METHALLIBURE, DELAYED PUBERTY AND THE PITUITARY–HYPOTHALAMIC COMPLEX OF FEMALE RATS

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Methallibure prevents the onset of puberty and ovulation and inhibits pituitary gonadotrophic activity in 30-day-old rats (Walpole, 1968). The compound also reduces food intake (Benson & Zagni, 1965) and body weight in adult rats (Labhsetwar & Walpole, 1972). Restriction of food intake *per se* causes a deficiency in LH secretion in adult rats (Howland, 1971), but Labhsetwar & Walpole (1972) have shown that the reduction in pituitary and plasma LH produced by methallibure in adult spayed rats is not secondary to its effect on food intake. It is due, presumably, to a primary effect on the hypothalamo-hypophyseal complex (Malven, 1971). In the present study, we explored the recurrence of delayed puberty in intact rats treated with methallibure (Ayerst).

Twenty-eight-day-old female rats of Sprague-Dawley strain from the Charles River Company were housed under standard conditions of lighting (07.00 to 21.00 hours) and temperature (71 to 72°F). One group, having free access to food, served as fully fed controls. The treatment group received methallibure (40 mg/kg/day in 0.2 ml of 0.5% Tween 80) orally for 20 days beginning at 28 days of age. The food consumption of these rats was measured daily and the same amount offered on the following day to those in the pair-fed group. (On the first day of the experiment, rats in the pair-fed group received an unrestricted quantity of food.) All the animals were weighed daily. Rats in a fourth, untreated, group were killed at 28 days of age following blood collection, under ether anaesthesia, into heparinized syringes. Blood was taken similarly from the remaining animals on the day following the last day of methallibure feeding. The pair-fed and fully fed rats were killed between 11.00 and 13.00 hours in such a way as to obtain representative samples during pro-oestrus and dioestrus. Organs were cleaned, and weighed on a torsion balance. Follicles and CL were counted after pressing ovaries between two slides and examining them under a microscope.

Plasma was stored frozen until assayed for LH, FSH and prolactin (Niswender Midgley, Monroe & Reichert, 1968) using NIH kits and the reference standards NIAMD-Rat-LH-1-3, Rat FSH-1-1 and Rat-Prolactin-1-1. The data were analysed by *t* test.

Table 1. The effects on organ weights of feeding methallibure to growing rats

Characteristics	Treatment groups		
	Fully fed	Pair-fed	Methallibure (40 mg/kg/day × 20)
No. of rats	15	16	17
Body wt (g)			
Initial	70 ± 1.4	72 ± 1.4	70 ± 1.2
Final	173 ± 2.0	145** ± 4.6	136** ± 8.0
Vaginal opening			
Age (days)	32 ± 0.42	41 ± 1.25**	—
Body wt (g)	94 ± 3.1	100 ± 5.6	—
Organ wt (mg/100 g body wt)			
Ovarian	35.3 ± 1.08	24.9** ± 1.56	10.9** ± 0.75 (**P-f)
Uterine	185 ± 11.3	118** ± 10.2	38** ± 1.6 (**P-f)
Pituitary	6.7 ± 0.27	5.8 ± 0.32	4.8** ± 0.22 (*P-f)
No. of follicles	12.4 ± 1.16	10.7 ± 1.07	12.8 ± 0.81
No. of CL	13.5 ± 1.45	8.3 ± 0.89*	N.D.** (**P-f)

* $P < 0.05$; ** $P < 0.01$; P-f=as compared to Pair-fed; N.D.=not detected.

Table 1 shows that dosing with methallibure caused a significant decrease in body growth ($P < 0.01$). Similar retardation of body weight gain also occurred in the pair-fed controls ($P < 0.01$). The restricted feeding alone delayed ($P < 0.01$), but did not entirely prevent, opening of the vagina as did dosing with methallibure. The methallibure treatment was effective in delaying puberty. Although the ovaries and the uteri of pair-fed animals were significantly lighter ($P < 0.01$) than those of fully fed females, the methallibure treatment

Table 2. The effect on plasma LH, FSH and prolactin of feeding methallibure to growing female rats

Hormone (ng/ml plasma)	Stage of cycle	Fully fed	Pair-fed	Methallibure	Prepubertal
LH (LER-1213A)	Dioestrus	a 16.1 ± 3.24 (6)	b 3.0 ± 1.91 (6)	**(**P-f) N.D. (9)	**(**P-f, **M) 71.6 ± 7.70 (10)
	Pro-oestrus	a 51.9 ± 5.19 (9)	** b 20.7 ± 4.58 (4)	—	—
FSH (RP-1)	Dioestrus	c 46.5 ± 6.15	d 61.1 ± 3.37	53.1 ± 2.49	**(**P-f, **M) 123.6 ± 11.07
	Pro-oestrus	c 66.0 ± 3.06	d 74.8 ± 8.87		
Prolactin (RP-1)	Dioestrus	c 12.5 ± 3.55	12.2 ± 1.04	** **P-f 3.3 ± 1.12	* *P-f, *M 9.0 ± 1.66
	Pro-oestrus	e 26.4 ± 2.47	14.6 ± 3.82	—	—

Common superscripts (a, b, c, d, e,) denote significantly different means. *, $P = 0.05$, **, $P = 0.01$. P-f, M=as compared to Pair-fed or Methallibure-treated. N.D.=not detected. Figures in parentheses denote the number of rats used for estimating LH, FSH and prolactin.

had a much more drastic effect ($P < 0.01$). The drug also significantly depressed pituitary weight ($P < 0.01$), whereas pair-feeding did not. Underfeeding is known to cause ovarian atrophy due to the deficiency of LH (Howland, 1971), and the number of CL was significantly reduced in our pair-fed rats. However, methallibure completely inhibited CL formation, confirming similar observations by Walpole (1968).

Neither methallibure nor pair-feeding significantly reduced the number of follicles. Restricted food intake does not alter the level of circulating FSH in the rat (Howland, 1971), and this may account for its failure to reduce the number of developing follicles. An unaltered follicular population in the methallibure-fed rats, however, might equally result from an absence of ovulation (Walpole, 1968) and subsequent lack of development of a new follicular crop. Although methallibure feeding in swine appears to inhibit both ovulation and follicular growth (Garbers & First, 1969), the latter seems to proceed unaltered in guinea-pigs with intrahypothalamic implants of methallibure (Malven, 1971).

Methallibure feeding completely inhibited the appearance of systemic LH (Table 2). The plasma level of LH was higher in the prepubertal than in either control group ($P < 0.01$). In the control groups, levels of LH were significantly higher during pro-oestrus than during dioestrus and the difference was significantly greater in the fully fed animals. In the latter, LH levels during pro-oestrus and dioestrus were significantly higher ($P < 0.01$) than in the pair-fed controls.

Levels of FSH in prepubertal rats were highest ($P < 0.01$) when compared to those in other groups. The levels in methallibure-fed rats did not differ from the dioestrus values in the controls. The evidence of a pro-oestrous surge of FSH was present in both control groups. Methallibure also reduced plasma prolactin levels, whereas pair-feeding did not. Prepubertal rats had levels of prolactin which were intermediate between those of control and methallibure-fed rats.

These results demonstrate that the postponement of puberty in methallibure-fed rats is associated with the inhibition of LH- and prolactin-producing components of the pituitary-hypothalamic complex. The drug may also directly modify thyroidal function (Archibald, Aschbacher & Tilton, 1973) and/or diminish ovarian sensitivity to circulating hormones. Undernutrition delays puberty till the rat reaches a body weight of 90 to 100 g. Since methallibure-fed rats fail to show signs of puberty even after they reach adult body weights, these effects of the drug do not seem to depend on a retardation of growth rate which is related to nutrition.

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