The Effects of Halothane on Ovulation in the Rat

In many types of neuroendocrinological experiments technical requirements call for the use of general anaesthesia, but it is often difficult to assess to what extent the anaesthetic per se is capable of altering the neural substrate under study. Urethane, for instance, has long been the anaesthetic of choice in electrophysiological investigations of neuroendocrine circuits, but recently it has been shown that this drug blocks ovulation in the rat when it is administered at appropriate times during the oestrous cycle[1,2]. A similar effect has been described for pentobarbital (Nembutal®)[3]; both drugs prevent ovulation by interfering with the neural triggering of pituitary LH release. More recently, halothane has become a popular anaesthetic, mainly because the degree of anaesthesia can rapidly be altered by changing inhalation rates. In this laboratory halothane (Fluothane® Ayerst) is used for an electrophysiological study of the action of estrogens on neural circuits implicated in the control of reproduction. It therefore became of interest to determine whether or not this anaesthetic would also interfere with spontaneous ovulation.

Experiments were performed on adult female rats of Wistar strain (200–280 g), maintained under a controlled lighting schedule (lights from 05.00 h to 19.00 h) and at a constant temperature of 22°C. Daily vaginal smears were taken, and only animals which displayed at least 3 consecutive 4-day oestrous cycles were used. On the day of prooestrus, the rats were exposed to ether for about 2 min between 13.15–13.45 h, intubated and anaesthetized with halothane. The halothane was administered in pure oxygen at a flow rate of 50 ml/min using a Fluotec Mark III vapourizer. At 16.30 h the anaesthetic was switched off and the animals allowed to recover. Each experimental animal was paired with a control animal injected with Nembutal (35 mg/kg i.p.) at the beginning of the experiment. This dose of Nembutal is known to block ovulation if administered prior to the 'critical period'[3]. Ovulation was assessed by counting the number of ova contained in the Fallopian tubes during the following morning (oestrus); if no ova were present the ovaries were immediately fixed and histological sections were made in order to confirm the absence of freshly ruptured follicles.

1% halothane was the lowest concentration which reliably induced sedation; at this dose level cutaneous sensitivity was still present. Therefore, in order to obtain a level of anaesthesia suitable for surgery it was necessary to increase the concentration of halothane to 1.5%. Ovulation was blocked in 7 of the 10 animals anaesthetized with 1% halothane during the 'critical period'; at a dose level of 1.5% halothane the ovulation blockade was complete (Table I). In view of these results it was of interest to determine whether or not a low concentration of halothane supplemented by subthreshold doses of Nembutal or urethane would allow ovulation to occur. A level of anaesthesia similar to that obtained with 1.5% halothane alone was induced by using 0.5% halothane in conjunction with either 17.5 mg/kg of Nembutal or 500 mg/kg of urethane; both combinations of anaesthetics blocked ovulation when administered during the 'critical period' (Table I).

In order to define the site of the ovulatory blockade rats anaesthetized with 1.5% halothane as described above were treated with ovine LH (NIH LH-S17) or synthetic Luteinizing Hormone Releasing Hormone (LRH; Beckman Instruments Inc., Spinco Division, Palo Alto, Calif., USA) at 16.00 h of the prooestrus phase. The LH was injected i.p. in 0.5 ml of saline and the LRH was injected s.c. in 0.5 ml of 0.01 *M* acetic acid in saline[4].

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Table I. Blockade of ovulation by halothane

Anaesthetic	No. of rats[a]	Ovulation blocked	Partial ovulation < 10 Ova	Full ovulation ⩾ 10 Ova
1% Halothane	10	7	1	2
1.5% Halothane	6	6	0	0
0.5% Halothane + 17.5 mg/kg Nembutal	4	4	0	0
0.5% Halothane + 500 mg/kg urethane	4	4	0	0

[a] Similar numbers of rats were concurrently injected with Nembutal, 35 mg/kg i.p.; ovulation was consistently blocked in these controls.

Table II. Effects of LH and LRH on halothane blockade

Anaesthetic	Hormones	No. of rats[a]	Full ovulation ⩾ 10 Ova	Partial ovulation < 10 Ova	Ovulation blocked
1.5% Halothane	50 µg LH	8	6	0	2
1.5% Halothane	100 µg LH	3	3	0	0
1.5% Halothane	500 ng LRH	10	9	0	1

[a] 21 rats were concurrently anaesthetized with 1.5% Halothane but did not receive any hormone treatment; ovulation was fully blocked in 17 and partially blocked in 3 controls.

Control animals were anaesthetized with 1.5% halothane and sham injected with the appropriate solvents. 18 out of 21 rats treated with LH or LRH shed a full complement of ova (Table II); this finding indicates a neural site of action of the anaesthetic.

Recent evidence suggests that the mechanism of halothane anaesthesia involves a decrease in the amount of transmitter released from nerve endings[5]; similar findings have been reported for barbiturates[6]. It is thus conceivable that both drugs interfere with ovulation by reducing the synaptic activation of neurons involved, directly or indirectly, in the production of LRH; this mechanism of action may be a general property of anaesthetics. Therefore, the exploration of neural mechanism underlying spontaneous ovulation in the rat is unlikely to yield physiologically meaningful results if carried out under the influence of any general anaesthetic.

Zusammenfassung. Halothan-Anaesthesie während der sogenannten «kritischen Periode» der Proöstrusphase bewirkt eine zentrale Hemmung der Spontanovulation bei der Ratte. Wie Barbiturate und Urethan ist deshalb Halothan als Anaesthetikum zur Erforschung neuroendokriner Mechanismen nur in beschränktem Masse geeignet.

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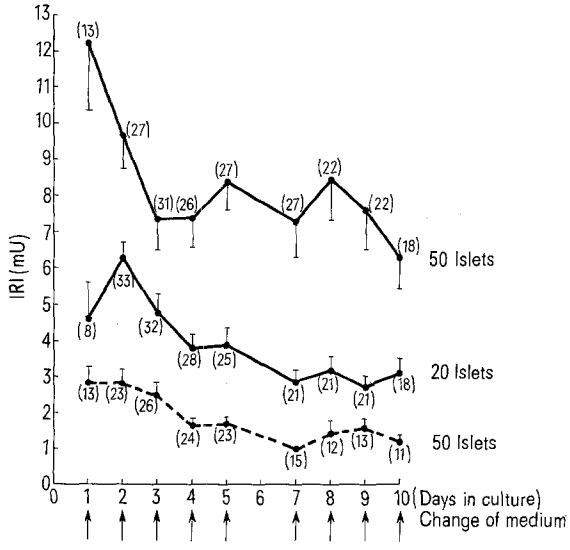
Insulin Release from Isolated Islets of Langerhans of the Rat in Organ Culture

The isolated islets of Langerhans of guinea-pig, mice and rabbits have been cultured in vitro[1–4]. There are only a few results on insulin secretion of cultured islets, especially on rat islets[5]. Our recent work attempts to study the possibilities of cultivation of adult rat islets, and to characterize the effect of different glucose concentrations in the nutritional medium on the insulin secretion within 24 h periods for a long duration of cultivation.

The islets of adult wistar rats (starved overnight), with a body weight of 160–180 g, were isolated by the modified method of Lacy and Kostianovsky[6]. The pancreas were aseptically excised, washed in Hank's salt solution and digested by shaking at 37 °C in Hank's solution containing 3.7 mg collagenase/ml for 20 min.

Enzyme treatment was interrupted by dilution with cold Hank's solution and the islets were subsequently sedimented at 4 °C and washed 5 times. The isolation of islets was carried out under sterile conditions by means of a stereomicroscope and the islets were collected in TC 199 medium (Difco Laboratories Detroit, Michigan).



Insulin (IRI) released into the medium by cultured rat islets [mU/culture bottle/24 h]. —, 16 m*M* glucose; ----, 5 m*M* glucose. Results as $\bar{x} \pm s\bar{x}$ with the number of observations in parentheses.

Groups of 20 or 50 islets were cultivated on glass fibre vlies ('Microlith', Schuller, Wertheim/Main) supported by metal grid in small culture bottles in 2 ml TC 199 with 10% inactivated calf serum, penicillin (100,000 U/l), streptomycin (145 mg/l) and hydrocortison (1 mg/l.) The glucose concentration was 5 m*M* or 16 m*M* during the whole cultivation period. The medium was changed every day.

The content of insulin in the medium was determined immunologically by back titration and alcohol precipitation[7], (standard: pork insulin Novo, 10–4000 μU/ml nutritional medium with 10% calf serum).

A high glucose concentration in the medium elicited a marked increase in the insulin release of cultivated islets (Figure) during the whole cultivation period ($P < 0.001$ for every day). With the high glucose concentration (16 m*M*) the medium insulin content of groups of 50 islets was significantly elevated in comparison with groups of 20 islets ($P < 0.01$).

The rate of insulin release calculated for one islet in groups with 50 or 20 islets was equal. There was no change, however, in the IRI values of groups of 20 or 50 islets after administration of a basal glucose concentration (Table), which means that the basal rate of insulin secretion calculated for one islet is diminished. The reason for this different behaviour is not clear. It may be that the negative feedback mechanism depends on the glucose concentration, on the time of incubation, and on the kind of medium or buffer.

With the duration of cultivation, the amount of insulin secretion is diminished. $P < 0.01$, first day, 50 islets; $P < 0.01$, second day, 20 islets. The present results agree well with the decreased secretion of monolayer

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