Hormonal consequences of subcutaneous 6-methoxy-2-benzoxazolinone pellets or injections in prepubertal male and female rats

M. K. Vaughan, J. C. Little*, G. M. Vaughan† and R. J. Reiter

Department of Cellular & Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284, U.S.A.; *Department of Biology, Abilene Christian University, Abilene, TX 79699, U.S.A.; and †Institute of Surgical Research, Ft. Sam Houston, TX 78234, U.S.A.

Summary. Prepubertal 27-day-old female rats maintained in a 14L:10D cycle (lights on 06:00 h) were injected s.c. at 13:00 h with saline or 2, 20 or 200 µg 6-methoxy-2-benzoxazolinone (6-MBOA) and killed 25–27 h later. No significant differences in body, pituitary or ovarian weight were noted. Differences in uterine weight (mg/100 g body weight) and in circulating free thyroxine index fit the pattern of a reduction after the lower doses with reversal of this effect after the highest dose. A dose-related rise in plasma prolactin concentration was accompanied by a significant increase in pituitary prolactin at the lowest (2 µg) dose.

When 27-day-old prepubertal male and female rats maintained in a 14L:10D cycle were implanted with a beeswax pellet or a wax pellet that contained 100 µg or 1 mg 6-MBOA and killed 3 days later between 14:00 and 16:00 h, body and absolute ventral prostate weights (but not weights of other accessory organs, testes or relative ventral prostate weights) in males were lower. Pituitary (but not plasma) prolactin concentrations were higher after the lower dose compared to the controls; pituitary and plasma values of LH and FSH were unchanged. In females, reproductive variables were unchanged except for a reduction of pituitary prolactin after the 1 mg dose. Triiodothyronine and its free index were elevated after the higher dose in males and the lower dose in females. The free thyroxine index appeared raised after the larger dose only in males.

The present results do not suggest a strong role of 6-MBOA in augmenting reproductive function in prepubertal laboratory rats over a period of 1–3 days at the doses used.

Keywords: 6-methoxy-2-benzoxazolinone; 6-MBOA; triiodothyronine; LH; FSH; prolactin; rat

Introduction

6-Methoxy-2-benzoxazolinone (6-MBOA), a plant-derived non-oestrogenic cyclic carbamate, stimulates reproduction in montane meadow voles (*Microtus montanus*) in field and laboratory conditions (Sanders *et al.*, 1981; Berger *et al.*, 1981). The compound is found in spring grass sprouts and is thought to trigger reproductive activity by initiating ovarian cyclicity in overwintering adult and subadult female voles. In regions where the growing season varies from year to year, the presence of 6-MBOA may serve as an important environmental cue for reproductive activity to ensure optimal survival of litters born at this time (Epstein *et al.*, 1986). Previous studies have shown that injections or feeding of 6-MBOA stimulate the reproductive tract of the female vole

leading to increased weight of the ovaries and uteri and increased numbers of large antral follicles, litters and young (Sanders *et al.*, 1981; Berger *et al.*, 1981, 1987).

The hormonal mechanisms by which 6-MBOA administration induces the rapid changeover from the prepubertal state to a cyclic adult have only been explored in female rats (Butterstein *et al.*, 1985) and gilts (Guthrie *et al.*, 1984) and not at all in males. Butterstein *et al.* (1985) observed that a single injection of 6-MBOA in prepubertal female rats had no effect on LH but elevated serum FSH concentrations and ovarian weight. Extended treatment with 6-MBOA also increased gonadal weight and the number of corpora lutea. In the present set of experiments, we have investigated further the effects of 6-MBOA injections and/or pellets on potential afternoon surge concentrations of plasma and pituitary LH and FSH in prepubertal female rats and have extended the observations to include prolactin and thyroid hormone concentrations. Additionally, we examined the reproductive and hormonal effects of 6-MBOA pellets in prepubertal male rats.

Materials and Methods

Animals. Prepubertal Sprague–Dawley male and female rats (Harlan, Houston, TX) arrived in our facility at 23 days of age. They were maintained throughout the experiments under controlled photoperiod (14 h light:10 h dark; 14L:10D; lights on 06:00 h) and temperature conditions ($22 \pm 2^\circ\text{C}$) and were given food (Tech Lab Chow, Winfield, Iowa) and water *ad libitum*. At 27 days of age, they were separated into two experimental protocols as outlined below. At the termination of each experiment the rats were weighed and quickly decapitated. Weights of the anterior pituitary and reproductive tracts (ovaries and uteri from females; testes, ventral prostates and accessory organs from males) were recorded. Plasma and anterior pituitary samples were maintained at -70°C until assayed for hormones.

Preparation of injections and pellets. 6-MBOA was purchased from Chem-Biochem (Lot #4CGA025, Salt Lake City, UT). For injections 6-MBOA was dissolved in warm 100% propylene glycol and then diluted with 0.15 M-NaCl so that the final mixture contained 95% saline:5% propylene glycol (v:v). For the pellets, 6-MBOA was mixed with 24 mg softened beeswax. Blank beeswax or 6-MBOA pellets were implanted subcutaneously on the back of ether-anaesthetized rats.

Experimental protocols. In Exp. 1, 4 groups (8/group) of 27-day-old female rats were given a s.c. injection of diluent or 2, 20 or 200 μg 6-MBOA at 13:00 h. Rats were killed between 14:00 and 16:00 h on Day 28.

In Exp. 2, 3 groups of male and female (8/group) 27-day-old rats were given implants on the morning of Day 27 with beeswax pellets or with wax pellets that contained 100 μg or 1 mg 6-MBOA. The rats were decapitated on Day 30 between 14:00 and 16:00 h.

Assays. Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin were measured in duplicate by double-antibody radioimmunoassays using reference preparations, standards and hormone for iodination provided by the National Institute of Arthritis, Digestive Diseases and Kidney (NIADDK). Thyroxine and triiodothyronine were measured by commercial kits from Diagnostic Products (Los Angeles, CA) using tubes provided with immobilized antibody. An inverse index of the fraction of thyroxine or triiodothyronine bound to plasma proteins was ascertained by determining the proportional triiodothyronine uptake (Diagnostic Products kit) *in vitro* from plasma onto immobilized antibody. Free thyroxine and triiodothyronine indices were obtained by calculating the product of the respective total thyronine concentration and the triiodothyronine uptake value. All samples for a given hormone were assayed together and intra-assay variation for all hormones was less than 10%.

Statistical analysis. Differences according to dose as a source of variation were assessed by ANOVA. If that was significant, the effect of dose was confirmed and characterized by comparisons of means (Student–Newman–Keuls test) or, in Exp. 1, by linear and quadratic regression against log dose or regression against the exponential of dose. In Exp. 2, there were too few dose levels to test meaningfully by regression the effect of dose beyond the results of the ANOVA and subsequent comparisons of means. The statistical principles and techniques are discussed in detail elsewhere (Dixon, 1983).

Results

In Exp. 1, a single injection of 2, 20 or 200 μg 6-MBOA had no effect on absolute uterine weights or body weight; however, the best-fit regression line for relative uterine weights (mg/100 g body weight) was significant ($P < 0.01$) and demonstrates an inhibitory effect of low doses (2 or 20 μg) of 6 MBOA and a return to near control levels with the highest (200 μg) dose (Fig. 1). Absolute (Table 1) or relative (not shown) weights of the pituitaries and ovaries were not altered. Similarly, no effect

Table 1. Organ weight and hormone data (values are mean $\pm$ s.e.) for female rats receiving a single injection of 6-MBOA

Treatment	No. of rats	Weight of:					LH		FSH			T ₃ uptake (%)	T ₃ (ng/dl)	T ₃ /T ₄ ratio
		Body (g)	Pituitary (mg)	Ovaries (mg)	Uterus (mg)	Plasma (ng/ml)	Pituitary (ng/ml)	Plasma (ng/ml)	Pituitary (ng/mg)	T ₄ (μg/dl)	FT ₄ I			
Controls	8	70 $\pm$ 2	3.58 $\pm$ 0.17	18.4 $\pm$ 1.3	68.5 $\pm$ 6.2	0.61 $\pm$ 0.10	678 $\pm$ 110	2.34 $\pm$ 0.38	117 $\pm$ 16	4.84 $\pm$ 0.15	2.39 $\pm$ 0.10	49.3 $\pm$ 0.7	115 $\pm$ 6	55.1 $\pm$ 3.3
2 μg	9	76 $\pm$ 3	3.57 $\pm$ 0.21	17.7 $\pm$ 0.9	55.6 $\pm$ 2.6	0.88 $\pm$ 0.19	697 $\pm$ 45	2.15 $\pm$ 0.26	132 $\pm$ 13	4.94 $\pm$ 0.26	2.35 $\pm$ 0.11	47.7 $\pm$ 0.9	120 $\pm$ 6	56.8 $\pm$ 2.1
20 μg	9	73 $\pm$ 2	4.00 $\pm$ 0.18	19.1 $\pm$ 1.3	53.1 $\pm$ 2.9	0.52 $\pm$ 0.10	678 $\pm$ 76	1.83 $\pm$ 0.22	131 $\pm$ 16	4.67 $\pm$ 0.26	2.21 $\pm$ 0.11	47.6 $\pm$ 1.3	116 $\pm$ 9	54.6 $\pm$ 2.1
200 μg	9	78 $\pm$ 2	3.64 $\pm$ 0.14	19.5 $\pm$ 0.4	66.1 $\pm$ 5.0	0.72 $\pm$ 0.17	745 $\pm$ 116	2.53 $\pm$ 0.24	150 $\pm$ 20	5.45 $\pm$ 0.16	2.69 $\pm$ 0.10	48.9 $\pm$ 0.5	112 $\pm$ 7	55.1 $\pm$ 3.9

T₄ = thyroxine; FT₄I = free thyroxine index; T₃ = triiodothyronine; FT₃I = free triiodothyronine index.

Table 2. Organ weight and hormone data for male rats implanted with 6-MBOA pellets

Treatment	No. of rats	Weight of:									
		Body (g)		Pituitary (mg)		Testes (g)		Accessory organs (mg)		Ventral prostate (mg)	
		(g)	(g)	(mg)	(mg)	(g)	(g/100 g)	(mg)	(mg/100 g)	(mg)	(mg/100 g)
Controls	9	104 $\pm$ 3	4.18 $\pm$ 0.32	1.30 $\pm$ 0.03	1.24 $\pm$ 0.02	31.9 $\pm$ 1.4	31.0 $\pm$ 1.2	45.5 $\pm$ 1.9	43.5 $\pm$ 1.4	0.78 $\pm$ 0.18	1355 $\pm$ 89
100 μg	8	91* $\pm$ 1	4.08 $\pm$ 0.15	1.17 $\pm$ 0.02	1.25 $\pm$ 0.04	31.6 $\pm$ 2.5	33.0 $\pm$ 1.1	35.6* $\pm$ 1.8	45.4 $\pm$ 2.8	0.75 $\pm$ 0.16	1233 $\pm$ 147
1 mg	8	100 $\pm$ 4	4.42 $\pm$ 0.15	1.26 $\pm$ 0.06	1.28 $\pm$ 0.03	34.1 $\pm$ 1.1	34.7 $\pm$ 2.9	45.4 $\pm$ 2.6	39.1 $\pm$ 2.0	0.56 $\pm$ 0.18	1395 $\pm$ 133

Values are mean $\pm$ s.e.

*P < 0.05 compared with controls.

was observed on pituitary LH or FSH concentrations or on circulating concentrations of LH, FSH, thyroxine, triiodothyronine, triiodothyronine uptake or the calculated free index of triiodothyronine concentrations (Table 1). However, the best-fit regression line of the free thyroxine index was significant ($P < 0.05$) and consistent with a depression at lower doses and significant negation of this effect at the 200 µg dose (Fig. 1). A dose-related rise in plasma prolactin concentration ($P < 0.01$) was observed and this was accompanied by a significant increase in pituitary prolactin content at the lowest (2 µg) dose (Fig. 1).

The presence of an implanted 6-MBOA pellet in female rats in Exp. 2 had no effect on body, ovarian or uterine weight; pituitary weights were significantly elevated ($P < 0.05$) in rats treated with the highest (1 mg) dose of 6-MBOA (Table 3). Pituitary LH and FSH and circulating concentrations of LH, FSH (Table 3), thyroxine and free thyroxine index (Fig. 2) were unaffected in the females with 6-MBOA implants. However, pituitary concentrations of prolactin were significantly depressed in females implanted with 1 mg 6-MBOA while plasma values were apparently unaffected (Fig. 2). Animals implanted with a pellet containing 100 µg 6-MBOA had significantly elevated circulating triiodothyronine, free triiodothyronine index (Fig. 2) and triiodothyronine/thyroxine ratio (Table 3) that were all approximately double the respective means of the controls with blank pellets or the animals receiving 1 mg pellets.

No significant differences in absolute or relative (mg/100 g body weight) weights of pituitary, testes or accessory organ (coagulating glands + seminal vesicles) weights were observed in male rats implanted with either dose of 6-MBOA although body weights were lower in animals receiving the lowest dose of 6-MBOA. Absolute ventral prostate weights were lower in rats receiving 100 µg 6-MBOA but when expressed relative to their already low body weights, this significance disappeared. No changes were observed in pituitary or plasma concentrations of LH (Table 2). However, males implanted with 100 µg 6-MBOA had higher pituitary prolactin concentrations with no detectable effect of the drug on circulating prolactin values (Fig. 3). The highest dose of 6-MBOA was associated with elevations in triiodothyronine, free triiodothyronine index, free thyroxine index and the triiodothyronine/thyroxine ratio (Fig. 3; Table 2).

Discussion

The maturation of the neuroendocrine-reproductive axis in the female rat has been divided into distinct developmental phases (Ojeda *et al.*, 1980) and the juvenile stage (Days 21–32) is pertinent to the present discussion. This phase in the female is marked by declining serum FSH and low LH concentrations, rising serum prolactin and growth hormone values, and increases in LH receptors and intraovarian oestradiol (Ojeda *et al.*, 1980); such changes result in a gradual increase in steroidal responsiveness to gonadotrophins. Serum prolactin concentrations during this stage normally rise in the afternoon (Ojeda *et al.*, 1976). Increased prolactin is known to hasten pubertal development in rats as demonstrated by studies in which prolactin was injected s.c. or implanted into the median eminence or in which chronic hyperprolactinaemia was induced by administration of sulpiride, a dopamine blocker (Clemens *et al.*, 1969; Wuttke *et al.*, 1976; Advis & Ojeda, 1978); however, hypoprolactinaemia induced by treatment with bromocriptine, a dopamine agonist, delays the onset of puberty (Advis *et al.*, 1980). In the present studies of juvenile female rats, a single injection of 6-MBOA induced a dose-related rise in plasma prolactin detected 25 h later. Pellets containing 6-MBOA depressed pituitary prolactin values but lack of an effect on circulating concentrations suggests that any effect of 6-MBOA on prolactin at this stage in this species is inconsistent or conforms to a pattern not yet clarified. However, prolactin appears to be a key hormone in the advancement of puberty in rats, as mentioned above, and whether an environmental (dietary) compound like 6-MBOA might exert its effects on puberty in wild species partly through a prolactin mechanism remains to be determined since 3 days of treatment with 6-MBOA were sufficient to elicit weight changes in the reproductive tract in voles (Berger *et al.*, 1981; Sanders *et al.*, 1981).

Fig. 1. Results of Exp. 1. Means $\pm$ s.e.m. and best-fit regression lines for uterine weight and FT_4I , respectively, as $y = 80.6 - 17.1x + 8.34x^2$ ($P < 0.01$ each for x and x^2 terms) and $y = 2.27 - 0.145x + 0.134x^2$ ($P < 0.05$ for x^2 term). In both cases, $x = \log$ dose, with controls computed as $\log 0.2 \mu\text{g}$ since zero dose does not exist on the log scale. Afternoon (14:00–16:00 h) concentrations of pituitary and plasma prolactin in 28-day-old female rats treated with saline (controls; 8/group), 2 (9/group), 20 (9/group) or 200 (9/group) μg 6-MBOA on Day 27 at 13:00 h. a, $P < 0.005$ compared with controls; b, $P < 0.025$ compared with 20 μg dose. The best-fit curve is shown for plasma prolactin as $y = -5.77e^{-0.212x} + 10.8$ ($P < 0.01$) in which $x = \text{dose}$ and controls are computed as $0.2 \mu\text{g}$.

Table 3. Organ weight and hormone data for female rats implanted with 6-MBOA pellets

Treatment	No. of rats	Weight of:				LH		FSH		T_3/T_4 ratio
		Body (g)	Pituitary (mg)	Ovaries (mg)	Uterus (mg)	Plasma (ng/ml)	Pituitary (ng/mg)	Plasma (ng/ml)	Pituitary (ng/mg)	
Controls	7	83 ± 1	3.74 ± 0.43	31.2 ± 1.2	147 ± 25	0.48 ± 0.13	655 ± 47	0.87 ± 0.19	99 ± 11	23.5 ± 2.0
100 μg	8	79 ± 3	3.91 ± 0.10	32.0 ± 1.9	109 ± 18	0.30 ± 0.08	691 ± 45	1.60 ± 0.25	113 ± 14	44.0† ± 2.4
1 mg	8	87 ± 4	4.86* ± 0.31	38.2 ± 3.3	130 ± 15	0.30 ± 0.07	530 ± 89	1.84 ± 0.44	94 ± 18	29.1 ± 2.0

Values are mean $\pm$ s.e.

* $P < 0.05$ compared with controls or 100 μg dose.

† $P < 0.001$ compared with controls or 1 mg dose.

Fig. 2. Afternoon concentrations of pituitary and plasma prolactin, FSH, thyroxine (T_4) and triiodothyronine (T_3) and their calculated free indices (FT_4I , FT_3I) are shown for 30-day-old female rats treated for 3 days with a blank beeswax pellet (controls; 7/group) or a pellet that contained 100 μ g (8/group) or 1 mg (8/group) 6-MBOA (Exp. 2). a, $P < 0.001$ vs controls or 1 mg 6-MBOA. * $P < 0.025$ vs controls. Values are mean $\pm$ s.e.m.

Fig. 3. Afternoon (14:00–16:00 h) concentrations of pituitary and plasma (ng/ml) prolactin, thyroxine (T_4), triiodothyronine (T_3) and the calculated free indices (FT_4I , FT_3I) are shown for 30-day-old male rats treated for the previous 3 days with a blank beeswax pellet (controls; 9/group) or a pellet that contained 100 μ g (8/group) or 1 mg 6-MBOA (8/group) (Exp. 2). a, $P < 0.025$ vs controls or 100 μ g 6-MBOA. b, $P < 0.025$ vs controls. Values are mean $\pm$ s.e.m.

Butterstein *et al.* (1985) reported that prepubertal female rats treated with a single injection or a Silastic implant of 6-MBOA for 3 days had increased ovarian and uterine weight and increased serum FSH concentrations. Although our experimental protocols were basically patterned like

those of Butterstein *et al.* (1985), we failed to find organ weight changes or alterations in circulating FSH concentrations although other hormones (prolactin and triiodothyronine) were affected. Such discrepancy between the hormonal results of the two sets of experiments may be due to the time of day of injections and/or death since this information was not given in the paper by Butterstein *et al.* (1985). Alternatively, the 14L:10D photoperiod used in the present study may have provided some overriding environmental cue not present in the experiments of Butterstein *et al.* (1985). Another source of variation between the two experiments concerned the type of pellet (Silastic or beeswax) used in the implantation experiment.

The modicum of effect in our organ weight data appears to be in an inhibitory direction with some doses of 6-MBOA in females (Fig. 1, relative uterine weights) rather than the stimulatory effects observed in the same dose range in a wild species (Sanders *et al.*, 1981; Ginther *et al.*, 1985) and in rats (Butterstein *et al.*, 1985). Sanders *et al.* (1981) examined a wide range of concentrations of 6-MBOA on uterine weight in montane voles (*Microtus montanus*) and noted that the threshold response occurred at 0.01 µg/day, became maximal at 5 µg/day, and was slightly inhibitory at the highest dose tested (200 µg/day). Our injection data might indicate an inhibitory effect at the low-dose end of the dose-response curve rather than at the high-dose end as reported by Sanders *et al.* (1981). However, adequate comparison of doses between our experiments and other studies is restricted by the unknown rate of exit of 6-MBOA from the subcutaneous pellets.

Morphological and hormonal changes associated with pubertal development in the male rat are grouped into several phases: infantile (7–21 days), juvenile (21–35 days), early pubertal (35–45 days), pubertal (45–60 days) and adult phases (Ojeda *et al.*, 1980). In the juvenile stage serum gonadal steroids, gonadal responsiveness and serum prolactin concentrations are increasing and the sensitivity to steroid negative feedback is still high (Ojeda *et al.*, 1980). It is during this stage that the serum FSH values are maximally elevated and, together with prolactin, induce an increase in testicular LH receptors (Negro-Vilar *et al.*, 1973; Ketelslegers *et al.*, 1978; Piacsek & Goodspeed, 1978; Zipf *et al.*, 1978). In the present experiment, pellets of 6-MBOA increased pituitary concentrations of prolactin in males (100 µg dose). However, undetectable changes in circulating pituitary hormones, though measured at a single timepoint, suggest absence of major stimulation of the pubertal process in these males induced during the 3-day period of exposure to 6-MBOA.

Several histological studies have indicated, and physiological studies corroborated, that thyroid output is maximal during early puberty and diminishes with age (Lansing & Wolfe, 1944; Grad & Hoffman, 1955; Kumaresan & Turner, 1966; Azizi, 1979). In rats, overall mean concentrations of circulating triiodothyronine and thyroxine peak during the 1st and 2nd months of age in both sexes and then decline (Segal *et al.*, 1982). The occurrence of high thyronine concentrations during the pubertal period might be simply the coincident maturation of two independent neuroendocrine systems. Similarly, the observed enhancement of triiodothyronine concentrations in the present experiment may not be directly facilitatory to pubertal development. Evidence for this is drawn from human studies in which primary hypothyroidism (but not hyperthyroidism) may cause sexual precocity (Blizzard *et al.*, 1972). Two hypotheses, which may need to be evaluated further, are whether the maturation of the two systems is linked in this species or whether high concentrations of triiodothyronine during puberty might indirectly facilitate the onset of ovarian cyclicity. In either case, the enhancement of triiodothyronine levels by 6-MBOA treatment could be involved in the mechanism to advance puberty in animals which are more sensitive to 6-MBOA. Whether longer treatment would advance puberty even in the rat through prolactin, thyroid, or other mechanisms is not known.

Considering that ingestion of plants that contain 6-MBOA is the normal route by which various species would come into contact with this compound in their natural habitat and that 6-MBOA activity is highest in early spring shoots and decreases throughout the growing season (Epstein *et al.*, 1986), one could envisage a scheme in which the higher triiodothyronine levels are an integral part of the overall reproductive process set in motion by 6-MBOA. The overwintering population of a species must provide the first litters in the new spring and it is this group of animals along with

these early litters that would consume the food sources highest in 6-MBOA and form the nucleus of the breeding population for a given year. Thus, 6-MBOA would elevate triiodothyronine concentrations and thereby increase general metabolic activity and, presumably, foraging behaviour which in turn would lead to increased caloric intake, increased fitness and, ultimately, to an earlier but possibly more productive reproductive performance. Such a scheme could be utilized to design further experiments.

This work was supported by NSF grant No. PCM 8410592 and the Abilene Christian University Research Council. We thank Mrs Sandy Coggins for help with the data analysis.

References

- Advis, J.P. & Ojeda, S.R.** (1978) Hyperprolactinemia-induced precocious puberty in the female rat: ovarian site of action. *Endocrinology* **103**, 924–935.
- Advis, J.P., Smith-White, S. & Ojeda, S.R.** (1980) Delayed puberty induced by chronic suppression of prolactin (PRL) release in the female rat. *Endocrinology* **106**, 207, Abstr. 531.
- Azizi, F.** (1979) Changes in pituitary and thyroid function with increasing age in young male rats. *Am. J. Physiol.* **237**, E224–E226.
- Berger, P.J., Negus, N.C., Sanders, E.H. & Gardner, P.D.** (1981) Chemical triggering of reproduction in *Microtus montanus*. *Science, N.Y.* **214**, 69–70.
- Berger, P.J., Negus, N.C. & Rowsemitt, C.N.** (1987) Effect of 6-methoxybenzoxazolinone on sex ratio and breeding performance in *Microtus montanus*. *Biol. Reprod.* **36**, 255–260.
- Blizzard, R.M., Thompson, R.G., Baghdassarian, A., Kowarski, A., Migeon, C.J. & Rodriguez, A.** (1972) The interrelationships of steroids, growth hormone, and other hormones on pubertal growth. In *Control of the Onset of Puberty*, pp. 342–359. Eds M. M. Grumbach, G. D. Grave & F. E. Mayer. John Wiley & Sons, New York.
- Butterstein, G.M., Schadler, M.H., Lysogorski, E., Robin, L. & Sipperly, S.** (1985) A naturally occurring plant compound, 6-methoxybenzoxazolinone, stimulates reproductive responses in rats. *Biol. Reprod.* **32**, 1018–1023.
- Clemens, J.A., Minaguchi, H., Storey, R., Voogt, J.L. & Meites, J.** (1969) Induction of precocious puberty in female rats by prolactin. *Neuroendocrinology* **4**, 150–156.
- Dixon, W.J.** (1983) *BMDP Statistical Software*. University of California Press, Berkeley.
- Epstein, W.W., Rowsemitt, C.N., Berger, P.J. & Negus, N.C.** (1986) Dynamics of 6-methoxybenzoxazolinone in winter wheat. Effects of photoperiod and temperature. *J. chem. Ecol.* **12**, 2011–2020.
- Ginther, O.J., Bergfelt, D.R., Scraba, S.T., Pivonka, P.R. & Nuti, L.C.** (1985) Effects of 6-MBOA on reproductive function in ponies, mice, rats and mink. *Theriogenology* **24**, 587–595.
- Grad, B. & Hoffman, M.M.** (1955) Thyroxine secretion rates and plasma cholesterol levels of young and old rats. *Am. J. Physiol.* **182**, 497–502.
- Guthrie, H.D., Pursel, V.G., Bolt, J.D. & Nachman, R.J.** (1984) Effect of gonadotropin-releasing hormone, stage of sexual maturation and 6-methoxybenzoxazolinone on plasma gonadotropins, ovarian development and uterine weight in prepubertal gilts. *Theriogenology* **22**, 683–692.
- Ketelslegers, J.M., Hetzel, W.D., Sherins, R.J. & Catt, K.J.** (1978) Developmental changes in testicular gonadotropin receptors: plasma gonadotropins and plasma testosterone in the rat. *Endocrinology* **103**, 212–222.
- Kumaresan, P. & Turner, C.W.** (1966) Effect of advancing age on thyroid hormone secretion rate of male and female rats. *Proc. Soc. exp. Biol. Med.* **124**, 752–754.
- Lansing, W. & Wolfe, J.M.** (1944) Structural changes associated with advancing age in thyroid gland of female rats with particular reference to alterations in connective tissue. *Anat. Rec.* **88**, 311–322.
- Negro-Vilar, A., Krulich, L. & McCann, S.M.** (1973) Changes in serum prolactin and gonadotropins during sexual development of the male rat. *Endocrinology* **93**, 660–664.
- Ojeda, S.R., Wheaton, J.E., Jameson, H.E. & McCann, S.M.** (1976) The onset of puberty in the female rat: changes in plasma prolactin, gonadotropins, luteinizing hormone-releasing hormone (LHRH) and hypothalamic LHRH content. *Endocrinology* **98**, 630–638.
- Ojeda, S.R., Andrews, W.W., Advis, J.P. & Smith-White, S.** (1980) Recent advances in the endocrinology of puberty. *Endocr. Rev.* **1**, 228–257.
- Piacsek, B.E. & Goodspeed, M.P.** (1978) Maturation of the pituitary gonadal system in the male rat. *J. Reprod. Fert.* **52**, 29–35.
- Sanders, E.H., Gardner, P.D., Berger, P.J. & Negus, N.C.** (1981) 6-methoxybenzoxazolinone: a plant derivative that stimulates reproduction in *Microtus montanus*. *Science, N.Y.* **214**, 67–69.
- Segal, J., Troen, B.R. & Ingbar, S.H.** (1982) Influence of age and sex on the concentrations of thyroid hormone in serum in the rat. *J. Endocr.* **93**, 177–181.
- Wuttke, W., Döhler, K.D. & Gelato, M.** (1976) Oestrogens and prolactin as possible regulators of puberty. *J. Endocr.* **68**, 391–396.
- Zipf, W.B., Payne, A.H. & Kelch, R.P.** (1978) Prolactin, growth hormone and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. *Endocrinology* **103**, 595–600.

Received 10 December 1987