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 (ADMX rats) and peripherally chemical-sympathectomized ADMX 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 ADMX rats to the stressful stimuli, however, resulted in lowered adrenocortical response with a lesser increase in serum CS concentration, while peripheral chemical-sympathectomy of ADMX 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 adrenocorticotropic 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 (ADMX rats). In this paper, we describe the changes in basal levels, diurnal variation of serum and adrenal
corticosteroids (CS), and response of adreno-
cortical gland to stressful stimuli on CS
elevation by peripheral administration of 6-
hydroxydopamine or guanethidine to intact
rats as well as ADMx 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
(ADMx 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 ADMx rat was confirmed
by histologic examination of the adrenal
gland (Fig. 1).

Chemical sympathectomy: ADMx rats or
intact rats were subjected to a peripheral
chemical-sympathectomy by giving 6-
hydroxydopamine into the tail vein 4 times
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 peri-
pheral chemical-sympathectomy (Fig. 2).

Determination of serum and adrenal corti-
costeroids: One week after chemical-
sympathectomy, rats were decapitated in-
stantaneously at a definite time between
10:00 and 10:30 a.m. with a guillotinetype
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.
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×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 (4–6°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 ADMx and chemically-sympathectomized ADMx rats: Resting levels of adrenal and serum CS of ADMx and chemically-sympathectomized ADMx 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 ADMx and chemically-sympathectomized ADMx 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 ADMx and chemically-sympathectomized ADMx rats in the stressful conditions: When ADMx 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
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 rats (Fig. 4). Peripheral chemical-sympathectomy with guanethidine of ADM rats also produced a higher increase in serum CS level than that of ADM rats under cold stress, such as was observed in peripherally chemical-sympathectomized with 6-hydroxydopamine ADM rats (Fig. 5).

Morphologic and histologic changes in spleen of chemically-sympathectomized rats: Spleens of ADM 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 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 hypothalmo-

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

| Rats                                      | N  | Adrenal (μg/100 mg) | Serum (μg/100 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    | 20 | 3.5±1.9            | 5.8±4.0           |

The values indicate the CS levels at around 10:00 a.m. as mean±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.
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 ADMx rats tended to recover in...
peripherally chemical-sympathectomized ADMx rats given stress.

The lowered response of the adrenal gland to stress in ADMx 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 ADMx rats under stress may be due to stimulation of hypothalamic-pituitary-adrenocortical functions. Enhancement of adrenocortical response to stress by chemical-sympathectomy in ADMx rats, however, seems difficult to explain. In the peripherally chemical-sympathectomized ADMx 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 hypothalamic-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 ADnx 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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