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RELATIONSHIP BETWEEN DURATION OF URETHANE OR PENTOBARBITONE ANAESTHESIA IN MALE AND FEMALE RATS AND ADENOHYPOPHYSEAL RESPONSE TO LUTEINISING HORMONE-RELEASING HORMONE

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1 Luteinizing hormone releasing hormone (50 ng/100 g body weight) was injected into male and female rats which had been anaesthetized with either urethane (1.2 g/kg) or pentobarbitone (45 g/kg) for 15, 100 or 240 min and serial blood samples were taken for estimation of plasma luteinizing hormone concentrations.

2 The rats anaesthetized with urethane tended to show a greater response to the releasing hormone than those given pentobarbitone.

3 The magnitude of the responses observed in male rats and in female rats anaesthetized with pentobarbitone did not change as the period of anaesthesia prior to injection of releasing hormone was lengthened from 15 min to 4 h. By contrast, in the groups of female rats anaesthetized with urethane the magnitude of the response to luteinizing hormone releasing hormone was related to the length of pretreatment anaesthesia. Thus both dioestrous and pro-oestrous rats given releasing hormone only 15 min after the onset of urethane anaesthesia had significantly ($P < 0.001$ and < 0.05 respectively) higher concentrations of luteinizing hormone (LH) in the plasma than rats treated with the releasing hormone after 240 min of anaesthesia.

4 These effects were not due to a differential action of the anaesthetics on the mechanism for clearing LH from the plasma.

Introduction

Urethane anaesthesia is often used in studies of hypothalamic control of pituitary hormone secretion. The drug has proved especially useful when the activity of single neurones is to be monitored with microelectrodes (for reviews see Cross & Silver, 1966; Dyer, 1974; Moss, 1976) and has provided a stable preparation for analysis of the milk-ejection reflex in the rat (e.g. Lincoln, Hill & Wakerley, 1973; Wakerley & Lincoln, 1973). However, urethane is less useful for investigation of hypothalamic control of pre-ovulatory gonadotrophin secretion since there is some evidence that the drug interferes with normal brain–pituitary–ovary function. The magnitude and site of action of this interference is not known fully, although there have been a number of studies directed towards providing the necessary data. For example, Haller & Barraclough (1968) found that normal ovulation occurred in rats anaesthetized with urethane early on the morning of pro-oestrus but these results were not reproduced by Lincoln & Kelly (1972) who showed that urethane, administered between 06 h 00 min and 14 h 00 min on the day of pro-oestrus, blocked ovulation in approximately 50% of the treated rats. However, Lincoln & Kelly (1972) reported that the dose of exogenous luteinizing hormone (LH) necessary to ensure ovulation was lower in urethane-anaesthetized rats than in those given barbiturate. In contrast, Blake & Sawyer (1972) found that a fivefold increase in exogenous LH was needed to produce ovulation in urethane-blocked rats when compared with the dose required for female rats treated with pentobarbitone. Blake & Sawyer suggested that urethane acted both centrally and on the ovary, but not on the pituitary. However, *in vitro* experiments with adenohypophyses obtained from young male rats have shown that urethane has a pronounced inhibitory action on the luteinizing hormone-releasing hormone (LH–RH) stimulated release of LH (Carter & Dyer, 1979).

In this laboratory we have had sympathy from time to time with various aspects of these conflicting data and have concluded that the action of urethane on the hypothalamic regulation of gonadotrophin secretion may change during the period of anaesthesia. Because we found that urethane has a direct effect on the pituitary (Carter & Dyer, 1979) we have tested our hypothesis by injecting a single dose of LH–RH into male and female rats preanaesthetized with urethane for various times and measuring by immunoassay the

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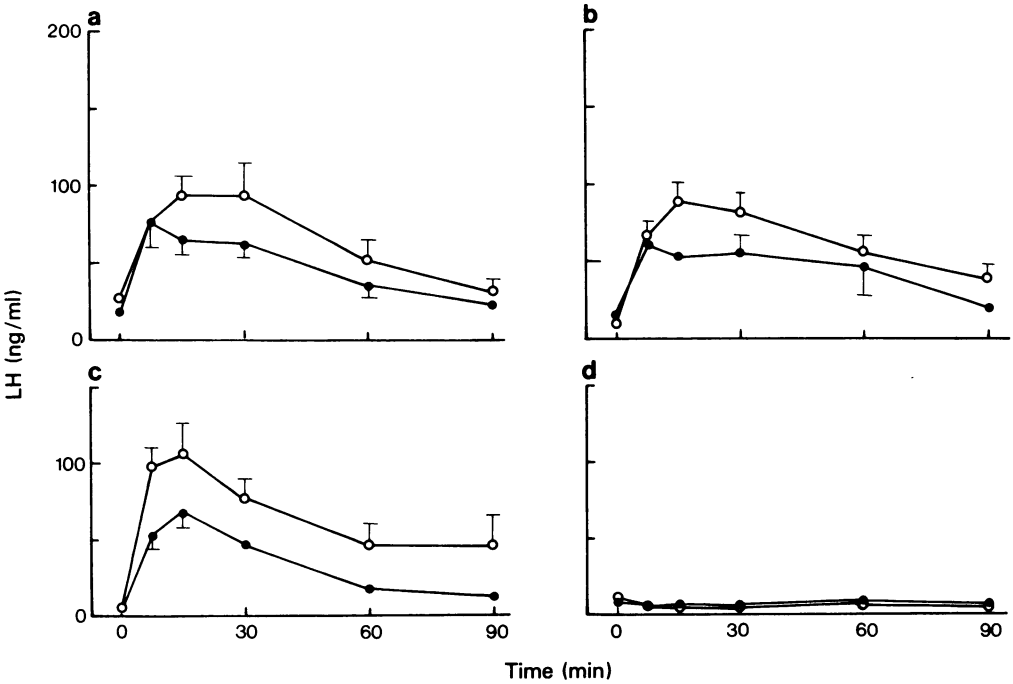

Figure 1 Plasma concentrations of luteinizing hormone (LH) (NIAMDD-RP1) obtained from male rats injected with luteinizing hormone-releasing hormone (50 ng/100 g body weight) at the time 0, 15 (a), 100 (b) or 240 (c) min after induction of anaesthesia with either urethane (○) or pentobarbitone (●). In (d) saline was injected instead of the decapeptide, 100 min after the onset of anaesthesia (n = 7 for all groups).

subsequent release of LH. The results obtained are presented in this paper and are compared with data obtained from similarly treated rats anaesthetized with sodium pentobarbitone.

Methods

Animals

Adult male and female rats from our own inbred Wistar strain were used for the experiments. The rats were kept in conditions of controlled lighting (14 h light:10 h dark; lights on at 05 h 00 min BST) and temperature (20° to 22°C) with food and water available *ad libitum*. Endocrine status was established for the female rats from daily vaginal smears and those animals with regular 4 day oestrous cycles were taken at pro-oestrus or dioestrus.

Experimental procedures

The rats were anaesthetized either with urethane (ethyl carbamate; 1.2 g/kg injected i.p. as a 25% w/v solution) or sodium pentobarbitone (Sagatal, May & Baker Ltd.; 45 mg/kg as a single i.p. injection with supplementary doses of 19 mg/kg when necessary). An external jugular vein was exposed for injection of LH–RH and withdrawal of blood samples. At 14 h 15 min and after 15, 100 or 240 min of anaesthesia, 0.7 ml of blood was withdrawn, for subsequent radioimmunoassay of LH, and the releasing hormone (LH–RH; 50 ng/100 g body weight) was injected. Further blood samples (0.7 ml) were taken 7.5, 15, 30, 60 and 90 min after this single injection.

Immunoassay procedure

Serum samples were stored at −20°C. The concentration of LH in these samples was measured in duplicate by a homologous double antibody radioimmunoassay system with kits provided by NIAMDD. Full details of the procedure and characteristics of this assay may be found in Carter & Dyer (1979).

Statistics

The mean and standard error of the mean were calculated for all values to allow graphical presentation of the data. Standard errors are not shown where they are smaller than the symbols used on the graphs. All

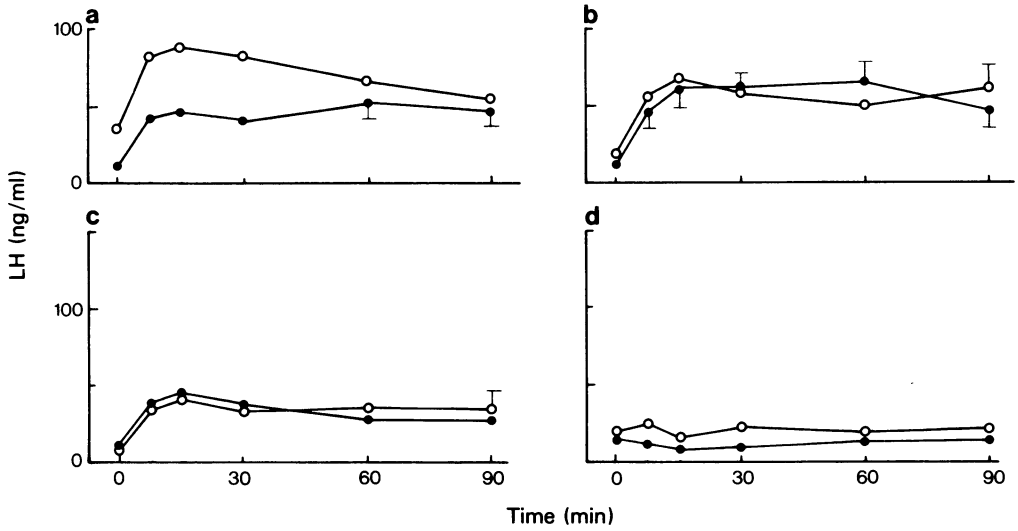

Figure 2 Plasma concentrations of luteinizing hormone (LH) (NIAMDD-RP1) obtained from female rats at dioestrus treated in the same way as the animals in Figure 1 ($n \geqslant 6$ for all groups).

probability values were calculated with the Mann–Whitney U test.

Results

Relationship between duration of anaesthesia and adenohypophysial response to luteinizing hormone-releasing hormone

In male rats the profiles of mean LH concentrations in the plasma were essentially the same for the group anaesthetized with either urethane or pentobarbitone and injected with LH–RH 15, 100 and 240 min after the onset of anaesthesia (Figure 1). However, the peak concentrations of LH were consistently higher in the rats anaesthetized with urethane. Similarly in the dioestrous and pro-oestrous female rats anaesthetized with pentobarbitone, the peak concentrations of LH in the plasma, resulting from the injection of releasing hormone, were not dependent on the duration of pre-injection anaesthesia. However, maximal concentrations of LH measured in pro-oestrous rats were significantly higher than those measured in dioestrous animals (Figures 2a–c and 3a–c; $P < 0.02$, 0.05 and 0.001 respectively) and there was a slower rise to peak concentrations in pro-oestrous rats after 100 min of pentobarbitone anaesthesia.

By contrast, the peak concentrations of LH measured in the plasma of urethane-treated pro-oestrous and dioestrous female rats were related to the duration of the anaesthesia preceding the injection of LH–RH. Thus in dioestrous rats, administration of the releasing hormone 15 or 100 min after the onset of urethane anaesthesia produced peak plasma concentrations of LH significantly higher than the maximal values obtained from animals injected after 240 min of anaesthesia (Figure 2; $P < 0.001$ and < 0.02 respectively). The rats anaesthetized with urethane 15 min before injection of LH–RH showed a significantly greater (Figure 2a; $P < 0.01$) rise in plasma luteinizing hormone concentrations than the group similarly treated but anaesthetized with pentobarbitone. Unlike male rats, this difference between groups of dioestrous females anaesthetized with pentobarbitone or urethane was no longer apparent at 100 or 240 min (Figures 2b and c).

For pro-oestrous rats the pituitary response to LH–RH was significantly greater in animals anaesthetized for only 15 min than those anaesthetized for 4 h (Figure 3; $P < 0.05$) and the response at 15 and 100 min was greater in the urethane-treated rats than those given pentobarbitone.

Effect of the anaesthetics on the half life of luteinizing hormone

The data presented above could have resulted in part from the faster clearance of LH from the plasma of rats anaesthetized with pentobarbitone. To investigate this possibility 2496 ng LH was injected intravenously in 1.3 ml of 1% phosphate buffered saline into pro-oestrous female rats which had been anaesthetized with either urethane or pentobarbitone 100 min earlier. Blood samples were taken, for estimation of plasma LH concentrations, as before. The results

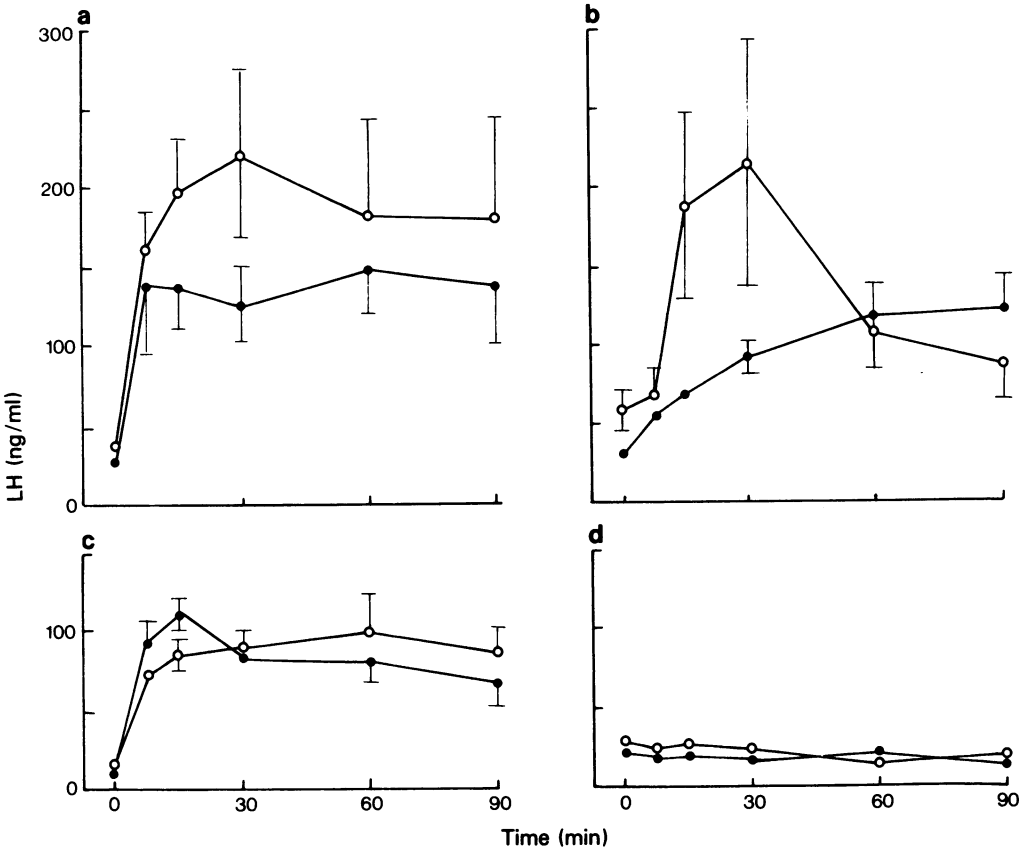

Figure 3 Plasma concentrations of luteinizing hormone (LH) (NIAMDD-RP1) obtained from female rats at pro-oestrus treated in the same way as animals in Figure 1 ($n \geqslant 6$ for all groups).

obtained demonstrated clearly that clearance of LH from the plasma of urethane-anaesthetized rats was not slower than in pentobarbitone-treated animals (Figure 4). Indeed, there was some indication that pentobarbitone increased the half life of LH. Thus any dissimilarity in half life of LH in urethane- and pentobarbitone-anaesthetized rats should have opposed the significant differences in LH secretion in these animals which resulted from a single injection of LH–RH.

Discussion

The experiments demonstrate clearly that in anaesthetized female rats the maximal concentration of plasma LH resulting from a single injection of LH–RH is related to the choice of anaesthetic. In addition, for female rats given urethane the magnitude of the pituitary response diminishes significantly when the period of anaesthesia before LH–RH injection is increased from 15 to 240 min. Thus the sensitivity of the pituitary to exogenous LH–RH decreases in female rats during the first 4 h of urethane anaesthesia. Since it is reasonable to anticipate a similar reduction in sensitivity to endogenous LH–RH the results support our hypothesis that a stable preparation for investigating hypothalamic control of pre-ovulatory gonadotrophin secretion is not obtained readily by anaesthetizing female rats with urethane. Our data may also provide some explanation for the conflicting views on the effects of urethane on the hypothalamus and/or pituitary (Haller & Barraclough, 1968; Lincoln & Kelly, 1972; Blake & Sawyer, 1972).

In contrast, the sensitivity of the adenohypophysis of male rats to exogenous LH–RH did not change when the pre-injection period of urethane anaesthesia was increased from 15 to 240 min. The reason for the greater stability of the urethane-anaesthetized male rats is not known. However, in the urethane-anaesthetized female rats the pituitary response to exogenous LH–RH may have been potentiated by continuing secretion of LH–RH. Some evidence for this suggestion is provided by the finding that the urethane-treated

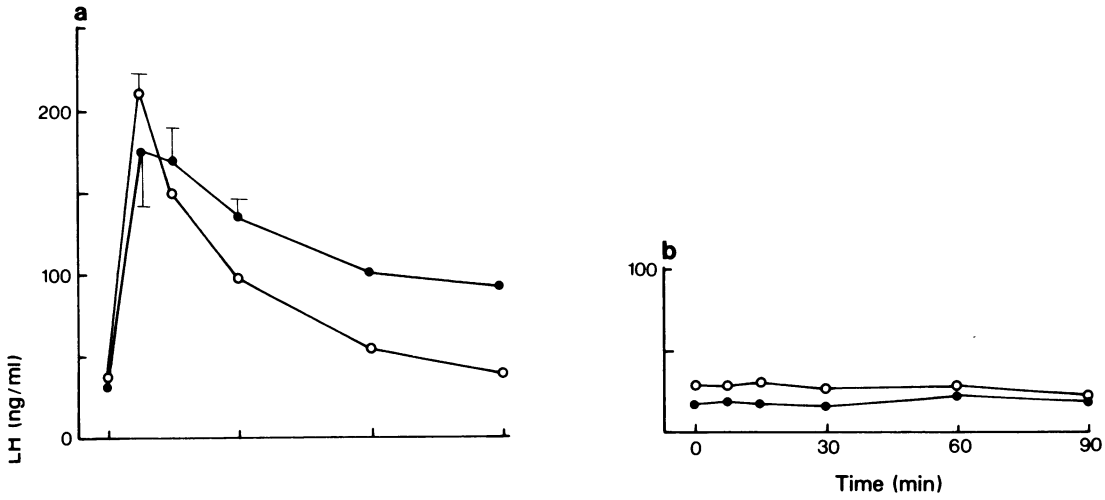

Figure 4 (a) Plasma concentrations of luteinizing hormone (LH) (NIAMDD-RP1) after intravenous injection at time zero of 2496 ng LH into pro-oestrous female rats anaesthetized 100 min earlier with either urethane (○) or pentobarbitone (●). (b) Control values for LH concentrations after intravenous injection of saline ($n \geqslant 5$ for all groups).

female rats showing the greatest response to LH–RH had slightly higher pre-injection concentrations of plasma LH than the equivalent pentobarbitone-anaesthetized rats. Potentiation of the pituitary response to LH–RH by previous exposure to this peptide occurs most readily in pro-oestrous rats (Fink, Chiappa & Aiyer, 1976) and this may be a reason for the different effects of urethane on the pituitary responsiveness to exogenous LH–RH described in this paper.

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