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Effect of Suppressing Prolactin in the Mouse on Liveweight, Food Intake and Ovulation Rate

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Abstract

The involvement, if any, of prolactin in the relationship between appetite and ovulation rate was studied in mice. Injections of 0, 50, 100 or 150 μg of bromocryptine were given twice-daily to 46-day-old virgin mice for a minimum of 15 days. Between days 5 and 12 of treatment, mice receiving either 50, 100 or 150 μg of bromocryptine consumed 3·1, 4·3 and 6·2 g more food, respectively, than did mice in the control group. Liveweights and liveweight gain, however, were unaffected by bromocryptine injections. From day 0 to 12 of treatment mice grew 0·16, 0·15, 0·21 and 0·16 g/day in the 0, 50, 100 and 150 μg bromocryptine groups, respectively, ($P > 0·05$). Plasma prolactin concentrations were suppressed, but ovulation rates were similar in the 50, 100 and 150 μg bromocryptine groups compared with the control (median prolactin concentrations and mean ovulation rates were 32·9, 32·5 and 31·6 ng/ml and 14·4, 15·1 and 15·7 ova, respectively, compared with 217·2 ng/ml and 14·9 ova in the control).

The results do not support the hypothesis that prolactin directly mediates a relationship between appetite and ovulation rate in the post-pubertal mouse.

Introduction

Studies of previously selected lines of mice have shown a relationship between appetite and ovulation rate (Brien *et al.* 1984), which holds for most of reproductive lifespan (Brien and Hill 1986) and is much greater than that expected from changes in liveweight (Brien *et al.* 1984). The physiological control of this relationship is unknown, but it is likely to involve hormone action. Changes in ovulation rate as a response to selection are probably mediated by a change in the secretory pattern of the gonadotrophins and/or modification of the ovarian sensitivity to their action. It is not known what has promoted these changes when selection has been practised for appetite.

There are differences in basal metabolic rate between the high and low appetite selection lines cited above (Bishop 1985; Bishop and Hill 1985) and it is therefore possible that differences in basal metabolic rate are responsible for the changes in ovulation rate rather than appetite *per se*. Prolactin could be involved because (1) it may be involved in the growth process in mice (Sinha *et al.* 1972; Bohnet and Friesen 1976; van Buul-Offers 1984); (2) injections of prolactin increase both appetite and growth rate in deer (Ryg and Jacobsen 1982); and (3) prolactin is involved in follicular growth and development in a number of mammals (McNeilly 1984). Furthermore, a lack of prolactin, but not of growth hormone, may retard sexual development in dwarf mice as it could be important in the induction of luteinizing hormone receptors in the ovary (Bohnet and Friesen 1976).

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The experiments reported here examined whether prolactin mediates a relationship between appetite and ovulation rate in the mouse. This was achieved by studying the effects of suppressing endogenous prolactin activity on appetite (food intake), liveweight and ovulation rate.

Materials and Methods

Endogenous prolactin activity was suppressed by treatment with bromocryptine [2-bromo-α-ergocriptine mesilate (CB154), Sandoz Ltd, Basel, Switzerland] which has been shown to suppress circulating prolactin levels in rats (Gosden *et al.* 1981) and pituitary prolactin levels in mice (Yanai and Nagasawa 1974).

Preparation and Use of Drugs

Concentrated stock solutions of bromocryptine were prepared for each dose level used. The solvent used was 70% (v/v) alcohol, slightly acidified with tartaric acid. Working concentrations of the drug were made up daily by diluting an aliquot of the stock solution with 0·9% (w/v) physiological saline. Bromocryptine was administered to mice subcutaneously in 0·1 ml of vehicle. Dose–response trials were conducted before the main experiment.

Animals

All female mice used were descendants of the G strain (Sharp *et al.* 1984); mice used in the dose–response trials were bred from the control populations, whereas mice used in the main experiment were bred from the high-appetite selection lines. Females were weaned at 3 weeks of age and housed in stock cages with four to six mice per cage and placed in a room with a 12-h photoperiod. After weaning, the mice were fed Rat and Mouse No. 3 Expanded Breeder Diet (Special Diet Services Limited, Witham, Essex, England).

First Dose–Response Trial

The females were weighed at 5·5 weeks of age and allocated in harems of three to a male in mating cages. Vaginal plugs were used to indicate the day of mating. Females with vaginal plugs were randomly allocated to one of five treatments: 0, 50, 100, 150 or 200 μg of bromocryptine. The zero dose treatment used was 0·9% (w/v) physiological saline.

Twice-daily subcutaneous injections were given to the mice, from the evening of day 5 of pregnancy, until the morning of day 9 (day 0 = day of vaginal plug). The animals were rapidly decapitated 4–5 h after the last injection and the trunk blood was collected via a heparinized glass funnel into a glass tube. Blood samples were centrifuged at 3500 rev/min for 20 min at 4°C and the plasma recovered and stored at −20°C. Once the blood sampling had been completed, reproductive tracts were examined for evidence of pregnancy and embryonic resorption.

Second Dose–Response Trial

Forty females were weighed at 5·5 weeks of age and randomly allocated within litters to four treatments; where possible each group contained one female from each litter. The treatments were 0, 50, 100 and 150 μg of bromocryptine injected twice-daily, at 0830 and 1930 h, for 2 days. The zero dose was the vehicle used to dissolve the drug. Mice were rapidly decapitated 7–7·5 h after the last injection, blood samples being collected, centrifuged, processed and stored as previously described. The start of injections was staggered within treatments so that it was only necessary to decapitate 10 animals on any one day. This minimized the possibility of a large release of prolactin due to disturbance stress (Döhler *et al.* 1977).

Main Experiment

At 6 weeks of age, the female mice were weighed and housed individually in cages designed to measure feed intake. After a 4-day adjustment period, the animals were weighed again and allocated at random within litters to one of four treatments—either 0, 50, 100 or 150 μg doses of bromocryptine given twice daily, starting at 0830 and 1930 h and continuing for a minimum of 15 days. The zero dose was the vehicle used to dissolve the drug. Mice were weighed every second day, and the amount of food consumed was measured every 4 days during the first 12 days of injections. On the 12th day, the mice were moved to metal cages with four individuals to each cage.

The female mice continued receiving twice-daily injections of the same quantities of bromocryptine,

as before. After they had spent 2 days in the metal cages, a male was introduced. Daily checks were made for vaginal plugs, and the female mice were rapidly decapitated 2 h after the injection given during the morning of the day a plug was found. Blood samples were collected, centrifuged, processed and stored as previously described. Ovulation rate was determined by counting the number of ova embedded in cumulus, using a binocular dissecting microscope.

Radioimmunoassay of Prolactin

Materials for assay of mouse prolactin were obtained from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK), Maryland, U.S.A. The specificity of the antiserum had already been established by Dr A. F. Parlow of the Pituitary Hormones Center, Torrance, California, U.S.A. The specificity of the method of radioimmunoassay for mouse prolactin has also been reported by Sinha *et al.* (1972). Samples were assayed in duplicate, using 25-μl aliquots of plasma. A separate assay run was used for each of the dose–response trials; a third assay run was used for the main experiment. Based on seven assay runs, the mean concentration for the high-quality control was 213·3 ng/ml, with a coefficient of variation of 14·4%. The intra-assay coefficient of variation averaged 6·0%. The minimum detectable dose was 0·16 ng/assay tube, or 6·4 ng/ml when 25 μl of plasma was used.

Results

First Dose–Response Trial

All the females had mated, as shown by the presence of a vaginal plug, and embryos were presumed to have implanted by the start of the injection regimes. Twice-daily doses of 100 μg of bromocryptine were enough to cause a significant decline in the frequency of mice with live embryos compared with the control group of mice injected with vehicle only (Table 1).

Prolactin concentrations in the plasma of mice injected with any of the doses of bromocryptine used were not lower than those of mice of the control group.

Table 1. Effects of dose of bromocryptine (CB154) on plasma prolactin concentration in both dose-response trials and on the number of mice with and without live embryos in the first trial

*$P < 0·05$; **$P < 0·01$ compared with control values within each trial (using χ^2 analysis for the numbers of animals with and without live embryos and Wilcoxon's test for rank of prolactin concentrations)

CB154 treatment (μg)	Total No. of mice	No. of mice pregnant	No. of mice not pregnant	Prolactin in plasma (ng/ml) Median	Range
		First trial, with mated mice			
Control	8	7	1	12·5	6·3–29·2
50	7	6	1	12·5	11·1–72·8
100	7	2**	5	14·0	9·5–17·9
150	7	2**	5	13·4	9·9–22·4
200	7	1**	6	14·6	11·4–102·5
		Second trial, with virgin mice			
Control	10	–	–	25·6	21·0–76·2
50	10	–	–	11·7**	10·5–20·0
100	10	–	–	15·0*	9·8–31·1
150	10	–	–	14·1**	11·2–36·1

Second Dose–Response Trial

All doses of bromocryptine significantly reduced the prolactin concentrations in plasma of the virgin female mice when compared with those in the control group (Table 1). However, relative to each other, the 50, 100 and 150 μg dose levels gave similar suppression of plasma prolactin concentration.

The first dose–response trial showed that 100 μg of bromocryptine reduced pregnancy rate, and the second trial demonstrated that this dose was effective in suppressing prolactin in virgin mice. Therefore, 0, 50, 100 and 150 μg doses of bromocryptine were used for the main experiment.

Main Experiment

All doses of bromocryptine significantly reduced the prolactin concentrations in plasma of the mice when compared with the control animals, with each dose giving similar suppression (Table 2). Ovulation rates were not affected by any dose of bromocryptine administered (Table 2). Animals receiving 100 μg of bromocryptine grew 0·06 g/day faster than mice in the other treatment groups, but this difference was not significant ($P > 0·05$) (Table 2).

Table 2. Least-squares means from analysis of variance for the amount of food eaten per mouse before and during the injection period, ovulation rate, and median and range of plasma prolactin level following administration of bromocryptine (CB154)

Means or medians within columns with unlike superscripts differ significantly ($P < 0·05$) (pair-wise comparisons used for food eaten and ovulation rate and for plasma prolactin level where Wilcoxon's rank test was used). Values in bold type are significantly different at $P < 0·01$ compared with control

CB154 treatment (μg)	No. of mice per group	Food eaten in the 4 days before injections (g)	Food eaten during injection period (g)				Ovulation rate	Plasma prolactin level (ng/ml)	
			Day 1–4	Day 5–8	Day 9–12	Day 5–12		Median	Range
Control	13	20·3[a]	22·5[a]	21·9[a]	22·2[a]	44·1[a]	14·9[a]	217·2[a]	30·3–366·2
50	14	19·8[a]	21·5[a]	22·9[ab]	24·3[ab]	47·2[ab]	14·4[a]	**32·9**[b]	16·3–150·5
100	14	20·2[a]	21·8[a]	23·4[ab]	25·0[b]	48·4[b]	15·1[a]	**32·5**[b]	15·5–161·3
150	13	20·2[a]	21·1[a]	24·3[b]	26·0[b]	**50·3**[b]	15·7[a]	**31·6**[b]	16·1–121·1
Standard error[A]		0·74	0·71	0·72	0·84	1·50	0·60		

[A] Standard error of a treatment mean based on residual within-treatment variance.

After a 4-day adjustment period, the higher the dose of bromocryptine given, the more food consumed by the mice, the dose–response relationship being almost linear (Table 2). The liveweights of mice in all groups increased from 26·8–28·1 g before injections began to 28·8–30·4 g at day 12, and there were no significant differences in growth rates between the treatment groups.

No evidence could be found of greater food wastage among animals receiving higher doses of bromocryptine.

Discussion

The results do not support the hypothesis that prolactin mediates a relationship between appetite and ovulation rate in the mouse because ovulation rate was unaffected by any dose of bromocryptine given. However, prolactin levels were not completely suppressed by bromocryptine (levels were still in the range of 16–161 ng/ml in the treated groups in the main experiment) and these detectable levels of the hormone may be sufficient to play some role. Furthermore, Shire (1976) regards plasma concentrations as unreliable reflections of hormone activity at the target organs. Despite this, it is unlikely that unphysiologically low levels of prolactin could

be involved in creating the large differences in ovulation rate found between lines of mice selected for high or low appetite (Brien *et al.* 1984), as mice of both lines would probably have prolactin levels in the normal physiological range.

This finding is in apparent contradiction to the suggestion that prolactin plays a role in inhibiting oocyte maturation (Baker and Hunter 1978). It is also at odds with a recent review; McNeilly (1984) concluded that evidence from a number of mammalian species indicated a role for prolactin at the ovarian level in influencing follicular growth and development.

Whereas prolactin is involved in the maintenance of the corpora lutea of pregnancy and pseudopregnancy in the mouse (Bartke 1973), its involvement in pre-ovulatory events in any species remains unclear. Prolactin may affect the quality of oocytes destined to ovulate without influencing the number that do. Alternatively, the concentration of prolactin may influence ovulation rate, but only after a long period of suppression.

Injections of pregnant mare serum gonadotrophin given to mice 43 h before oestrous induction can markedly influence ovulation rate (Fowler and Edwards 1960; Land 1965), this suggests that ovulation rate is determined during the preceding oestrous cycle. As the minimum duration of bromocryptine administration in the present study was 15 days for any individual mouse, there should have been enough time for the lowered prolactin concentrations to influence ovulation rate.

Bromocryptine could directly influence other hormones other than prolactin; for instance the drug is a dopamine receptor agonist (Mehta and Tolis 1979) and there is some evidence that dopamine may inhibit gonadotrophin secretion (Evans *et al.* 1982), but this is inconclusive (Sirinathsinghji and Martini 1984). Clayton and Bailey (1984) have reported that bromocryptine does not affect LH and FSH concentrations despite its ability to modulate GnRH receptors independently of changes in serum prolactin. However, in the main experiment reported here, ovulation rate was unaffected by any dose of bromocryptine given.

Suppression of prolactin in the main experiment was associated with increases rather than decreases in food intake. This was contrary to expectation, as Ryg and Jacobsen (1982) have shown that injections of prolactin increase appetite in deer. In addition, food intakes and growth rates of sheep, cattle and deer are higher in summer months, a time of the year when prolactin activity is also high, although there is no proof that prolactin is causally involved (Forbes 1982).

It is possible that the stimulation of food intake by bromocryptine found in the present study was not connected to prolactin suppression. Bromocryptine affects the hypothalamus, the central and peripheral nervous systems and the gastrointestinal tract, see Flückiger (1976) and a review of Mehta and Tolis (1979). Dopamine is an important catecholamine, and when injected into the lateral hypothalamus of hungry rats, it has been shown to suppress feeding behaviour (Leibowitz 1976). A decrease in dopamine turnover from bromocryptine administration therefore might reduce satiety in mice, which could explain the results of this study.

Bromocryptine could have influenced appetite in the treated mice by other mechanisms. It has been observed to increase spontaneous motor activity in mice in a dose-dependent fashion (dose range 2·5–10 μg/g of liveweight, Flückiger 1976), and it can also induce hypothermia in mice (Flückiger 1976) and rats (Calne *et al.* 1975) at high dose levels (5–20 μg/g of liveweight, Calne *et al.* 1975). There are also dopamine receptors in the gut, which probably mediate the inhibitory effect

of dopamine on gastric emptying in man (Harrington *et al.* 1983). The actual mechanism or mechanisms of action remain obscure.

The alterations bromocryptine may cause to motor activity and/or thermoregulation could help account for the lack of effect of the greater food intake on liveweight in treated mice, if the extra food consumed was being utilized to compensate for alterations in thermogenesis. However, as mentioned above, bromocryptine may act at many sites in the body, so there could be other possible explanations.

In the first dose–response trial, the interruption of pregnancy in many animals may have jeopardized the opportunity to demonstrate differences in plasma prolactin activity due to treatment, differences which were clearly demonstrated in the second trial with virgin mice. There may have been two opposing influences on prolactin activity in the first trial: on the one hand prolactin concentrations may have been returning to the higher levels found in non-pregnant than in pregnant mice (Murr *et al.* 1974; Sinha *et al.* 1975) and on the other a lowering of plasma prolactin concentration may have resulted from the administration of bromocryptine.

The lack of change in ovulation rate whilst appetite increased in the main experiment tends to discredit the 'flushing' hypothesis put forward by Brien *et al.* (1984) to explain the high ovulation rates in lines of mice selected for high appetite. However, caution is required before fully extrapolating the findings of an experiment with bromocryptine, with its possible pharmacological side effects, to help explain the results of a selection experiment run under 'normal' husbandry conditions. Further experimentation is still needed to explain the relationship between appetite and ovulation rate in mice.

The post-pubertal mouse was used as a model in this experiment, as pre-pubertal animals may have failed to become sexually mature under bromocryptine administration (Advis *et al.* 1981). However, the relationship discovered between appetite and ovulation rate arose from mice selected for the amount of food eaten between 4 and 6 weeks of age (adjusted phenotypically for 4-week liveweight), a period during which the females are going through the onset and attainment of puberty. A different physiological background may exist during this neopubertal period than afterwards, and the role of prolactin could be more important at this time than in the post-pubertal stage, as was examined in this experiment.

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