Slowed Turnover of Norepinephrine in the Brain Associated with an Acute Elevation in Blood Pressure of Rats*

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SUMMARY

Acute responses of blood pressure and turnover of norepinephrine in the brain to intracisternally injected guanethidine were studied in rats. Systolic blood pressure in guanethidine-treated rats showed a dose-relating rise for over 9 hrs, while norepinephrine contents in cortex-cerebellum, brain stem and heart were not affected.

The endogenous norepinephrine in brain samples, however, did not decrease against α -methyl-p-tyrosine to result in regression coefficients significantly different from those in the saline-treated group whereas norepinephrine in heart was reduced similarly in the 2 groups, indicating a slowed turnover of norepinephrine selectively in the brain. The results may indicate that the retarded norepinephrine turnover in the brain is causatively related with an acute elevation in blood pressure.

Additional Indexing Words:

Rat Brain Heart Guanethidine α -methyl-p-tyrosine
Blood pressure Norepinephrine turnover

THE brain amines including norepinephrine (NE) and serotonin (5HT) are known to concentrate in the specific neuronal systems originated in the brain stem,¹⁾ where the central regulatory function of blood pressure has been also demonstrated.²⁾

Although these biogenic amines have been recently suggested to bear important roles in the central regulation of blood pressure,³⁾⁻⁵⁾ their contribu-

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tion in the initiation and maintenance of various types of experimental hypertension remains so far obscure. Yamori et al^{6),7)} in rats with spontaneous hypertension and Nakamura et al⁸⁾ in those induced by DOCA-saline loading both reported a reduction in turnover of the brain NE without significant alteration in the content at a stage of sustained hypertension, imputing the reduced turnover could be causatively related with those hypertensive mechanisms.

Indeed, NE turnover dynamically balancing over synthesis, storage, catabolism, release or uptake^{9),10)} would be related more profoundly with roles of the brain NE as a central neurotransmitter than its relatively static level.

However, interpretation of the reduced turnover of the brain NE in experimental hypertension⁶⁾⁻⁸⁾ is difficult, partly because it remains unknown if slowing of NE metabolism selectively in the brain could raise the level in blood pressure.

The present experiment was, therefore, undertaken to study the possibility in rats with use of guanethidine which has been reported to concentrate positively into the adrenergic neurones and inhibit release of catecholamines, especially of NE, blocking the response to sympathetic nerve activity and sensitizing effectors to NE, without significant affect on 5HT metabolism.¹¹⁾⁻¹³⁾

METHODS

Sprague-Dawley male rats, weighing 150–200 Gm, were used throughout steps in the present study.

Blood pressure response to intracisternal (i.C.) injection of guanethidine

Either 500 μ g/Kg or 1 mg/Kg of guanethidine sulfate (CIBA-Geigy <Japan>, Ltd, Tokyo) solved in 25 μ l of 0.9% saline just before use was i.C. injected according to the method by Schanberg et al¹⁴⁾ in respectively 6 or 5 rats, and response in the systolic blood pressure was compared with that in rats administered i.C. with the vehicle, 25 μ l of 0.9% saline.

Systolic blood pressure before and for 8 hrs after the injection was monitored by a tail-plethysmographic cuff method with recording on discoid papers (Ueda Inst Co Ltd, Tokyo: UsM-105) in conscious state.

The experiment was started after confirming the blood pressure became lowered and stabilized during preconditioning of animals for 6 days to the apparatus as described elsewhere.³⁾

Effect of i.C. injection of guanethidine on the level and turnover of NE in brain and heart (Fig. 1)

After 3 hrs of i.C. pretreatment with either 1 mg/Kg of guanethidine sulfate in 20 rats or 25 μ l of 0.9% saline in 19 and after 9 hrs of the former in 4, experimental and control rats in each 5 were decapitated under light ether anesthesia. Brain

Fig. 1. Side-view of the rat brain being illustrated the sphere of the samples including cortex-cerebellum and brain stem.

and heart were immediately taken out, and the former was divided into cortex-cerebellum and brain stem on an ice-cold glass plate, being kept frozen until the time of assay. As illustrated in Fig. 1, brain stem was separated rostrally along anterior commissure and superior colliculi, laterally at cerebellar peduncles and caudally at dorsal origin of the 1st cervical nerves so that the sample in the present study was expected to consist roughly of thalamus, hypothalamus, midbrain, pons, and medulla oblongata.^{8),15)}

NE was eluted and determined by the modified method of Barchas et al¹⁶⁾ with use of IRC-50 columns.

Another 29 rats received hereafter intraperitoneal (i.p.) injection every 3 hr with 250 mg/Kg of DL- α -methyl-p-tyrosine (Nakarai Chem, Ltd, Kyoto), a specific depletor of tyrosine hydroxylase, suspended in 1 ml of 0.9% saline. The animals, each 4 to 5 in either group, were then sacrificed as described above after 3, 6, and 9 hrs of the 1st injection, and NE in samples were similarly decided. Turnover of the endogenous NE was estimated on the regression co-efficient (k)¹⁷⁾ of decline of the levels after block of synthesis by α -methyl-p-tyrosine.^{8),9)}

Results were expressed as means $\pm$ S.E.M., and Fisher's t-test was performed for all statistical analyses.

RESULTS

Blood pressure response to i.C. injection of guanethidine (Fig. 2)

Intracisternally applied guanethidine caused a dose-related elevation in blood pressure. Systolic blood pressure in 5 rats injected i.C. with 1 mg/Kg guanethidine significantly rose after 2 hrs from 113 ± 4 mmHg, reached at the maximum of 142 ± 5 mmHg after 5 hrs and remained elevated even after 8 hrs. Five hundred μ g/Kg of the drug also evoked a similar increase from 116 ± 2 mmHg to 141 ± 7 mmHg, however, ceasing gradually.

Whereas, the i.C. administration of the vehicle revealed no significant change.

Fig. 2. Blood pressure response to intracisternal (i.C.) injection of guanethidine. Either 500 µg/Kg or 1 mg/Kg of guanethidine in 25 µl of 0.9% saline was i.C. injected in rats to be compared the systolic blood pressure reaction with that in those treated with the vehicle.

Table I. Wet Weight and Initial Level of Norepinephrine in Brain and Heart

	No.	Guanethidine -treated	No.	Saline-treated
Wet weight, mg				
Brain stem	20	498.7 ± 8.6	19	503.1 ± 7.3
Cortex-Cerebellum	20	1,273.1 ± 20.4	19	1,251.7 ± 14.4
Heart	20	706.5 ± 25.9	20	721.1 ± 24.2
Level of norepinephrine, ng/Gm				
Brain stem	5	386.1 ± 31.8	4	416.6 ± 70.5
Cortex-Cerebellum	5	141.7 ± 14.7	5	188.4 ± 21.4
Heart	5	649.4 ± 54.4	5	622.2 ± 56.8

Wet weight and norepinephrine level as $\text{mean} \pm \text{S.E.M.}$ in the tissue samples after 3 hrs of intracisternal administration with either 1 mg/Kg of guanethidine or 25 µl of 0.9% saline are listed. None of them are significantly different between the 2 groups.

Effect of i.C. injection of guanethidine on level and turnover of NE in brain and heart (Tables I and II, Fig. 3)

Wet weight and NE level were not affected after 3 hrs of i.C. administration with 1 mg/Kg of guanethidine (Table I).

The endogenous levels in samples of the saline-treated animals gradually

Fig. 3. Regression of endogenous levels of norepinephrine in tissue samples following α -methyl-p-tyrosine.

After 3 hrs of pretreatment with either 1 mg/Kg of guanethidine or 25 μ l of 0.9% saline ($\uparrow$), 250 mg/Kg of 3-methyl-p-tyrosine was intraperitoneally injected in every 3 hrs ($\downarrow$).

Table II. Co-efficient of Norepinephrine Regression in Brain and Heart

	k (mean $\pm$ S.E.M.)/hr			
	No.	Guanethidine-treated	No.	Saline-treated
Brain stem	13	+0.183 $\pm$ 0.027*	14	-0.199 $\pm$ 0.026
Cortex-Cerebellum	15	+0.115 $\pm$ 0.013*	14	-0.160 $\pm$ 0.021
Heart	15	-0.206 $\pm$ 0.020	15	-0.181 $\pm$ 0.021

Co-efficient (k/hr) of norepinephrine regression during 9 hrs after 250 mg/Kg of α -methyl-p-tyrosine intraperitoneally applied in every 3 hrs was calculated on the tissues of rats illustrated in Fig. 3.

* $P < 0.001$, different from the saline-treated group.

decreased after i.p. injection of α -methyl-p-tyrosine during observation for 9 hrs, while those in brain stem and cortex-cerebellum in the guanethidine-applied group did not show any decline (Fig. 3).

NE contents in ng/Gm of wet weight of the brain samples in 5 rats listed in Fig. 3 who had received i.C. guanethidine and subsequent i.p. α -methyl-p-tyrosine were 185.4 ± 14.1 in cortex-cerebellum and 553.8 ± 90.5 in brain stem after 9 hrs of guanethidine treatment, whereas those in 4 rats who had been injected only guanethidine were 201.6 ± 23.8 in the former and 570.1 ± 62.5 in the latter, showing the levels not significantly but even more augmented than in the 5 rats.

On the other hand, NE in heart was reduced similarly by α -methyl-p-tyrosine in animals pretreated i.C. with either guanethidine or saline (Fig. 3).

Co-efficients of NE regression illustrated in Fig. 3 were significantly different in brain samples between the 2 groups, while similar in heart (Table II).

DISCUSSION

Inhibition of the neurogenic release of endogenous NE and presumably subsequent block of the sympathetic nerve activity by guanethidine¹¹⁾⁻¹³⁾ have been reported to develop in an early stage of administration when tissue levels of NE are not yet significantly eliminated.^{11), 18)} The present study was designed with use of this initial effect of guanethidine which is also known to hardly pass through the blood-brain-barrier,^{11), 18), 19)} expecting an influence of the drug on NE metabolism selectively in the brain.

As illustrated in Fig. 2 and Table I, the i.C.-applied guanethidine revealed a dose-relating elevation in blood pressure for over 9 hrs after injection, during which NE levels in brain portions and heart showed no significant change.

Turnover of endogenous NE in these tissues was estimated on the content regression after inhibition of the rate-limiting step for synthesis by α -methyl-p-tyrosine,⁸⁾⁻¹⁰⁾ since the effect of guanethidine on the membrane transport mechanism^{12), 13)} might interfere with entry of labelled amines into the brain as already pointed out on the dopaminergic neurones and glial cells.⁹⁾

In the present study, i.C. injected guanethidine blocked the decline of NE content induced by α -methyl-p-tyrosine,^{11), 20)} resulting in significantly different coefficients in brain samples but not in heart, to indicate a slowered turnover of NE selectively in the brain.

Although the suppression of monoamine oxidase and amine uptake by the drug^{13), 19), 20)} would also mimic the retardation of NE metabolism, the present reduction in NE turnover of the brain appears to be derived mainly in block of NE release by the centrally administered guanethidine.^{11)-13), 18)-20)}

When considering the depressor response to centrally applied reserpine²¹⁾ which is known to facilitate turnover and release of the brain NE,^{9),20)} the present elevation in blood pressure seems to be elicited by the suppression of NE turnover in the brain perhaps due to block of the releasing mechanism.

The data would also support the blunted NE metabolism in the brain of rats with experimental hypertension⁶⁾⁻⁸⁾ being ascribed to be causatively related with their hypertensive mechanisms, although the sustained state of hypertension would not be identical with acute pressor response as seen in the present study.

The results also appear not to be inconsistent with the speculation on the synaptic activity of the brain NE as inhibitory α -adrenergic.^{22),23)} However, a lot of evidences indicating (1) the diphasic dose-response relations in blood pressure change by the centrally administered α -sympathomimetic amines including NE,²¹⁾ (2) conversion of the relation into a simple dose-pressor reactions by preceding block of the central sympathetic activity,^{4),21),24)} (3) alteration of the response by depth of anesthesia,²²⁾ and (4) possible competition between α - and β -adrenergic drives in the medullary cardiovascular areas⁴⁾ all suggest the central adrenoreceptor mechanism being far more complicated, as described elsewhere,²⁶⁾ to interpret conclusively on the present data.

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