Induction of ovulation in adult mice with a synthetic LH-RH (AY-24,031) and an analogue (AY-25,205)

U. K. Banik and M. L. Givner

*Department of Endocrinology and Immunochemistry, Ayerst Research Laboratories,
P.O. Box 6115, Montreal, Canada*

Ovulation can be induced in mice and rats by various types and combinations of gonadotrophins, as well as by synthetic LH-RH following pretreatment with PMSG (see Stern & Schuetz, 1970; Gosden & Readhead, 1974). The present work was undertaken to establish whether LH-RH and an analogue could induce ovulation in dioestrous mice without pretreatment with PMSG.

Adult (30 ± 5 g) virgin Swiss albino mice were maintained in an air-conditioned room ($24\text{--}25^\circ\text{C}$; 45–50% humidity) and exposed to equal periods of light and darkness. The decapeptides used were: (1) synthetic LH-RH (AY-24,031): H-Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂, and (2) LH-RH analogue (AY-25,205): H-Pyr-His-Trp-Ser-Tyr-[D-Ala]-Leu-Arg-Pro-NH-CH₂-CH₃. The freeze-dried decapeptides were injected i.p. in 0.2 ml of 0.1% gelatin/0.9% saline between 15.00 and 15.30 hours on the day of dioestrus which was determined from examination of vaginal smears and recognition of the day of pro-oestrus. No distinction was made between animals with 4-day and 5-day cycles. Control mice received injections of the vehicle only. Evidence for ovulation was obtained by counting the eggs in the oviducts on the morning after the injection. The results were assessed by the fourfold contingency test tables of Mainland & Murray (1952).

Table 1. The induction of ovulation in dioestrous mice (10/group) with a synthetic LH-RH (AY-24,031) or an analogue (AY-25,205)

AY-24,031			AY-25,205		
Dose (ng/mouse)	No. of mice ovulating	No. of ova† (mean $\pm$ S.E.M.)	Dose (ng/mouse)	No. of mice ovulating	No. of ova† (mean $\pm$ S.E.M.)
0 (vehicle only)	0	0	0 (vehicle only)	0	0
10	0	0	0.625	0	0
20	0	0	1.25	2	11.0
40	2	6.5	2.5	4*	3.4 $\pm$ 1.9
80	0	0	5.0	10**	10.5 $\pm$ 1.2
100	5*	8.0 $\pm$ 2.1	10.0	10**	10.4 $\pm$ 1.3
200	4*	8.0 $\pm$ 2.5	20.0	10**	10.1 $\pm$ 1.3
500	4*	4.5 $\pm$ 2.0	100.0	9**	10.4 $\pm$ 1.2
1000	5*	7.8 $\pm$ 2.2	1000.0	10**	9.8 $\pm$ 1.2

Significantly different from controls: * $P < 0.05$; ** $P < 0.01$.

† For ovulating mice only.

As shown in Table 1, AY-24,031 was unable to induce consistent ovulation and the numbers of eggs were lower than those characteristic for this colony (10.1 ± 0.7 (S.E.M.)). Premature ovulation of normal numbers of eggs was induced by low and high doses of AY-25,205. The ability of AY-24,031 and AY-25,205 to induce ovulation in mice at other stages of the oestrous cycle was also examined. A few oestrous and metoestrous mice ovulated after a large dose (1000 ng/mouse) of AY-24,031 or AY-25,205. Most pro-oestrous mice responded to doses as low as 1 ng AY-25,205 and 100 ng AY-24,031 and ovulation numbers were normal even after a dose of 1000 ng LH-RH or analogue. Since a small amount of AY-25,205 was a highly effective inducer of ovulation in dioestrous mice but a larger quantity of AY-24,031 was only partly effective, it appears that this analogue is not only

more potent than AY-24,031 but also has a greater ability to stimulate the hypophysial-ovarian system. Compound AY-25,205 has also been shown to release LH and FSH in rats and women and to induce ovulation in rats (see Banik & Givner, 1975).

The present study also demonstrates that LH-RH and its analogue can induce ovulation in dioestrous mice which have not been pretreated with PMSG. The results of Gosden & Readhead (1974) in mice were therefore probably influenced more by PMSG than LH-RH. Since large doses of AY-24,031 and AY-25,205 did not induce superovulation in mice at any stage of the cycle, these or similar compounds may be more useful for inducing ovulation in women than the gonadotrophins which have been associated with multiple ovulations (see Taymor, 1974; Spadoni, Cox & Smith, 1974).

We thank Dr H. Immer, Dr V. R. Nelson and Dr K. Sestanjan for synthesizing AY-24,031 and AY-25,205; Mr P. Gauthier and Mr W. Vanleenhoff for technical help; and Dr R. Deghenghi for his interest.

References

- BANIK, U.K. & GIVNER, M.L. (1975) Ovulation-induction and antifertility effects of an LH-RH analogue (AY-25,205) in cyclic rats. *J. Reprod. Fert.* **44**, 87-94.
- GOSDEN, R.G. & READHEAD, C. (1974) Induction of ovulation in mice by synthetic luteinizing hormone releasing hormone. *J. Endocr.* **62**, 417-418.
- MAINLAND, D. & MURRAY, I.M. (1952) Tables for use of fourfold contingency tests. *Science, N.Y.* **116**, 591-594.
- SPADONI, L.S., COX, D.W. & SMITH, D.C. (1974) Use of human menopausal gonadotropin for induction of ovulation. *Am. J. Obstet. Gynec.* **120**, 988-993.
- STERN, S. & SCHUETZ, A.W. (1970) Asynchrony of ovulation and mating in mice treated with gonadotrophins. *J. Reprod. Fert.* **23**, 257-261.
- TAYMOR, M.L. (1974) The use of luteinizing hormone-releasing hormone in gynecologic endocrinology. *Fert. Steril.* **25**, 992-1005.

Received 13 August 1975