

# Effect with time of a norepinephrine synthesis inhibitor (U-14,624) on hypothalamic catecholamine and plasma gonadotrophin concentrations in the ovariectomized rat

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**Summary.** Injection of the dopamine hydroxylase inhibitor U-14,624, to ovariectomized rats resulted in the reduction of hypothalamic norepinephrine concentration over a 48-h period and the elevation of hypothalamic dopamine concentration over a 24-h period. Plasma LH and FSH levels were markedly suppressed up to 48 h after drug treatment. These results suggest that U-14,624 is a much more potent inhibitor of gonadotrophin secretion than has been previously reported.

## Introduction

Specific catecholamine synthesis inhibitors have been used to investigate the relationship between these neurotransmitters and pituitary gonadotrophin release (Coppola, Leonardi & Lippman, 1966; Kalra, Kalra, Chen & Clemens, 1978; Weiner & Ganong, 1978). 1-Phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624), which acts upon dopamine- $\beta$ -hydroxylase to prevent norepinephrine synthesis from its precursor dopamine, is one such drug whose effects have been extensively studied (Johnson, Boukma & Kim, 1970; Kalra & McCann, 1973; Drouva & Gallo, 1976; Kalra, 1977; Kalra *et al.*, 1978). This pharmacological agent suppresses gonadotrophin secretion, implicating norepinephrine as being facilitatory to gonadotrophin release (Drouva & Gallo, 1976; Kalra, 1977; Kalra *et al.*, 1978). However, little is known about the time course of effects of U-14,624 on hypothalamic catecholamines and plasma gonadotrophin levels. In the present investigation, this drug was used to study the effects of norepinephrine depletion on plasma LH and FSH levels in the ovariectomized rat over a period of 48 h.

## Materials and Methods

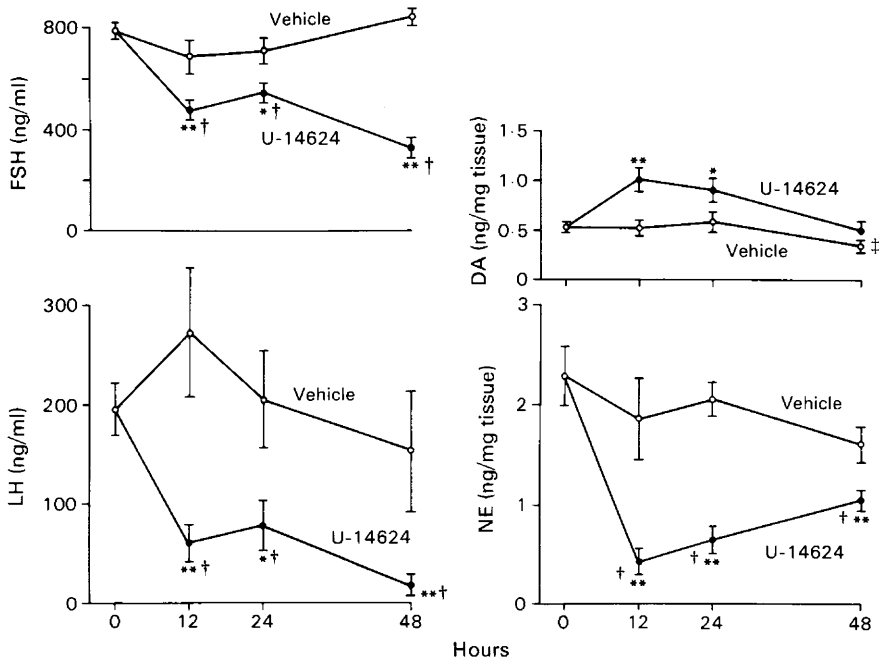
Adult female rats (Sprague–Dawley, 200–250 g, Simonsen) were housed 5 per cage under controlled lighting (lights on from 05:00 to 19:00 h) with free access to Purina lab chow and water. From 1 week after arrival, vaginal smears were taken 6 days per week. Only rats that had shown at least 2 consecutive 4- or 5-day oestrous cycles were used.

Ovariectomy was performed on all animals on the day of vaginal diestrus 72 h before the start of the experiment, and animals were randomly assigned to one of 7 groups: those killed at time zero as a normal control group, with no drug or vehicle injection, those injected with U-14,624 (Sigma, 200 mg/kg *i.p.*) dissolved in dimethylsulphoxide (DMSO) 12, 24, or 48 h

before being killed, and those injected with DMSO alone, 12, 24 or 48 h before being killed. Animals were killed by rapid decapitation at the appropriate time. Trunk blood was collected in heparinized test tubes, which were centrifuged to obtain the plasma component. Plasma was stored at  $-20^{\circ}\text{C}$  until assay for LH and FSH. Brains were rapidly removed and the medial basal hypothalamus was dissected out as previously described by Carrillo & Sheridan (1980), weighed and frozen in solid  $\text{CO}_2$  for subsequent catecholamine determination. The medial basal hypothalamus was chosen as the site to measure catecholamines because it is well established that noradrenergic and dopaminergic fibres as well as the LH-RH terminals terminate in the median eminence (Weiner & Ganong, 1978; Barry, 1979; McCann, 1980). Plasma LH and FSH were determined by radioimmunoassay, using the kit provided by NIAMDD (A. F. Parlow) with rat LH-RP1 and FSH-RP1 as reference preparations. These preparations have a biological potency of  $0.03 \times \text{NIH-LH-S1}$  for LH and  $2.1 \times \text{NIH-FSH-S1}$  for FSH. All samples were determined in a single assay which had an intra-assay coefficient of variation of 4.0% for LH and 5.0% for FSH. Catecholamine determination was done by the radioisotope-enzymic method according to the protocol described by Palkovitz, Brownstein, Saavedra & Axelrod (1974). This assay procedure is routinely carried out (Morgan & Herbert, 1980) and has a sensitivity of 20 pg per tube for both norepinephrine and dopamine. Statistical probabilities were determined by two-way analysis of variance for repeated measures and the Student-Newman-Keuls test (Sokal & Rohlf, 1969).

### Results

Animals treated with U-14,624 showed a significant increase in hypothalamic dopamine levels at 12 and 24 h when compared to the vehicle treated controls but by 48 h there was no significant difference between the two groups (Text-fig. 1). There were significant decreases in



**Text-fig. 1.** Plasma levels of FSH and LH and medial basal hypothalamic content of dopamine (DA) and norepinephrine (NE) in non-injected female rats (0 h) and in rats treated with U-14,624 or DMSO (vehicle). Each point represents the mean  $\pm$  s.e.m. for 5–8 animals/group. \* $P < 0.05$ ; \*\* $P < 0.01$  when compared to the vehicle-injected group † $P < 0.01$ , ‡ $P < 0.05$  when compared to the value at 0 h.

norepinephrine and FSH and LH concentrations at 12, 24 and 48 h when compared to the 0 h group. Values in animals treated with DMSO alone did not differ from those at 0 h except for hypothalamic dopamine concentration at 48 h.

### Discussion

These results are in agreement with those reported by Johnson *et al.* (1970) for the effects of U-14,624 on norepinephrine and dopamine levels in the whole rat brain, and with those of Drouva & Gallo (1976) who reported that U-14,624 reduced the norepinephrine concentration in the medial basal hypothalamus and the plasma levels of gonadotrophins 18 h after treatment in long-term ovariectomized rats. In contrast to the results presented here, however, Drouva & Gallo (1976) found that dopamine in the medial basal hypothalamus was not significantly elevated in the drug-treated animals. These discrepancies may be the result of the differences in the length of time that the animals were ovariectomized or the route of injection: Drouva & Gallo (1976) used a suspension, whereas we dissolved the drug in DMSO before injection and thus may have resulted in more of the drug getting into the rat. Kalra *et al.* (1978) and Kalra (1977) also reported a reduction in circulating LH values in long-term ovariectomized rats 24 h after treatment with U-14,624. Whereas in many of these previous reports only one time point was measured, the results presented here demonstrate that U-14,624 will suppress hypothalamic norepinephrine concentration as well as pituitary gonadotrophin for at least 12–48 h.

Because U-14,624 is very insoluble, other investigators have administered this drug in a methylcellulose or propylene glycol suspension. Our initial experience with injecting U-14,624 in suspension proved unreliable, because it was very difficult to determine the amount of drug that the rats received. DMSO was the only solvent that readily dissolved U-14,624; however, there is very little known about the effects of DMSO on hypothalamic catecholamines concentration and gonadotrophin secretion, although its use has been reported in neuroendocrine studies (Clemens, Flaugh, Parli & Sawyer, 1980). In our study DMSO had very little effect on the catecholamine concentration in the medial basal hypothalamus or on plasma levels of LH and FSH, suggesting that this solvent for U-14,624 may be the most suitable.

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