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Pineal function cannot prevent the occurrence of castration cells in male rats

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Summary. The relationship between the pineal gland and the pituitary gland was investigated in male rats. The results indicate that the hypothalamo-adenohypophysial-gonadal axis is affected by the pineal gland, but the appearance of castration cells following gonad ablation may be only slightly modified by alterations in pineal gland function.
Key words. Rat pineal; rat pituitary; castration cells; functional activation of pineal; gonadectomy.

It is believed that the pineal gland produces some anti-gonadotrophic substances, e.g., melatonin, arginine vasotocin (AVT), etc., and alters the secretion of gonadotrophins via the hypothalamus and/or adenohypophysis[1-5]. Above all, Martin and Klein[6] reported that melatonin can act directly on the pituitary to suppress the release of LH induced by LHRH and that melatonin may act both at the hypothalamic level and at the pituitary level to regulate LH secretion.

It is known that pinealectomy sometimes results in pituitary hypertrophy[7], and usually enhances the pituitary contents[5-8]. However, to our knowledge, there are few reportes concerning the relationship between the pineal and the ultrastructural features of gonadotrophs. Clementi et al.[9] demonstrated a remarkable dilation of the endoplasmic reticulum (ER) associated with cellular hyperfunction following pinealectomy.

The present study was performed in order to learn whether a functionally activated pineal gland, induced by blinding and olfactory bulbectomy in male rats, prevents the occurrence of castration cells or modifies the ultrastructure of gonadotrophs following gonadectomy in the rat.

Materials and methods. 25 Male rats of a Wistar-derived strain were obtained at 35 days of age, separated into 5 groups with 5 animals in each, and surgically prepared for the experiment: group 1 was sham-operated (control-group); group 2 was gonadectomized (G-group); group 3 was gonadectomized, blinded and olfactory bulbectomized (GB-group); group 4 was gonadectomized, blinded, olfactory bulbectomized and pinealectomized (GBP-group); group 5 consisted of rats which were gonadectomized, blinded, olfactory bulbectomized, and injected with 5 µg melatonin mixed 10% gelatin solution twice a day for 1 month (GBM-group). All animals were anesthetized with nembutal during the operations. The animals were sacrificed after 1 month by decapitation following anesthesia with ether. The anterior pituitary glands were removed and cut into halves. One half was fixed in Bouin's solution for immunocytochemistry and the other was fixed in 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer for 2 h and then in 1% osmic acid for 1 h prior to processing for EM examination. For immunocytochemistry, the unlabeled antibody method of Sternberger et al.[10] was used to identify gonadotrophs. Antiserum to rat LH was a gift from NIADDK, NIH, USA.

Results. The control group (sham-operated) showed no alteration of ultrastructure of gonadotrophs in the anterior pituitary (fig. 1) as compared with that of normal intact rats. Removal of testes for 30 days induced numerous hypertrophic gonadotrophs as seen in figure 2; likewise, removal of testes, olfactory bulbs and eyeballs for 30 days brought about various

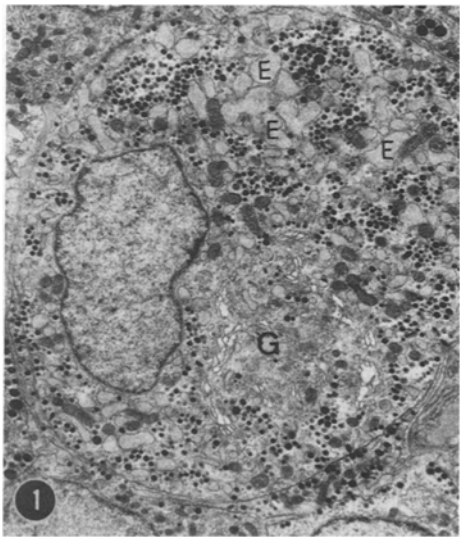

Figure 1. A typical gonadotroph (Type-I gonadotroph) of the rat. E: Endoplasmic reticulum, G: Golgi body. × 4500.

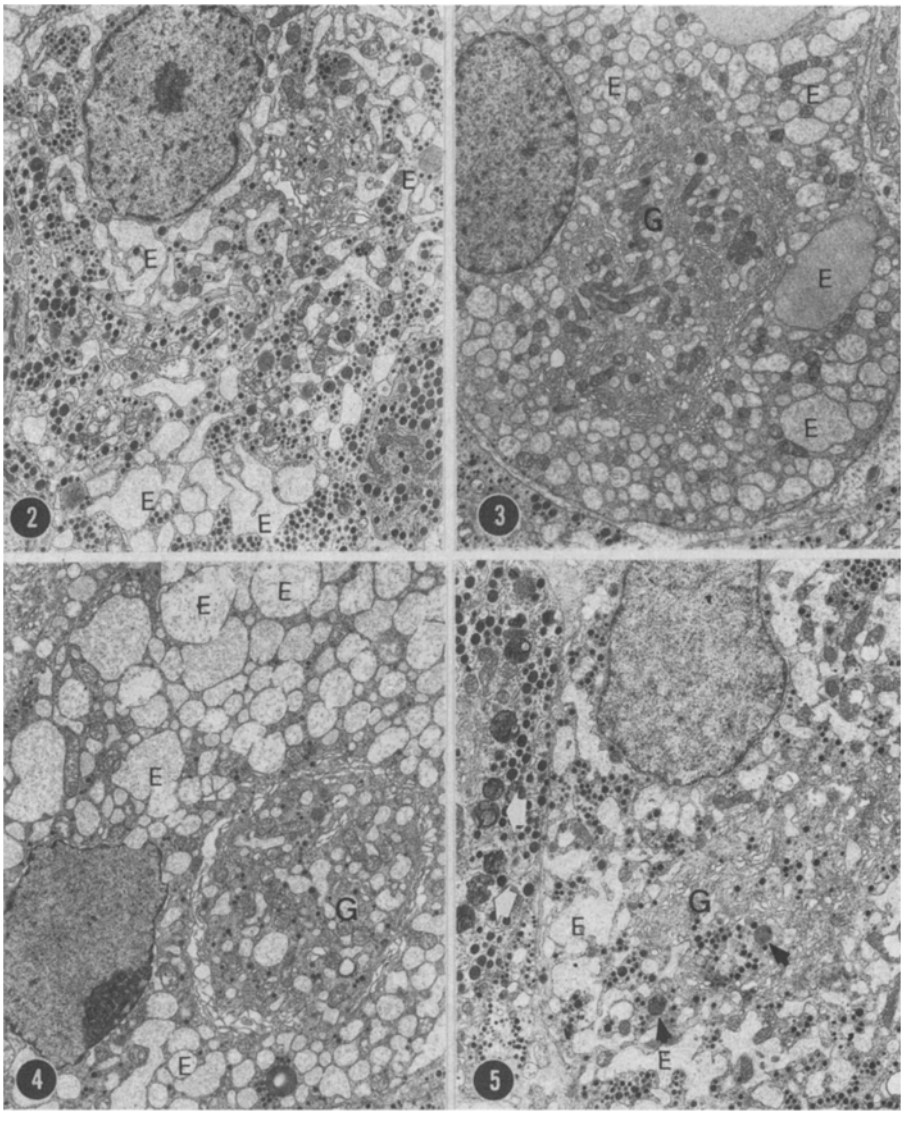

Figure 2. An example of hypertrophic gonadotroph of male rat castrated for 30 days. Figure 3. An example of a gonadotroph in which numerous vesicular endoplasmic reticulum and very scant secretory granules are contained. The animals were castrated, olfactory bulbectomized, pinealectomized and blinded for 30 days. Figure 4. An example of gonadotroph of castrated, pinealectomized, blinded and olfactory bulbectomized male rat. Figure 5. An example of gonadotroph of castrated, olfactory bulbectomized, blinded and melatonin-treated male rat. Endoplasmic reticulum are dilated but many lysosomes are seen (arrows). Secretory granules wer scattered and numerous than are in figures 2 or 3. E, Endoplasmic reticulum, G, Golgi body. × 4500.

dilations of the ER and variant localization of secretory granules in the gonadotrophs. In addition, some dilated ER-containing gonadotrophs in this group showed scanty secretory granules, as shown in figure 3. However, when pineal, testes, eyeballs and olfactory bulbs were completely removed for 30 days an even more dilated ER and more prominent Golgi bodies were seen in most of the gonadotrophs than was the case for the other groups (fig. 4). Following the administration of melatonin to gonadectomized, blinded and olfactory bulbectomized rats, the conspicuous dilation of ER and the development of Golgi bodies seen in gonadotrophs of the GBP-group were much less pronounced. In this group, numerous lysosomes were observed in the hypertrophic gonadotrophs as seen in figure 5.

Discussion. It is evident that castration cells occur owing to an elevation of hypothalamic LHRH after gonadectomy, which follows the lack of secretion of sex hormone(s)[11]. Shiino[12] reported that LHRH could induce hypertrophy of gonadotrophs in vitro when synthetic LHRH was added to the culture medium. On the other hand, it was reported that pinealectomy in the rat resulted in an elevation of serum FSH[5] and LH[8] after the operation as well as hypertrophy of the gonadotrophs.

When mammals are placed in darkness or short photoperiods, the pineal is generally assumed to have anti-gonadotrophic effects[13,14]. As anti-gonadotrophic substances, melatonin and AVT are thought to be most important by endocrinologists[15–17]. If the pineal gland regulates the function of the pituitary gland by depressing the secretion of LHRH, it should be interesting to observe the ultrastructure of gonadotrophs following gonadectomy in blinded rats which are known to have a highly stimulated pineal function. Shiino et al.[18] demonstrated clear-cut changes in the ultrastructure of prolactin cells in blinded and olfactory bulbectomized rats. They explained the cellular changes of prolactin cells as due to an alteration by the pineal in the secretion or action of prolactin inhibiting factor.

When castration, pinealectomy, olfactory bulbectomy, and blinding were performed at the same time, and the animal examined after 30 days, the occurrence of castration cells were prominent, but we assume that the combined operation of gonadectomy, olfactory bulbectomy and blinding only partially prevented the occurrence of typical castration cells although many enlarged gonadotrophs contained only very scarce secretory granules in the cytoplasm. Melatonin administration to gonadectomized and blinded rats tended to prevent the full development of castration cells and their development of cytoplasmic organelles. These results indicate that the hypothalamo-adenohypophysial-gonadal axis may be partially regu-

lated by the pineal gland, but that stimulating release of hypothalamic LHRH induced by severe lack of sex hormones after gonadectomy cannot be prevented by a stimulation of pineal function.

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Ethanol preference in rats with a prior history of acetaldehyde self-administration

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Summary. Peripherally self-injected acetaldehyde in interaction with environmental and nutritional variables significantly enhances alcohol drinking in rats and suggests an involvement of acetaldehyde in voluntary alcohol intake.
Key words. Rat; ethanol preference; acetaldehyde self-administration.

The importance of acetaldehyde in the regulation of voluntary ethanol intake in experimental animals is the subject of considerable debate[1-8]. Recently we reported that naive rats will self-inject i.v. significant amounts of acetaldehyde when placed at 80% reduced body weight (80% b.wt) on a 1-h daily FI 60-sec food delivery schedule over a period of 10 days[9]. This finding led us to consider the possibility that i.v. self-injection of acetaldehyde may affect an animal's consumption and preference for ethanol. To test this possibility rats were given the opportunity to self-infuse i.v. either acetaldehyde or saline before being presented with a choice of alcohol and water. In such situations the animals could choose ethanol for its pharmacological effects and/or as an alternative liquid or energy source, the amount of ethanol consumed being determined by a variety of regulatory mechanisms[7,9,10]. We report here an alteration of alcohol drinking in the rat by peripherally self-administered acetaldehyde. We suggest an involvement of acetaldehyde in the development of an animal's preference for alcohol.

Details of the procedure for inducing i.v. acetaldehyde self-administration have been reported elsewhere[9]. Two groups of 9 male Long-Evans hooded rats, surgically implanted with jugular catheters and reduced to 80% of their free feeding body weights were maintained on a 12:12 light/dark cycle (dark period beginning at 12.00 h) and allowed to self-inject a 1% v/v acetaldehyde solution (2.32 mg/kg/infusion) for a period of 20 days. Two similar groups were allowed to self-inject saline during this period.

In this procedure the rats were placed individually in operant chambers for 1 h/day over the 20 consecutive days, at the same time each day, with acetaldehyde or saline available i.v. through bar pressing. When an animal pressed the operant bar, the pump was activated for 5 sec and an infusion of fluid (0.07 ml) was delivered into the jugular vein. During the 5-sec infusion interval, additional presses did not reactivate the pump and were not recorded. All infusions during each 1-h test session were automatically monitored on cumulative recorders. Throughout the entire 20-day test period a FI 60-sec food delivery schedule was in operation with Noyes food pellets (45 mg) delivered non-contingently at the rate of 1 pellet per min. All rats were tested within 5 h after onset of the dark period and under red light conditions. At the end of 20 days plus a 2-day period without drug available for self-administration, 1 acetaldehyde and 1 saline group maintained at 80% b.wt were tested over 10 days for their preference for ethanol with tap water as an alternative fluid. During the 10-day period, the ethanol (95%) solutions offered to the animals were increased systematically in concentrations from 3 to 30% using a 3-bottle 2-choice technique[11]. The 2nd acetaldehyde and saline control groups were placed on free feeding conditions 2 days prior and during the 10-day ethanol preference sequence. A 2-way analysis of variance (groups × days, with repeated measures over the days factor) carried out on the self-injection data, showed significant main effects of drug treatments ($F(3,24) = 7.429$, $p < 0.01$) and days ($F(19,456) = 3.039$, $p < 0.001$) at a type 1 error rate of 0.05. Post hoc analysis with Newman-Keul's comparisons showed that animals in acetaldehyde (AcH) group 1 self-injected significantly more acetaldehyde ($p < 0.01$) than saline relative to animals in control groups 1 and 2. A significant difference ($p < 0.05$) was also found between animals in acetaldehyde group 2 and animals in both saline control groups. No significant differences between the two acetaldehyde groups or between the 2 saline control groups were shown. Animals with malfunctioning catheters were excluded from the analysis. Data showing the mean number of acetaldehyde and saline self-infusions by the animals over 20-day test period are presented in figure 1.

A 2-way analysis of variance (groups × days, with repeated measures over the days factor) performed on the ethanol