Determination of Catecholamines and Their Metabolites in Adrenals of Stress-Loaded and Wild Suncus (Suncus murinus)

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Abstract—The contents of catecholamines and their metabolites were measured in the adrenals of 3 groups of suncus. The first group was naive housed suncus; the second, suncus with stress-loading; and the third, wild ones. There were no significant differences in dopamine, norepinephrine and normetanephrine levels among the control, stress and wild groups. The content of epinephrine, however, decreased in the stress and wild group to 57% and 18%, respectively. These results suggest that housed suncus can easily react to stressful circumstances.

The suncus (Suncus murinus, Insectivora) is a new experimental animal (1, 2). In our previous paper, catecholamines (CAs) and their metabolites were measured in the urine, brain, heart and adrenal of the suncus as indexes of sympathoadrenal activity (3, 4). In comparison with rats, the contents of dopamine (DA) and its metabolites were lower in suncus urine. On the contrary, both norepinephrine (NE) and epinephrine (EPI) metabolite levels were rather higher. Although the contents of CAs and their metabolites in the brain and heart were almost similar between the suncus and rat, CAs contents in suncus adrenal were very markedly higher than those of the rat (NE: 9.2 times and EPI: 3.8 times). Enlargement of the medulla (ca. 3 times in percentage of medulla volume) was observed in suncus adrenal, which was considered to be a key point for the understanding of high levels of CAs contents.

We hypothesized that Suncus adrenal usually releases CAs at a very low rate because of a relatively stress-free condition, and the suncus is able to release CAs at a high rate when it is exposed to stressful stimuli. In this report, to test this hypothesis, we have measured the contents of CAs and their metabolites in the adrenals of the suncus under 3 different situations: control, stressed and wild suncus. Intraperitoneal saline administration was employed as a stress because suncus are so nimble in movement and savage that we empirically noticed that holding them to administer drugs would produce more stress than in the case of rats or mice.

Male suncus weighing 45–80 g were housed in a temperature-controlled room at 24±1°C and supplied with water and laboratory chows (special food for suncus, Central Institute for Experimental Animals, Tokyo) ad libitum (control group). Suncus in the stress group were administered intraperitoneally with 2 ml/kg and 1 ml/kg of physiological saline at 36 hr and 12 hr before sacrifice, respectively. Wild suncus were captured with traps at poultry farms in the suburbs of Tainan, Taiwan on January 31–February 7, 1988. The traps, being set from 5 to 6 in the evening and recovered early in the next morning, were brought to a laboratory in Chia-Nan Junior College of Pharmacy, Tainan. Then the captured, suncus was anesthetized with ether immediately, and its
pair of adrenals were excised onto dry ice at that college.

The frozen adrenal was homogenized in 1.0 ml of 0.1 M perchloric acid containing 5 mM EDTA and then centrifuged at 3,000 rpm for 30 min at 4°C. A 50-μl aliquot of the supernatant was directly injected to a reversed-phase HPLC system using a TSK gel ODS-80TM (250×4.6 mm, Tosoh Co., Ltd., Tokyo), and native fluorescence (315 nm) of CAs and their metabolites with excitation (280 nm) was continuously measured with a spectrofluorometer (PF-210, JASCO, Tokyo). A linear gradient elution was used from 100 mM acetate-Na buffer (pH 4.7) to 100 mM acetate buffer plus methanol (3:1, v/v) over 30 min, followed by 100 mM acetate buffer plus methanol (1:1, v/v) for 30 min.

The weight of a pair of adrenals was about 10 mg, being equal in both housed and wild suncus, and there was no relationship between adrenal weight and body weight, as shown in Fig. 1. This result suggests that the sympathoadrenal system of the suncus used in this study was already mature.

The contents of CAs and their metabolites are given in Table 1. There were no significant differences in the levels of DA, NE and normetanephrine (NMN) between the control, stress and wild groups. The content of EPI, however, decreased in the stress and wild groups to 57% and 18% of that in the control group, respectively. Metanephrine (MN) content in the wild group also decreased to 64% of that in the control group. These results indicate that housed suncus was able to excrete a very large amount of EPI from the adrenals during a relatively short period, 36 hr, and that the store of EPI was almost exhausted in wild suncus. In addition, there were no significant differences concerning the contents of CAs and their metabolites between male and female wild suncus (lower 2 rows of Table 1).

There can be two interpretations for the remarkable decrease of adrenal EPI content in wild suncus. The first explanation is that the wild suncus is always surrounded by stressful stimuli, which promote adrenal EPI release. As a result, in the wild suncus, adrenal EPI storage is smaller than in the housed ones. Consequently, the production of EPI is always a matter of great urgency, and storage of lays amounts of CAs is required. Enlargement of the medulla is desirable for these purposes. The second explanation emphasizes the acute stress given to wild suncus during capturing. Captured wild suncus were restrained in a small trap for 13 hr at the longest, transported by car over rough roads, and immediately their adrenals were excised under anesthesia. These con-

![Fig. 1. Relationship between body weight and adrenal weight of suncus. Symbols △: housed male, ●: wild male, and ○: wild female.](image)

Table 1. Contents of catecholamines and their metabolites in suncus adrenal

| Group   | Sex | DA       | NE       | EPI       | NMN       | MN       |
|---------|-----|----------|----------|-----------|-----------|----------|
| Control | Male (n=6) | 71.8± 8.5 | 4970±470 | 9480±870 | 9.44±0.60 | 7.81±0.25 |
| Stress  | Male (n=6) | 99.2±19.3 | 3890±400 | 5390±780* | (not determined) |        |
| Wild    | Male (n=7) | 123± 23   | 3400±620 | 1700±330** | 9.01±1.69 | 4.97±0.83* |
| Wild    | Female (n=7) | 201± 37   | 3000±630 | 1130±160 | 6.82±0.83 | 4.48±0.58 |

Values are expressed as pmol/mg tissue (mean±S.E.M.). Significant difference from the control (*t-test, between males): *P<0.05, **P<0.01, ***P<0.001. aDOPA, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and vanillylmandelic acid were not detected.
ditions might have induced acute EPI release from the adrenals. This series of experiments can not discriminate between these two possibilities. If captured wild suncus are housed for a while, the acute stress effect of capturing itself would disappear, and we could differentiate between the two possibilities.

Our results indicate that simple stress such as intraperitoneal saline administration can cause a dramatic decrease of EPI contents in housed suncus adrenals. In housed suncus, Maruoka et al. observed higher tyrosine hydroxylase activity of adrenals, much higher contents of CAs and greater enlargement of its medulla than observed in rats (4). These results suggest that housed suncus can easily react to stressful circumstances.

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