Endocrinological responses during suckling in Hatano high- and low-avoidance rats

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Abstract

Hatano high-avoidance (HAA) and low-avoidance (LAA) animals were originally selected from Sprague–Dawley rats for good and poor active avoidance learning in a shuttle box. We studied the endocrinological profile in lactating rats to determine the effect of suckling during mid-lactation in HAA and LAA rats. The pups were separated from their mother rats 6 h before the onset of suckling and blood samples were drawn from unanaesthetized mother rats via a jugular cannula at 0, 5 and 15 min after the suckling stimulus and then 15, 45 and 105 min after pups were removed.

Plasma concentrations of oxytocin in HAA rats were significantly higher than in LAA rats during the suckling period. Plasma concentrations of prolactin and ACTH in HAA rats were significantly higher than in LAA rats during the suckling period, and at 15 min and 45 min after the pups were removed. However, there were no strain differences in circulating corticosterone between the two lines, indicating that the response of the hypothalamo–pituitary axis to the suckling stimulus was greater in HAA

rats than in LAA rats, whereas the ACTH-induced adrenal response of corticosterone release was higher in LAA rats than in HAA rats. Since dopamine from the median eminence inhibits prolactin secretion from the lactotrophs of the anterior pituitary, and tuberoinfundibular dopaminergic neurones are partially regulated by the level of circulating prolactin, we evaluated the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis. TH, measured by the accumulation of 3,4-dihydroxyphenylalanine, was significantly higher in HAA rats than in LAA rats before the suckling stimulus. After the suckling stimulus, TH activity in HAA rats was significantly lower than before suckling, whereas TH activity in LAA rats was not changed.

These findings clearly demonstrated that apparent differences between the two Hatano lines exist in endocrinological profiles during suckling. These strain differences probably originate from neurotransmitter changes, such as dopamine.

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Introduction

Pups obtain necessary nourishment and cause a variety of hormonal changes in the mother by suckling (Voogt 1978). The suckling stimulus increases the plasma concentrations of prolactin (Taya & Greenwald 1982, Grosvenor *et al.* 1986, Taya & Sasamoto 1989, 1990), adrenocorticotrophic hormone (ACTH), corticosterone (Voogt *et al.* 1969) and oxytocin (Higuchi *et al.* 1985, 1986, Grosvenor *et al.* 1986), and decreases luteinizing hormone and follicle-stimulating hormone (Taya & Greenwald 1982, Taya & Sasamoto 1989, 1990).

The Hatano rat lines have been genetically selected and bred from Sprague–Dawley rats to estimate the

pharmacological and toxicological effects of chemicals using the performance shuttle-box test. This learning test assesses the acquisition of avoidance ability by the rats escaping to the safe chamber after a conditioned stimulus of sound and light. When animals fail to avoid the stimulus they receive an electric shock. The Hatano rat lines were selected to resolve the high variability in the data using this test. High-avoidance animals (HAA) quickly acquire the active avoidance response, whereas low-avoidance rats (LAA) fail to acquire this response (Ohta *et al.* 1995). Besides the divergence in active avoidance behaviour, we have shown various other phenotypic differences. Adrenal weights are heavier in HAA rats than in LAA rats, and plasma concentrations of ACTH are higher in HAA rats

than in LAA rats (Ohta *et al.* 1999). HAA rats and LAA rats show differences in follicular development, luteal function and pattern of hormonal secretion during the oestrous cycle (Asai *et al.* 2002). The body weights of the HAA pups are heavier than the LAA pups during the latter half of lactation. HAA dams retrieve their pups faster and eject more milk than LAA dams (Ohta *et al.* 2002).

However, differences in the endocrinological profiles during suckling between the two Hatano lines have not been studied. To test the hypothesis that strain differences exist in the profiles of the hypothalamo–pituitary–adrenal axis and milk secretion after a suckling stimulus in Hatano rat lines, we examined the secretory pattern of plasma hormones after the suckling stimulus and the activity of the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH), in the stalk–median eminence (SME) in order to investigate the regulation of prolactin secretion.

Materials and Methods

Animals

Nulliparous female HAA rats ($n=20$) and LAA rats ($n=20$) at 70–100 days of age were used. Animals were maintained under a 12 h light:12 h darkness cycle (light period from 0700 to 1900 h), at a temperature of 21–25 °C and relative humidity of 50–65%. Food (CE-2; Clea Japan, Inc., Tokyo, Japan) and water were available *ad libitum*. Mating was allowed to occur within each strain. All pregnant rats were housed individually in plastic cages (35 × 40 × 18 cm) with wood chip bedding and allowed to deliver spontaneously. The litter size was adjusted to eight pups per dam on lactation day 1 (day of parturition = day 0 of lactation). All experimental procedures involving animals were carried out 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 Use Committee at the Hatano Research Institute of Food and Drug Safety Center.

Plasma hormonal responses after the suckling stimulus in lactating rats

One day before each experiment (lactation day 12–14), a silicon cannula (Kaneka Medix Co., Osaka, Japan) was inserted into the atrium via the external jugular vein in each rat under ether anaesthesia, to be used to withdraw blood samples. The cannula was kept patent with heparinized saline whenever blood was not being removed.

On lactation day 13–15, the pups were separated from their mothers at 6 h before the onset of suckling. Blood samples were drawn from a jugular cannula at 0, 5 and 15 min after suckling began, followed by sampling at 15,

45 and 105 min after the pups were removed. Blood was collected in heparinized tubes containing aprotinin and centrifuged immediately, and plasma was separated and stored at –60 °C until assayed for ACTH, corticosterone, prolactin and oxytocin.

RIA

Plasma concentrations of ACTH (Kanesaka *et al.* 1992) and corticosterone (Tomabechei *et al.* 1994) in plasma were measured by double-antibody RIAs using ¹²⁵I-labelled radioligands as described previously. Synthetic rat ACTH 1–39 (Sigma Chemical Co., St Louis, MO, USA) was used as the reference standard. The intra- and interassay coefficients of variation were 11.3% and 11.9% for ACTH and 9.5% and 16.4% for corticosterone respectively.

Plasma concentrations of oxytocin were also measured by double-antibody RIAs using ¹²⁵I-labelled radioligands. Synthetic oxytocin (Sigma Chemical Co.) was used as the reference standard. Anti-oxytocin serum (rabbit) was purchased from Chemicon International, Inc. (Temecula, CA, USA), and the radioligand oxytocin (NEX 187) was purchased from New England Life Science Products, Inc. (Boston, MA, USA). Plasma samples were purified and separated from plasma proteins using Sep-Pak Plus C₁₈ cartridges (Waters Co., Milford, MA, USA) as described previously (Stock & Uvnas-Moberg 1988). The intra- and interassay coefficients of variation for oxytocin were 4.4% and 6.8% respectively.

Plasma concentrations of prolactin were measured using NIDDK kits (NIH, Bethesda, MD, USA) for rat prolactin. Hormone for iodination was rat prolactin-I-5. The anti-serum used was anti-prolactin-S-9. Results are expressed in terms of NIDDK rat prolactin-RP-2. The intra- and interassay coefficients of variation for prolactin were 3.4% and 5.2% respectively.

TH activity before and after the suckling stimulus in lactating rats

Mother rats were injected with *m*-hydroxybenzylhydrazine dihydrochloride (NSD 1050; 50 mg/kg body weight *i.p.*; Sigma), an L-aromatic amino acid decarboxylase inhibitor, at 30 or 15 min before the onset of suckling. Rats were killed by decapitation 30 min after administration of NSD 1050. The SME was dissected with a pair of fine scissors under a stereoscopic microscope, homogenized in 150 µl 0.1 M perchloric acid, and centrifuged at 10 000 *g* for 10 min (Demarest 1980). The content of 3,4-dihydroxyphenylalanine (DOPA) in the supernatant was determined by HPLC with electrochemical detection. The pellet was solubilized in 0.5 M PBS and analysed for protein content by the method of Bradford (Bradford 1976).

Milk ejection and pup weight

All pups were removed from their dams on the morning of the experiment (lactation day 13–15). After 6 h of

Figure 1 Effects of the suckling stimulus (solid bars) on plasma concentrations of (a) prolactin, (b) ACTH, (c) oxytocin and (d) corticosterone in HAA and LAA rats. Data are expressed as the means $\pm$ S.E.M. of six rats. * $P < 0.05$ versus HAA rats (Student's *t*-test or Mann-Whitney *U* tests). + $P < 0.05$ versus presuckling values (two-way ANOVA followed by Tukey-Kramer tests).

isolation, urine was manually expressed from the bladders of the pups. Pups were weighed and returned to their dams for 15 min of suckling and then reweighed to determine the amount of milk yield they had obtained from the dam.

Statistical analyses

All values are expressed as means $\pm$ S.E.M. Significant differences were analysed between HAA rats and LAA rats by Student's *t*-test when uniformity of variance was confirmed by *F*-test. When the variance was not uniform, Mann-Whitney *U* test was used. Plasma concentrations of prolactin, ACTH, corticosterone and oxytocin, and concentrations of DOPA were analysed using two-way ANOVA followed by Tukey-Kramer test. $P < 0.05$ was considered statistically significant.

Results

Circulating hormone levels after the suckling stimulus in lactating rats (Fig. 1)

The suckling stimulus induced an increase in plasma concentrations of prolactin, ACTH, oxytocin and corticosterone in both strains. The plasma concentrations of

prolactin and ACTH in HAA rats were significantly higher than in LAA rats during the suckling period and 15 and 45 min after the pups were removed (Fig. 1a and b). Plasma oxytocin levels before the suckling stimulus in HAA rats were significantly higher than in LAA rats. Concentrations of plasma oxytocin in HAA rats were significantly higher than in LAA rats during the suckling period (Fig. 1c). There was no strain differences in the concentrations of corticosterone between the two lines during the period studied (Fig. 1d).

TH activity in the SME before and after the suckling stimulus (Fig. 2)

The HAA and LAA rats received the i.p. injection of NSD 1050 either 30 min before the return of the pups or 15 min before the onset of suckling followed by suckling for 15 min. In all cases, rats were killed 30 min after NSD 1050 was injected. TH activity was measured by the accumulation of DOPA at 30 min after an injection of NSD 1050. TH activities before the suckling stimulus in HAA rats were significantly higher than in LAA rats. After the suckling stimulus, TH activity in HAA rats was significantly lower than before suckling, whereas TH activity in LAA rats was not changed compared with that before suckling.

Figure 2 DOPA accumulation at 30 min after an injection of NSD 1050 in lactating HAA and LAA rats. Rats received the injection of NSD 1050 15 min before the onset of suckling and then suckling was allowed for 15 min. Data are expressed as the means $\pm$ S.E.M. of seven rats. Bars with different letters are significantly different from one another at $P < 0.05$ as determined by two-way ANOVA followed by the Tukey–Kramer test.

Milk ejection and body weight (Fig. 3)

Milk yield was significantly lower for LAA rats than for HAA rats. Body weights of LAA pups were lower than HAA rats after the experiment.

Figure 3 Milk yield in HAA and LAA dams determined by gain in pup weight during a 15-min period of suckling following a 6-h separation * $P < 0.05$ versus HAA rats (Student's *t*-test).

Correlations between ACTH and corticosterone (Fig. 4)

There was a positive correlation between plasma concentrations of ACTH and corticosterone in HAA rats ($n=6$, $r^2=0.63$, $P < 0.05$) and LAA rats ($n=6$, $r^2=0.38$, $P < 0.05$). The data showed that there was a clear difference between the two strains in the stress response to the suckling stimulus of the hypothalamo–pituitary and adrenal glands in the secretion of ACTH and corticosterone respectively. The hypothalamo–pituitary response of ACTH secretion to the suckling stimulus in HAA rats was higher 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 that there are differences between HAA rats and LAA rats in the pattern of hormonal secretion during suckling. The concentrations of plasma prolactin, ACTH and oxytocin in LAA rats were significantly lower than in HAA rats during the suckling period. The weights of LAA pups were lower than those of HAA rats. Milk yield was significantly lower in LAA rats than in HAA rats. Our previous report showed LAA pups with reduced weight gain compared with HAA pups (Ohta *et al.* 1998), LAA dams with a lowered prolactin secretion 15 min after suckling compared with HAA rats, and LAA dams with a lowered milk yield during a 15-min period of suckling compared with HAA dams (Ohta *et al.* 2002). We also reported that there were no strain differences in the levels of milk ejection following oxytocin injection between HAA and LAA rats (Ohta *et al.* 2002). Furthermore, we investigated the weight of the pups in a previous cross-fostering study (Ohta *et al.* 1998) where half the litters from HAA dams were fostered onto LAA dams, and half the litters from LAA dams were fostered onto HAA rats. Body weight was lower for HAA offspring fostered by LAA dams than for pups fostered by HAA dams, whereas it was greater for LAA offspring fostered by HAA dams than pups fostered by LAA dams (Ohta *et al.* 1998). The present study in conjunction with two previous papers (Ohta *et al.* 1998, 2002) findings suggested that there are no strain differences in milk synthesis and strength of suckling by pups but the differences indicated that LAA rats are more insensitive to the suckling stimulus than HAA rats.

TH activity was higher in HAA than in LAA rats before the onset of suckling. TH activity decreased at 15 min after the onset of suckling in HAA rats, whereas it did not change in LAA rats in response to suckling. Prolactin acts on the epithelial cells of the mammary gland to stimulate milk synthesis. Complex neuronal pathways involved in translating the suckling stimulus to increased prolactin secretion exist. It is well known that dopamine is a physiologically important prolactin-inhibitory factor and is secreted into hypothalamic portal blood to inhibit

Figure 4 Correlation between plasma concentrations of ACTH and corticosterone in HAA rats (left-hand graph) and LAA rats (right-hand graph) during suckling and at 15 min after suckling. The number of animals (n) and correlation coefficients (r^2) are as indicated.

prolactin release from lactotrophs tonically (MacLeod & Login 1976, Matsuzaki *et al.* 1997). In early lactation, the prolactin-induced increase in TH activity leads to negative feedback, but this effect is lost by mid-lactation. The injection of MMQ cells (which are anterior pituitary tumour cells known to secrete large amounts of prolactin in the rat) inhibited plasma prolactin levels in dams on day 6 of lactation, but not on day 13 (Arbogast & Voigt 1996). Treatment with an s.c. injection of prolactin increased TH activity in the SME on day 6, but not on day 13 (Voigt *et al.* 2001). Twenty-four hours after pup removal, the tuberoinfundibular dopamine neuronal activity and TH mRNA expression increased. Following removal of the suckling stimulus, prolactin decreased via changes in the activity of tuberoinfundibular dopamine neurones (Dermarest & Moore 1980, Arbogast & Voigt 1996). Removal of tonic dopamine inhibition is not sufficient to account for the high levels of prolactin attained during lactation, and additional releasing factors are probably involved. Thyrotrophin-releasing hormone, vasoactive intestinal peptide and oxytocin are some of the mediators of the prolactin response induced by suckling (de Greef *et al.* 1981, 1987, Jaworski *et al.* 1997, Matsuzaki *et al.* 1997, Watanobe *et al.* 2000). In this study, no clear differences between the strains were found in concentrations of prolactin before the suckling stimulus, but TH activity in HAA rats was significantly higher than in LAA rats. Suckling generally increases dopamine activity after pup removal. However, dopamine activity in LAA rats did not increase after pup removal. These strain differences in prolactin response may be due to regulation of the prolactin-inhibiting factor, dopamine, in addition to the prolactin-releasing factor during lactation. Further studies are necessary to clarify the differences in the mechanisms.

In conclusion, the results of the present study indicated clearly that HAA and LAA rats exhibit marked differences in the response of prolactin, oxytocin and ACTH secretion during suckling.

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