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RESEARCH NOTE

Effects of pantothenic acid supplement on secretion of steroids by the adrenal cortex in female rats

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Abstract
Purpose The effect of pantothenic acid (PaA) supplementation on adrenal secretion of corticosterone and progesterone in female rats was investigated.
Methods An in-vitro primary adrenal cell culture system was used. Pregnant rats were given 0.03% PaA in their drinking water throughout pregnancy and the period of lactation. In the first experiment, after weaning, female rats continued to receive 0.03% PaA treatment until 10 weeks of age. The animals were then decapitated and adrenal cells were cultured in the absence or presence of rat adrenocorticotropic hormone (ACTH) for 4 h. In the second experiment, adrenal cells from lactating rats on day 5 of lactation were cultured in the absence or presence of rat ACTH for 4 h.
Results The effect of ACTH at 10^{-10} M on corticosterone and progesterone release was greater for PaA-treated cyclic rats than for control cyclic rats. The effect of ACTH at 10^{-10} M on corticosterone release was greater for PaA-treated lactating rats than for control lactating rats. Circulating ACTH and corticosterone levels in PaA-treated and control cyclic and lactating rats were no different.
Conclusions These results indicate that PaA supplementation induced hyperresponsiveness to ACTH stimulation in cyclic and lactating female rats. These results clearly demonstrated that PaA is an essential factor in adrenal steroidogenesis of female rats.

Keywords Adrenal steroidogenesis · Corticosterone · Cyclic rats · Lactating rats · Pantothenic acid

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Introduction

Pantothenic acid (PaA), a component of coenzyme-A (CoA), was reported to be linked to the main stress regulatory center, the hypothalamus–pituitary–adrenal (HPA) axis, as early as the 1950s by Hurley and Morga [1]. In the 1980s, Pietrzik [2] demonstrated that the greatest retention and most intensive metabolism of PaA in rats occurred in the adrenal glands. Jaroenporn et al. [3] showed that PaA enhanced the secretion of corticosterone and progesterone in the adrenal cortex cells of male rats in vitro. Yamamoto et al. [4] demonstrated that the plasma level of testosterone and several measures of sperm motility were significantly reduced in rats maintained on a PaA-deficient diet compared with control rats. These results clearly showed that PaA is an essential factor in adrenal and testicular endocrinology in male rats. The flexibility of the HPA axis was confirmed by Kappor et al. [5] who reported that

fluctuating maternal and prenatal glucocorticoid levels modified HPA activity and even life-long stress-related behavior of the offspring [6]. The report also emphasized the importance of the early living environment. However, whether or not early and long-term administration of PaA affect the HPA axis of the offspring is still poorly understood. This study was undertaken to determine the effect of PaA treatment on the function of the adrenal cortex in cyclic and lactating female rats.

Materials and methods

Materials

Dulbecco's modified Eagle's medium (DMEM), MEM non-essential amino acids solution, penicillin and streptomycin (Invitrogen, Burlington, ON, Canada), HEPES (Dojindo, Gaitherburg, MD, USA), collagenase type V, deoxyribonuclease, fetal bovine serum (Sigma–Aldrich, St Louis, MO, USA), rat adrenocorticotropic hormone (ACTH) (AFPRFR7890, NIDDK, Torrance, CA, USA), PaA (calcium pantothenate) (Daiichi Fine Chemical, Tokyo, Japan), and aprotinin (Sigma–Aldrich) were purchased.

Animals

Wistar Imamichi rats from the Imamichi Institute for Animal Reproduction (Kasumigaura, Ibaraki, Japan) were used in this study. Adult female rats were kept in a room under controlled temperature (25 ± 2°C), humidity (50 ± 10%), and lighting (light on 0500–1900 hours) conditions. Vaginal smears were examined daily. At 1700 hours on the day of proestrus each female was transferred to the cage of a single male and left overnight. The occurrence of mating was checked next morning by the presence of sperm in the vaginal smear, this day was designed as day 0 of pregnancy. The day of parturition was designed as day 0 of lactation. For lactating rats, all litters were adjusted to nine young on the day of parturition and the young were weaned at 21 days of age. A rat diet (MR-Breeder; Nosan Corporation, Yokohama, Japan) was given to all animals ad libitum.

Experimental design

In the first experiment, on day 0 of pregnancy, pregnant rats were treated with 0 (control) or 0.03% PaA in the drinking water for the day of the experiment, ensuring rats were exposed to PaA for their whole lives. Female rats aged approximately 70 days treated with PaA or water were killed at 1100 hours on the day of estrus by decapitation, and the adrenal glands were immediately removed. The whole glands were used and the procedures were performed in accordance with previously described methods [3]. To examine the effects of PaA on ACTH-stimulated release of corticosterone and progesterone, the adrenal cells were incubated in the absence or presence of rat ACTH (AFPRFR7890 10^{-13} to 10^{-10} M) for 4 h. At the end of the 4-h incubation period, the supernatant was decanted and stored at −20°C until assayed for corticosterone and progesterone. 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, and progesterone.

In the second experiment, the effects of PaA on lactating rats were investigated. Adult female rats aged 70 days were treated with 0 (control) or 0.03% PaA in the drinking water from day 0 of pregnancy until day 5 of lactation. All rats were killed by decapitation at 1100 hours on day 5 of lactation, and the adrenal grands were used for primary cell-culture experiments in the same was as for experiment I. Concentrations of corticosterone in the media were measured. Trunk blood was collected in the same way as for experiment I.

All procedures were carried out in accordance with the guidelines established by Tokyo University of Agriculture and Technology for use of laboratory animals.

Radioimmunoassay

Concentrations of ACTH, corticosterone, and progesterone in plasma were determined by double-antibody radioimmunoassay (RIA) with ^{125}I-labeled radioligands as described elsewhere [7–9]. The antigen against progesterone (GDN 377) was kindly provided by Dr G.D. Niswender (Colorado State University, Fort Collins, CO, USA).

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by use of the Statistical Package for the Social Sciences (SPSS) 16.0. One way ANOVA and the unpaired-sample *t* test were used for comparison of plasma hormone levels, hormone secretion levels in cell culture medium, and organ weights. *P* values of less than 0.05 were considered to be statistically significant.

Results

Experiment I: cyclic rats

Body weights for PaA-treated rats (222.6 ± 5.66 g, $n = 16$) were significantly lower than for control rats

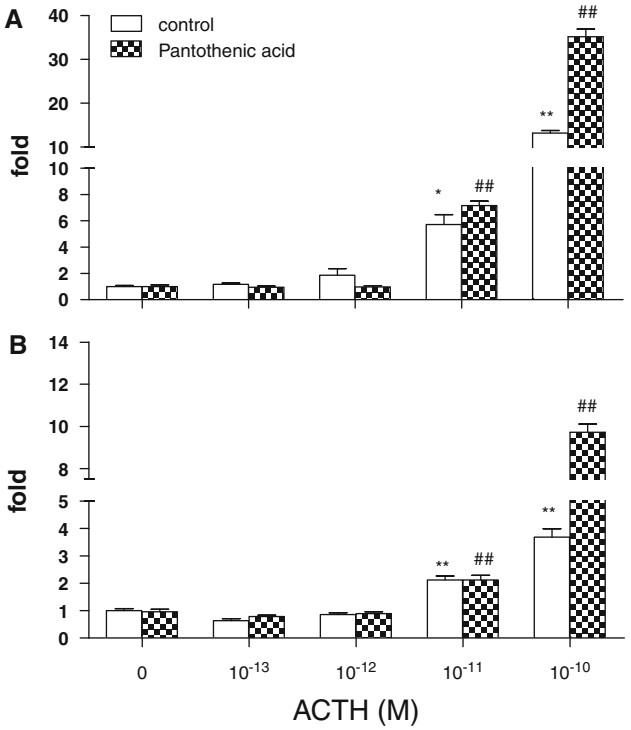

Fig. 1 Effects of ACTH (10^{-13} to 10^{-10} M) on release of corticosterone (**a**) and progesterone (**b**) by primary adrenal cells from adult cyclic female rats treated with 0 (control) or 0.03% PaA. Data were normalized to no treatment of ACTH in the control group and are the mean ± SEM from quadruplicate samples. $*P < 0.05$, $**P < 0.01$, compared within the control group; $^{\#\#}P < 0.01$, compared within the PaA group

(258.4 ± 4.43 g, $n = 16$). The relative weight of the adrenal gland in the PaA-treated group (0.40 ± 0.025 g, $n = 16$) was not statistically significantly different from that in the control group (0.35 ± 0.014 g, $n = 16$).

Basal plasma concentrations of ACTH (control rats: 513.0 ± 80.0 pg/ml, $n = 5$ vs. PaA-treated rats: 786.0 ± 160.0 pg/ml, $n = 5$), corticosterone (control rats: 290.6 ± 45.4 ng/ml, $n = 16$ vs. PaA-treated rats: 245.9 ± 39.5 ng/ml, $n = 16$), and progesterone (control rats: 14.6 ± 1.8 ng/ml, $n = 16$ vs. PaA-treated rats: 17.5 ± 1.7 ng/ml, $n = 16$) were not significantly different between the two groups.

Administration of ACTH (10^{-13} to 10^{-10} M) to cultured adrenal cells resulted in a clear dose-dependent increase in corticosterone and progesterone for both control and PaA-treated cyclic rats (Fig. 1). Corticosterone and progesterone release in response to ACTH (10^{-10} M) were markedly higher for adrenal cells from PaA-treated rats than for those from control rats. A significant difference was observed between the two groups (Fig. 1).

Experiment II: lactating rats

Plasma concentrations of corticosterone were not significantly different between the control lactating (70.67 ± 9.75 ng/ml, $n = 5$) and PaA-treated lactating rats (115.70 ± 25.65 ng/ml, $n = 5$).

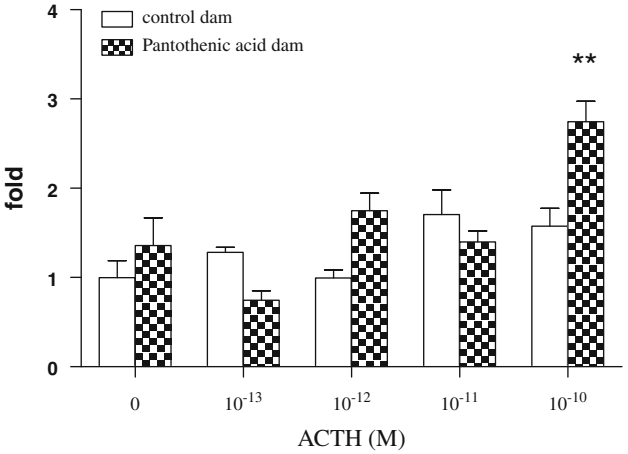

Fig. 2 Effects of ACTH (10^{-13} to 10^{-10} M) on release of corticosterone by primary adrenal cells from lactating female rats treated with 0 (control) or 0.03% PaA. Data were normalized to no treatment of ACTH and are the mean ± SEM from quadruplicate samples. $**P < 0.01$, as compared within the PaA group

Administration of ACTH (10^{-13} to 10^{-10} M) resulted in no corticosterone-release response for both control and PaA-treated rats (Fig. 2). In contrast, after administration of ACTH (10^{-10} M) a significant increase in corticosterone was observed from cultured adrenal cells from the PaA-treated rats but not from those from the control rats.

Discussion

This study demonstrated that treatment with PaA enhanced secretion of corticosterone by adrenal cells of cyclic and lactating female rats. On ACTH stimulation (10^{-10} M), cells originating from the adrenals of cyclic female rats treated with PaA released corticosterone and progesterone at levels significantly higher than those from control adrenals. For lactating rats, the adrenocortical cell culture from PaA-treated lactating rats was also more sensitive to ACTH stimulation, as indicated by higher corticosterone release. The adrenals of PaA-treated rats are more sensitive to stimulation with ACTH than adrenals from control rats, although there was no difference in the plasma concentrations of ACTH between two groups. These results suggest that PaA acts directly on the adrenal cortex but does not affect hypothalamus or pituitary levels. Consistent with this study, our previous work demonstrated that ACTH-stimulated release of corticosterone and progesterone from adrenal cells was greater for PaA-treated male rats than for control male rats [3]. Our previous study also clearly showed a decrease in circulating corticosterone and testosterone in PaA-deficient male rats [9]. This study

demonstrated that PaA is an essential factor for maintenance of adrenal cortex function in female rats, in agreement with previous studies of male rats [3]. These results demonstrated that, in the long-term, PaA supplementation induced adrenal hyperresponsiveness to ACTH stimulation in female rats. In this study, body weight was significantly lower for PaA-treated rats than for control rats, although the exact mechanism was not clear. These results also showed that PaA supplementation during pregnancy clearly enhanced the ability of adrenal cells to secrete corticosterone in response to ACTH in early lactating rats. Our previous work has also demonstrated that the adrenal gland is essential for maintenance of normal secretion of gonadotropins and prolactin and for maintenance of ovarian function during lactation in the rat [10]. The suckling stimulus suppresses secretion of gonadotropin-releasing hormone (GnRH), LH, and follicle-stimulating hormone (FSH), and follicular development in lactating rats. These effects are primarily mediated by endogenous corticotrophin-releasing hormone (CRH) and opioid peptides [11]. Although suckling is maintained, plasma concentrations of LH and ovarian follicular development increase gradually as lactation progresses [12–14]. These gradual increases in gonadotropins during lactation in rats, however, were not observed when adrenalectomy was performed [10]. Abolishing the increase in plasma concentrations of LH and FSH in the plasma by adrenalectomy therefore prevented maturation of the new set of follicles usually seen during the second half of lactation in rats. Thus the adrenal glands modify the secretion of LH during lactation and, as a consequence, are important in establishing ovarian function during lactation.

In this study, PaA supplementation stimulated the ability of adrenal cortex cells from early lactating rats to secrete corticosterone in response to ACTH. These results showed that PaA supplementation is one useful method of recovery of ovarian function in lactating females.

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