Blockade of Ovulation After Atropine Implants in the Lateral Hypothalamus of the Rat

It is known that atropine blocks spontaneous and reflex ovulation by acting on the neural phase of lutein hormone (LH) secretion[1]. Atropine also inhibits male and female sexual behavior in rats of both sexes[2–4]. These findings suggest that parasympathetic mechanisms are involved in the nervous control of gonadotrophic secretion and reproductive behavior[5].

Some authors[6,7] have shown in rats the existence of a cholinergic system which arises from the anterior mesencephalon and runs rostrally to basal subcortical zones, reaching the hypothalamic areas. LEONARDELLI[8] showed subtle changes of the acetylcholinesterase activity in the lateral and dorsal hypothalamic cells of cats, guinea-pigs and rats. These changes were related to the different seasons of the year, the estral cycle, and castration. Since large doses of parenterally administered atropine are necessary to block ovulation in the rat, which makes its specificity controvertible, we decided to microimplant the drug in the anterior lateral hypothalamus and to study the changes in estral cycles and sexual organs.

The experiments were carried out on albino rats of our strain, weighing 150–200 g and having had at least 3 regular 4–5 days estral cycles. The animals were kept on a 14 h light – 10 h dark schedule, and water and food were available ad libitim. Atropine sulphate (150–250 µg) was tamped into capillary tubes (OD 300 µ, ID 150 µg), and the tips of the tubes were sealed with a thin layer of sucrose. The animals were anesthetized with sodium pentobarbital (30 mg/kg), and the tubes were implanted stereotaxically, according to the DE GROOT atlas for the rat brain[9], in the anterior lateral and posterior hypothalamus and in the anterior amygdaloid area. The tubes were secured to the skull surface with dental acrylic.

In a control group, tubes containing paraffin were implanted in the anterior lateral hypothalamus. Daily vaginal smears were taken in the post-operative period. At the end of the experiment the animals were killed under ether and the ovaries, uteri, adrenals, thyroids and pituitaries were carefully dissected out and weighed on a torsion balance. Histological sections of ovaries and uteri were performed. The brains were fixed in 10% formalin solution and serial sections were made and examined to determine the precise location of the implants.

Transfixion of the uterine horns was performed on the fourth day of the post-operative diestrous to elucidate the formation of deciduoma.

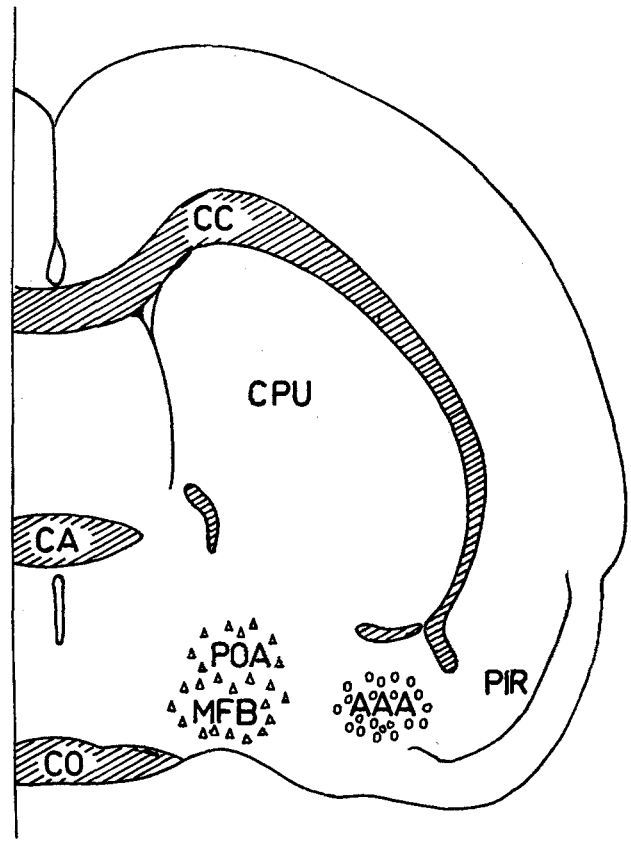
A total of 34 out of 37 rats with atropine implants in the anterior lateral hypothalamus (Figure) had disturbed cycles, with prolonged diestrous up to 12 days, absence of deciduoma, and a diminution of ovarian weight. The histology of the ovaries after this long diestrous showed lack of young corpora lutea and an increased number of growing follicles. In 40% of the implanted animals there was a change in the spontaneous behavior which lasted for 5–6 days and was characterized by restlessness, aggressivity, and disruption of the sleep-wakefulness cycle[10].

Seven out of a control group of 16 animals implanted with paraffin in the anterior lateral area showed disturbed estral cycles. Only 1 of the 10 rats implanted with atropine in the anterior amygdaloid area (Figure) showed a prolonged diestrous.

It is concluded that atropine implants cause prolonged diestrous and inhibit ovulation by disrupting the neural phase of the ovulatory hormone (OH) secretion. The absence of uterine deciduoma during the early diestrous, the decrease of ovarian weight and the absence of mammary hypertrophy, all indicate that there is no pseudopregnancy.

Prolonged diestrous without traumatic deciduoma has been found after hypothalamic lesions of the medial eminence not interrupting the portal system[11], and also after lesions of the mesencephalic reticular formation at the level of the ventral tegmental system[12]. The latter system belongs to the caudal pole of the cholinergic system described by SHUTE and LEWIS[7].

The reappearance, after 30 days, of normal cycles in animals implanted with atropine in the lateral hypo-



Transversal section of the rat hypothalamus (A 7.4, DE GROOT[9]). △, area where atropine implants inhibit ovulation; ○, area where atropine implants were negative.

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thalamus, as well as the absence of response in those implanted in the anterior amygdaloid area and posterior hypothalamus suggests that the drug has a specific and short-lasting effect by blocking the cholinergic mechanisms related to the release of the ovulatory quota of gonadotrophins. The decrease of the ovarian weight as well as the inhibition of compensatory hypertrophy after unilateral castration by hypothalamic implants of atropine (unpublished observations) would suggest the blockade of the folliculotrophic hormones as a whole (FSH-LH).

Résumé. Des implantations d'atropine dans l'hypothalamus antérieur et latéral de la rate provoquent un diestrus prolongé et une diminution du poids de l'ovaire sans réponse utérine au déciduome traumatique. On conclut que l'atropine agit sur des voies colinérgiques en relation avec la sécrétion des gonadotrophines.

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Do the Products of Messenger RNA Hydrolysis Cause 'Cloudy Swelling'?

The accumulation of intra-cellular water by cells subjected to toxic stresses was first noted by Virchow[1] who termed the process 'cloudy swelling' and believed it to be one of the primary causes of cell death. Its pathogenesis is still not fully understood[2]. However, it is clear that it involves a failure of the cellular osmotic control mechanisms[3]. During recent experiments on RNA stability in *Tetrahymena* we noticed that these ciliated protozoons were subject to slight hydropic changes during periods of increased RNA hydrolysis caused by synchronizing heat-shifts. These cells are quite viable at synchronizing temperatures provided the duration of exposure is limited[4]. If Actinomycin D (ActD) is added to the medium during these temperature shifts a more perceptible increase in cell size is consistently noted. We have examined this swelling phenomenon in some detail. The results suggest that it is a result of *net* hydrolysis of unstable RNA. If the hydrolysis is allowed to proceed at a rapid rate and to a sufficient degree the cells will die of severe hydropic degeneration. The cytological changes seen in such cells are virtually identical to those found in classical 'cloudy swelling'. Since the effects of ActD and temperature are synergistic and both are known to induce net RNA degradation it appears that the edema may be initiated by the RNA hydrolysis products.

Material and method. To follow the rate of degradation of unstable RNA, the cells were synchronized and resuspended in inorganic medium. This facilitates rapid RNA labeling and has no effect on either cell viability or cell division[5]. At the beginning of the experiment 100 µc of H^3 uridine was added to the medium and the cells were labeled for 20 min. ActD (50 µg/ml) was then added and the culture divided into 3 parts. One portion was incubated at 29 °C (the optimum growth temperature), one at 34 °C, and one at 37 °C[6]. The rate of decay of prelabeled RNA was followed using the direct filter paper disc procedure[7]. After 60 min a small sample was removed from each culture, fixed with saturated $HgCl_2$, and photographed. Control samples lacking ActD were also incubated at each of the 3 temperatures. Previous experiments have shown that the uptake of exogenous uridine ceases at 34 °C[5]. This inhibition of uridine uptake is presumably due to the expansion of intra-cellular nucleotide pools[8].

Results and discussion. The effect of adding ActD to the medium after pulse labeling with uridine is seen in Figure 1. At 29 °C the uptake of label slows and decay begins after a short delay. At 34 and 37 °C decay begins almost immediately and proceeds at rates roughly proportional to the degree of temperature elevation. The effects on cellular morphology are seen in Figure 2. These

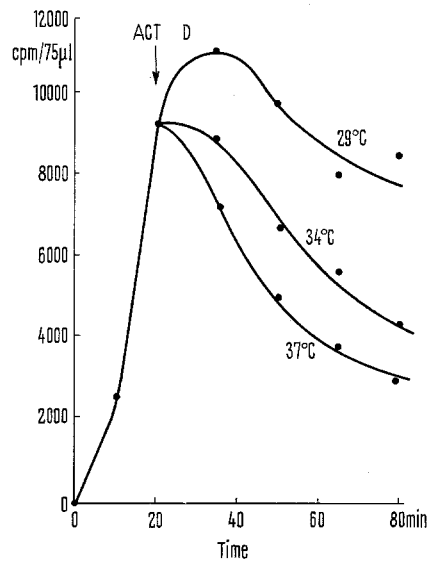

Fig. 1. Effect of temperature variation of the rate of decay of prelabeled RNA. Synchronized *Tetrahymena pyriformis* GL were labeled with H^3 uridine for 20 min as described in the text. Actinomycin D was then added (50 µg/ml) and the culture divided into 3 parts and incubated at the temperatures indicated. The rate of decay is roughly proportional to the temperature for values above the optimum growth temperature (29 °C).

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