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

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Monoamine synthesis inhibitors have been used extensively to unravel the role of neurotransmitters in the hypothalamic regulation of pituitary LH release (Coppola, Leonard & 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, Anagonste, 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 radiiodination 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 radioimmunoaassay kits supplied by NIAMDD. The LH results were expressed in terms of the NIAMDD-Rat-LH-RP1 Standard (0.03 × 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
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 (Halász, 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.

This work was supported by grants from the Population Council and NIH (HD-08364). I thank Ms Lyn Thomas for typing the manuscript.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>LH-RH (pg)</th>
<th>POA (pg)</th>
<th>Serum (pg/ml)</th>
<th>LH (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>15.0 ± 3.8</td>
<td>504.5 ± 56.5</td>
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<tr>
<td>U-14,624</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>2.23 ± 1.63†</td>
<td>261.0 ± 90.1*</td>
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<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>1368.8 ± 118.9</td>
<td>513.0 ± 35.1</td>
<td>6.27 ± 0.43</td>
<td>803.4 ± 74.4</td>
</tr>
<tr>
<td>U-14,624</td>
<td>11</td>
<td>1135.7 ± 138.5</td>
<td>519.0 ± 32.8</td>
<td>4.22 ± 0.36‡</td>
<td>280.0 ± 90.2‡</td>
</tr>
</tbody>
</table>

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.

References


Received 14 September 1976