EFFECTS OF ADRENAL DEMEDULLATION AND PERIPHERAL NORADRENALINE-DEPLETING AGENTS ON ADRENOCORTICAL FUNCTION AND SPLEEN IN RATS

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Abstract—Adrenocortical functions of adrenal-demedullated rats (ADM_x rats) and peripherally chemical-sympathectomized ADM_x rats were studied by examining changes in the levels of serum and adrenal corticosteroids (CS). Resting levels of serum and adrenal CS were not influenced by adrenal-demedullation and peripheral chemical-sympathectomy with 6-hydroxydopamine. Diurnal variation in serum CS concentration was also unchanged, suggesting that peripheral adrenergic systems do not influence the basal function of hypothalamo-pituitary-adrenocortical axis. Exposure of ADM_x rats to the stressful stimuli, however, resulted in lowered adrenocortical response with a lesser increase in serum CS concentration, while peripheral chemical-sympathectomy of ADM_x rats with 6-hydroxydopamine or guanethidine caused a significant enhancement of adrenocortical response to the stress with elevation of the serum CS concentrations. These findings suggest that increased peripheral adrenergic activity may suppress the activation of the hypothalamo-pituitary-adrenocortical system as the animals were exposed to the stressful stimuli. Adrenal-demedullation produced no increase in spleen weight while chemical-sympathectomy by peripheral administration of 6-hydroxydopamine did produce a significant increase in the weight of this organ. Histological features following chemical-sympathectomy are described.

Functional participation of the peripheral adrenergic system for regulation of hypothalamo-pituitary-adrenocortical function has been discussed by a few investigators (1, 2) in contrast to the extensive research on the regulation mechanisms of adrenocorticotrophic hormone (ACTH) secretion by central noradrenergic system (3). Advancement in the development of specific chemical agents which affect preferentially the synthesis, storage, release and uptake of neurotransmitters has provided additional knowledge on the control mechanism of ACTH release. 6-Hydroxydopamine reportedly

destroys peripheral adrenergic nerve endings leading to a suppression of peripheral adrenergic function in adult rats (4). Chronic administration of guanethidine to rats also produces similar effects (5). These agents, however, did not decrease the levels of catecholamines in the adrenal medulla. To assess the contribution of the peripheral adrenergic system to regulation of the pituitary-adrenocortical function, we gave these chemical agents to adrenal-demedullated rats (ADM_x rats). In this paper, we describe the changes in basal levels, diurnal variation of serum and adrenal

corticosteroids (CS), and response of adrenocortical gland to stressful stimuli on CS elevation by peripheral administration of 6-hydroxydopamine or guanethidine to intact rats as well as ADM_x rats. Changes in the weight and histologic features of the spleen are also described.

MATERIALS AND METHODS

Chemicals: 6-Hydroxydopamine and guanethidine were purchased from Sigma Chemical Company and pentobarbital sodium from Abbott Laboratories. Alumina (Alumina Woelm N. Akt. 1) was purchased from Woelm Pharma, washed with 2 N hydrochloric acid and water, and activated by heating at 120°C for 60 min and then at 200°C for 120 min (6). Organic solvents of reagent grade were purchased from Wako Pure Chemicals.

Animals: Male Donryu rats weighing 50–100 g were purchased from Nippon Rat Co., Saitama, Japan. They were fed a laboratory chow diet (CE-2, Nihon Clea Co., Tokyo) and water *ad libitum* in an airconditioned animal room (23±1°C) illuminated from 6:00 to 18:00 o'clock. These rats were handled when weighed daily between 9:00–10:00 a.m.

Adrenal-demedullation: When these rats weighed 150–200 g, the adrenal medullae were enucleated under pentobarbital anesthesia (50 mg/kg body weight, i.p.). These so-prepared rats were kept on saline instead of water for one week, in the separate cages. Then 5 adrenal-demedullated rats (ADM_x rats) of the same litter were put together in one cage and fed laboratory chow and water for one week. Complete absence of adrenal medulla in ADM_x rat was confirmed by histologic examination of the adrenal gland (Fig. 1).

Chemical sympathectomy: ADM_x rats or intact rats were subjected to a peripheral chemical-sympathectomy by giving 6-hydroxydopamine into the tail vein 4 times

Fig. 1. Defective medullary tissue in a medullectomized rat adrenal gland. Evidence of the regenerated adrenocortical tissue without a medulla 2 weeks after enucleation. Procedure of adrenal demedullation is described in the text. H-E stain $\times 50$.

in 2 weeks according to Angeletti (4). Control rats were given saline instead of 6-hydroxydopamine. Guanethidine was also used for peripheral chemical-sympathectomy by chronic i.p. administration according to Johnson and O'Brien (5). Decrease in abrupt elevation of the levels of blood catecholamines after decapitation was valid proof for adrenal demedullation and peripheral chemical-sympathectomy (Fig. 2).

Determination of serum and adrenal corticosteroids: One week after chemical-sympathectomy, rats were decapitated instantaneously at a definite time between 10:00 and 10:30 a.m. with a guillotine-type cutter. In an experiment to examine diurnal variation of blood CS concentration, the animals were killed between 10:00 and 10:30 a.m. and between 4:00 and 4:30 p.m.

Fig. 2. Time-course of increases in adrenaline and noradrenaline concentrations in blood collected immediately after decapitation of rats. Trunk blood was collected in 3–4 conical centrifuge tubes continuously from the decapitated rat. Procedure for determination of adrenaline and noradrenaline is described in the text. a: Adrenaline, b: Noradrenaline.

Trunk blood was collected in the conical centrifuge tubes in ice-cold water and the adrenal glands quickly removed. Serum and adrenal CS were determined fluorometrically by a modified method (7) of Guillemin et al. (8). Corticosterone was used as reference steroid.

Determination of serum catecholamines:

To 0.5 ml aliquots of serum was added 2 ml of 0.4 N perchloric acid and the preparation was centrifuged at $10,000\times g$ for 20 min at 0°C . To 1.5 ml aliquots of the supernatant was added 2 ml of EDTA solution containing 200 mg, the pH adjusted to 5–6 with diluted ammonia solution, and finally 100 mg of activated alumina was added. Catecholamines in the reagent mixture were absorbed to alumina by constant shaking of the mixture for 15 min at room temperature, eluted with acidic methanol from alumina, and separated into adrenaline and noradrenaline using Shimadzu's LC-1 type of high-speed liquid chromatograph. Adrenaline and noradrenaline

were determined fluorometrically by the THI method. One ng each of the reference adrenaline and noradrenaline was run simultaneously before and after the samples.

Exposure to stressful stimuli: Rats were exposed to cold environment ($4\text{--}6^{\circ}\text{C}$) for 30 min (cold stress). Forcing the rats to move a distance of about 50 m on a carrier (transfer stress) or forced-swimming for 5 min in 25°C -water (forced-swimming stress) was also used in other experiments. Rats were killed immediately after being exposed to such stress.

Examination of spleen: Soon after decapitation, the spleen was removed, cleaned, weighed and fixed in 10% formalin. Sections of tissue were stained with hematoxylin-eosin for histologic examination.

RESULTS

Resting levels of adrenal and serum CS in ADM_x and chemically-sympathectomized ADM_x rats:

Resting levels of adrenal and serum CS of ADM_x and chemically-sympathectomized ADM_x rats at the period of time between 10:00 and 10:30 a.m. were not significantly different from that of the control intact rats (Table 1). Resting levels of serum CS at the period between 4:00 and 4:30 p.m. of ADM_x and chemically-sympathectomized ADM_x rats were not significantly different from that of the control intact rats. The levels in the afternoon were significantly higher than those in the morning in the 3 groups of rats (Fig. 3). Thus diurnal variation of serum CS concentration was not affected either by adrenal demedullation or by peripheral chemical-sympathectomy.

Changes in the levels of serum CS of ADM_x and chemically-sympathectomized ADM_x rats in the stressful conditions:

When ADM_x rats were exposed to stressful stimuli such as transfer-, cold-, and forced-swimming stress, significant increase in serum CS was evident, but the extent of this increase was

Table 1. Resting levels of adrenal and serum corticosteroids in the intact, adrenal demedullated, and peripherally chemical-sympathectomized adrenal demedullated rats

Rats	N	Corticosterone	
		Adrenal ($\mu\text{g}/100\text{ mg}$)	Serum ($\mu\text{g}/100\text{ ml}$)
Intact	18	2.3 ± 1.6	4.2 ± 2.2
Adrenal demedullated	16	2.9 ± 1.7	6.5 ± 4.8
Peripherally chemical-sympathectomized adrenal demedullated	20	3.5 ± 1.9	5.8 ± 4.0

The values indicate the CS levels at around 10:00 a.m. as mean $\pm$ S.D. Adrenal demedullation and peripheral chemical-sympathectomy were carried out according to the methods described in the text. No significant differences were noted between the levels of 3 different groups.

Fig. 3. Resting levels of serum corticosterone at 10 a.m. and 4 p.m. in intact, adrenal demedullated (ADM_x), and peripherally chemical-sympathectomized ADM_x rats. [□]: Control intact rats, [▤]: ADM_x rats, [▦]: Peripherally chemical-sympathectomized ADM_x rats. *: Significantly different from the levels at 10 a.m. time ($p<0.05$). —: S.D.

much less than that of the control intact rats. An addition of peripheral chemical-sympathectomy with 6-hydroxydopamine, however, resulted in enhancement in increase of serum CS which was low in ADM_x rats (Fig. 4). Peripheral chemical-sympathectomy with guanethidine of ADM_x rats also produced a higher increase in serum CS level than that of ADM_x rats under cold stress, such as was observed in peripherally

chemical-sympathectomized with 6-hydroxydopamine ADM_x rats (Fig. 5).

Morphologic and histologic changes in spleen of chemically-sympathectomized rats: Spleens of ADM_x rats examined 10 days after peripheral chemical-sympathectomy with 6-hydroxydopamine were deeply reddish near brown in contrast to the bright red color in the control intact rats. The shape was round and thickset, while the shape of the spleen from intact rats was long with a sharp edge and thin. The weight of the spleen of peripherally chemical-sympathectomized rats was significantly heavier than that of the control intact rats (Table 2). Light microscopic examination revealed that the white pulp of the spleen of peripherally chemical-sympathectomized rats was remarkably enlarged and surrounded by red pulp containing more red blood cells than in the case of the control intact rats (Fig. 6 a, b).

DISCUSSION

The present study with ADM_x rats indicated that the basal secretion of corticosteroids (CS) was not affected by inhibition of the function of the peripheral adrenergic nervous system by 6-hydroxydopamine. Diurnal variation of the serum CS level was not affected by peripheral chemical-sympathectomy, suggesting that hypothalamo-

Table 2. Spleen weights of intact, adrenal demedullated (ADM_x) and peripherally chemical-sympathectomized ADM_x rats

Rat	N	Spleen Weight (g) (mean±S.D.)
Intact	36	0.58±0.36
ADM _x	33	0.68±0.22
ADM _x +Chemical-sympathectomized	40	0.79±0.18*

*Significantly different from intact rats, $p < 0.05$ **Fig. 4.** Changes in the levels of serum corticosterone of rats produced by exposure to stress. □: Control intact rats, ▤: Adrenal demedullated rats (ADM_x rats), ▦: Peripherally chemical-sympathectomized ADM_x rats. R: Resting level, S: Level immediately after exposure to stress. *, **: Significantly different from the resting level, * $p < 0.05$, ** $p < 0.01$.

pituitary-adrenocortical function is not influenced by changes in the function of peripheral adrenergic nervous system in the resting condition. Under conditions of stress, however, CS secretion from the adrenal gland was remarkably affected by suppression of

peripheral adrenergic functions. The significant increase in serum CS in stressed intact rats was significantly suppressed by adrenal demedullation. On the hand, the decrease of adrenocortical response in serum CS of ADM_x rats tended to recover in

Fig. 5. Larger increases in serum corticosterone by exposure to cold stress in adrenal demedullated (ADM_x) rats sympathectomized peripherally with 6-hydroxydopamine or guanethidine, as compared to ADM_x rats. : Adrenal demedullated (ADM_x) rats, : ADM_x+chemically sympathectomized rats with 6-hydroxydopamine. : ADM_x+chemically sympathectomized rats with guanethidine. R: Resting level, S: Level after exposure to cold stress. *: Significantly different from R, $p < 0.05$.

(a)

(b)

Fig. 6-a,b. Histology of the spleen of intact control rats (a) and peripherally chemical-sympathectomized adrenal-demedullated rats (b). H-E stain $\times 50$

peripherally chemical-sympathectomized ADM_x rats given stress.

The lowered response of the adrenal gland to stress in ADM_x rats may be explained by the absence of adrenaline which will stimulate ACTH secretion from the anterior pituitary gland in rats. A lower but significant increase in the serum CS of ADM_x rats under stress may be due to stimulation of hypothalamo-pituitary-adrenocortical functions. Enhancement of adrenocortical response to stress by chemical-sympathectomy in ADM_x rats, however, seems difficult to explain. In the peripherally chemical-sympathectomized ADM_x rats, the response of adrenocortical tissue to the exogenous ACTH was to the same extent as seen in the intact rats, therefore the demedullated adrenal gland itself may not be responsible for the phenomenon described above. It may be assumed that noradrenaline from peripheral adrenergic

neurons works as an inhibitory factor on the hypothalamo-pituitary-adrenal axis *via* afferent influences from peripheral organs or tissues that are stimulated by noradrenaline. Although Naumenko (2) reported that the peripherally acting adrenergic agent, naphtyzin, stimulated ACTH release from anterior pituitary gland in the guinea pig, it has been speculated that functional changes in the peripheral adrenergic structure would relate to the function of the central nervous system and both stimulatory and inhibitory effects on release of corticotropin-releasing factor from the hypothalamus would be produced through receptor mechanisms involved in the peripheral tissues (9). Atrial receptors may also be involved in control mechanisms related to ACTH secretion (10).

Morphologic and histologic changes were observed in the spleen of peripherally chemical-sympathectomized ADM_x rats and such alterations were probably due to noradrenaline depletion in the peripheral adrenergic system following chemical-sympathectomy with 6-hydroxydopamine.

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