DEPRESSED PITUITARY PROLACTIN LEVELS IN BLINDED ANOSMIC FEMALE RATS: RÔLE OF THE PINEAL GLAND

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Summary. The effect of blinding combined with anosmia on pituitary prolactin levels was studied in prepuberal female rats. Pituitary prolactin content and concentration were significantly depressed and the animals also evinced a noticeable retardation in the growth of their reproductive organs. Pinealectomy, when performed concurrently with blinding and olfactory bulb removal, prevented the fall in prolactin levels and ovarian weights and resulted in uterine weight levels between those of blind anosmic and untreated animals.

The combination of bilateral orbital enucleation and olfactory bulb removal in prepuberal rats has been shown to delay puberty and result in a state of severe reproductive retardation. This inhibition of sexual maturation is prevented if blind-anosmic animals are also pinealectomized (Reiter, Klein & Donofrio, 1969; Reiter & Ellison, 1970). Based on the premise that pineal substances act at the neural level to inhibit gonadotrophins (Quay, 1969), levels of FSH and LH have been studied in rats sensitized to pineal activity. Levels of both hormones were depressed but to a statistically insignificant degree, due to the variation among individual samples (Reiter & Sorrentino, 1971). This work was undertaken to determine if another important reproductive hormone, prolactin, might be affected under similar experimental conditions.

Sprague-Dawley rats at 25 days of age were operated upon to give the following three groups: (1) untreated (control), (2) blind-anosmic, (3) blind-anosmic-pinealectomized. Blinding was accomplished by bilateral orbital enucleation, while olfactory bulb removal followed the technique of Reiter *et al.* (1969) and pinealectomy, that of Hoffman & Reiter (1965). The animals were housed four or five per clear plastic cage in temperature ($23 \pm 2^\circ \text{C}$) and light (14 hr light/10 hr dark) controlled rooms. All animals were allowed unrestricted access to food and water; some food was always placed on the floor of the cages.

Ten weeks after the initial operative procedure, all animals were killed by decapitation using a small animal guillotine. Trunk blood was collected, centrifuged (2500 rev/min at 10°C) and the plasma fraction stored at -20°C . The anterior pituitaries were dissected free, weighed, and homogenized in 0.5 ml

0.05 M-phosphate buffer and frozen until the time of assay. Body, uterine and ovarian weights were also recorded at this time. A double antibody radio-immunoassay kit for rat prolactin supplied by the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Distribution Program was used to determine the level of prolactin in each pituitary and plasma sample. Hormonal values were expressed in terms of NIAMD-Rat Prolactin-I-1.

Numerical data were subjected to an analysis of variance and, where appropriate, a *t* test between means was performed.

Mean body and organ weights are listed in Table 1. Both 'treated' groups had significantly smaller anterior pituitary glands when compared to control animals. Blind-anosmic rats had ovaries that differed significantly from those of untreated animals, whereas blind-anosmic-pinealectomized animals did not.

TABLE 1
BODY, ANTERIOR PITUITARY, OVARIAN AND UTERINE WEIGHTS OF 95-DAY-OLD RATS
SHOWING THE EFFECTS OF BLINDING, ANOSMIA AND PINEALECTOMY

Groups (no. of rats)	Body weight (g)	Mean organ weights*		
		Anterior pituitary	Ovaries	Uterus
Untreated controls (8)	245 ± 7	12.9 ± 0.8 (5.3 ± 0.3)	72.8 ± 6.4 (29.5 ± 2.1)	434 ± 40 (180 ± 17)
Blind-anosmic (9)	216 ± 9	7.7 ± 1.0 ^c (3.5 ± 0.4) ^b	47.9 ± 5.7 ^{a, d} (21.7 ± 1.9) ^a	207 ± 47 ^{b, d} (92 ± 19) ^b
Blind-anosmic-pinealectomized (8)	260 ± 11	9.6 ± 0.5 ^b (3.7 ± 0.1) ^c	63.1 ± 4.1 (24.6 ± 1.8)	318 ± 24 ^a (124 ± 10) ^a

Values expressed as means ± S.E.

* Absolute weights (mg) with relative weights (mg/100 g body weight) in parentheses.

^a *P* < 0.05 compared with controls.

^b *P* < 0.01 compared with controls.

^c *P* < 0.001 compared with controls.

^d *P* < 0.05 compared with blind-anosmic-pinealectomized rats.

The combination of blinding and anosmia resulted in a marked decrease in uterine weight when compared to the untreated condition. Again, removal of the pineal gland in the blind-anosmic animal returned this parameter toward normal, though the uteri of these rats still weighed significantly less than those of untreated animals. Text-figure 1 indicates that both the pituitary content and the concentration of prolactin was strikingly decreased from normal levels in the blind-anosmic animals. Normal pituitary prolactin titres were found when pineal gland removal accompanied the above operative procedures. Due to the great amount of variation within each group (controls 2.9 ± 4.2 $\mu\text{g/ml}$, blind-anosmic 1.6 ± 0.7 $\mu\text{g/ml}$, blind-anosmic-pinealectomized 4.7 ± 2.1 $\mu\text{g/ml}$), there were no significant differences ($F = 1.46$; $P > 0.05$) between the various groups with respect to plasma levels of prolactin. It should be noted that the effect of bilateral orbital enucleation, olfactory bulb removal or pinealectomy alone were not studied, as these procedures, when performed singly, have proved incapable of profoundly altering the reproductive physiology of the rat (see Reiter & Sorrentino, 1971, for references).

Reiter & Ellison (1970) have reported that the ovaries of blind-anosmic rats contain noticeably smaller corpora lutea, as well as fewer vesicular follicles. Plasma levels of prolactin have been shown to exhibit a cyclic variation during the oestrous cycle, being highest at pro-oestrus (Niswender, Chen, Midgley, Meites & Ellis, 1969) and since this hormone is capable of synergizing with LH in promoting steroidogenesis in the rat corpus luteum (Armstrong, Miller & Knudsen, 1969), it is possible that prolactin might play an active rôle in the normal functioning of the ovary in the cyclic animal. The depressed titres of prolactin in the blind-anosmic rat may, either by itself or in combination with a

TEXT-FIG. 1. Comparison of pituitary prolactin levels. Figures in parentheses indicate the number of animals per group.

* $P < 0.02$ compared with controls or blind-anosmic-pinealectomized rats.

** $P < 0.05$ compared with controls or blind-anosmic-pinealectomized rats.

decrease in the levels of other gonadotrophins, be responsible for the morphological condition of the ovaries of this animal. A reduction of the LH and/or FSH levels alone may even be the critical determinant.

However, Chen & Meites (1970) have shown that ovariectomy results in decreased prolactin secretion, probably due to a lack of oestrogen. While the ovaries presented here (Table 1) for blind-anosmic animals are significantly less in weight than normals, they nevertheless are in no way equivalent to being absent as in the castrate condition. Indeed, such animals have even been shown to be capable of breeding, albeit with some difficulty (Reiter, Sorrentino & Ellison, 1970). On the other hand, uterine weights in the blind-anosmic animals

do indicate a probable depression of oestrogen titres in these rats. It would be of considerable interest, therefore, to determine if the effect on prolactin seen in such animals is a direct consequence of blinding and anosmia or rather a secondary condition related to a primary retardation of the reproductive organs of these females.

In the light of the fact that reduced food intake and decreased body weight can result in reduced pituitary prolactin levels (Meites & Reed, 1949), it should be noted that Reiter & Sorrentino (1971) have found that food intake in blind-anosmic female rats is not significantly depressed from levels seen in normals nor from levels evinced by blind-anosmic-pinealectomized animals. This would indicate that the results found herein do not result from a change in this parameter. The apparent though not significant depressions in body weight of the blind-anosmic animals may be related to the suppression of growth hormone known to exist after such treatment (Sorrentino, Reiter, Schalch & Donofrio, 1971).

To the authors' knowledge, this work represents the first positive indication that the pineal gland may be capable of depressing pituitary prolactin levels in the rat.

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