Strain Difference in Pituitary-adrenal Axis between Wistar-Imamichi and Long Evans Adult Male Rats

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Abstract: In the present study, we used closed colony-Wistar-Imamichi (WI), inbred WI and Long Evans (LE) adult male rats to examine the secretion of ACTH and corticosterone in response to restraint stress. Blood (0.3 ml) was withdrawn through a jugular cannula at 0, 15, 30, 60 and 120 min after the onset of restraint stress. Plasma concentrations of ACTH and corticosterone increased after stress in all groups, but the responses of ACTH and corticosterone secretion were higher in LE rats than in WI rats. Present data suggest that the LE rat might be a good model as a high-response strain and the closed colony or the inbred WI rat might be a good model as a low-response strain in restraint stress experiments.

Key words: rat, strain difference, stress response

Stress has been related to various diseases, including depression and has been known to increase the activity of the hypothalamic-pituitary-adrenal (HPA) axis [1, 11]. The identification of differences in the HPA axis between strains of rats might be important for investigating biological responses to stress, such as depression of the immune system, behavior and hypertension. There have been several studies reporting strain differences in the HPA axis among stress in rats, but most of them have compared the activity of the HPA axis among Fisher 344 (F344), Lewis (LEW), Spontaneously Hypertensive (SHR) and Wistar Kyoto (WKY) rats. These reports suggest that F344 shows a higher response in the HPA axis than LEW rats [2, 3, 5, 6]. Long Evans (LE) and Wistar strains are widely used for multipurpose experiments. However, there are few studies reporting the strain differences in stress response including these two strains in detail. In the present study, we used Wistar-Imamichi (WI) as a Wistar strain and LE adult male rats, and examined plasma concentrations of ACTH and corticosterone after the onset of restraint stress. Inbred WI rats recently have been established from a closed colony WI at the Institute for Animal Reproduction. In addition to closed colony WI rats, inbred WI rats were also used for the stress experiment in the present study.

Adult male closed colony or inbred WI (328 ± 7.61g) rats and LE (324 ± 9.23 g) rats were obtained from the Institute for Animal Reproduction (Ibaraki, Japan). Rats were housed under controlled temperature and lighting.
(lights on from 0700 to 1900 h) conditions and supplied with food and water ad libitum. Five to six animals were used in each experiment. Forty-eight hours before each experiment, a silastic cannula was inserted into the right atrium via the external jugular vein [8] in each rat for drawing blood samples. On the day of the experiment, animals were stressed by immobilization [9] in a small disposable restrainer (DecapiCone, Braintree Scientific Inc., MA, U.S.A.) for 120 min. Blood (0.3 ml) was withdrawn through the atrial cannula at 0, 15, 30, 60 and 120 min after the onset of restraint stress and was replaced with an equal volume of heparinized saline. All procedures were approved by the Animal Care and Use Committee of Dokkyo University School of Medicine. Plasma concentrations of ACTH [8–10] and corticosterone [4, 9] were determined by double-antibody radioimmunoassay using 125I-labeled radioligands as described previously. Antisera to ACTH and corticosterone were obtained from IgG Co. (Nashville, TN, U.S.A.) and Scantibodies Laboratory Inc. (Santee, CA, U.S.A.), respectively. The intra- and inter-assay coefficients of variation were 6.3 and 11.9 for ACTH and 6.8 and 9.5 for corticosterone, respectively. All results are expressed as the mean ± SEM. The data were analyzed using two-way analysis of variance (ANOVA) followed by Fisher’s protected least significant difference (PLSD) test; a value of P<0.05 was considered significant.

Plasma concentrations of ACTH and corticosterone increased in all groups after the onset of restraint stress (Fig. 1). However, the increases in plasma ACTH (LE vs. closed colony; F=8.47, P=0.0196 and LE vs. inbred; F=14, P=0.0057) and corticosterone (LE vs. closed colony; F=7.695, P=0.0242 and LE vs. inbred; F=6.206, P=0.0375) in response to restraint stress were smaller in both (closed colony and inbred) WI strains than in LE rats (Fig. 1). There was no significant difference in the responses of ACTH (F=0.362, P=0.564) and corticosterone (F=0.247, P=0.6324) secretion between closed colony and inbred WI rats.

It is well established that various stresses, such as restraint, forced swimming and fasting stress, increase the secretion of corticotrophin releasing hormone (CRH), ACTH and corticosterone from the hypothalamus, pituitary and adrenal cortex, respectively [1,11]. Previous studies have examined the strain differences in ACTH or corticosterone secretion among F344, LEW, SHR and WKY rats [2, 3, 5, 6], but few studies report the difference of the HPA axis between strains including WI and LE rats in detail. In the present study, the secretion of ACTH and corticosterone in response to restraint stress clearly showed a difference between strains, and the responses of ACTH and corticosterone secretion were higher in LE than in WI rats. Only one study has examined corticosterone secretion in response to restraint stress in Wistar and LE rats, reporting that there was no difference of corticosterone secretion between Wistar and LE rats [7]. The difference of result
in corticosterone response between this earlier report and ours might be due to the way of taking blood samples. Blood was drawn from a clipped tail vein while the rat was restrained and the plasma concentration of corticosterone before stress showed high values (over 200 ng/ml) as a basal level [7]. In the present study, 48 h before each experiment, a silastic cannula was implanted in jugular vein in each rat for drawing blood samples and the plasma concentration of corticosterone before stress showed a reasonable value. Another factor possibly causing the different result might be the difference between Wistar and WI rats. Other studies have mentioned that strain differences were found in the expression of CRH mRNA and in plasma concentrations of ACTH and corticosterone at 3 h after restraint stress between F344 and LEW rats [2, 6]. Preliminary findings from our laboratory have demonstrated that ACTH and corticosterone responses in F344 rats were similar to those in LE rats and the responses in SD rats were mid-way between WI and LE rats. Further research is necessary to clarify the strain differences of the HPA axis including other major strain such as SD and F344. Our results suggest that comparison between WI and LE rats in response to stress would provide a good experimental model for various researches of the HPA axis as well as comparison between F344 and LEW rats. The growth curves of WI and LE are similar and their adult sizes are bigger than F344 or LEW rats. In experiments requiring large sample volumes, it would be advantageous to use WI and LE rats.

In conclusion, the secretion of ACTH and corticosterone in response to restraint stress was higher in LE than in WI rats. The LE rat might be a good model as a high-response strain and the WI rat might be a good model as a low-response strain in restraint stress experiments.

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**References**