EFFECTS OF CHRONIC INGESTION OF ANTHRANILIC ACID ON LACTATION IN MICE

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Summary

Treatment of mice with 0.04% anthranilic acid (AnA) as drinking water resulted in an apparent stimulation of pup's growth and food intake of mothers in their first lactations associated with an increased rearing rate and no alteration in plasma prolactin level. AnA showed no significant effects on the day of vaginal opening, the pattern of estrous cycles, plasma growth hormone level and endocrine organ weights of the female and male offspring. The growth of male offspring was significantly retarded by AnA, however, their reproductivity was quite normal. The results indicate that AnA can stimulate lactation of mice through little modulation of endocrine systems.

(Key Words: Anthranilic Acid, Body Growth, Food Intake, Lactation, Mouse)

Introduction

Nagasawa et al. (1988) previously found that the chronic ingestion of anthranilic acid (AnA) as drinking water stimulated significantly RNA content and RNA/DNA ratio of mammary glands in virgin female mice fed normal diet. Since a prior mammary gland growth is an essential factor for the subsequent lactation, the results tempted us to examine the effect of AnA on lactation.

This paper deals with this problem. The effect of AnA on post-weaned growth of offspring was also checked.

Materials and Methods

Animals and treatments

A highly inbred strain of C3H/He mice maintained in our laboratory were used. They were kept in aluminium cages (18x30x13 cm) with wood shavings, maintained in a windowless animal room which was air-conditioned (22-24°C and 55-75% relative humidity) and artificially illuminated (14 hours of light from 5:00 AM to 7:00 PM) and provided with a commercial diet (Lab MR Breeder: Nihon Nosan Kogyo KK, Yokohama, Japan) *ad libitum*.

Experiment I

Each female litter were divided into two groups at days 70-90 of age. The 1st group was given AnA as drinking water at the concentration of 0.04% (400 mg/ℓ tap water) throughout the experiment and the 2nd group received tap water only as the control. Simultaneously, female mice in each group were placed with males and pregnant mice were caged individually and the number of pups was reduced to 6 when they were more than 6 on the day of parturition (day 0 of lactation). They were normally nursed until day 20 of lactation when weaned.

Lactational performance. Lactational performance was estimated by average pup's weights and pup's growth rates on days 12 and 20 of age.

Reproduction. Pregnancy rate, delivery interval, mother weight at parturition, litter size, rearing rates on days 12 and 20, rate of still born-pups and still-birth rate were employed as the indices of reproductivity as detailed previously (Nagasawa and Furukoshi, 1985).

Food and water intakes. Food and water intakes were estimated by the difference in weights of food box and water bottle, respectively, be-

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tween days 12 and 15 of pregnancy and between days 8 and 11 of lactation and expressed in terms of g/day.

Plasma prolactin level. On the morning (8:30-9:30 AM) of day 12 of lactation, mother mice were bled by orbital puncture under the light ether anesthesia. Blood was centrifuged at 1,000 g for 20 min at 4°C and plasma was kept at −20°C. Plasma prolactin level was determined by homologous radioimmunoassay.

Experiment II

Body growth, vaginal opening and estrous cycle of offspring. Female and male offspring in each group of Experiment I were weaned on day 20 of age, maintained 4-6 each, given the same water and categorized to the same groups as those of their mothers and weighed every 7 days from the 1st to the 13th weeks after weaning. Vaginal smears were also examined every day in some females from the day of vaginal opening to day 60 of age.

Plasma growth hormone (GH) level and organ weights. On the morning (8:30-9:30) when the mice were 90 days old, all females and some males of both groups were bled by decapitation under the light ether anesthesia and plasma GH level was determined by radioimmunoassay. Anterior pituitary, adrenals, ovaries, testes, ventral prostate, lung, liver and kidneys were weighed and expressed in terms of mg/30g body weight.

Mammary gland growth. At autopsy, the bilateral third thoracic mammary glands of females were also removed and prepared for the whole-mount preparations, which were examined under the 10-fold magnification and the degree of mammary end-bud formation was rated from 1 to 7 in increments of 1 (Nagasawa et al., 1980).

Male reproductivity. Remaining male mice of both groups were placed with normal females for 30 days and examined the rate of pregnancy, delivery interval, litter size, and average pup's weight on day 0 as the indices of male reproductivity.

Results

Experiment I

Lactation (figure 1). Average weight and growth rate of pups on day 12 were significantly higher in the experimental group given AnA than in the control ($p < 0.05$). Quite similar, but not-significant, trends were also observed on day 20.

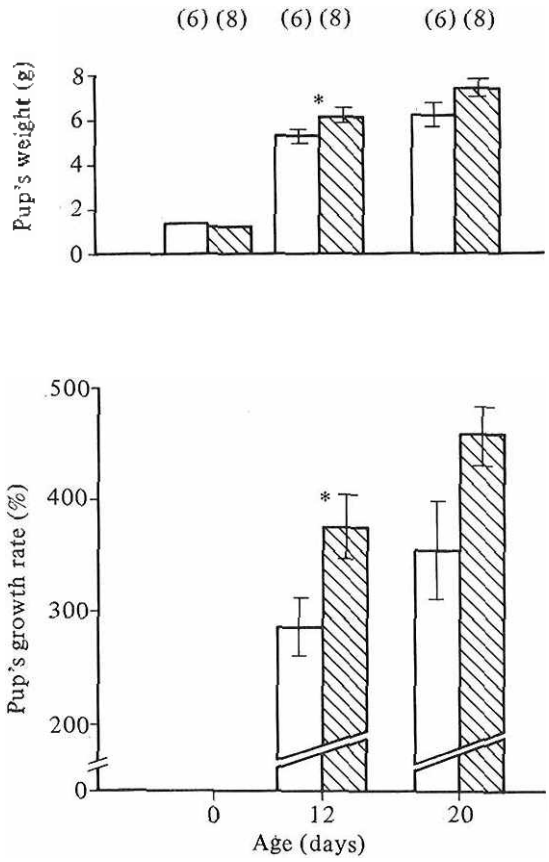

Figure 1. Effect of anthranilic acid (AnA) on lactation (Experiment I). ☐ Control, ▨ Experimental (AnA). Number of mice is in the parentheses. *Significantly different between groups at $p < 0.05$.

Reproduction (table 1). While delivery interval was significantly prolonged in the experimental group than in the control ($p < 0.05$), all mice even in the former delivered normally within 33 days after placing with males. Litter size, still-birth rate and rate of still born pups were affected little by AnA. Rearing rates on both days 12 and 20 were

TABLE 1. EFFECT OF ANTHRANILIC ACID (AnA) ON REPRODUCTION (MEAN±SEM) (EXPERIMENT I)

	Control	Exptl (AnA)
Number of mice	8	9
Delivery interval (days)	20.6±0.4	24.9±0.7*
Litter size	5.3±0.7	6.1±0.4
Mother wt. at parturition (g)	26.1±0.6	28.0±0.7
Rearing rate (%)		
day 12	73.3±0.8	85.2±11.0
day 20	52.9±11.4	79.6±11.7
Rate of still-born pups (%)	8.5	4.5
Still-birth rate (%)	0	0

*Significantly different between groups at $p < 0.05$.

apparently higher in the experimental group than in the control, although the difference was not statistically significant.

Food and water intakes (table 2). There was little difference between groups in the intake of either food or water during pregnancy. Meanwhile, food intake during lactation was significantly higher in the experimental group than in the control.

Plasma prolactin level. No significant difference was observed between the experimental and the control groups in plasma prolactin level on day 12 of lactation (data not shown).

Experiment II

Body growth of offspring after weaning (figure 2). In males, body weight of the experimental mice given AnA was lower than that of the control at any age examined and the difference was statistically significant after 3 weeks ($p < 0.05$). The growth pattern of females was similar in both groups, while the difference in weight was statistically significant at some ages.

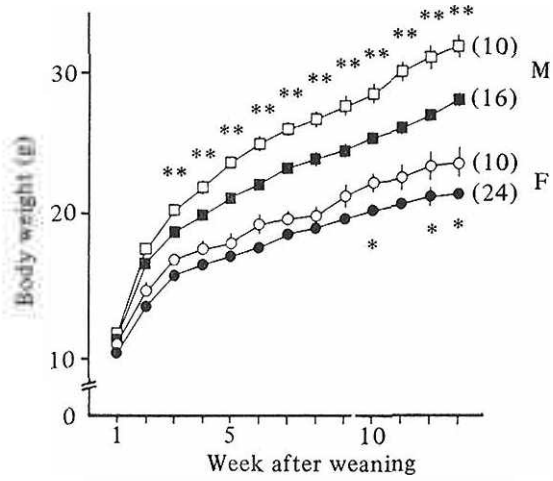

Figure 2. Effect of anthranilic acid (AnA) on growth of offspring after weaning (Experiment II). □, ○ Control, ■, ● Experimental (AnA). *and **significantly different between groups at $p < 0.05$ and 0.01, respectively.

Vaginal opening, estrous cycle, mammary growth, organ weights and plasma GH level. There was little difference in any parameter between the experimental and the control groups in either females or males (data not shown).

Male reproductivity (table 3). No difference between groups was observed in all parameters examined as the indices of male reproductivity.

TABLE 2. EFFECT OF ANTHRANILIC ACID (AnA) ON FOOD AND WATER INTAKES (MEAN + SEM) (EXPERIMENT I)

Period	Group	No. of mice	Food intake (g/day)	Water intake (g/day)
Days 12-15 of pregnancy	Control	8	4.9±0.3	6.5±0.1
	Exptl (AnA)	9	5.3±0.3	6.6±0.4
Days 8-11 of lactation	Control	7	9.4±0.6	10.4±0.8
	Exptl (AnA)	8	11.6±0.4*	11.8±0.6

*Significantly different between groups at $p < 0.05$.

TABLE 3. EFFECT OF ANTHRANILIC ACID (AnA) ON MALE REPRODUCTIVITY (MEAN ± SEM) (EXPERIMENT II)

	Control	Exptl (AnA)
Number of mice	10	10
Delivery interval (days)	24.3±1.5	21.9±0.8
Mother wt. at parturition (g)	28.5±0.9	27.4±0.6
Litter size	7.0±0.7	5.9±0.4
Rate of still-born pups (%)	4.1	7.8
Still-birth rate (%)	0	0

Discussion

This study shows that chronic ingestion of AnA stimulated lactation as estimated by pup's growth on days 12 and 20, which was associated with the increased rearing rate. In our previous study (Nagasawa et al., 1988), AnA elevated RNA content and RNA/DNA ratio in the mammary glands and an increase by AnA of food intake during lactation was observed in this study. The present results also show that mammary lobulo-alveolar growth was affected little by AnA in virgin mice, which is in good accord with our previous finding that AnA had little effect on mammary DNA content (Nagasawa et al., 1988). Thus, stimulated lactation by AnA would primarily be ascribed to the synergism of enhanced mammary gland cell activity for milk synthesis with an increased nutrient supply.

The causes of retardation by AnA of pregnancy and growth of male offspring are not clear at present. However, the effects would be only slight, since all female mice delivered normally within 33 days after placing with these males, which indicates that the reproductivity of male offspring is quite normal.

There were little differences between the experimental and the control groups in plasma prolactin level during lactation, the age of vaginal opening, the pattern of estrous cycle, endocrine organ weights and plasma GH levels of offspring. These observations suggest that the effects of AnA on lactation and body growth are not through its modulation of endocrine system.

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