Suppression of serum LH-RH and LH in rats by an inhibitor of norepinephrine synthesis

S. P. Kalra

*Department of Obstetrics and Gynecology, University of Florida, College of Medicine,
Gainesville, Florida 23610, U.S.A.*

Monoamine synthesis inhibitors have been used extensively to unravel the role of neurotransmitters in the hypothalamic regulation of pituitary LH release (Coppola, Leonardi & Lippmann, 1966; Kordon & Glowinski, 1972; Kalra, P.S. & McCann, 1973). Inhibition of tyrosine hydroxylase by α -methyl-paratyrosine (Corrodi & Hansen, 1966) or dopamine- β -hydroxylase by sodium diethyldithiocarbamate (Goldstein, Anagnoste, Lauber & McKereghan, 1964) blocked the preovulatory discharge of LH and ovulation (Kalra & McCann, 1974). These pharmacological agents and a brain norepinephrine depletor, U-14,624 (1-phenyl-3-(2-thiazolyl)-2 thiourea (Johnson, Boukma & Kim, 1970), have been shown to suppress the release of pituitary LH induced by ovarian steroids (Kalra, Kalra, Krulich, Fawcett & McCann, 1972; Kalra, P.S. & McCann, 1973). These results were viewed as evidence for the involvement of a hypothalamic norepinephrine system in controlling LH release as suggested by Sawyer (1952). A single intraventricular injection of 6-hydroxydopamine to deplete norepinephrine and dopamine (Kostrzewa & Jacobowitz, 1974) reduced the LH-RH content of the hypothalamus (Kalra, 1975), but little other information is available to show that the depletion of brain catecholamines, and specifically norepinephrine, by these drugs results in modification of LH-RH secretion. In the present study the inhibition of pituitary LH release by the norepinephrine depletor, U-14,624, was investigated in ovariectomized rats.

Adult Charles River CD rats, maintained in controlled temperature and light (14 h light, 10 h dark) conditions, were used 4 weeks after bilateral ovariectomy. The rats were then injected i.p. at 11.00 h with either U-14,624 (200 mg/kg; Regis Chemical Co. Morton Grove, Illinois, U.S.A.) in 1% Tween-80 or vehicle alone. The rats were killed by decapitation 24 h later and the serum from the trunk blood was frozen at -20°C for subsequent analysis of LH and LH-RH. The brains were quickly removed and blocks encompassing the medial basal hypothalamus (MBH) and the preoptic area (POA) were dissected out as described in detail elsewhere (Kalra, 1976a, b). The neural tissues were homogenized in 2 ml 0.1 N-HCl and kept frozen at -20°C . The extracts were neutralized with 1 N-NaOH before the LH-RH estimations. LH-RH in the hypothalamic and serum samples was analysed by the radioimmunoassay techniques described by Nett, Akbar, Niswender, Hedlund & White (1973). The antiserum (donated by Dr T. Nett and Dr G. D. Niswender) was LH-RH-BSA (No. 42). Synthetic LH-RH (Beckman Instrument Company, Palo Alto, California, U.S.A.) was used for radioiodination and as the reference standard. In the first experiment 200 μl serum from each rat were assayed for LH-RH activity (sensitivity of the assay 0.5 pg/assay tube), while in Exp. 2, 1.2 ml serum from each rat were extracted with methanol and processed for LH-RH estimation (sensitivity of assay 0.8 pg/assay tube) as described by Kalra (1976b). The LH activity in the serum was estimated with the radioimmunoassay kits supplied by NIAMDD. The LH results were expressed in terms of the NIAMDD-Rat-LH-RP1 Standard ($0.03 \times \text{NIH-LH-S1}$). The statistical comparisons were made between groups using Student's *t* test.

The results from the two experiments are summarized in Table 1. Serum LH-RH was significantly reduced in the treated rats in both experiments. These low circulating concentrations of LH-RH in rats treated with U-14,624 were accompanied by significantly depressed serum LH levels. The amounts of LH-RH in the MBH and the POA were not significantly altered following U-14,624 injection.

Drouva & Gallo (1976) found that treatment of ovariectomized rats with the same dose of U-14,624 produced a 90% reduction in norepinephrine levels without any noticeable effect on dopamine

Table 1. The effect of U-14,624 on LH-RH and LH in ovariectomized rats

Treatment	No. of rats	LH-RH			LH
		MBH (pg)	POA (pg)	Serum (pg/ml)	Serum (pg/ml)
Experiment 1					
Control	6	—	—	15.0 ± 3.8	504.5 ± 56.5
U-14,624	5	—	—	2.23 ± 1.63†	261.0 ± 90.1*
Experiment 2					
Control	11	1368.8 ± 118.9	513.0 ± 35.1	6.27 ± 0.43	803.4 ± 74.4
U-14,624	11	1153.7 ± 138.5	519.0 ± 32.8	4.22 ± 0.36‡	280.0 ± 90.2‡

Values are mean ± S.E.M.; those for Experiment 2 are uncorrected for extraction losses (20–25 %).

Values significantly different (*t* test) compared with controls: **P* < 0.05; †*P* < 0.02; ‡*P* < 0.01.

in the hypothalamus, and that the pulsatile discharge of LH which is normally observed in ovariectomized rats was abolished. The present results are the only ones known to suggest that the disruption of norepinephrine systems in the hypothalamus leads to impaired release of LH-RH, although LH-RH content in the MBH and the POA did not appear to be affected. This was, however, a short-term experiment; intraventricular injection of 6-hydroxydopamine was found to have reduced the LH-RH activity in the MBH 8 days later (Kalra, 1975). Furthermore, electrochemical stimulation of the POA of rats pretreated with brain norepinephrine depletors also caused reduced LH release (Kalra, S.P. & McCann, 1973). Taken together, these data strongly support the hypothesis that the hypothalamic catecholamines, probably norepinephrine, may regulate the secretion of LH-RH from the peptidergic neurones in the hypothalamus. LH-RH-producing cell elements are known to be present in the entire preoptic-tuberal pathway (Barry, Dubois & Carette, 1974; Kizer, Palkovits & Brownstein, 1976), and contrary to earlier beliefs (Halašz, 1969), it is now apparent that LH-RH from the POA is involved in the tonic and the cyclic discharge of pituitary LH during the oestrous cycle (Kalra, 1976b). Since U-14,624 pretreatment blocks the LH surge induced by ovarian steroids (Kalra *et al.*, 1972) as well as the basal release of LH in the ovariectomized rats (present results), it would appear that the secretory activity of the LH-RH-producing elements in the preoptic-tuberal pathway is markedly affected by the subnormal levels of brain norepinephrine induced by the catecholamine synthesis inhibitor.

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References

- BARRY, J., DUBOIS, M.P. & CARETTE, B. (1974) Immuno-fluorescence study of the preoptico-infundibular LRF neurosecretory pathway in the normal, castrated or testosterone-treated male guinea pig. *Endocrinology* **95**, 1416–1423.
- COPPOLA, J.A., LEONARDI, R.G. & LIPPMANN, W. (1966) Ovulatory failure in rats after treatment with brain norepinephrine depletors. *Endocrinology* **78**, 225–228.
- CORRODI, H. & HANSEN, L.C.F. (1966) Central effects of an inhibitor of tyrosine hydroxylase. *Psychopharmacologia* **10**, 116–125.
- DROUVA, S.V. & GALLO, R.B. (1976) Catecholamine involvement in episodic luteinizing hormone release in adult ovariectomized rats. *Endocrinology* **99**, 651–658.
- GOLDSTEIN, M., ANAGONSTE, B., LAUBER, E. & MCKEREGHAN, M.R. (1964) Inhibition of dopamine-β-hydroxylase by disulfiram. *Life Sci.* **3**, 763–767.
- HALAŠZ, B. (1969) The endocrine effects of isolation of the hypothalamus from the rest of the brain. In *Frontiers of Neuroendocrinology*, pp. 307–342. Eds W. F. Ganong & L. Martini. Oxford University Press.
- JOHNSON, G.A., BOUKMA, S.R. & KIM, E.G. (1970) *In vivo* inhibition of dopamine-β-hydroxylase by 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624). *J. Pharmacol. exp. Ther.* **171**, 80–87.
- KALRA, P.S. & MCCANN, S.M. (1973) Involvement of catecholamines in feedback mechanisms. *Prog. Brain Res.* **9**, 185–198.

- KALRA, P.S., KALRA, S.P., KRULICH, L., FAWCETT, C.P. & MCCANN, S.M. (1972) Involvement of norepinephrine in transmission of the stimulatory influence of progesterone on gonadotropin release. *Endocrinology* **90**, 1168–1176.
- KALRA, S.P. (1975) Modification by 6-hydroxydopamine (6-OHDA) of ovarian steroid feedback action on LH release mechanisms. *Proc. A. 57th Meeting Endocrine Soc.*, p. 100.
- KALRA, S.P. (1976a) Circadian rhythm in luteinizing hormone-releasing hormone (LH-RH) content of preoptic area during the rat estrous cycle. *Brain Res.* **104**, 354–358.
- KALRA, S.P. (1976b) Tissue levels of luteinizing hormone releasing hormone in the preoptic area and hypothalamus and serum concentration of gonadotropins following anterior hypothalamic deafferentation and estrogen treatment of the female rat. *Endocrinology* **99**, 101–107.
- KALRA, S.P. & MCCANN, S.M. (1973) Effect of drugs modifying catecholamine synthesis on LH release induced by preoptic stimulation in the rat. *Endocrinology* **93**, 356–362.
- KALRA, S.P. & MCCANN, S.M. (1974) Effects of drugs modifying catecholamine synthesis on plasma LH and ovulation in the rat. *Neuroendocrinology* **15**, 79–91.
- KIZER, J.S., PALKOVITS, M. & BROWNSTEIN, M.J. (1976) Releasing factors in the circumventricular organs of the rat brain. *Endocrinology* **98**, 311–317.
- KORDON, C. & GLOWINSKI, J. (1972) Role of hypothalamic monomeric neurones in the gonadotropin-release regulating mechanisms. *Neuropharmacology* **11**, 153–162.
- KOSTRZEWA, R.M. & JACOBOWITZ, D.M. (1974) Pharmacological actions of 6-hydroxydopamine. *Pharmac. Rev.* **26**, 199–288.
- NETT, T.M., AKBAR, A.M., NISWENDER, G.D., HEDLUND M.T. & WHITE, W.F. (1973) A radioimmunoassay for gonadotropin releasing hormone (GH-RH) in serum. *J. clin. Endocr. Metab.* **36**, 880–885.
- SAWYER, C.H. (1952) Stimulation of ovulation in the rabbit by intraventricular injection of epinephrine and norepinephrine. *Anat. Rec.* **112**, 285.

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