA (250 μg×2)	27.5±3.0	22.5±5.3**
α-MPT (200 mg/kg)	25.8±7.8	26.6±7.2

Reserpine and α-MPT (α-methyl-*p*-tyrosine) were administered intraperitoneally and 6-OHDA (6-hydroxydopamine) was administered intracerebroventricularly. Each value represents mean±S.E.M. of 6 experiments. * $p<0.05$, ** $p<0.01$, significantly different from control.

Table 3. Effects of pretreatment with adrenolytic drugs on the cardiovascular responses to clonidine which was microinjected into the nucleus tractus solitarius

	Decrease in BP (mmHg)		Decrease in HR (beats per min)	
	Clonidine		Clonidine	
	(300 pmol)	(1 nmol)	(300 pmol)	(1 nmol)
Control	26.7±3.8	34.8±3.7	26.3±9.7	30.2±10.2
Reserpine (2 mg/kg)	22.0±2.7	29.6±7.2	20.5±3.2	23.6±7.2
6-OHDA (250 μg×2)	33.2±2.6*	46.5±3.6**	33.0±7.2	40.0±6.8*
α-MPT (200 mg/kg)	33.0±4.0	39.8±5.7	24.2±4.3	28.3±3.3

6-OHDA; 6-hydroxydopamine, α-MPT; α-methyl-*p*-tyrosine. Each value represents mean±S.E.M. of 6 experiments. * $p<0.05$, ** $p<0.01$ significantly different from control.

was consistently potentiated as shown in Table 3. In this case, the effect of α-MPT or reserpine was less in degree in comparison to 6-OHDA.

Effects of pretreatment with 5, 7-DHT

The responses to 5-HT, α-MNE and clonidine were compared with those of control, following 5,7-DHT pretreatment (200 μg, i.c.v.). The responses to α-MNE and clonidine observed following pretreatment with 5,7-DHT were not significantly different from control, but the response to 5-HT was significantly potentiated (Fig. 5).

Baroreflex sensitivity

Changes in heart period (reciprocal of heart rate expressed in milliseconds) produced by vasoconstrictor, phenylephrine and vasodilator, sodium nitroprusside were plotted against changes in blood pressure induced by these drugs as shown in Fig. 6. 6-OHDA and 5,7-DHT were injected i.c.v. 5 days before experiments, respectively. Otherwise, 6-OHDA (8 μg) or 5,7-DHT (10 μg) was microinjected into the NTS bilaterally 4 days before experiments to confirm the toxic effect of these neurotoxins. The slopes of linear regression lines of experimental groups exposed to the neurotoxins were less steep in comparison to vehicle.

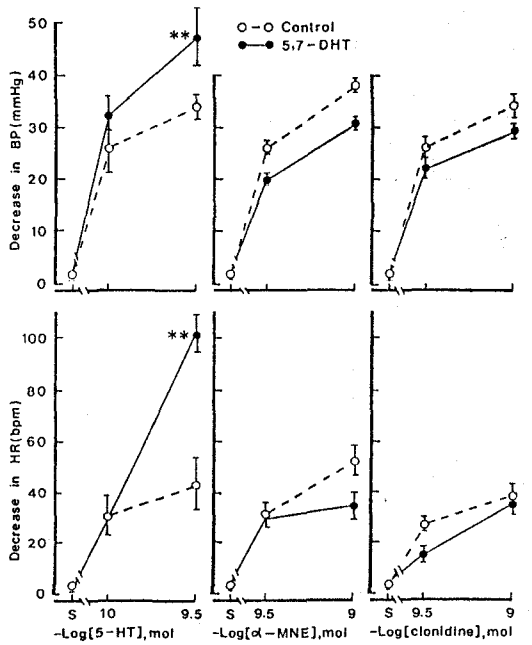

Fig. 5. Effect of pretreatment with 5,7-dihydroxytryptamine (5.7-DHT; 200 μg, i.c.v.) on cardiovascular responses to 5-HT (n=6), α-methylnoradrenaline (α-MNE, n=6) and clonidine (n=6) which were microinjected into the nucleus tractus solitarius. Each value represents decreases in blood pressure (BP) and heart rate (HR). ** $p<0.01$, significantly different from control. S; saline.

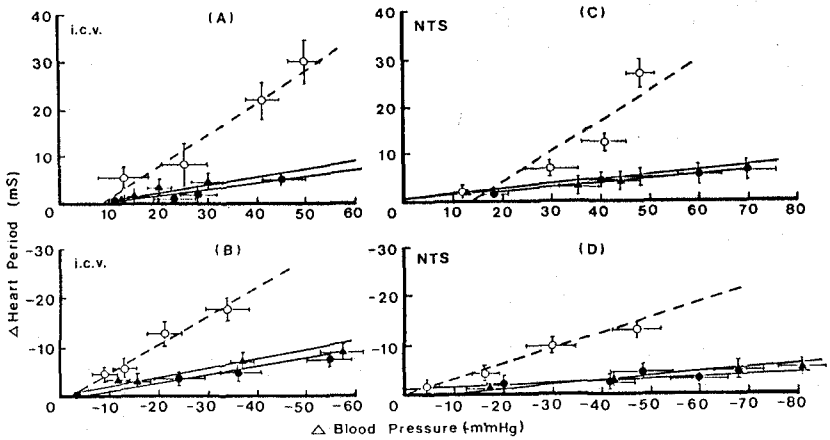

Fig. 6. Changes in heart period were plotted against the changes in blood pressure produced by phenylephrine (A and C) and sodium nitroprusside (B and D) following 6-hydroxydopamine (●–●) or 5,7-dihydroxytryptamine (▲–▲) i.c.v. injection (A and B) and injection into the nucleus solitarius (C and D). Vehicle (○–○). Each point represents mean±S.E.M. of 6 experiments.

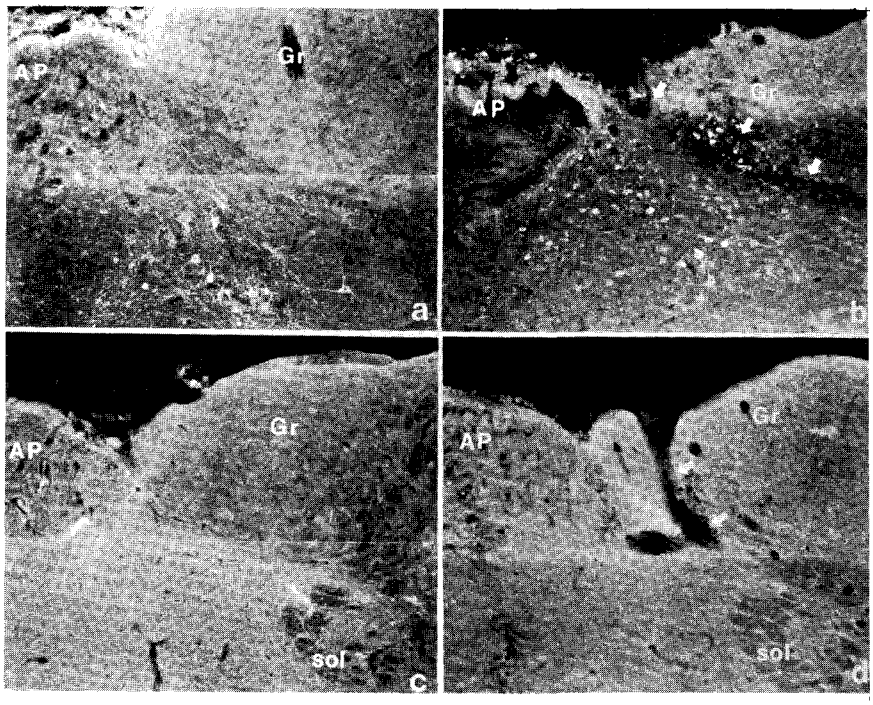

Fig. 7. Immunofluorescent micrograph of nucleus tractus solitarius in normal and rat treated with 6-hydroxydopamine (6-OHDA) and 5,7-dihyroxytryptamine (5,7-DHT). Reduction of catecholaminergic cell bodies was not observed but the axon terminal varicosities were markedly reduced (b) in comparision to normal rat (a). The varicosity of serotonergic axon terminal was markedly reduced (d) in comparision to normal rat (c). 6-OHDA (8 μg) and 5,7-DHT (10 μg) were respectively microinjected into the NTS bilaterally 4 days before experiments. Injection sites are indicated by arrows. AP; area postrema, Gr; gracile nucleus, Sol; solitary tract. Magnification, $\times 100$.

Immunohistochemical determinations

Neuronal changes of the NTS after injection of neurotoxins into the NTS were compared with normal rats (Fig. 7). In normal rats, catecholaminergic cell bodies, axon terminal varicosities and serotonergic axon terminals were seen in the NTS as shown in Fig. 7 a and c. The reduction of catecholaminergic cell bodies was not observed, whereas the axon terminal varicosities were markedly reduced by injection of 6-OHDA. Similarly, the varicosity of serotonergic terminals was markedly reduced by injection of 5,7-DHT (Fig. 7 b and d).

DISCUSSION

NTS is one of the most important sites regulating the cardiovascular function in the brain and it is the major site of termination of afferents of the ninth and tenth cranial nerves (Miura and Reis, 1972; Palkovits and Zaborszky, 1977). Further, it is characteristically related to the baroreceptor function (Brown, 1981; Spyer, 1981).

In the present study, microinjection of 5-HT and 5-HTP into the NTS produced decreases in blood pressure and heart rate. These are in agreement with the reports of Laguzzi (1984) and Schvaloff (1986). Characteristically, a depressor response to 5-HT was observed biphasically at femtomolar level of 5-HT as well as at picomolar concentrations of 5-HT. These biphasic effects of 5-HT on the blood pressure and heart rate were blocked similarly not only by the ketanserin or ritanserin, but also by methysergide, suggesting that the receptors of the NTS responding to both the femtomolar and picomolar concentrations of 5-HT are pharmacologically identical. If this

observation is true, it remains to be clarified why there are two different binding affinity sites.

It is widely reported that NTS is densely innervated by serotonin-containing neurons (Gaudin-Chazal *et al.*, 1982; Pickel et al., 1984; Steinbusch, 1981), in which high levels of aromatic L-amino acid decarboxylase is found (Jaeger *et al.*, 1984). Thus, a question arises as to why the depressor response to the exogenous 5-HT are clearly manifested in spite of the presence of the endogenous 5-HT within NTS. Following an injection of 5,7-DHT, a selective destroyer of serotonergic neuron (Chalmer *et al.*, 1984), the response to 5-HT was highly exaggerated. However, upon pretreatment with PCPA, a tryptophan hydroxylase inhibitor that decreases the content of 5-HT in the brain (Koe and Weissen, 1966), the effect of 5-HT and 5-HTP was not exaggerated. Based on these results, it is postulated that the exogenous 5-HT injected may have acted preferentially on the postsynaptic sites.

In the present study, α_2-adrenoceptor agonists, α-MNE and clonidine were demonstrated to inhibit the cardiovascular function. These results are consistent with the previous reports (Kubo and Misu, 1981; 1987; Zandberg *et al.*, 1979). After injection of 6-OHDA for chemical sympathectomy (Jonsson and Sachs, 1971; Kubo and Hashimoto, 1978), the inhibitory effect of clonidine was also enhanced as was observed with 5-HT upon destruction of serotonergic neuron with 5,7-DHT. Following pretreatment with either reserpine (Araki *et al.*, 1981) or α-MPT (Green *et al.*, 1983), the inhibition of cardiovascular responses by clonidine was not affected. Interestingly, depressor effect of 5-HT (300 pmol) was reduced when pretreated either with reserpine or with 6-OHDA. Therefore, it can be deduced form these results that as the inhibition by clonidine is mediated by postsynaptic α_2-adrenoceptors the inhibitory effects of 5-HT when injected into the NTS may be mediated by the activation of postsynaptic serotonergic receptors. Thus, it is suggested that the action of serotonergic neuron may be interdigitated with α_2-adrenoceptor activation in the NTS.

Activation of baroreflex induced by either phenylephrine or sodium nitroprusside was characteristically attenuated by pretreatment with 6-OHDA or 5,7-DHT. This finding was consistently evidenced when both neurotoxins were injected into the NTS directly or i.c.v. As reported for the catecholaminergic neuron (Armstrong *et al.*, 1981; Bucholtz and Nathan, 1984; Snyder *et al.*, 1978), the obtained results indicated that the serotonergic neuron also plays a pivotal role in the activation of baroreflex.

In the NTS, the serotonergic axon terminals were associated with catecholaminergic cell bodies and axon terminals (Koda and Bloom, 1983; Palkovits and Jacobowitz. 1974). According to Pickel (1982; 1984), catecholaminergic neurons receive direct synapses from 5-HT-containing axon terminals in NTS. It has been demonstrated that the pathway from catecholaminergic axon terminals of NTS to A1 catecholaminergic neuron group of rostral ventral medulla (Blessing *et al.*, 1982; Sawchenko and Swansson, 1982) may have a close relevance to baroreflex activation. Consequently, the reduction of axon terminal varicosity of catecholaminergic neuron by 6-OHDA may cause impairment of baroreflex sensitivity. Likewise, the change in serotonergic neurons may lead to change in baroreflex sensitivity. However, a further study is required as to how the two neurons couple each other in controlling peripheral cardiovascular responses.

In conclusion, it is suggested that (1) stimulation of serotonergic receptors in the NTS leads to decreases in blood pressure and heart rate, (2) the responsible serotonergic and catecholaminergic receptors are located postsynaptically, and (3) both serotonergic and catecholaminergic neurons in the NTS may have a close relevance to the baroreflex activation.

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REFERENCES

Araki H, Aihara H, Watanabe S, Yamamoto T and Ueki S: *Effects of reserpine, α-methyl-p-tyrosine, p-chlorophenylalanine and 5,7-dihydroxytryptamine on the hippocampal kindling effect in the rats. Japan J Pharmacol 33:1177-1182, 1983*

Armstrong DM, Pickel VM, Joh TH, Reis DJ and Miller RJ: *Immunocytochemical localization of catecholamine synthesizing enzymes and neur-*

opeptides in area postrema and medial nucleus tractus solitarius of rat brain. J Comp Neurol 196:505-517, 1981

Blessing WW, Jaeger CB, Ruggiero DA and Reis DJ: *Hypothalamic projections of medullary catecholamine neurons in the rabbit: a combined catecholamine fluoresecence and HRP transport study. Brain Res Bull 9:279-286, 1982*

Brown AM: *What the baroreceptors tell the brain in hypertension. In Perspectives in Cardiovascular Research. Vol 6, edited by JP Buckley, and CM Ferrario, Raven Press, New York, pp 1-9, 1981*

Bucholz RA and Nathan MA: *Chronic lability of the arterial blood pressure produced by electrolytic lesions of the nucleus tractus solitarius. Circ Res 54:227-238, 1984*

Chalmer JP, Minson JB and Choy V: *Bulbospinal serotonin pressor pathways and hypotensive action of methyldopa in the rat. Hypertension 6(Suppl. II): 16-21, 1984*

Coons AH: *Fluorescent antibody methods. In General Cytochemical Methods. edited by JF Danielli, Academic press, New York, pp 399-422, 1958*

De Jong W and Nijkamp FP: *Centrally induced hypotension and bradycardia after administration of α-methylnoradrenaline into the area of the nucleus tractus solitarius. Br J Pharmacol 58:593-598, 1976*

Gaudin-Chazal G, Seyfritz N, Araneda S, Bigier D and Puizillout JJ: *Selective retrograde transport of ^{3}H-serotonin in vagal afferents. Brain Res Bull 8:503-508, 1982*

Goldstein DS: *Arterial baroreflex control of heart rate in the conscious rats. Am J Physiol 238: H515-H519, 1980*

Green AR, Johnson P and Nimgaonka VL: *Increased 5-HT receptor number in brain as a probable explanation for the enhanced 5-hydroxytryptamine mediated behavior following repeated electroconvulsive shock administration to rats. Br J Pharmacol 80:173-177, 1983*

Healy DP, Jew Balck AC and Williams TH: *Bradycardia following injection of 6-hydroxydopamine into the intermediate portion of nucleus tractus solitarius medialis. Brain Res 206:415-420, 1981*

Hökfelt T, Elde R, Johnsson O, Goldstein M, Luft R, Efendic S, Wilsson G, Terenius L, Ganten D, Jeffcoate SL, Rehfeld J, Said S, Perez De La Mora M, Teran L and Palacios R: *Aminergic and peptidergic pathways in the nervous system with special reference to the hypothalamus. In The Hypothalamus. edited by Reichlin S, Baldessarini R, Martin JB, Raven Press, New York, pp 69-135, 1978*

Jaeger CB, Ruggiero DA, Albert VR, Park DH, Joh TH and Reis DJ: *Aromatic L-amino acid decarboxylase in the rat brain: Immunocytochemical localization in neurons of the brain stem. Neuroscience 11(No 3):691-713, 1984*

Jonsson G and Sachs CH: *Uptake and accumulation of ^{3}H-6-hydroxydopamine in adrenergic nerves. Eur J Pharmacol 16:55-62, 1971*

Kalia M and Welles RV: *Brain stem projections of the aortic nerve in the cat: A study using tetramethyl benzidine as the substrate for horseradish peroxidase. Brain Res 188:23-32, 1980*

Koda LY and Bloom EE: *Distribution of catecholamine-containing cell bodies and blood vessles in the rat nucleus tractus solitarius. Brain Res 289: 71-78, 1983*

Koe BK and Wissman A: *p-Chlorophenylalanine: A specific depletor of brain serotonin. J Pharmacol Exp Ther 154:499-516, 1966*

Kubo T and Hashimoto M: *Effects of intracerebroventricular and intraspinal 6-hydroxydopamine on blood pressure of spontaneously hypertensive rats. Arch Int Pharmacodyn 232: 166-176, 1978*

Kubo T, Kihara M, Hata H and Misu Y: *Cardiovascular effects in rats of alpha-1 and alpha-2 adrenergic agents injected into the nucleus tractus solitarii. Naunyn-Schmiedeberg's Arch Pharmacol 335:274-277, 1987*

Kubo T and Misu Y: *Pharmacological characterzation* of the α-adrenoceptors responsible for a decrease of blood pressure in the nucleus tractus solitarius of the rat. Naunyn-Schmiedeberg's Arch Pharmacol 137:120-125, 1981

Kuhn DM, Wolf WA and Lovenberg W: *Review of the role of the central serotonergic neuronal system in blood pressure regulation. Hypertension 2:243-255, 1980*

Laguzzi R, Reis DJ and Talman WT: *Modulation of cardiovascular and electrocortical activity through serotonergic mechanisms in the nucleus tractus solitarius of the rat. Brain Res 304:321-328, 1984*

Laguzzi R, Talman WT and Reis DJ: *Serotonergic mechanisms in the nucleus tractus solitrius may regulate blood pressure and behavior in the rat. Clin Sci 63:323s-326s, 1982*

Miura M and Reis DJ: *Termination and secondary projections of carotid sinus nerve in the cat brain stem. Am J Physiol 217:142-153, 1969*

Palkovits M, Brownstein M and Saavedra JM: *Ser-*

otonin content of the brainstem nuclei in the rat. Brain Res 80:237-249, 1974

Palkovits M, De Jong W, Zandberg P, Versteeg DHG, Vander Guyten J and Leranth CS: *Central hypertension and nucleus tractus solitarii catecholamines after surgical lesions in the medulla oblongata of the rat. Brain Res 127:307-312, 1977*

Palkovits M and Jacobowitz DM: *Topographic atlas of catecholamines and acetylcholinesterase-containing in the rat brain, II. Hindbrain (mesencephalon, rhombencephalon). J Comp Neurol 157:29-42, 1974*

Palkovits M and Zaborsky L: *Neuroanatomy of central cardivascular control. Nucleus tractus solitarii; afterent and efferent neuronal connections in relation to the baroreceptor reflex arc. In Hypertension and Brain Mechanisms, Progress in Brain Research. Vol. 47, edited by W De Jong, AP Provoost and AP Shapiro, Elservier, Amsterdam, pp9-34, 1977*

Pickel VM, Joh TH, Chan J and Baudet A: *Serotonergic terminals: ultrastructure and synaptic interaction with catecholamine-containing neurons in the medial nuclei of the solitary tract. J Comp Neurol 225:291-301, 1984*

Pickel VM, Chan J, Joh TH and Beaudet A: *Serotonergic terminals in the nucleus tractus solitarius: ultrastructure and synaptic association with catecholaminergic neurons. Soc Neurosci Abstr 8:122-128, 1982*

Reis DJ, Perrone MH and Talman WT: *Glutamic acid is the neurotransmitter of baroreceptor afferents terminating in the nucleus tractus solitarius (NTS): possible relationship to neurogenic hypertension. In Central Nervous System Mechanisms in Hypertension. Vol 6, edited by JP Buckley, CM Ferrario, Raven Press, New York, pp37-48, 1981*

Sawchenko PE and Swanson LW: *The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res 4:275-325, 1982*

Schvaloff A and Laguzzi R: *Seotonin receptor in the rat nucleus tractus solitarius and cardiovascular regulation. Eur J Pharmacol 132:283-288, 1986*

Snyder DW, Nathan MA and Reis DJ: *Chronic lability of arterial pressure produced by selective destruction of the catecholamine innervation of the nucleus tractus solitarius in the rat. Circ Res 43(4):662-671, 1978*

Spyer KM: *Neural organization and control of the baroreceptor reflex. Rev Physiol Biochem Pharmacol 88:24-124, 1981*

Steinbusch HW: *Distribution of serotonin immunoreactivity in the central nervous system of the rat cell bodies and terminals. Neuroscience 6(4):557-681, 1981*

Wing MH and Chalmer JP: *Participation of central serotonergic neurons in the control of the circulation of the unanesthetized rabbit. Circ Res 35:504-513, 1974*

Zamboni L and De Martino C: *Buffered picric acid-formaldhyde: A new, rapid fixative for electron microscopy. J Cell Biol 35:148A, 1967*

Zandberg P, De Jong W and De Wiew D: *Effect of catecholaminergic receptor stimulating agents on blood pressure after local application in the nucleus tractus solitarius of medulla oblongata. Eur J Pharmacol 55:43-56, 1979*

＝국문초록＝

흰쥐의 혈압 및 심박동수 조절에 대하여 Nucleus Tractus Solitarius 부위의 Serotonin성 기전의 역할

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혈압 및 심박동수 조절에 대한 serotonin성 기전의 역활을 흰쥐 뇌의 NTS에서 검토하고 catecholamine성 기전과 비교하여 다음과 같은 결과를 얻었다.

1) 5-HT를 NTS에 주사시 혈압 및 심박동수는 감소되었고, 5-HT 300 pmol을 NTS에 주사하였을 때 가장 현저하게 하강하였다. α-MNE과 clonidine도 마찬가지로 혈압과 심박동수의 감소를 일으켰다. 5-HT의 효과는 5-HT 수용체 길항제인 ritanserin, methysergide 및 ketanserin의 전처치에 의하여 봉쇄되었다.

2) Reserpine과 6-OHDA를 전처치하였을 때는 5-HT에 의한 혈압 및 심박동수 감소가 현저하게 약화되었다.

3) 5,7-DHT 전처치 후에는 5-HT에 의한 혈압 및 심박동수 감소는 증가하였다. 한편 6-OHDA 전처치에 의하여서도 clonidine의 혈압 및 심박동수 감소 현상은 항진하였다.

4) 5,7-DHT와 6-OHDA를 i.c.v.로 전처치하였을 때 phenylephrine과 sodium nitropursside에 의한 압반사 감수성은 현저히 저하하였고, 이들을 NTS로 주사시도 압반사 감수성이 손상되었다.

5) Immunohistochemistry에 의해 NTS에서 catecholamine성 세포체, 신경축색 말단과 serotonin성 신경축색 말단을 관찰하였고, 6-OHDA 주사후에 catecholamine성 신경축색 말단의 varicosity는 현저히 감소하였다. 한편 5,7-DHT처치에 의하여서는 serotonin성 신경축색 말단의 varicosity가 현저히 감소하였다.

이상의 실험 결과, NTS에 있는 serotonin성 수용체의 활성에 의하여 (1) 혈압 및 심박동수 감소가 일어나며, (2) 이는 catecholamine성 신경계와 관련되어 야기되며, (3) serotonin과 catecholamine성 수용체는 NTS의 postsynaptic site에 존재하고, (4) serotonin과 catecholamine성 신경세포는 압반사 조절에 있어서 중요한 역활을 하는 것으로 사료된다.