Differential responses of the hypothalamo–pituitary–adrenocortical axis to acute restraint stress in Hatano high- and low-avoidance rats

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Abstract

The high- and low-avoidance animal (HAA and LAA respectively) strains of Hatano rats were originally selected and bred from Sprague–Dawley rats for their performance in the shuttle-box task. The present study focused on the activity of the hypothalamo-pituitary–adrenocortical (HPA) axis of HAA and LAA rats in response to restraint stress. The restraint stress induced an elevation in plasma concentrations of ACTH, prolactin, corticosterone and progesterone. Peak levels of plasma ACTH during stress conditions were significantly higher in HAA rats than in LAA rats, while peak levels of prolactin were significantly lower in HAA rats than in LAA rats. Under stress conditions, ACTH and prolactin synthesis in the anterior pituitary glands was significantly higher in HAA rats compared with LAA rats. The peak plasma concentrations of corticosterone, during restraint stress, were significantly higher in LAA rats compared with HAA rats. These results indicate that the response of the hypothalamo-pituitary axis to acute restraint stress is greater in HAA rats than in LAA rats, whereas the ACTH-induced adrenal response of corticosterone release is higher in LAA rats than in HAA rats. On the other hand, prolactin secretory activity is higher in LAA rats compared with HAA rats. These differences in endocrine responses to stress may be involved in the regulation of the avoidance responses in the shuttle-box task.


Introduction

The two-way active avoidance learning test is generally used for evaluating the effects of chemicals in pharmacological and toxicological studies. However, this test often produces large individual variations in data. To settle this issue, Hatano rat lines have been genetically selected and bred from Sprague–Dawley rats on the basis of their performance in a shuttle-box task (Ohta et al. 1995). The shuttle-box test produces individual differences and, therefore, high-avoidance animals (HAA) could be selected on the basis of their high rate of avoidance response and low-avoidance animals (LAA) for their low rate of response. It is also known that Roman and Syracuse rats, selectively bred for differential shuttle-box acquisition, originated from Wistar and Long–Evans strains respectively (Bignami 1965, Brush et al. 1979).

The restraint stress induced the facilitation of the hypothalamo–pituitary–adrenocortical (HPA) axis and the down-regulation of the hypothalamo–hypophysial–gonadal (HPG) axis (Tohei et al. 1997). Further comparisons of these two Hatano lines, using the behavioral tests used routinely in experimental neurobehavioral teratology, have revealed additional differences in activity levels, in maze performance (Ohta et al. 1995) and in behavioral development (Ohta et al. 1998). Furthermore, the plasma concentrations of adrenocorticotropic hormone (ACTH) have been higher in HAA rats than in LAA rats after shuttle-box testing (Ohta et al. 1999). The adrenal weights have been heavier in HAA rats than in LAA rats in both sexes of the Hatano rat (Ohta et al. 1999). Strain differences have been observed as an increase in cortical thickness, but not medullary area, of adrenal glands in HAA rats (Ohta et al. 1999). Strain differences have also been reported in the hormonal responses to stress and in adrenal gland weight (Gentsh et al. 1982, Walker et al. 1989, Brush et al. 1991, Driscoll et al. 1998) when comparing high-avoidance rats, such as Roman high-avoidance (RHA) rats and Syracuse high-avoidance (SHA) rats, and low-avoidance rats such as Roman low-avoidance (RLA) rats and Syracuse low-avoidance (SLA) rats.

The present study was conducted to further clarify strain differences, using the restraint-stress model, the endocrinological characteristics of the HPA axis in Hatano rat strains.

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Materials and Methods

Animals and experimental procedures

Adult male Hatano rats (8–11 weeks) from each strain were used (HAA, n=40; LAA, n=40). Animals were maintained under a 12 h light:12 h darkness cycle (light period from 0700 to 1900 h), at a temperature of 23–25 °C and a relative humidity of 55 ± 5%. Food (CE-2, Clea Japan, Inc., Tokyo, Japan) and water were available ad libitum. Animals were stressed by immobilization in a plastic bag (DecapiCone, Braintree Scientific Inc., MA, USA). Animals were killed by decapitation at 0, 5, 15, 30, 60, 120 or 180 min after restraint stress and then after 120 min of resting after 180 min stress. All experimental manipulations were performed between 1000 and 1600 h. Trunk blood was collected in heparinized tubes containing aprotinin and centrifuged immediately; plasma was separated and stored at −20 °C until assayed for ACTH, corticosterone, prolactin and progesterone. Anterior pituitary glands were homogenized in 1 ml 0.85% (w/v) NaCl solution and centrifuged at 25 000 g for 30 min at 4 °C. These supernatants were stored at −20 °C until assayed for ACTH, prolactin, corticosterone and progesterone. The experimental protocol was in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology, and approved by the Animal Care and Use Committee at the Hatano Research Institute of Food and Drug Safety Center.

RIA

Plasma prolactin concentrations were measured using NIDDK kits for rat prolactin. Hormones for iodination were rat prolactin–I–5. The antiserum used was anti-prolactin–S–9. Results were expressed in terms of National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) rat prolactin–RP–2. The intra- and inter-assay coefficients of variation for prolactin were 3.4 and 5.2% respectively.

Concentrations of ACTH (Kanesaka et al. 1992), corticosterone (Tomabechi et al. 1994) and progesterone (Taya et al. 1985) in plasma were measured by double-antibody RIAs using 125I-labelled radioligands as described previously. The intra- and inter-assay coefficients of variation were respectively: 11.3 and 11.9% for ACTH, 9.8 and 17.5% for corticosterone and 9.5 and 16.4% for progesterone.

Statistical analysis

All data are expressed as s.e.m. Significant differences between HAA rats and LAA rats were analyzed by Student’s t-test when uniformity of variance was confirmed by the F-test. When the variance was not uniform, the Mann–Whitney U-test was used. When more than two means were compared, ANOVA was carried out and the significance of the differences between means was determined by the Tukey–Kramer multiple-comparison test. P<0.05 was considered to be statistically significant.

Results

Concentrations of pituitary and adrenal hormones in plasma and pituitary before stress

There were no significant differences in plasma concentrations of ACTH, prolactin, corticosterone and progesterone between HAA and LAA rats, and no significant strain differences were found in concentrations of adenohypophyseal ACTH and prolactin (Table 1).

Changes in concentrations of ACTH and prolactin, corticosterone and progesterone in circulation and in pituitary glands

The restraint-stress challenge test induced a sharp increase in plasma concentrations of ACTH in both strains. The peak levels of ACTH were observed 60 min after stress in both strains, but the peak of ACTH was significantly higher in HAA rats than in LAA rats (Fig. 1a). Pituitary concentrations of ACTH in HAA rats increased at 30 and 60 min after stress and these values were significantly higher than basal levels; concentrations of ACTH in HAA rats were significantly higher at 5, 30, and 60 min after stress than the levels in LAA rats (Fig. 2a).

The peak levels of plasma prolactin were observed 15 min after stress in both strains, whereas the peak concentrations in LAA rats were significantly higher than in HAA rats (Fig. 1b). Pituitary concentrations of prolactin in HAA rats at 15 and 180 min after stress were significantly higher than the basal level and were also significantly higher than levels in LAA rats (Fig. 2b).

Table 1 Profile of pituitary and adrenal hormones in adult male Hatano HAA and LAA rats

<table>
<thead>
<tr>
<th>Hormones</th>
<th>HAA</th>
<th>LAA</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>144.2 ± 75.1</td>
<td>123.9 ± 14.3</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>51 ± 2.3</td>
<td>16.4 ± 7.8</td>
</tr>
<tr>
<td>Corticosterone (ng/ml)</td>
<td>27.6 ± 3.3</td>
<td>59.2 ± 9.0</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.16 ± 0.08</td>
<td>0.22 ± 0.11</td>
</tr>
<tr>
<td>Pituitary glands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH (ng/mg tissue)</td>
<td>22.3 ± 3.2</td>
<td>22.8 ± 5.4</td>
</tr>
<tr>
<td>Prolactin (ng/mg tissue)</td>
<td>141.0 ± 62.5</td>
<td>203.2 ± 46.7</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. of five rats.

No significant differences were found for LAA versus HAA rats (Mann–Whitney U-test or Student’s t-test).
During restraint stress, plasma concentrations of corticosterone increased in both strains. The peak levels of corticosterone were observed 30 min (in HAA rats) and 60 min (in LAA rats) after stress. The peaks of corticosterone concentration were significantly higher in LAA rats than in HAA rats. Thereafter, plasma concentrations of corticosterone slowly decreased in LAA rats approaching basal levels by 300 min; in contrast, the peak levels were maintained until 300 min later in HAA rats (Fig. 1c).

During restraint stress, plasma concentrations of progesterone increased in both strains, but no significant difference was found between HAA and LAA rats (Fig. 1d).
Correlations between ACTH and corticosterone

There was a positive correlation between plasma concentrations of ACTH and corticosterone in HAA rats \((n=40, r=0.49, P<0.05)\) and LAA rats \((n=40, r=0.48, P<0.05)\) (Fig. 3). The data showed that there was a clear difference between the two strains in the stress response of the pituitary and adrenal glands, i.e. secretion of ACTH and corticosterone respectively. The pituitary stress response of ACTH secretion was higher in HAA rats than in LAA rats, whereas the adrenal response of corticosterone secretion was higher in LAA rats than in HAA rats.

Discussion

The present study clearly demonstrated differences in endocrine responses between HAA and LAA rats in the HPA axis during stress. The present study clearly showed that plasma concentrations of ACTH and corticosterone were higher in LAA rats than in HAA rats during stress, whereas plasma concentrations of ACTH were lower in LAA rats than in HAA rats (Fig. 1). In the HPA axis, these results indicate that the pituitary response of ACTH release during stressful conditions was higher in HAA rats than in LAA rats. However, basal levels of plasma corticosterone tended to be higher in LAA rats than in HAA rats and the peak plasma corticosterone level during stress conditions was also significantly higher in LAA rats than in HAA rats. These results indicate that ACTH-induced adrenal response of corticosterone release during restraint stress was higher in LAA rats than in HAA rats. Our previous study (Ohta et al. 1999) demonstrated that in female LAA rats, basal levels of plasma corticosterone were also significantly higher than in HAA rats, whereas adrenal gland weights were heavier in HAA rats than in LAA rats in both sexes. These results, therefore, suggest that HAA rats are more sensitive to stress compared with LAA rats. In previous papers, RHA rats were found to have large adrenal glands compared with RLA rats (Gentsch et al. 1981, 1982, Walker et al. 1989, Castanon et al. 1994, Aubry et al. 1995), whereas SHA rats had smaller adrenal glands that contained more basal corticosterone than SLA rats (Del Paine & Brush 1990, Brush 1991, Brush et al. 1991). On the other hand, in Roman and Syracuse rats, the response of the HPA axis is lower in high-avoidance rats than in low-avoidance rats during stress (Gentsch et al. 1981, 1982, Walker et al. 1989, Castanon et al. 1994, Brush 1991, Brush et al. 1991, Aubry et al. 1995). These results show that the genetic selection for avoidance behavior does not correlate withconsistent differences in the activity of the HPA axis.

It has been reported that plasma concentrations of prolactin increased in response to acute and chronic stress (Ajika et al. 1972, Euker et al. 1975, Turpen et al. 1976). In the present study, for both lines of Hatano rats, plasma concentrations of prolactin also increased under restraint stress. The peak concentrations of plasma prolactin were significantly higher in LAA rats than in HAA rats. In RHA and RLA rats, the plasma prolactin levels under various stresses were lower in RHA rats than in RLA rats as well as in Hatano rats (Gentsch et al. 1981, Steimer et al. 1997). It is well known that dopamine inhibits prolactin secretion. Our findings might suggest that concentrations of dopamine in HAA rats are higher than in LAA rats. \(\alpha\)-dopa infusion for 2 weeks increased plasma concentrations of dopamine, corticosterone and deoxycorticosterone, and also dopamine contents in the adrenal zona glomerulosa (Inglis & Kenyon 1992), indicating that dopamine plays a key role outside the central nervous system. Circulating ACTH increased in dopamine D2 receptor (D2R)-null mice compared with wild-type

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**Figure 3** Correlation between plasma concentrations of ACTH and corticosterone in HAA (a) and LAA rats (b) during restraint stress. Number of animals \((n)\), correlation coefficients \((r^2)\) and \(P\) values are as indicated.
controls. The adrenal glands in D2R-null mice show the characteristic hypertrophy of the adrenal cortex (Saiardi & Borrelli 1998). These reports suggest that dopamine is involved in adrenal steroid biosynthesis directly and indirectly. In addition, dopamine agonist has been reported to be involved in memory. The induction of working memory deficits has become linked to the D1 receptor, whereby acute blockade evoked working memory deficits. For example, infusions of the selective D1 receptor antagonists SCH23390 or SCH39166 into the prefrontal cortex of monkeys (Sawaguchi & Goldman-Rakic 1991) or rats (Seamans et al. 1998) impaired spatial working memory performance. Memory and learning, therefore, may be influenced by neuromodulators such as dopamine.

In conclusion, the results of the present study indicate that HAA and LAA rats exhibit marked differences in the response of their HPA axis and in prolactin secretion during acute restraint stress. These strain differences may alter their performance in the shuttle-box task.

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