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SOMATOTROPIN AS THE NON-ACTH FACTOR OF ANTERIOR PITUITARY ORIGIN FOR THE MAINTENANCE OF ENHANCED ALDOSTERONE SECRETORY RESPONSIVENESS OF DIETARY SODIUM RESTRICTION IN CHRONICALLY HYPOPHYSECTOMIZED RATS [1)]

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Evidence for a non-ACTH effect of the pituitary on the aldosterone hypersecretory response to dietary sodium restriction in the rat was recently reported by the authors of the present paper (1) and by Palmore and Mulrow (2). ACTH treatment in rats hypophysectomized prior to sodium-deprivation, albeit maintained normal glucocorticoid secretory activity and responsiveness of the adrenal, failed nonetheless to increase the aldosterone secretion rates of treated animals to above the basal levels of intact, sodium-repleted animals (1, 2), or restored to treated animals the selectively enhanced aldosterone secretory responsiveness of the adrenal (1) which recent evidence also indicate to be characteristic of the normal adrenocortical response to prevailing states of sodium-deficiency among animals of various mammalian species studied (3), including the rat (1, 4). Similar impairments of aldosterone secretory responsiveness were also observed of adeno-

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hypophysectomized, but not neuro-hypophysectomized, rats subjected to dietary sodium restriction (1), thus indicating that the postulated non-ACTH pituitary factor affecting aldosterone secretion resided in the anterior lobe of the pituitary. Treatment of chronically hypophysectomized, sodium-deprived rats with either whole rat pituitary powder (1, 2) or bovine anterior pituitary powder alone (1) effected dose-related increases in aldosterone secretion and, with the higher dosage of anterior pituitary powder used, eventuated in the complete restoration of an enhanced responsiveness of the aldosterone-secreting portions of the adrenal normally observed of intact, sodium-deprived control animals.

Somatotropin, or growth hormone, of various species origin has been reported to be without any direct aldosterone-stimulating effects on the adrenals of normal or chronically hypophysectomized rats when added into the *in vitro* incubation media (5, 6, 7), but were capable of preventing the depression in or even slightly increasing the *in vitro* aldosterone productions of the adrenals of chronically hypophysectomized rats when subacutely administered *in vivo* to chronically hypophysectomized rats maintained on a sodium-replete diet (5, 6). In view of these demonstrated effects of somatotropin, we have therefore undertaken the present study to investigate the possibility that somatotropin may be the non-ACTH anterior pituitary factor which, in its absence, impairs or prevents the aldosterone hypersecretory response to dietary sodium restriction in chronically hypophysectomized rats.

Methods

Female albino rats of the Wistar strain bred in our own laboratory and weighing between 120-130 g. initially were subjected to 14 days of dietary sodium restriction. The low-sodium powder

diet (Natrinon^{R}, Nutricia N.V., The Netherlands) used contained approximately 9 μEq Na/g and was given ad lib. Demineralized water was provided as drinking fluid.

The animals of the two chronically-hypophysectomized groups were hypophysectomized via the trans-auricular route on the seventh day of dietary sodium restriction, and were thereafter given 2 U of a long-acting preparation (8) of ACTH (β^{1-24}ACTH(Zn)) subcutaneously every 48 hrs to maintain adrenal weight and the glucocorticoid secretory responsiveness of the adrenal (1). In addition, one of these two groups of animals received subcutaneously injected doses of 250 μg of a purified bovine somatotropin (STH) preparation dissolved in 7% gelatin twice daily throughout the postoperative period, while the remaining group of hypophysectomized animals, plus another group of sham-operated animals to be subsequently subjected to acute-hypophysectomy at 18-20 hrs prior to the terminal experiment, received twice daily injections of 0.2 ml 7% gelatin. The purified bovine somatotropin preparation was obtained from N.V. Organon (Oss, The Netherlands) and contained a mean potency of 1.9 U/mg powder (confidence limits: 1.3 - 2.8 U/mg). β^{1-24}ACTH(Zn) was last administered to the animals at least 40 hrs prior to the terminal experiment, and completeness of hypophysectomy was determined by autopsy. The adrenals of animals which showed the presence of pituitary remnants upon inspection of the sella turcica were discarded. Animals were weighed before surgical hypophysectomy or sham-hypophysectomy on the seventh day of dietary sodium restriction (preoperative weight) and again on the day prior to the terminal experiment (postoperative weight).

The adrenocortical sensitivities of response of the glucocorticoid and mineralocorticoid secreting portions of the adrenal

were assessed by measuring the plasma corticosterone concentrations and the *in vitro* corticosteroid secretion rates of animals in response to a challenge dose of 16 mU ACTH(A_1) given intravenously 15 minutes prior to decapitation (1).

Plasma corticosterone concentrations were determined spectrofluorometrically (9) and measurements of the total corticoid and aldosterone secretion rates *in vitro* were performed according to methods previously described (10). Briefly, paired adrenal glands from each animal, after having been removed, dissected clean, weighed and quartered immediately after decapitation of the animal, were incubated for 1 hr in 2 ml Krebs-Ringer-bicarbonate buffer medium containing 200 mg glucose/100 ml buffer in a Dubnoff metabolic incubator at 37°C under oxygen containing 5% carbon dioxide. The corticosteroids produced were next extracted from the incubation medium with 2 ml methylenechloride, and the total corticosteroid production was determined spectrophotometrically by measuring the difference in optical densities at 240 and 260 mμ. Aldosterone contained in the pooled extracts plus 2 ml methylenechloride washings of two incubation media of paired adrenals was then separated from other corticosteroids and identified against reference standards by thin-layer chromatography, and quantitatively determined by measuring its intensity of alkaline fluorescence on paper in a Locarte fluorometer.

To facilitate ready comparison with the results of our earlier study (1), the aldosterone secretion rates *in vitro* are herein similarly expressed as μg/hr and not as μg/100 mg/hr, because the latter parameter could not be appropriately compared with or adequately describe the state of aldosterone secretion in chronically hypophysectomized animals not treated with β^{1-24}ACTH(Zn) and bearing atrophic adrenals reported in our previous study (1).

Statistical analysis of the results were performed using the standard "Student's" t-test (11).

Results

The chronically hypophysectomized, sodium-deprived rats treated only with β^{1-24}ACTH(Zn) failed to respond to the acute challenge of 16 mU ACTH(A_1), and the mean *in vitro* aldosterone secretion rate (Table 1) observed of this group of animals corresponded to the range of mean rats of secretion (0.5 - 0.8 μg/hr) generally observed in our earlier study (1) in β^{1-24}ACTH(Zn)-treated, chronically hypophysectomized rats (whether with or without ACTH challenge and whether fed the low-sodium or standard diets) and acutely hypophysectomized, sodium-repleted rats. The chronically hypophysectomized, sodium-deprived rats treated with 500 μg of bovine STH daily, however, responded to acute ACTH challenge with a characteristic and similar magnitude of response as the acutely hypophysectomized, sodium deprived control animals. Their mean rates of aldosterone secretion *in vitro* were nearly indistinguishable from each other, and were significantly greater ($p < 0.005$ for both groups) than that of the chronically hypophysectomized, sodium-deprived animals not treated with bovine STH.

The mean *in vitro* total corticoid secretion rates in response to ACTH challenge for all three groups of animals did not differ significantly from each other, but for reasons not well understood the mean plasma corticosterone concentration of the control group of chronically hypophysectomized, β^{1-24}ACTH(Zn)-treated animals demonstrated a greater ($p < 0.025$) magnitude of response than did those of either of the other two groups.

None of the mean body weights of any of the groups measured preoperatively on the seventh day of dietary sodium restriction differed significantly from each other ($p > 0.1$ between all

groups). STH-treatment of chronically hypophysectomized animals prevented the postoperative body weight loss seen in untreated, control animals, and promoted partial weight gain in treated animals to the observed postoperative mean weight level which approached, though statistically different ($p < 0.01$) from, that of sham-operated, acutely hypophysectomized animals.

Discussion

Chronic absence of the anterior pituitary in the rat impairs the ability of the adrenals to secrete increased amounts of aldosterone in response to dietary sodium restriction (1, 2), and causes the adrenals of chronically hypophysectomized rats to be refractory to stimulation (1) even under prevailing and persistent states of sodium-deficiency which, in intact animals, selectively enhances the sensitivity of response of the adrenal to stimuli which affect aldosterone secretion (3, 4). Treatment of chronically hypophysectomized, sodium-deficient rats with somatotropin, as demonstrated in the present study, effectively and virtually completely restored to treated animals the enhanced aldosterone secretory responsiveness characteristic of the adrenals of intact animals subjected to dietary sodium restriction. We submit, therefore, that while other anterior pituitary hormones could not be unconditionally and completely disregarded, the present finding presents strong supportive evidence for the hypothesis that the non-ACTH anterior pituitary factor affecting the adrenal mineralocorticoid secretory response to dietary sodium restriction indicated in previous studies (1, 2) is somatotropin.

Somatotropin treatment of intact, sodium repleted rats *in vivo* has been reported to have no stimulating effects on aldosterone secretion *in vivo* (12). For lack of readily available supply, the effect of somatotropin on aldosterone secretion in this animal

preparation was not investigated in the present study. However, if somatotropin is purportedly the relevant non-ACTH anterior pituitary factor involved, then our earlier observation (unpublished) that treatments with anterior pituitary powder were devoid of any demonstrable aldosterone-stimulating activity in intact, sodium-repleted rats suggests that the apparent adrenoglomerulotropic effect of somatotropin in sodium-deficient rats is mediated indirectly. This interpretation is consistent with the demonstrations that somatotropins of various species origin, including the somatotropin of bovine origin used in this study, were without any aldosterone-stimulating activity on rat adrenals *in vitro* when added into the incubation media (5, 6, 7), or on perfused calf adrenals *in vivo* when added to the perfusate (13). Also, it does not appear reasonably likely that somatotropin effects the direct release of adrenoglomerulotropic substances. Suggestive evidence is that it does not increase the aldosterone secretion rates *in vivo* of rats (12), or *in vitro* of mouse (14), when administered acutely or subacutely to intact, sodium-repleted animals. Although it has been shown that somatotropin induced slight increases in the *in vitro* aldosterone secretion rates of treated, chronically hypophysectomized (11-12 days) rats maintained on a sodium-replete diet (6), it is possible that these animals were not completely restored to sodium-balance and may have had relative but significant net losses of urinary sodium secondary to the uncontrolled state of diabetes insipidus during the postoperative period in spite of the probably irrelevant but known extra-adrenal sodium-retaining effects of somatotropin (15). Somatotropin prepared by the method of Raben and Westermeyer (16) has been reported to increase aldosterone excretion in human subjects (17). However, whether or not aldosterone excretion under

this condition reflects actual secretion is uncertain, and recent studies showed that infusions of somatotropin prepared by the method of Raben and Westermeyer into isolated perfused adrenal pouches of hypophysectomized dogs could not stimulate aldosterone secretion in vivo without also stimulating 17-hydroxycorticoid and corticosterone secretions (18), thus indicating that STH prepared by the method of Raben and Westermeyer may be contaminated with amounts of adrenal cortical stimulating substances which could be ACTH or other polypeptides with ACTH-like action. Our preparation of STH used contained no apparent contaminating ACTH-like activity. In a non-related study, postoperative administrations of 200 μg or 400 μg of this same STH preparation daily for 12 days in chronically hypophysectomized rats maintained on a standard laboratory diet failed to affect the postoperative course of degenerate changes in adrenal weight and plasma corticosterone concentration which remained indisdinguishable from those of placebo-treated, control animals (De Wied, unpublished data). Nonetheless, even should our preparation of STH have contained any detectable amounts of ACTH-like substances the presumed effect of somatotropin observed on the aldosterone secretory responsiveness studied could not have been explained by the presence of these contaminants. This is evident in the demonstration that while β^{1-24}ACTH(Zn) treatment of control rats effectively maintained normal adrenal weight and glucocorticoid secretory responsiveness, it failed to have any parallel effects on aldosterone secretory responsiveness observed in somatotropin-treated rats studied under otherwise identical experimental conditions. On the basis of these available evidence discussed, we are therefore inclined to interpret our data to indicate that, while somatotropin may not be itself the direct impetus or causal

factor for aldosterone secretion, its presence is imperative for the normal functioning of agencies which mediate the aldosterone hypersecretory response to dietary sodium restriction in the rat, and that it is probably the non-ACTH anterior pituitary factor affecting aldosterone secretion indicated in the rat (1, 2), and may also be in the dog (19).

TABLE 1

The adrenocortical responses to acute ACTH (16 mU) challenge of chronically hypophysectomized, sodium-deprived rats treated with bovine somatotropin (STH) and β^{1-24}ACTH(Zn). Values represent the mean ± the standard error of the mean.

	Treatment		
Low-sodium diet	Hypox ACTH(Zn)	Hypox ACTH(Zn)+STH	Acute Hypox
In vitro secretion rates:			
Aldosterone, μg/hr	0.80±0.04	1.58±0.07	1.66±0.04
Total Corticoids, μg/100 mg/hr	19.7±1.6	18.9±1.0	21.7±1.4
Plasma Corticosterone, μg/100 ml	32.0±1.7	23.9±3.1	25.3±3.8
Adrenal Weight, mg	36.1±1.8	37.8±1.6	36.8±1.3
Body Weight, g:			
preoperative	131.4±1.6	129.1±2.4	133.5±2.3
postoperative	126.3±3.7	137.7±2.1	142.4±2.7

SUMMARY

Somatotropin treatment in chronically hypophysectomized, sodium-deprived rats effectively restored to treated animals the distinct and enhanced aldosterone secretory responsiveness of the adrenal which characterizes the adrenals of intact rats subjected to dietary sodium restriction, but absent in chronic and non-treated, adenohypophysectomized or totally hypophysectomized rats subjected to similar conditions of dietary sodium restriction.

Treatment with β^{1-24}ACTH(Zn) alone was ineffective, albeit adrenal weight and glucocorticoid secretory responsiveness were effectively maintained. The observed efficacious effect of somatotropin is similar and indistinguishable from the previously demonstrated effect produced by treatment of anterior pituitary powder in chronically hypophysectomized, sodium-deprived rats. The earlier findings that somatotropin is without any direct or specific stimulatory effect on aldosterone secretion and that treatment of intact, sodium-repleted rats with anterior pituitary powder is devoid of any adrenoglomerulotropic influence on the observed refractoriness of the aldosterone secretory response to stimulation justify the inference that somatotropin is the relevant non-ACTH anterior pituitary factor imperative for the presumed normal maintenance of agencies which mediate the characteristic adrenocortical changes in aldosterone secretory capacity in intact, sodium-deprived rats.

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