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Blockade of the Postorchidectomy Increase in Gonadotropins by Implants of Atropine into the Hypothalamus (39347)

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Atropine sulphate can suppress gonadotropin release in rats of both sexes following its subcutaneous (1–3) or intraventricular injection (2, 3). The aim of the present study was to localize the regions involved in the blocking action of atropine by implanting a small amount of the alkaloid in discrete brain regions.

Material and methods. Adult male rats (270–320 g body wt) obtained from Simonsen Laboratories (Gilroy, Calif.) were maintained in an air-conditioned room with controlled lighting (on 5 AM, off 7 PM). Purina rat chow and water were provided *ad libitum*.

Using ether anesthesia, stainless steel cannulae (23-gauge, 20 mm in length) were implanted bilaterally in various hypothalamic loci or into the anterior pituitary using the Kreig Johnson stereotaxic instrument. Cannulae had been previously filled with the mixture to be implanted by tamping the tips into the mixture. This mixture consisted of equal amounts of atropine sulphate (Sigma) mixed with cholesterol (w:w) (50% atropine), 1 part of atropine to 19 parts of cholesterol (5% atropine) or pure cholesterol as a control. Cannulae were implanted into one of the following loci: suprachiasmatic-anterior hypothalamic region; the middle hypothalamus, which was in the vicinity of the arcuate median eminence region; the posterior hypothalamus, in the premammillary and mammillary regions; the frontal cortex at a depth of 2 mm from the dura; or into each lobe of the anterior pituitary. Cannulae were secured to the skull surface with dental cement.

Then, a 1-ml blood sample was withdrawn from the external jugular vein and this was followed by bilateral orchidectomy via the scrotal route at 4:30–6:30 PM. Sixteen hours later (8:30–10:30 AM on the following day) a second blood sample was withdrawn as before while the animals were etherized.

Verification of the location of the tips of the cannulae was accomplished readily by examining the base of the brain at autopsy and in some instances these localizations were confirmed after fixing the brain in 10% formalin and cutting serial frontal sections through the hypothalamic region at 20 μm which were then stained with cresyl violet. To measure the amount of drug delivered from the cannulae, they were filled in the routine manner and then using a mandril, the drugs were pushed out of the cannula and weighed. At sacrifice, the amount of drug remaining in the cannulae was similarly weighed so that the quantity delivered while the cannula was implanted could be estimated.

LH in plasma was measured by the method of Niswender *et al.* (4) and FSH using the kits provided by NIAMDD.[3] Results were expressed in terms of NIH-LH-S1 in the case of LH and the RP1 reference preparation in the case of FSH. The paired Student's t test was used to calculate the significance of changes in hormone titers produced by a treatment. When the means of two groups were compared, analysis was by Student's t test.

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Results. Cerebral cortex. Implants containing 50% atropine sulphate in the frontal cortex failed to block the increase in plasma LH and FSH titers which follows castration in the male rat (Figs. 1 and 2).

Anterior hypothalamic implants. Implants of cholesterol in the suprachiasmatic-anterior hypothalamic region were associated with elevations in both plasma LH and FSH following castration which were similar to those observed in animals with atropine implants in the cerebral cortex. In contrast, implants of 50% atropine sulphate completely abolished the postcastration rise of both LH (Fig. 1) and FSH (Fig. 2). The increase in both gonadotropins in this group was significantly less than the increase in the animals bearing cholesterol implants ($p < 0.05$). The implants of 5% atropine sulphate had only a slight and nonsignificant suppressive effect on the postcastration rise in both gonadotropins.

Mid-Hypothalamic implants. Results in this group of animals were similar to those in animals bearing anterior hypothalamic implants in that a postcastration rise in both gonadotropins occurred in cholesterol-implanted controls and no significant rise occurred in animals bearing implants of the higher but not the lower dose of atropine sulphate.

Posterior hypothalamic implants. Results in this group were also similar to those in the other hypothalamic groups except that there were no significant rises in plasma FSH in any of the implanted groups in this series. In the case of LH a postcastration rise occurred in animals implanted with cholesterol or the lower dose of atropine but no significant increase occurred in animals implanted with 50% atropine sulphate.

Intrapituitary implants. A significant rise in LH occurred in the castrates with implants of either cholesterol or the high dose of atropine in the anterior pituitary, but FSH failed to rise significantly following implantation of either cholesterol or atropine into the pituitary.

Quantity of atropine delivered by the implants. Weighing the material within the cannula before and after implantation showed that approximately 50 μg disappeared from each cannula or a total of 100 μg from both cannulae. In the case of 5% atropine implants this would mean that 5 μg of atropine sulphate was delivered to the brain. Since less than one-half of the weight of atropine sulphate is atropine, only about 2.2 μg of atropine would be released from the 5% atropine sulphate cannulae during the 16-hr period, a dose of 130 ng/hr. The quantity of atropine released in the case of

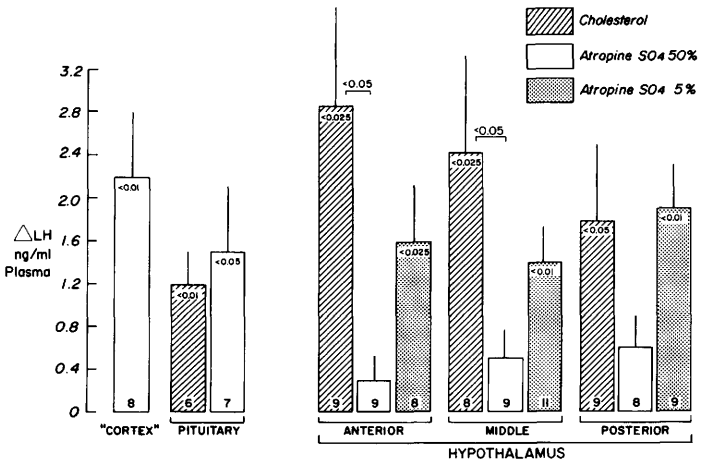

FIG. 1. The change in plasma LH 16 hr after castration in rats bearing implants of cholesterol or atropine sulphate in various hypothalamic loci, the cerebral cortex, or the anterior pituitary. In this and the subsequent figure, the height of the bar indicates the mean response and the vertical line 1 standard error of the mean. Significance of the change from the precastration plasma titer is indicated within each bar, and the number of animals is indicated at the bottom of each column.

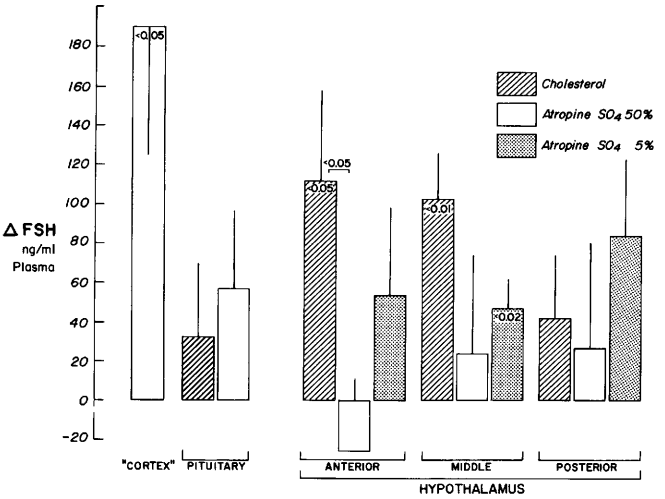

FIG. 2. The increase in FSH titers in plasma following castration in rats bearing various implants within the brain or pituitary.

the 50% atropine sulphate implants would be 1.3 μg/hr.

Discussion. The present results indicate that a small amount of atropine sulphate delivered to the basal hypothalamus can abolish the postcastration rise in both LH and FSH. On the other hand, implants within the cerebral cortex or pituitary failed to block the increase in plasma LH. It was not possible with this technique to localize definitively the most sensitive area within the hypothalamus since implants were effective whether implanted in the anterior, middle, or posterior hypothalamus; however, the most dramatic inhibition was found with implants in the anterior and middle hypothalamus which is the region in which changes in acetylcholine metabolism can be related to altered gonadotropin release (5). The ability of implants in such a wide area of the hypothalamus to inhibit the postcastration rise in LH may be related to diffusion of the atropine to a considerable distance from the cannula tip. Our results are in agreement with earlier work in which atropine implants into the anterior and middle hypothalamus were found capable of blocking ovulation in female rats (6), and it is noteworthy that similar intrahypothalamic implants of atropine can also block ACTH secretion (7, 8).

In the case of FSH, atropine implants in the hypothalamus but not in the cerebral cortex also blocked the postcastration rise. Since implants into the pituitary of cholesterol alone as well as atropine suppressed the postcastration rise in this gonadotropin, we cannot be sure that the action of atropine to inhibit FSH release was not mediated on the anterior pituitary by uptake of the alkaloid into portal vessels and subsequent delivery to the gland. This is unlikely in view of the failure of pituitary implants of atropine to inhibit the LH rise. Probably the failure of FSH to rise following implantation of cannulae into the pituitary is related to mechanical disruption of anterior pituitary FSH release by the implantation procedure since it was equally pronounced with cholesterol implants.

In conclusion, the present results support earlier studies which suggest that acetylcholine may play a role as a synaptic transmitter to stimulate release of LH-releasing hormone (LHRH) which would in turn increase gonadotropin release. The postulated cholinergic synapses would be located in the anterior and middle hypothalamus.

Summary. Bilateral implants of atropine sulphate were placed in various loci in the brain or into the anterior pituitary in male rats and the effects of the implants on the postcastration rise in plasma FSH and LH was determined. The increase in both gonadotropins at 16 hr after castration still occurred in animals with implants in the

cerebral cortex. The postcastration rise of both FSH and LH was blocked by atropine implants in the anterior, middle, or posterior hypothalamus but was not interfered with by control implants of cholesterol. Bilateral implants of either cholesterol or atropine into the anterior pituitary failed to alter the increase in plasma LH following castration but both types of implants interfered with the postcastration rise in FSH, possibly because of trauma to the pituitary from the cannulae. It is suggested that hypothalamic cholinergic synapses may play a role in stimulating the increased LHRH release which induces the postcastration rise in gonadotropins.

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