The Effects of Intracerebroventricular Administration of Adrenergic Agonists and Antagonists on Adrenaline Secretion from the Adrenal Medulla in Stressed Conscious Rats*

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The effects of intracerebroventricular (i.c.v.) administration of adrenergic agonists and antagonists on the increase in serum adrenaline (Ad) induced by immobilization stress were examined in unanesthetized, unrestrained rats. The serum Ad of rats showed a significant linear increase as time elapsed after the induction of immobilization stress. This immobilization stress-induced increase was inhibited by the i.c.v. administration of a small amount of noradrenaline (NA) and phenylephrine. Iso-proterinol and clonidine failed to inhibit the immobilization stress-induced increase. The inhibition of the immobilization stress-induced increase by i.c.v. administration of NA was antagonized by pretreatment with phentolamine and prazosin, but not by pretreatment with yohimbine and propranolol. These results suggested that NA administered *via* an i.c.v. route may inhibit the stress-induced increase in adrenomedullary Ad secretion by an action on the central α_1 -adrenoceptor.

Keywords — catecholamine; adrenaline secretion; stress; central adrenoceptor; adrenal medulla

Introduction

We have performed a study of the central regulating mechanism for adrenomedullary adrenaline (Ad) secretion in rats. For this purpose the effects of centrally acting agents on changes in peripheral blood Ad levels were investigated. When blood samples were obtained by decapitation or cardiac puncture, rapid increases in blood Ad levels were reported.¹⁻⁷⁾ Because of error induced by the stress of collection, the Ad levels of these samples did not reflect the true levels.¹⁾ Blood Ad levels were reported to rise due to even minor stress of anesthesia.⁸⁾ It appears to be necessary to collect blood samples in a non-stressful fashion from unanesthetized and unrestrained animals to assess true blood Ad levels. A method for this type of blood sampling has been described previously.¹⁾ Various drugs were administered by an intracerebroventricular (i.c.v.) route to elucidate the central effects on adrenomedullary Ad secretion. Conventionally, animals are restrained for a short period of time during i.c.v. drug administra-

tion.⁹⁾ The handling required for i.c.v. drug administration may be an adequate stress to cause changes in the blood Ad levels. The present study minimized the stress to which the animals were subjected by employing a method of drug administration to rats in an unanesthetized and unrestrained condition using an i.c.v. route from outside the cage.

Using this method, basal plasma Ad levels were determined from blood samples collected from conscious, unrestrained rats. Various drugs were administered to the animals *via* an i.c.v. route and they were then immediately immobilized in a dorsal posture to cause stress. The effect on stress-induced increases in serum Ad levels of various drugs given to rats by an i.c.v. route without adding additional stress was investigated.

Materials and Methods

1. Test Animals — Male Donryu rats weighing 300 to 350 g were used after at least two weeks of standardized handling. Solid chow

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(CA-2, Nippon CLEA Co.) and tap water were given *ad libitum*. The temperature of the feeding room was controlled at $23 \pm 1^\circ\text{C}$, and the light-dark cycle was 12 h with the light period from 6:00 AM to 6:00 PM.

2. Test Drugs — Pentobarbital sodium (Abbot Lab.), noradrenaline (NA) hydrochloride (Sigma), phenylephrine hydrochloride (Sigma), clonidine hydrochloride (Boehringer Ingelheim), isoproterenol hydrochloride (Sigma), phentolamine mesylate (Ciba-Geigy, Takeda), prazosin hydrochloride (Taito-Pfizer), yohimbine hydrochloride (Sigma), propranolol hydrochloride (Sumitomo Kagaku), and heparin sodium (Shimizu) were used.

3. Insertion of Cannula into the Lateral Ventricle — At 72 h prior to the administration of test drugs, each rat was anesthetized with an intra-peritoneal injection of sodium pentobarbital (35 mg/kg), and placed in a stereotaxic apparatus (Krieg-Model No. 51200, Stoelting Co.). A fine, stainless, L-shaped tube was implanted into the right lateral ventricle and secured to the skull with dental cement. A silicone tube of 0.3 mm in inner diameter was connected to one end of this stainless pipe, tunneled through the cervical subcutaneous region, and then exited the cage through a protective spring (Fig. 1).

4. Intravenous Catheterization — At 24 h after catheter insertion into the lateral ventricle, a venous catheter with an 0.8 mm inner diameter (Atom Venous Catheter 4 Fr) for blood sample collection was inserted into the right jugular vein under pentobarbital anesthesia. The collection port exited the cage after passing

through a protective spring (Fig. 1).¹⁾

5. Collection of Blood from Unanesthetized and Unrestrained Rats, i.c.v. Administration of Drugs and Subsequent Stress Loading — From the unanesthetized and unrestrained rats, 2 ml blood samples were collected at appropriate intervals, and basal blood levels of Ad and NA were determined. Two hours later various drugs were administered by an i.c.v. route using the method previously described. Immediately after i.c.v. drug administration, the rats were taken from the cage and stressed with forced immobilization in a dorsal posture. The forced immobilization stress consisted of fixing the four limbs and upper teeth with a rope and immobilizing the rat in a dorsal posture on a fixing plate. During stress, 2 ml blood samples were collected through the venous catheter at 10, 20 and 30 min intervals. The animals were tested in groups of five or six.

6. Determination of Ad and NA Levels — From blood samples collected, 1.0 ml of serum was separated by centrifugation ($20000 \times g$) with 2.0 ml of 0.4 N perchloric acid for protein removal. Serum catecholamines (CA) were adsorbed onto alumina by the method described by Anton and Sayre¹⁰⁾ and eluted with acidic methanol from a small elution pipette. From these prepared specimens, Ad and NA were separated using high-pressure liquid chromatography (Shimadzu, type LC-1) and measured fluorometrically by the trihydroxyindole method.²⁾ Serum CA levels were corrected on the basis of hematocrit values determined at the time of blood sample collection and expressed in ng/ml. The student's *t*-test was employed for an evaluation of statistical significance of the difference against a control group which received an i.c.v. administration of physiological saline.

Results

1. The Effect of Blood Collection on Serum CA Levels in Conscious, Unrestrained Rats

After 25 μl of physiological saline was administered by the i.c.v. route in six conscious, unrestrained rats, 2 ml blood samples were collected at 10 min intervals four times in succession to investigate the effect of the blood collection on

Fig. 1. Illustration of a Catheterized Conscious Rat

Fig. 2. Time-Related Changes in Serum Catecholamine Concentrations in Conscious Unrestrained Rats ($n = 6$)

● — ●, Ad; ○ --- ○, NA.

serum CA level. No changes occurred in serum Ad and NA levels for at least 60 min after administration (Fig. 2).

2. Time-Related Changes in Serum Ad Levels Induced by Immobilization Stress Following i.c.v. Administration of Physiological Saline in Conscious, Unrestrained Rats

Immediately after an i.c.v. administration of 25 μ l of physiological saline, a stress of forced immobilization in a dorsal posture was constantly applied. During stress exposure, serum Ad levels increased significantly to a level of 5.58 ± 0.80 ng/ml (mean $\pm$ standard error, $p < 0.01$, $n = 8$) after 10 min, a 16-fold increase

Fig. 3. Time-Related Changes in Serum Ad Levels after Forced Immobilization Stress ($n = 8$)

Each point $\bar{x}$ represents mean $\pm$ S.E.M. $a) p < 0.01$ compared to conscious unrestrained rats. Saline i.c.v. administration (25 μ l).

Fig. 4. Effect of i.c.v. NA Administration (1 ng, $n = 6$), Phenylephrine (1 ng, $n = 6$) and Clonidine (1 ng, $n = 6$ and 1 μ g, $n = 5$) on Immobilization Stress-Induced Serum Ad Increase

Each point represents mean $\pm$ S.E.M. $a) p < 0.05$ compared to stressed rats after i.c.v. saline injection. ● — ●, saline i.c.v. (control); ○ --- ○, NA i.c.v. (1 ng); × --- ×, phenylephrine i.c.v. (1 ng); Δ --- Δ , clonidine i.c.v. (1 ng); $\blacktriangle$ --- $\blacktriangle$, clonidine i.c.v. (1 μ g).

over the basal level of 0.35 ± 0.03 ng/ml ($n = 15$). The level increased to 10.30 ± 1.84 ng/ml, a 30-fold increase, after 20 min ($p < 0.01$, $n = 8$), displaying a linear increase (Fig. 3).

3. Time-Related Changes in Serum Ad Levels Induced by Immobilization Stress after i.c.v. Administration of NA

In rats exposed to immobilization stress im-

Fig. 5. Effect of i.c.v. Isoproterenol Administration (1 ng, $n = 5$) on an Immobilization Stress-Induced Serum Ad Increase

Each point represents mean $\pm$ S.E.M. ● — ●, saline i.c.v. (control); Δ --- Δ , isoproterenol i.c.v. (1 ng).

Fig. 6. Effect of Phentolamine (1 μ g, $n = 6$) and Propranolol (1 μ g, $n = 5$) on the Inhibition of Immobilization Stress-Induced Serum Ad Increase after i.c.v. NA Administration

Each point represents mean $\pm$ S.E.M. a) $p < 0.05$ compared to stressed rats after i.c.v. NA injection. $\bullet - \bullet$, saline i.c.v. (control); $\circ - \circ$, NA i.c.v. (1 ng); $\triangle - \triangle$, phentolamine i.c.v. (1 μ g) + NA i.c.v. (1 ng); $\blacktriangle - \blacktriangle$, propranolol i.c.v. (1 μ g) + NA i.c.v. (1 ng).

mediately after i.c.v. administration of 1 ng of NA, the serum Ad level was 2.60 ± 0.41 ng/ml after 10 min, significantly lower ($p < 0.05$, $n = 6$) than the level recorded in control animals exposed to the immobilization stress after an administration of physiological saline. The serum Ad levels after 20 and 30 min were 5.07 ± 1.55 and 7.36 ± 1.10 ng/ml, respectively, significantly lower than the level recorded for the control group ($p < 0.01$, $n = 6$, Fig. 4).

4. Time-Related Changes in Serum Ad Levels Induced by Immobilization Stress after i.c.v. Administration of Phenylephrine, Clonidine and Isoproterenol

In rats exposed to the immobilization stress immediately after an i.c.v. administration of 1 ng of phenylephrine, an α_1 -agonist, serum Ad levels 10, 20 and 30 min after immobilization were lower than those recorded for the control group, with statistically significant differences ($p < 0.01$, $n = 6$). The lower increase in serum Ad levels was almost of similar magnitude to that observed after an i.c.v. administration of 1 ng of NA (Fig. 4). I.c.v. administration of 1 ng as well as 1 μ g of clonidine, an α_2 -agonist, and 1 ng of isoproterenol, a β -agonist, failed to inhibit the immobilization stress-induced increase in

Fig. 7. Effect of Prazosin (1 μ g, $n = 5$) and Yohimbine (1 μ g, $n = 6$) on the Inhibition of Immobilization Stress-Induced Serum Ad Increase after i.c.v. NA Administration

Each point represents mean $\pm$ S.E.M. a) $p < 0.05$ compared to stressed rats after i.c.v. NA injection. b) $p < 0.01$ compared to stressed rats after i.c.v. NA injection. $\bullet - \bullet$, saline i.c.v. (control); $\circ - \circ$, NA i.c.v. (1 ng); $\triangle - \triangle$, prazosin i.c.v. (1 μ g) + NA i.c.v. (1 ng); $\times - \times$, yohimbine i.c.v. (1 μ g) + NA i.c.v. (1 ng).

serum Ad levels (Figs. 4 and 5).

5. Effect of adrenergic α - and β -Blockers on the Inhibition of Immobilization Stress-Induced Increases in Serum Ad Levels by i.c.v. Administration of NA

When 1 ng of NA was administered by an i.c.v. route immediately after an i.c.v. administration of 1 μ g of phentolamine, an α_1 - and α_2 -antagonist, the inhibition of the immobilization stress-induced increase in serum Ad levels caused by the i.c.v. administration of NA was antagonized, with a recovery of serum Ad levels almost to the level of the control group ($p < 0.05$, $n = 6$, Fig. 6). This recovery of serum Ad levels after the i.c.v. administration of phentolamine was statistically significant at 10, 20 and 30 min after immobilization as compared to levels observed in the NA-administered group. When 1 μ g of phentolamine alone was administered by an i.c.v. route, the immobilization stress induced a marked increase in serum Ad levels similar to that observed in the control group. Moreover, pretreatment with 1 μ g of prazosin, an α_1 -antagonist, also antagonized the NA effect (Fig. 7). However, 1 μ g of yohimbine, an α_2 -antagonist, did not reverse the NA-related inhibition of the stress-induced increase in serum

Fig. 8. The Effect of an i.c.v. Administration of Prazosin ($1 \mu\text{g}$, $n = 6$) and Yohimbine ($1 \mu\text{g}$, $n = 5$) on Immobilization Stress-Induced Serum Ad Increase after i.c.v. NA Administration

Each point represents mean $\pm$ S.E.M. a) $p < 0.05$ compared to stressed rats after i.c.v. saline administration. b) $p < 0.01$ compared to stressed rats after i.c.v. saline administration. ● — ●, saline, i.c.v. (control); ○ --- ○, prazosine, i.c.v. ($1 \mu\text{g}$); △ --- △, yohimbine, i.c.v. ($1 \mu\text{g}$).

Ad levels (Fig. 7). On the other hand, an i.c.v. administration of 1 ng of NA after pretreatment with $1 \mu\text{g}$ of propranolol, a β -antagonist, did not restore the inhibition of immobilization stress-induced increases in serum Ad levels (Fig. 6). The stress-induced increase in serum Ad levels was not inhibited by i.c.v. administration of prazosin ($1 \mu\text{g}$) alone but was inhibited significantly by yohimbine ($1 \mu\text{g}$) alone given by the same route (Fig. 8).

Discussion

Many investigators have commented that blood CA levels of rats were quickly increased when animals were sacrificed by decapitation.³⁻⁷⁾ According to previous reports^{2,3)} a comparison of CA levels in blood samples from decapitated rats to those from conscious, unrestrained rats revealed that serum NA levels were about five times higher and Ad levels about 25 times higher in the blood samples obtained by decapitation. Previously reported CA levels in blood collected from decapitated rats did not reflect true blood CA levels. It is difficult to detect delicate changes in CA levels using a decapitation technique for blood sample collection.

It has also been reported that blood CA levels of rats were elevated markedly in response to minor stresses such as touching the animals or opening the cage door.^{5,7,11)} In view of these facts, we attempted to devise a method to collect blood samples without directly touching the rats, and with the rats in a conscious, unrestrained condition.¹⁾ Conventionally, i.c.v. drug administration has been performed in immobilized animals under ether anesthesia. Blood CA levels, however, rose significantly in rats after such procedures as reported by Kvetnanski and Mikulaj.¹¹⁾ We developed a method of i.c.v. drug administration in conscious, unrestrained animals from outside of the cage which required no touching of the animals (Fig. 1).

Using our method, $25 \mu\text{l}$ of physiological saline administered to the rats by an i.c.v. route had little significant influence on serum Ad levels at 10 min intervals after the injection for at least 60 min (Fig. 2). This method of i.c.v. administration and the amount, as well as the frequency of blood sampling, had little or no influence on serum Ad levels. The drugs were always administered in a volume of $25 \mu\text{l}$, and blood samples were collected in a volume of 2 ml from conscious, unrestrained rats.

It has been reported previously that peripheral serum Ad decreased to almost unmeasurable levels in adrenalectomized rats, whereas peripheral serum NA showed little more elevation than in intact rats. It is considered that most of the peripheral serum Ad in rats comes from the adrenal medulla, while peripheral serum NA comes chiefly from peripheral sympathetic nerves.^{1,3)}

On the basis of results obtained from these basic studies on serum Ad levels in conscious, unrestrained rats, time-related changes in serum Ad levels were examined after i.c.v. administration of physiological saline, immediately followed by immobilization in a dorsal posture (Fig. 3). After forced immobilization stress, serum Ad levels showed a significant continuous linear increase reaching levels 16, 30 and 50 times higher than basal levels at 10, 20 and 30 min after stress introduction.

The stress-induced serum Ad increases were inhibited significantly by an i.c.v. administration

of a trace amount (1 ng) of NA (Fig. 4). An i.c.v. administration of 1 ng of phenylephrine, an α_1 -agonist, also inhibited these increases significantly (Fig. 4). However, an i.c.v. administration of 1 ng and 1 μ g of clonidine, an α_2 -agonist, or 1 ng of isoproterenol, a β -agonist, was ineffective in the inhibition of stress-induced serum Ad increases (Fig. 4). An i.c.v. administration of clonidine was previously reported to inhibit a metabolic turnover of NA in central nervous system (CNS)¹²⁻¹⁴⁾ with lowering blood pressure in rats. One possible interpretation is that an i.c.v. administration of NA inhibits a stress-induced increase in adrenomedullary Ad secretion. In other words, stress-induced increase in central NA turnover might be suppressed by preadministration of NA (i.c.v.). In order to test this possibility, phentolamine, an α_1 - and α_2 -adrenergic antagonist, was preadministered to intact rats (i.c.v., 1 μ g) followed by administration of NA (i.c.v., 1 ng) which might suppress the stress-induced increase of NA turnover in CNS leading to an increase in adrenomedullary Ad secretion. In fact, the pretreatment of phentolamine (i.c.v.) followed by administration of 1 ng of NA (i.c.v.) caused a recovery of serum Ad decrease due to NA (i.c.v.) administration alone during immobilization stress exposure (Fig. 6). In addition, the inhibition caused by an administration was restored to the increased levels observed in the control group (Fig. 6). The inhibition caused by an i.c.v. NA administration was also reversed by pretreatment with 1 μ g of prazosin, an α_1 -antagonist, while 1 μ g of yohimbine, an α_2 -antagonist, was not an effective antagonist of NA inhibition. Pretreatment with 1 μ g of propranolol, a β -antagonist, also did not influence the NA inhibition of stress-induced increases in blood Ad levels (Fig. 6). The increases in serum Ad level induced by forced immobilization stress were inhibited by central α_1 -receptor stimulation, according to these findings.

Although the administration of 1 μ g of prazosin alone did not inhibit the increase in serum Ad levels, an i.c.v. administration of 1 μ g of yohimbine alone produced a significant inhibition. This finding may be explained as follows; yohimbine would inhibit the accelerated turn-

over of central NA, and the stress-induced increase of central NA turnover might be inhibited at the α_2 -receptor level. It is then supposed that yohimbine administered *via* i.c.v. route prior to exposure to immobilization stress, would inhibit the accelerated NA turnover in CNS due to stress by action on α_2 -receptor. It is also proposed that a degree of acceleration of metabolic turnover of central NA may be regulated on α -adrenergic receptor level.

It remains unknown, however, by what mechanism i.c.v. NA inhibits stress-induced increases in serum Ad levels. Noble *et al.* administered [3 H]NA in rats by an i.c.v. route and investigated the distribution of the drug at various sites within the brain. They found a rapid distribution of [3 H]NA from the ventricle to various sites within the brain, with the highest 3 H level in the hypothalamus.¹⁵⁾ Struyker, Boudier *et al.*, on the other hand, reported that the administration of 3 to 100 nmol of NA into the anterior-hypothalamic preoptic regions in rats under anesthesia with pentobarbitol was followed by a dose-dependent fall in blood pressure and heart rate.¹⁶⁾ Furthermore, Nathan and Reis suggested, on the basis of the results of experiments on the effects of anterior hypothalamus stimulation on blood pressure and heart rate, that the Ad release from the adrenal medulla was regulated by the adrenergic nervous system, which passes through or starts from the anterior hypothalamus.¹⁷⁾ According to these results, α_1 -adrenergic drugs administered by an i.c.v. route in conscious, unrestrained rats may inhibit the stress-induced increases in Ad secretion from the adrenal medulla by an action on the hypothalamus.

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