

Failure of Harderian Gland Removal to Alter Pituitary-Adrenal Function

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The influence of the Harderian gland on pituitary-adrenal function was assessed by subjecting intact control and Harderianectomized (Hx) rats to an experimental protocol for determining non-stress and stress plasma corticosterone levels. Three months after surgical removal of the Harderian gland, morning (0800 h) and afternoon (1600 h) non-stress plasma corticosterone levels of Hx rats were not different from those of controls. Likewise, stress-evoked increases in plasma corticosterone levels did not differ between groups. Vaginal cyclicity was not abolished by Harderianectomy, and at autopsy, body and organ weights of experimental animals were not different ($P > 0.05$) from those of controls.

INTRODUCTION

The Harderian glands are large ocular glands that secrete a lipid by a merocrine mechanism. Classically the gland has been assigned a lubricating function, most likely for the nictitating membrane, but studies undertaken during the last decade have suggested that the gland has more than one function (1). It has been implicated in endocrine function (2), and more recently it has been suggested that it may be involved in immune function (3). However, to date definitive information regarding an endocrine function has not come forth.

In light of the gland's involvement in endocrine function (2), its recent association with immune function (3), and our recent observation of stress-induced lymphocytic infiltration of the Harderian gland (Norvell, unpublished observation), it seemed appropriate to evaluate the role of the Harderian gland in pituitary-adrenal function.

MATERIALS AND METHODS

Thirty-four adult female (180 - 200 g) Sprague-Dawley rats (Charles River, CD) housed 3/cage and acclimated to controlled lighting (fluorescent illumination from 0400 to 1800) and temperature ($26 \pm 2^\circ\text{C}$) were used in this study. Food and water were available *ad libitum*.

Rats were divided into two groups, Harderian gland removed (Hx) and intact controls (C). Harderian glands were surgically removed subsequent to pentobarbital anesthesia supplemented with methoxyflurane (Metofane). A 1.5 - cm incision was made above the eye such that the back of the eyeball and the opening of the orbit were exposed. The eyeball was then pulled forward so as to expose the superior lobe of the Harderian gland. The gland was removed by holding the superior lobe with a pair of forceps, pulling it upwards and outwards, and cutting away connective tissue. Care was taken to avoid damage to the optic nerve and accompanying blood vessels. Upon complete removal of the gland, surgical gel foam was placed in the orbital cavity. Following wound closure and recovery from anesthesia, the rats were returned to the animal quarters. Post-surgery animals were housed 2/cage and body weights were recorded every two weeks. Vaginal smears were taken five days a week for ten weeks. Approximately 90 days after surgery, non-stress and stress aspects of pituitary-adrenal function were evaluated.

Three days before the evaluation, the rats were moved into individual cages. The animal quarters were locked and not entered for 18 hr preceding the evaluation. All bleeding and collecting procedures were done outside the animal quarters. Beginning at 0800 h or 1600 h, jugular blood samples were obtained for determining pre-stress or post-stress plasma cor-

ticosterone levels. In brief, non-stress samples were obtained by moving a rat from its home cage to an adjoining room where it was quickly anesthetized with methoxyflurane. The external jugular vein was then exposed and 1.5 mL blood was withdrawn into a heparinized syringe (< 3 min from the time of cage opening). The corticosterone concentration in this sample was used as an index of pre-stress pituitary-adrenal function. Stress samples were obtained by moving a rat from its cage to the adjoining room where it was gently restrained for two minutes. The rat was then placed in an individual holding cage, and 12 min later (i.e., 15 min from time of cage opening), the rat was anesthetized as above and a jugular blood sample obtained. The corticosterone concentration in the second sample was used as an index of stress-induced pituitary-adrenal activity.

Six days later rats were sacrificed via rapid decapitation at 1200 or 2400 h. Trunk blood was collected into heparinized centrifuge tubes for subsequent evaluation of plasma corticosterone levels. Following decapitation, the carcass was laparotomized and the liver, spleen, adrenals, and ovaries were removed, cleaned, and weighed to the nearest 0.2 mg. Prior to sacrifice, wounds made for previous jugular bleedings were examined for differential healing and other abnormalities. After the decapitation, heads of Hx animals were saved and the orbit examined for remnants of the Harderian gland.

The microfluorometric assay of Glick et al. (4) was used to determine plasma corticosterone concentrations. Statistics were derived from analysis of variance.

RESULTS

All rats upon recovering from surgery appeared normal and active. No observable differences in behavior were noted between the two groups; they responded similarly to daily handling. Tests for pupillary response showed good pupillary constriction in both eyes and a consensual pupillary response in all except two animals. Differential response in wound healing was not noted. At autopsy none of the Hx animals showed gland remnants and the optic nerves of all Hx rats were intact. Histological evaluation of the vaginal smears showed no alteration in vaginal cyclicity. Body weights of both groups increased significantly throughout the duration of the study and at necropsy body and organ weights of experimental animals were not different ($P > 0.05$) from those of the intact controls.

As indicated in Fig. 1, pituitary-adrenal function as measured by plasma corticosterone was not altered by Hx. Non-stressed rats, both intact and Hx, showed a marked diurnal variation in plasma corticosterone levels. Neither AM nor PM non-stress values of Hx rats were different from those of intact rats. However, AM and PM stress responses of both controls and Hx rats were significantly greater ($P < 0.01$) than their respective non-stress levels, but neither the AM nor the PM response of Hx animals was different from the response of the intact rats. Plasma corticosterone levels of trunk blood of Hx rats collected at autopsy were not different ($P > 0.05$) from those of the control group.

DISCUSSION

Although the Harderian gland has been implicated in endocrine function (2), few morphometric changes or variations in the endocrine parameters have been observed following removal of the gland. Reiter and Klein (5) reported an increase in uterine weight, but vaginal cyclicity, body weight, and the weights of ovaries, adrenals, and the pituitary of rats housed in alternating light/dark conditions were not altered by Harderianectomy. In agreement with the above, we did not observe

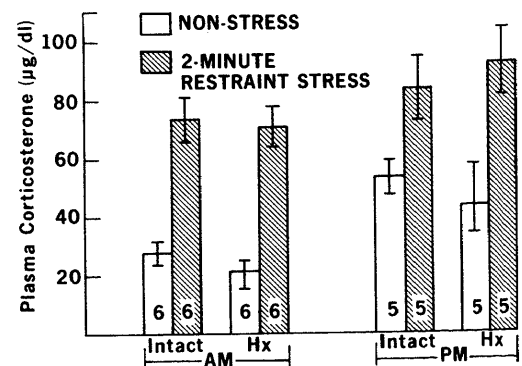


Figure 1. Effect of Harderian gland removal (HX) on plasma corticosterone levels. The group mean is indicated by the columns, the vertical lines indicate the standard error of the mean, and the number of animals per treatment is indicated near the base in each column.

differences in body weight or weight of those organs examined. Additionally, Harderianectomy did not produce an alteration in pituitary-adrenal function. Morning and afternoon non-stress plasma corticosterone levels of Hx rats were not different from those of controls. Likewise stress-evoked increases in plasma corticosterone of Hx rats did not differ from those of controls.

Thus it seems unlikely that the Harderian gland of the albino rat is essential for pituitary-adrenal or pituitary-ovarian function. However, the gland may be more important for hormonal control in other animals, especially those that exhibit sexual dimorphism of the gland such as golden hamsters (1). To clearly demonstrate the role of the Harderian gland in endocrine function it may be necessary to study a less inbred animal under experimental conditions that resemble the natural environment more closely.

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