and pathology. *Edited by* I. M. Arias, H. Popper, D. Schacter, and D. A. Shafritz. Raven Press, New York. pp. 663–673.

GREENWAY, C. V., and R. STARK. 1971. Hepatic vascular bed. Physiol. Rev. **51**: 23–65.

GREENWAY, C. V., R. D. STARK, and W. W. LAUTT. 1969. Capacitance responses and fluid exchange in the cat liver during stimulation of the hepatic nerves. Circ. Res. **25**: 277–284.

LAUTT, W. W. 1979*a*. Neural activation of adrenoreceptors in glucose mobilization from liver. Can. J. Physiol. Pharmacol. **57**: 1037–1039.

——— 1979*b*. Autonomic neural control of liver glycogen metabolism. Med. Hypotheses, **5**: 1287–1296.

——— 1980. Hepatic nerves: a review of their functions and effects. Can. J. Physiol. Pharmacol. **58**: 105–123.

——— 1981. Evaluation of surgical denervation of the liver in cats. Can. J. Physiol. Pharmacol. **59**: 1013–1016.

——— 1983. Afferent and efferent neural roles in liver function. Prog. Neurobiol. (N.Y.). **21**: 323–348.

LAUTT, W. W., and M. G. CÔTÉ. 1976. Functional evaluation of 6-hydroxydopamine-induced sympathectomy in the liver of the cat. J. Pharmacol. Exp. Ther. **198**: 562–567.

——— 1977. The effect of 6-hydroxydopamine-induced hepatic sympathectomy on the early hyperglycemic response to surgical trauma under anesthesia. J. Trauma, **17**: 270–274.

LAUTT, W. W., E. S. MARTENS, and D. J. LÉGARÉ. 1982. Insulin and glucagon response during hemorrhage induced hyperglycemia. Can. J. Physiol. Pharmacol. **60**: 1624–1629.

LAUTT, W. W., and C. WONG. 1978. Hepatic parasympathetic neural effect on glucose balance in the intact liver. Can. J. Physiol. Pharmacol. **56**: 679–682.

LOUIS-SYLVESTRE, J., J. M. SERVANT, R. MOLIMARD, and J. LEMAGNEN. 1980. Effect of liver denervation on the feeding pattern of rats. Am. J. Physiol. **239**: R66–R70.

NATHAN, P. W., and T. A. SEARS. 1960. Effects of phenol on nervous conduction. J. Physiol. (London), **150**: 565–580.

RICHARDSON, P. D. I., and P. G. WITHRINGTON. 1981. Liver blood flow. I. Intrinsic and nervous control of liver blood flow. Gastroenterology, **81**: 159–173.

SAWCHENKO, P. E., and M. I. FRIEDMAN. 1979. Sensory functions of the liver—review. Am. J. Physiol. **236**: R5–R20.

SCHAUMBERG, H. N., R. BYCK, and R. O. WELLER. 1970. The effect of phenol on peripheral nerves. A histological and electrophysiological study. J. Neuropathol. Exp. Neurol. **29**: 615–630.

SKANDALAKIS, J. E., S. W. GRAY, R. E. SORIA, J. L. SORG, and J. S. ROWE. 1980. Distribution of the vagus nerve to the stomach. Am. Surg. **46**: 130–139.

WOOD, K. M. 1978. The use of phenol as a neurolytic agent: a review. Pain, **5**: 205–229.

YAMAGUCHI, N., and D. GARCEAU. 1980. Correlations between hemodynamic parameters of the liver and norepinephrine release upon hepatic nerve stimulation in the dog. Can. J. Physiol. Pharmacol. **58**: 1347–1355.

Chronic treatment with [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide reversibly delays puberty in the female rat[1]

KATHLEEN M. LANDYMORE AND MICHAEL WILKINSON[2]

Departments of Physiology and Biophysics and the Department of Obstetrics and Gynaecology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7

Received October 6, 1983

LANDYMORE, K. M., and M. WILKINSON. 1984. Chronic treatment with [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide reversibly delays puberty in the female rat. Can. J. Physiol. Pharmacol. **62**: 853–855.

Luteinizing hormone-releasing hormone (LHRH) agonist analogs have been suggested as a useful treatment for precocious puberty, though there is some concern that long-term treatment might be deleterious to normal sexual development. We have taken advantage of the very short maturation period of the female rat (approximately 35 days from birth) to examine the effects of chronic (daily) treatment with [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide. We have observed that this treatment (either 1 or 2 μg/day) from day 5 after birth significantly delays sexual maturation but does not affect subsequent sexual cycles.

LANDYMORE, K. M., et M. WILKINSON. 1984. Chronic treatment with [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide reversibly delays puberty in the female rat. Can. J. Physiol. Pharmacol. **62**: 853–855.

On a suggéré l'utilisation d'analogues agonistes de l'hormone de libération de la lutéostimuline (LHRH) comme traitement utile à la puberté précoce et ce, malgré la crainte qu'un traitement à long terme puisse vraisemblablement être délétère au développement sexuel normal. Nous avons profité de la très courte période de maturation du rat femelle (approximativement 35 jours après la naissance) pour examiner les effets d'un traitement chronique (quotidien) avec du [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH éthylamide. Nous avons observé que ce traitement (1 ou 2 μg par jour) retardait significativement la maturation sexuelle à partir du jour 5 de la naissance, mais qu'il n'influençait pas les cycles sexuels subséquents.

[Traduit par le journal]

Introduction

The gonadotropin-releasing hormone (GnRH) and its synthetic analogs enjoy widespread utility in a variety of clinical applications (Yen 1983; Ory 1983). For example, the native hormone is used for induction of ovulation and spermatogenesis. The superactive analogs on the other hand are capable of desensitizing the pituitary to such an extent that gonadotropin secretion is potently suppressed. This property allows the use of the analogs in male and female contraception and more recently, in the management of precocious puberty (Laron et al. 1981; Comite et al. 1981; Comite et al. 1982; Pescovitz et al. 1983). In children with true precocious puberty

[1]Supported by the Medical Research Council of Canada and the Atlee Endowment Fund.

[2]Author to whom all correspondence should be sent at the following address: The Department of Physiology and Biophysics, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

the analog [D-Trp^6-Pro^9-NEt]-luteinizing hormone – releasing hormone (LHRH) induces regression of breast size, testicular volume, pubic and facial hair as well as a fall in serum estradiol and testosterone. Growth and bone age appeared normal. These data have suggested that GnRH analogs are an effective therapy for precocious puberty. Some concern has been expressed, however, at the possible deleterious effects of long-term analog treatment during an extended critical developmental period (MacGillivray 1982). For example, pituitary desensitization during infancy and childhood could interfere with the normal development of the reproductive neuroendocrine system (Styne and Grumbach 1978). Thus, the long-term effects of any treatment schedule performed prepubertally, extended or otherwise, need to be carefully evaluated in terms of reversibility.

In the present study, we have taken advantage of the very short maturation period of the female rat (approximately 35 days following birth) to examine the long-term influence of multiple injections of the analog [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide (GnRH-A). We have observed that daily treatment, from day 5 after birth, with GnRH-A significantly delays puberty but does not affect subsequent sexual cycles.

Methods

Female Sprague–Dawley rats were obtained as litters of eight pups (4 days of age) with their mothers from Canadian Breeding Farm and Laboratories (St. Constant, Qué.). The litters were pooled and housed eight per cage under fixed lighting conditions (0700–1900). Treated animals, from day 5, received either 1 or 2 μg of GnRH-A dissolved in 0.1 mL of 0.9% saline. Injections were made subcutaneously into the dorsocervical skinfold. Half the pups from each litter served as controls and received a daily needle prick in the same area. The pups were weaned at 21 days of age. All animals were then examined daily for vaginal opening (VO) and, after this occurred, vaginal smears were obtained each day before 1100. Body weights were recorded at 2- to 3-day intervals.

Two additional treatment groups received 4 μg GnRH-A as a subcutaneous injection each day starting at either 23 or 30 days of age. In the experiment in which a 1-μg dose was used, treatment was stopped when all the control rats exhibited VO (day 38). However, in all subsequent experiments, treatment was continued until all the rats, treated and untreated, showed VO. Statistical analysis was by Student's *t*-test. *P* values less than 0.05 were taken to denote significant differences.

Results

None of the treatments had any effect on the normal body weight gain. For example, Fig. 1 illustrates the growth curves for the experiment with daily injections of 2 μg. Table 1 shows the effect of analog treatment on vaginal opening. Both doses of analog (1 and 2 μg per day) given from day 5 onwards significantly delayed the onset of sexual maturation. Figure 2 illustrates the data obtained for the 2-μg treatment. There was no consistent pattern observed in the type of vaginal smear obtained at VO, though there was a slight preponderance of estrous smears. Nevertheless, all animals immediately began to cycle normally (4- to 5-day cycles).

Table 1 also shows that the shorter periods of treatment with a higher dose (4 μg) (from day 23 and day 30 after birth) had no significant influence on the mean time of VO. However, in the experiment in which injections were begun at day 23 after birth, 80% of control rats showed VO by day 34 compared with only 40% of the treatment group, though the overall means were not different. This suggests that treatment from day 23 does modify sexual maturation but not to any significant degree.

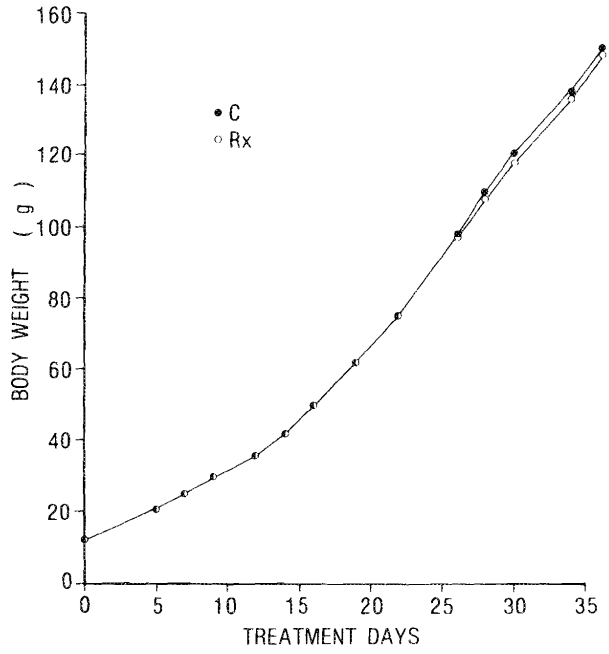

FIG. 1. Growth curves for immature female rats injected with 2 μg/day of GnRH-A (○), or untreated (●). Standard errors were all less than 5% of the mean.

TABLE 1. Effects of [D-Ala^6, des Gly-NH$_2^{10}$]-LHRH ethylamide on timing of vaginal opening (VO) in immature female rats

Treatment started	Dosage (μg)	Day of VO (days after birth)	
Day 5	0	35.9±0.9	$P < 0.005$
	1.0	40.1±1.2	
Day 5	0	35.8±0.7	$P < 0.005$
	2.0	42.9±1.6	
Day 23	0	33.7±0.6	NS
	4.0	35.2±0.7	
Day 30	0	35.3±0.8	NS
	4.0	35.2±1.1	

NOTE: Values are means ± SEM for groups of 12 animals. NS, not significant.

Discussion

Our results indicate that in the immature female rat a relatively small daily dose of GnRH-A causes a significant delay in puberty when treatment is started in the neonate. Interestingly, with continuous therapy throughout the peripubertal period, these animals eventually reach vaginal opening in spite of continued injections of analog, which suggests that sufficient estradiol is produced to permit vaginal canalization to take place. Moreover, our vaginal smear data show that immediately following VO and cessation of treatment these rats begin to exhibit normal cycles. In contrast, a higher dose of GnRH-A (4 μg/day) given from 23 or 30 days of age failed to affect the timing of VO. These results imply that a constant dose level of analogue (1 or 2.0 μg/rat) becomes less effective as the rats rapidly increase their body weight.

In some respects our results are complementary to those obtained previously. Johnson et al. (1976), using a very similar analog, reported inhibition of normal ovarian and uterine growth, a delay of VO and a brief absence of normal cycles. This latter observation, in contrast with our own results, is

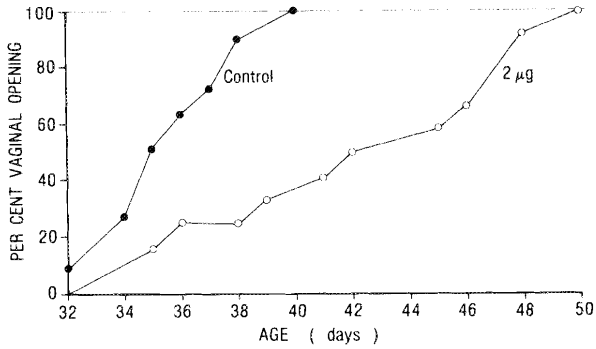

FIG. 2. Effect of daily injection of GnRH-A (2 μg) on timing of vaginal opening. Control animals were untreated.

almost certainly due to the twice-daily injections of analog (0.5 and 3.0 μg/day). Indeed, further studies by Corbin et al. (1978) using a very high dosage of GnRH-A (1 mg·day $^{-1}$·rat^{-1}; i.e., almost 1000 times the amount used in our experiments) also demonstrated a delay of VO. Note that in both these reports, treatment was not begun until 22–25 days after birth. Experiments in which injections of [D-Trp^6]-LHRH (0.05 and 1.0 μg/day) were not started until 30 days after birth have been reported by Vilchez-Martinez et al. (1979). These authors also noted a delay in VO after treatment with the higher dose levels. All of these experiments, including our own, employed Sprague–Dawley rats. It seems likely, therefore, that the discrepancies observed are very likely due to either the analog used or the dosage–treatment schedules.

An important additional difference between our experiments and those previously reported (Johnson et al. 1976; Corbin et al. 1978; Vilchez-Martinez et al. 1979) is that we can say with certainty that long-term treatment with GnRH-A from the neonatal period to the time of puberty, has no adverse effects either on body growth or on subsequent estrus cycles. The onset of normal cycles, despite a significant delay in VO, is in keeping with previous studies in rat (Johnson et al. 1976), monkey (Fraser 1983), and the human female (Bergquist et al. 1982) in which ovulatory cycles were restored following long-term agonist treatment.

Our results suggest that pituitary desensitization during a critical developmental period has no untoward influence on final sexual maturation. Whether this is true for the immature human remains to be determined.

BERGQUIST, C., S. J. NILLIUS, and L. WIDE. 1982. Long-term intranasal LHRH agonist treatment for contraception in women. Fertil. Steril. **38**: 190–193.

COMITE, F., G. B. CUTLER, J. RIVIER, W. W. VALE, D. L. LORIAUX, and W. F. CROWLEY. 1981. Short-term treatment of idiopathic precocious puberty with a long-acting analogue of luteinizing hormone-releasing hormone. N. Engl. J. Med. **305**: 1546–1550.

COMITE, F., K. HENCH, A. MCNEMAR, and A. DWYER. 1982. LHRH analog therapy in 15 children with true precocious puberty: effect on somatic growth and bone maturation. Endocrinology (Baltimore), **110**(Suppl.): 153.

CORBIN, A., C. W. BEATTIE, J. TRACY, R. JONES, T. J. FOELL, J. YARDLEY, and R. W. A. REES. 1978. The antireproductive pharmacology of LHRH and agonistic analogues. Int. J. Fertil. **23**: 81–92.

FRASER, H. M. 1983. Effect of treatment for one year with an LHRH agonist on ovarian, thyroidal and adrenal function and menstruation in the stumptailed monkey (*Macaca arctoides*). Endocrinology (Baltimore), **112**: 245–253.

JOHNSON, E. S., R. L. GENDRICH, and W. F. WHITE. 1976. Delay of puberty and inhibition of reproductive processes in the rat by a gonadotropin-releasing hormone agonist analog. Fertil. Steril. **27**: 853–860.

LARON, Z., R. KAULI, Z. B. ZEEV, A. M. COMARU-SCHALLY, and A. V. SCHALLY. 1981. D-Trp^6-analogue of luteinizing hormone-releasing hormone in combination with cyproterone acetate to treat precocious puberty. Lancet, **II**: 955–956.

MACGILLIVRAY, M. H. 1982. Treatment of idiopathic precocious puberty. N. Engl. J. Med. **306**: 1109–1110.

ORY, S. J. 1983. Clinical uses of luteinizing hormone-releasing hormone. Fertil. Steril. **39**: 577–591.

PESCOVITZ, O. H., L. POTH, K. HENCH, A. MCNEMAR, G. B. CUTLER, and F. COMITE. 1983. True precocious puberty complicating cogenital adrenal hyperplasia: treatment with LHRH analog. Endocrinology (Baltimore), **112**(Suppl.): 314.

STYNE, D. M., and M. M. GRUMBACH. 1978. Puberty in the male and female: physiology and disorders. *In* Reproductive endocrinology. *Edited by* S. C. C. Yen and R. B. Jaffe. W. B. Saunders Co., Philadelphia, PA. pp. 189–240.

VILCHEZ-MARTINEZ, J. A., E. PEDROZA, A. ARIMURA, and A. V. SCHALLY. 1979. Paradoxical effects of D-Trp^6-LHRH on the hypothalamic-pituitary gonadal axis in immature female rats. Fertil. Steril. **31**: 677–682.

YEN, S. S. C. 1983. Clinical applications of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs. Fertil. Steril. **39**: 257–266.