

Compensatory Increase in Adrenal Weight and Dopamine- β -hydroxylase Activity after Unilateral Adrenalectomy: Experimental Evidence for a Pineal-Dependent Contribution (41206)

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Abstract. Changes in adrenal weight and adrenomedullary dopamine- β -hydroxylase (DBH) activity following either unilateral adrenalectomy and pinealectomy separately or both operations combined were examined in adult male rats. Animals were acclimated in continuously darkened animal quarters from 10 days prior to surgery to sacrifice 10 days postoperatively. Adrenal DBH activity was measured by a coupled radioenzymatic method. Compensatory adrenal hypertrophy following unilateral adrenalectomy was associated with a significant increase in adrenal DBH activity. However, when unilateral adrenalectomy was combined with pinealectomy, this change in DBH activity no longer occurred. Pinealectomy alone did not bring about any alteration in adrenal DBH activity. Absolute and relative adrenal wet weights were significantly increased after unilateral adrenalectomy, either with or without pinealectomy. These results suggest that, at least in darkness, the pineal contributes to the mechanism whereby compensatory increase in adrenal DBH activity occurs, but not in that involving increase in adrenal weight.

An intact pituitary–adrenocortical axis has been shown to be important for the maintenance of normal levels of activity of adrenomedullary catecholamine biosynthetic enzymes. Thus, hypophysectomy leads to significant reduction in the activities of adrenal tyrosine hydroxylase (TH), dopamine- β -hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT) in the rat (1–3). However, the response of adrenal DBH differs markedly from that of adrenal TH and PNMT to pituitary or adrenal hormone replacement after pituitary extirpation. Whereas the activities of adrenal TH and PNMT in hypophysectomized animals can be restored to near normal levels by ACTH (1, 2), restoration of adrenal DBH activity by ACTH in hypophysectomized animals is only partial (3). Furthermore, although administration of dexamethasone in hypophysectomized animals restores adrenal PNMT activity (1), it does not restore adrenal DBH activity (3).

Attempts to establish a relationship between the pineal and the adrenal gland have been directed mostly to the evaluation of changes in adrenocortical function following manipulation of the pineal (4–7). The

possibility of pineal–adrenomedullary endocrine interrelations has been examined only recently (8, 9). We present here the effects of pinealectomy and unilateral adrenalectomy, either alone or combined, on the weight and DBH activity of the adrenal gland. DBH is the enzyme that β -hydroxylates dopamine to form norepinephrine (10).

Materials and Methods. Twenty-seven-day-old male Spargue–Dawley rats were obtained (Laboratory Supply Co., Indianapolis, Ind.) and acclimated in continuously darkened animal quarters with access to Purina rat chow and water *ad libitum*. A dim (20-W) continuous red light was placed in one corner of the room to allow necessary minimum visibility for animal handling and care. After 10 days of acclimation, matched groups of rats were (a) sham-unilaterally adrenalectomized [SUAX], (b) unilaterally adrenalectomized [UAX], (c) sham-pinealectomized [SPX], (d) pinealectomized [PX], (e) sham-unilaterally adrenalectomized + sham-pinealectomized [SUAX + SPX], and (f) unilaterally adrenalectomized + pinealectomized [UAX + PX]. An unoperated group was included

also. Surgery was performed under mild ether anesthesia. Adrenalectomy was performed on the left side following the conventional lumbar approach; pinealectomy followed the procedure of Quay (11). Animals were sacrificed 10 days postoperation by decapitation and the right adrenals were collected, freed of fat, weighed, and immediately frozen in tubes kept on dry ice. The brains of SPX and PX animals were dissected open to check for the presence or absence of the pineal and also for postoperative signs of physical injury, if any. Those animals showing any visible edema or injury in the brain were discarded.

DBH activity in the adrenal was estimated according to the radioenzymatic method of Molinoff *et al.* (12) with some modifications. Adrenals were homogenized individually in 400 vol of 0.005 M Tris-HCl buffer, pH 7.5, containing 0.1% Triton X-100. Homogenates were centrifuged at 10,000g for 10 min at 4° and lipids removed by aspiration. Two-hundred microliters of supernatant fluid was added in a 15-ml centrifuge tube containing a reaction mixture of 25 μ l of 0.048 M ascorbic acid, 20 μ l of 0.5 M pargyline (MAO inhibitor), 25 μ l of 0.5 M sodium fumarate, 10 μ l of 0.03 M phenylethylamine (substrate), 10 μ l of 1.0 M Tris-HCl buffer and 1500 units of catalase. Ascorbate, fumarate, phenylethylamine, and tris were adjusted to a lower pH (5.5) than pH 6.0 (12) since, in agreement with other investigators (13, 14) we also noted that a lower pH is conducive to greater apparent DBH activity. To inactivate the endogenous inhibitors of DBH (15), a final copper concentration of 1.7×10^{-5} M was used (12). The total reaction mixture of 310 μ l was incubated at 37° in a shaking bath for 20 min, when phenylethylamine was β -hydroxylated to phenylethanolamine by DBH. The reaction was stopped and the second part of the assay was initiated by adding 100 μ l of a mixture containing 10 μ l of partially purified PNMT, 10 μ l of (1 μ mol) *S*-[methyl-¹⁴C]adenosylmethionine (New England Nuclear, Boston, Mass.) and 80 μ l of 1.0 M Tris-HCl buffer, pH 8.6, to each tube. This was incubated for 30 min at 37° after which the reaction was stopped by the addition of 0.5 ml of 0.5 M borate

buffer, pH 10, to each tube. The radiolabeled product, *N*-[¹⁴C]methylphenylethanolamine, was then extracted by vigorous shaking on a Vortex mixer into 6 ml of toluene containing 3% isoamyl-alcohol. After a brief centrifugation at low speed, 4 ml of the organic phase was transferred to a counting vial containing 10 ml of phosphor. The radioactivity was measured by means of a liquid scintillation counter. Blanks and internal standards were prepared by heating tissue homogenates at 95° for 5 min. One-hundred nanograms of phenylethanolamine was added in standard tubes. Both blanks and standard tubes contained all the reaction mixtures. All assays were run in triplicate. DBH activity was expressed as nanomoles of product formed per right adrenal gland (rather than adrenal weight, since significant changes in the weight of the cortex can occur during experimental manipulations) per hour.

PNMT used in the second part of the assay was prepared and partially purified from bovine adrenal glands according to the method of Axelrod (16) as modified by Molinoff *et al.* (12). This involved Sephadex gel column chromatography and subsequent collection of fractions. Those fractions having PNMT activity were pooled and concentrated using Amicon ultrafiltration. The concentrated form contained about 5 mg of protein/ml and was kept frozen at -20° in small aliquots.

Statistical analysis. The analysis of variance (ANOVA) for a single-factor completely randomized design and the Waller-Duncan (17) multiple comparison test were used to determine differences in adrenal weight and adrenal DBH activity among the various surgical groups.

Results. Absolute adrenal weight. The effects of different surgical procedures on the absolute adrenal wet weight are shown in Fig. 1. ANOVA indicated a significant ($P < 0.001$) difference in absolute weight according to surgical treatment. The Waller-Duncan procedure indicated that the UAX group had significantly ($P < 0.05$) greater adrenal weight compared to that of the unoperated or the SUAX groups. Absolute adrenal weight of the UAX + PX group was significantly ($P < 0.05$) greater

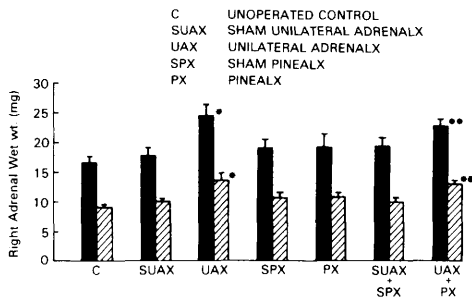


Fig. 1. Changes in absolute (■) and relative (▨) adrenal (right) wet weights in rats following various surgical procedures. Vertical bars represent Mean \pm SE. * $P < 0.05$ when compared to respective absolute or relative adrenal weight of the SUAX group; ** $P < 0.05$ when compared to the respective absolute or relative adrenal weight of the SUAX + SPX group. There were five to six animals in each group.

than those of the unoperated, SUAX, SPX, and SUAX + SPX groups. No significant differences were noted in absolute weights among the intact control, SUAX, SPX, PX, and SUAX + SPX groups.

Relative adrenal weight. The results in terms of relative adrenal weight were shown by ANOVA to contain significant ($P < 0.001$) differences according to surgical treatment (Fig. 1). The Waller–Duncan procedure indicated that the relative adrenal weight in the UAX group was significantly ($P < 0.05$) greater than that of the intact control or the SUAX groups. UAX + PX animals showed significant ($P < 0.05$) higher relative adrenal weight compared to the intact control, SUAX, SPX, and SUAX + SPX groups; however, there was no significant difference in adrenal weight between the UAX and UAX + PX groups. Relative adrenal weights in the unoperated, SUAX, SPX, PX, and SUAX + SPX were not significantly different from each other.

Adrenal DBH activity. ANOVA indicated a significant difference ($P < 0.005$) in adrenal DBH activities according to surgical treatments. The Waller–Duncan analysis showed that the UAX group had significantly ($P < 0.01$) greater DBH levels than the SUAX or the unoperated groups. However, when animals were both unilaterally adrenalectomized and pinealectomized, UAX-induced increase in adrenal DBH ac-

tivity was no longer evident; DBH activity in the UAX + PX group was significantly ($P < 0.01$) reduced compared to that of the UAX group. Furthermore, the enzyme activity in the UAX + PX group did not differ significantly from that of the matched doubly sham-operated SUAX + SPX group. Pinealectomy alone had no effect on the adrenal DBH activity (Fig. 2).

Discussion. It has been known for a long time that unilateral adrenalectomy leads to compensatory hypertrophy of the remaining adrenal (18, 19). Our data on the effects of various surgical manipulations on adrenal weight confirm this, but also provide some important additional insight concerning the role of the pineal. UAX, as expected, resulted in significant increases in both absolute and relative adrenal weights (Fig. 1) which are expected to be chiefly cortical in localization. Engeland *et al.* (20) have shown that in the rat of the same strain, sex, body weight, length of post-operative period, and side of adrenal surgery as in the present experiment, UAX led to significant gains in weight of the remaining adrenal. Our experiments were similar to those of Engeland *et al.*, except that our experimental animals were exposed to continuous near-darkness and not a light–dark cycle (20). Our results thus show that the absence of white light or of light–dark cycles does not interfere with the mechanism of compensatory adrenal growth.

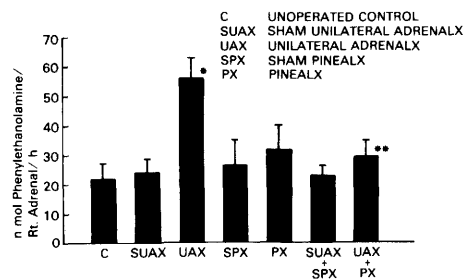


Fig. 2. Effects of various surgical manipulations on the adrenal (right) dopamine- β -hydroxylase activity in rats. Vertical bars represent Mean \pm SE. * $P < 0.01$ when compared to the SUAX group; ** $P < 0.01$ when compared to the UAX group, but not significantly different from SUAX + SPX group. There were five to six animals in each group.

In the present experiment surgical extirpation of the pineal, either alone or in combination with UAX, did not bring about any change in absolute or relative adrenal weight. Vaughan *et al.* (6) have shown that pinealectomy leads to significant increase in adrenal weight in mice kept in a light-dark cycle. A significant increase in adrenal weight was also evident in pinealectomized rats maintained in a near-darkness condition (21). It is to be noted, however, that in works dealing with the effects of pinealectomy on adrenal weight, there exists some inconsistency or disagreement; decrease, increase and no apparent effect have been reported (22–24). These differences are probably due, at least in part, to factors such as differences in strain, age, photoperiodic regimen, and postoperative period prior to sacrifice of animals.

A major and new finding in this work is that UAX-induced compensatory hypertrophy of the adrenal was associated with a significant increase in adrenal DBH activity. Furthermore, although surgical extirpation of the pineal did not effect any change in adrenal DBH activity, PX combined with UAX led to significant inhibition of the increase in DBH activity noted in the UAX group with intact pineals. It is noteworthy that pinealectomy alone did not bring about any significant change in adrenal DBH activity, since in earlier studies a decrease in adrenal DBH activity in the S_1 rat (8) and an increase in adrenomedullary DBH activity in the hamster (9) were noted following pinealectomy. These differences in results may be suggestive of strain and/or species-distinctive differences. It is interesting to note that in an earlier report, Ciaranello *et al.* (25) showed that the activity of another adrenomedullary enzyme, PNMT, is increased following unilateral adrenalectomy.

Compensatory adrenal hypertrophy is thought to be the result of a hypocortical signal with subsequent increased secretion of pituitary ACTH. Levels of activity of adrenomedullary biosynthetic enzymes such as TH, DBH, and PNMT have been shown to be regulated, in part, by the pituitary-adrenocortical axis (1–3). All three enzymes are significantly reduced following

hypophysectomy. While administration of dexamethasone to hypophysectomized animals restores adrenal PNMT activity (1), it is incapable of restoring adrenal DBH activity (3). ACTH is more potent than dexamethasone in bringing about greater recovery of DBH activity levels following pituitary ablation (2). Thus, in the present experiment in which a UAX-induced increase in adrenal DBH activity was observed, it remains unclear whether this is due to increased levels of corticoids or of ACTH.

In contrast to the general belief that UAX-induced adrenal hypertrophy follows from a hypocorticotid condition, evidence has grown recently to indicate that this may not be the case. Thus, a neural rather than hormonal regulation of compensatory adrenal growth has been suggested by Dallman and associates (26). Engeland *et al.* (20) have shown that the presence of an intact pituitary is not necessary for compensatory adrenal hypertrophy to occur following removal of one adrenal. Our present observation that the UAX-induced increase in adrenal DBH activity was significantly inhibited in the UAX + PX (but not in the PX) group, may suggest that in darkness the pineal contributes to the mechanism of neural mediation of compensatory hypertrophy whereby the compensatory increase in adrenal DBH activity occurs, but not in that involving the increase in adrenal weight. The anatomical and/or physiological basis for such a pineal mediation of adrenal DBH activity in the UAX condition, however, remains to be determined.

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