Central serotonergic stimulation of aldosterone secretion.

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Research Article

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Central Serotonergic Stimulation of Aldosterone Secretion

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Abstract

Serotonin stimulates aldosterone secretion both in vitro and in vivo, and serotonin antagonism decreases plasma aldosterone levels in patients with idiopathic aldosteronism. This study was designed to assess the effects of the serotonin precursor, 5-hydroxytryptophan (5HTP), upon aldosterone secretion in man, and to determine whether stimulatory effects of 5HTP are mediated through the central nervous system. Oral 5HTP, administered as a single 200-mg dose, increased plasma aldosterone levels from 4.7±0.6 to 13.3±2.8 ng/dl in dexamethasone-pretreated, normal volunteers. Peripheral inhibition of decarboxylation of 5HTP, achieved by pretreatment with carboxydopa, 25 mg three times daily for 3 d, significantly increased the stimulatory effects of 5HTP on aldosterone levels ($P < 0.001$). No change in aldosterone levels occurred in subjects who received placebo after pretreatment with dexamethasone and carboxydopa. Increased aldosterone was not accompanied by increases in plasma levels of renin activity, potassium, or ACTH. Plasma levels of 5HTP were markedly increased by carboxydopa pretreatment, but peak plasma levels of serotonin were not significantly altered. Four patients with idiopathic aldosteronism all had an increase in plasma aldosterone levels after 5HTP administration, whereas the response in four patients with aldosterone-producing adenoma was variable.

Incubation of isolated human and rat adrenal glomerulosa cells with serotonin resulted in increased aldosterone secretion by both sets of cells, whereas 5HTP was ineffective in stimulating aldosterone secretion in vitro. We conclude that central serotonergic pathways are involved in the stimulation of aldosterone induced by administration of 5HTP. This mechanism may be an important etiologic factor in the hypersecretion of aldosterone that occurs in patients with idiopathic aldosteronism.

Introduction

Serotonin stimulates aldosterone secretion both in vitro and in vivo (1–5), and cyproheptadine, a serotonin antagonist, has been shown to decrease plasma aldosterone levels in patients with idiopathic aldosteronism (6). The mechanisms by which serotonin and cyproheptadine alter aldosterone secretion in vivo have not been established.

Several pituitary hormones have been shown to stimulate aldosterone secretion. These include β-endorphin (7), β-lipotropin (8), α-melanocyte-stimulating hormone (MSH)[1] (9, 10), γ-MSH (11), and aldosterone-stimulating factor (12, 13). As the secretion of many pituitary-derived hormones is influenced by serotonergic mechanisms (14–18), serotonergic stimulation of aldosterone secretion in vivo may occur through centrally mediated as well as through direct adrenal effects.

We have administered L-5-hydroxytryptophan (5HTP), the immediate precursor of serotonin, to normal subjects and patients with aldosteronism to further characterize serotonergic effects on aldosterone secretion. Normal subjects also received 5HTP after pretreatment with carboxydopa, a peripheral inhibitor of the conversion of 5HTP to serotonin, in order to assess a possible central effect of serotonin on aldosterone secretion.

Methods

Human studies. 15 male volunteers ages 20–35 were studied. All were within 10% of ideal body weight with the exception of one 22-yr-old volunteer whose weight was 20% below ideal body weight. Supine blood pressure was below 140/80 on two separate occasions. Blood counts were normal, and none of the subjects was taking any medications. The study was approved by the human studies committee of the University of Michigan Hospital.

10 volunteers were studied on two separate occasions. They were pretreated with dexamethasone, 0.5 mg four times daily, for 3 d before each study. During the second study carboxydopa, 25 mg three times daily for 3 d, was added to the pretreatment regimen. The last dose of each medication was taken ~1 h before the beginning of the study. During the preparatory period volunteers maintained their regular diet, and during the last 24 h they collected urine for sodium determination. Five other volunteers were studied as a placebo group. They were studied after pretreatment with dexamethasone and carboxydopa. Subjects were fasted for at least 10 h before the start of the study. At 8:00 a.m. on the day of the study a butterfly needle was inserted intravenously and the line was kept open with heparinized saline. The total amount of heparin used did not exceed 500 U.

At 8:30 a.m. (−30 min) the first blood samples were drawn and at 9:00 a.m. the volunteers took placebo or 200 mg of 5HTP orally. All subjects were supine, awake, and fasting during the entire study. Blood pressure and heart rate were monitored hourly. Two basal samples were drawn 30 min apart for plasma renin activity (PRA) and aldosterone. A single basal sample was drawn for electrolytes, cortisol, ACTH, 5HTP, and serotonin. Subsequent blood samples for PRA, aldosterone, electrolytes, and cortisol were drawn hourly. Samples for ACTH, 5HTP, and serotonin were drawn every 2 h. Samples were put on ice (with the exception of electrolytes) and centrifuged within 1 h. Samples for 5HTP and serotonin were centrifuged immediately. The plasma was separated and frozen at −20°C. Cortisol, ACTH, PRA, and aldosterone were measured by radioimmunoassay (19–22). Serotonin and 5HTP were separated by reverse-phase liquid chromatography and measured by electrochemical detection using the method of Lyness et al. (23). The sensitivity of both the serotonin and 5HTP assays was 0.5 ng/ml, intraassay variation was

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; MANOVA, multivariate ANOVA; MSH, melanocyte-stimulating hormone; PRA, plasma renin activity; 5HTP, L-5-hydroxytryptophan.

10% for both assays, and interassay variation was 15% for serotonin and 22% for 5HTP.

Eight patients with aldosteronism were studied using the same protocol without carboxydopa pretreatment. All patients studied had elevated plasma (>18 ng/dl) and urinary (>17 μg/24 h) aldosterone levels after 3 d of a 150-meq sodium diet. Upright PRA was suppressed (<1.0 ng/ml per h) after 3 d of a 10-meq sodium diet. Patients were studied with high resolution computerized tomography and iodocholesterol (NP-59) scintiscanning. Four patients (three with adenoma and one with idiopathic aldosteronism) underwent selective venous catheterization during ACTH infusion for determination of adrenal venous levels of aldosterone and cortisol. Four patients (two women and two men) had the diagnosis of aldosterone-producing adenoma confirmed by subsequent adrenalectomy. Four men with the diagnosis of idiopathic aldosteronism were treated medically. The criteria for the diagnosis of idiopathic aldosteronism have been previously reported (24).

In vitro studies. Male Sprague-Dawley rats, 180–220 g, were maintained on a low sodium diet containing 0.006% NaCl (ICN Nutritional Biochemicals, Cleveland, OH) for 3 d. 20–25 rats were killed by decapitation between 8:00 and 9:30 a.m. The adrenal glands were removed and the capsular portion separated under a dissecting microscope. Cells were dispersed in medium 199 (25) containing collagenase (2 mg/ml), using 1 ml for four capsules, and incubated with shaking for 30 min at 37°C under 95% O_2, 5% CO_2. The tissue was disrupted by repeated aspiration into a siliconized Pasteur pipette. The supernatant was filtered through nylon gauze and collected. A smaller volume of medium 199 containing DNAse (0.05 mg/ml) was added to the remaining tissue. The disruption procedure was repeated twice with DNAse. The three filtered cell suspensions were pooled and centrifuged at 200 *g* for 10 min at room temperature. The cell pellet was washed in medium 199 and recentrifuged twice. The final cell pellet was resuspended in 4 ml of medium 199 and the number of cells was determined with a hemocytometer. The cells were diluted in an appropriate volume of medium 199 containing 2 mg/ml bovine serum albumin (BSA) to yield 250,000 cells/0.95 ml. Serotonin or 5HTP in various concentrations was dissolved in 0.05 ml of medium 199 and added to polypropylene test tubes. Cell suspension, 0.95 ml, was then added to each tube. Incubation of isolated cells was carried out at 37°C under 95% O_2 and 5% CO_2 with shaking for 2 h. After incubation, the tubes were put in an ice bath and centrifuged at 3,400 *g* for 10 min. The supernatants were transferred to glass test tubes, corked, and frozen for later aldosterone radioimmunoassay. This experiment was performed on two separate occasions.

Adrenal glands were removed from two patients operated on for aldosteronoma and from one patient with renal cell carcinoma. They were placed immediately in medium 199 at 4°C. The normal tissue and the tumor were separated and the capsular portions of the normal gland were removed using a microtome. Isolated cells were then prepared and incubated as described above for rat glomerulosa cells.

Collagenese was purchased from Worthington Biochemical Corp. (Freehold, NY), medium 199 with Earle's salt was obtained from Gibco (Grand Island, NY), and BSA, DNAse, serotonin, and 5HTP were obtained from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis. Data were calculated as mean±SE. Individual time points after carboxydopa pretreatment were compared with the corresponding time points without pretreatment using a two-tailed paired *t* test with Bonferroni protection. The response to 5HTP after carboxydopa was compared with the response without carboxydopa using multivariate analysis of variance (MANOVA). Both stages were also separately analyzed by repeated measures of analysis of variance (ANOVA) and the time points were compared with basal using a two-tailed Dunnet's test with an alpha level of 0.05. The data for serotonin and 5HTP levels were analyzed using two-tailed paired Wilcoxon ranked sign test with Bonferroni protection to compare individual time points between the two studies. Two-tailed paired Wilcoxon ranked sign test with Bonferroni protection was used to compare individual time points with basal.

In vitro studies using rat adrenal cells were analyzed using a two-tailed *t* test with Bonferroni protection to compare stimulated aldosterone levels with basal levels. For studies with human cells a two-tailed *t* test with Bonferroni protection was used. The data were analyzed using the CLINFO system.

Results

Effect of 5HTP in normal volunteers. The only observed side effects were nausea and vomiting, which occurred in four volunteers when 5HTP was administered after carboxydopa pretreatment. Two volunteers had increased cortisol levels due to the stress of vomiting and were excluded from statistical analysis. Two other volunteers vomited once, and the apparent stress was minimal. Cortisol and ACTH levels remained suppressed in these subjects throughout the course of the study. There was no difference in the basal levels of aldosterone during the two studies with 5HTP (Fig. 1). An increase in aldosterone levels occurred during both studies. In the absence of carboxydopa, the increase was significant ($P < 0.025$, ANOVA), and the peak occurred after 2 h.

After carboxydopa pretreatment, the aldosterone response to 5HTP was more prominent ($P < 0.001$, ANOVA), and was significantly greater than the response without carboxydopa pretreatment ($P < 0.0001$, MANOVA). Within 1 h after 5HTP administration, aldosterone levels were significantly higher than basal. The peak was reached after 3 h, and aldosterone levels remained significantly elevated after 6 h. Aldosterone levels at 3, 5, and 6 h after 5HTP were significantly higher after carboxydopa than without carboxydopa pretreatment. No significant change in plasma aldosterone levels occurred after administration of placebo to volunteers pretreated with dexamethasone and carboxydopa (Table I). Basal levels of aldosterone in this group were similar to those in the subjects that received 5HTP.

Serum potassium levels were not significantly altered from basal during either study (Fig. 2). Potassium levels were higher during the initial phase of study after carboxydopa pretreatment than without carboxydopa, and the levels were significantly different between the two studies ($P < 0.05$, MANOVA). During the last 3 h of study, potassium levels were similar during the two studies. There was no significant correlation between the level of potassium and the level of aldosterone during either stage of study. PRA remained stable throughout both studies, and there was no significant difference in PRA between the two studies (Fig. 2). Urinary sodium level was 178±34 meq/24 h

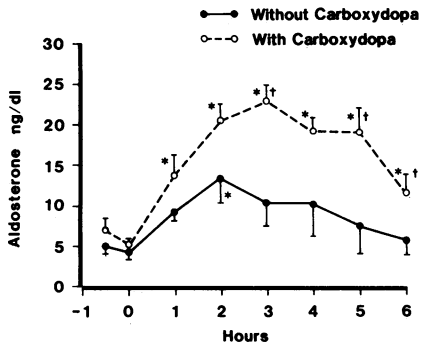

Figure 1. Plasma aldosterone levels after administration of 5HTP in normal volunteers. † $P < 0.05$ using two-tailed paired *t* test with Bonferroni protection for comparison between the two studies. * $\alpha = 0.05$ compared with basal (−30 and 0 min combined) by two-tailed Dunnet's test using ANOVA.

Table I. Plasma Aldosterone Levels in Normal Volunteers After Placebo Administration

Time (*h*)	−0.5	0	1	2	3	4	5	6
Plasma aldosterone (*ng/dl*)	4.2±1.2	4.7±0.5	2.1±0.7	2.2±0.7	1.9±0.6	3.1±0.7	2.7±0.6	6.8±2.0

without carboxydopa pretreatment and 170±22 meq/24 h after carboxydopa.

There was no significant change in mean blood pressure (Fig. 3) or heart rate (not shown) during either study. Mean pressure was higher after carboxydopa pretreatment than without pretreatment, but the overall difference was not significant (MANOVA). Serum sodium levels did not change during either study, and were not different between the two studies. ACTH levels were suppressed throughout both studies (normal ACTH at 0800: 60–90 pg/ml) (Fig. 3). Cortisol levels were below 5 μg/dl in all subjects throughout the study. Most samples were below the lower limit of detectability (<0.78 μg/dl).

Serotonin levels without carboxydopa pretreatment reached a peak 1 h after 5HTP administration (Fig. 4). With carboxydopa pretreatment, the highest serotonin levels occurred 3 h after 5HTP administration. Peak serotonin levels were higher without pretreatment than with carboxydopa, but the difference was not significant. Plasma levels of 5HTP increased significantly more after carboxydopa than without pretreatment. There was no correlation between plasma levels of 5HTP or serotonin and plasma levels of aldosterone.

Effects of 5HTP in patients with aldosteronism. Aldosterone levels increased in all four patients with idiopathic aldosteronism after 5HTP administration (Fig. 5 *A*). In two of the four patients with adenoma, aldosterone levels changed <25% compared with basal (Fig. 5 *B*). ACTH, cortisol, and PRA were suppressed in all patients and did not change throughout the study. Potassium levels in the patients with adenoma were lower than in patients with idiopathic aldosteronism and did not change in either group during the study.

Effect of serotonin and 5HTP in vitro. Serotonin stimulated aldosterone secretion by rat glomerulosa cells in a dose-dependent fashion. There was no significant effect of 5HTP on aldosterone secretion in any of the concentrations tested (Fig. 6). Because of the variability in basal aldosterone secretion between different experiments, results are expressed as percentage change from basal. Serotonin also stimulated aldosterone secretion by

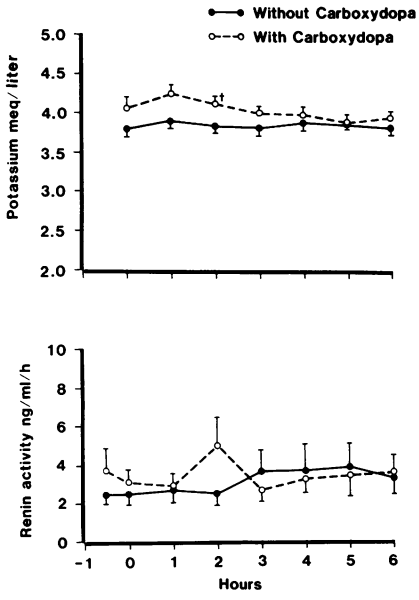

Figure 2. Potassium and PRA after 5HTP administration in normal volunteers. † As in Fig. 1.

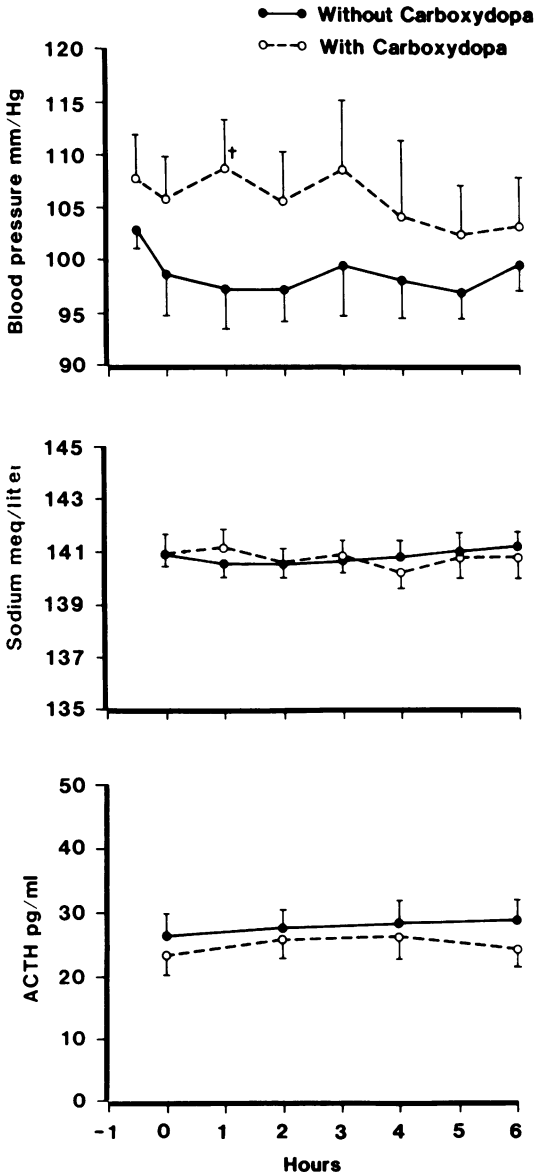

Figure 3. Mean blood pressure, serum sodium, and ACTH after 5HTP administration in normal volunteers. † As in Fig. 1.

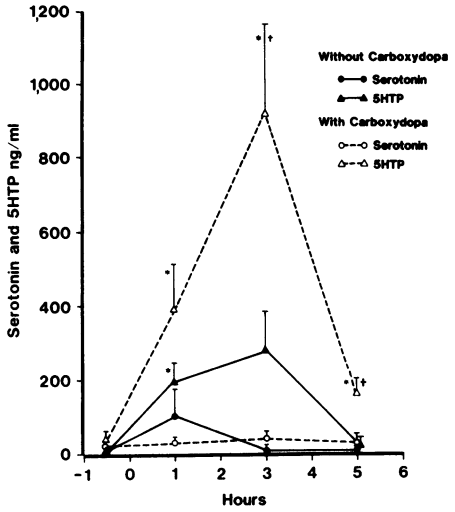

Figure 4. 5HTP and serotonin levels after 5HTP administration in normal volunteers. † $P < 0.05$ using two-tailed paired Wilcoxon ranked sign test with Bonferroni protection for comparison between the two studies. * $P < 0.05$ compared with basal using two-tailed paired Wilcoxon ranked sign test with Bonferroni protection.

cells from normal human adrenal tissue and by cells from aldosterone-secreting adenomata (Fig. 7). 5HTP was ineffective in both groups of cells.

Discussion

The prominent increase in aldosterone levels induced by 5HTP and its augmentation by carboxydopa cannot be explained by changes in the classic aldosterone secretogogues. ACTH levels were suppressed, as expected after dexamethasone pretreatment, and PRA remained stable throughout the study. The slight increase in serum potassium that was present during the first half of the study after carboxydopa pretreatment is unlikely to be the cause of the increased aldosterone response during that stage. Potassium levels did not show any significant change during the study, and tended to decrease after the peak levels which occurred at 1 h. Peak aldosterone levels during this portion of the study were not achieved until 3 h after 5HTP administration. During the last 3 h of study with carboxydopa, plasma aldosterone levels remained significantly elevated even though plasma potassium levels were not different from those without carboxydopa pre-

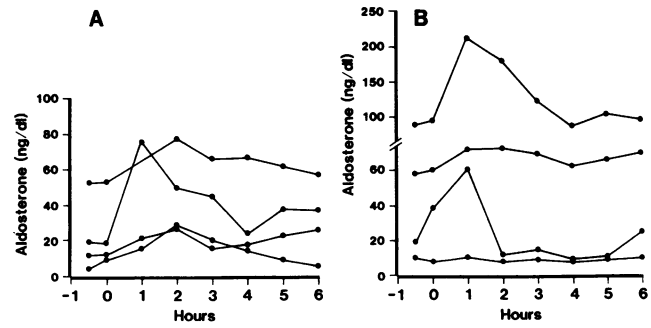

Figure 5. Aldosterone levels after administration of 5HTP in patients with idiopathic aldosteronism (*A*) and aldosterone-producing adenoma (*B*).

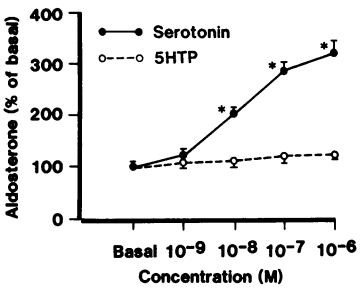

Figure 6. Aldosterone levels after incubation of rat glomerulosa cells in vitro with 5HTP or serotonin. The results represent two experiments with a total number of 10 tubes for basal and 5 tubes for each other point. * $P < 0.05$ compared with basal using two-tailed t test with Bonferroni protection.

treatment. Moreover, in three volunteers whose potassium levels were equal during the two studies, carboxydopa pretreatment resulted in the same prominent enhancement of 5HTP-induced aldosterone secretion. None of these features is consistent with potassium-mediated stimulation of aldosterone (26–29).

The increase in plasma aldosterone after administration of 5HTP is unlikely to be a result of spontaneous variation (6, 30). Previous studies in dexamethasone-suppressed volunteers have shown no significant changes in plasma aldosterone during the period from 0900 to 1630 (30). Similarly, the present studies showed no significant changes in aldosterone levels after placebo administration to subjects pretreated with dexamethasone and carboxydopa.

Unlike the stable PRA observed in the present study, Modlinger et al. (4) found a rise in renin activity after oral tryptophan administration. Two studies in rats have also shown a stimulatory effect of serotonin and 5HTP on PRA (31, 32), and the stimulation appears to be peripherally mediated, as decarboxylase inhibition blocked the effect of 5HTP on PRA (32). Our results are similar to those of Mantero et al. (5), who reported no effect of intravenous serotonin administration on PRA. The discrepant PRA results between Modlinger's study (4) and our own may be explained by effects of other metabolites of tryptophan on renin secretion. Different levels of serotonin may also have been achieved in these studies, as postulated by Mantero (5).

Peripheral inhibition of tryptophan decarboxylase with carboxydopa has been used in combination with 5HTP in the treatment of myoclonus. This combination allows for greater central serotonergic effects with a lower dose of 5HTP (33–37). The

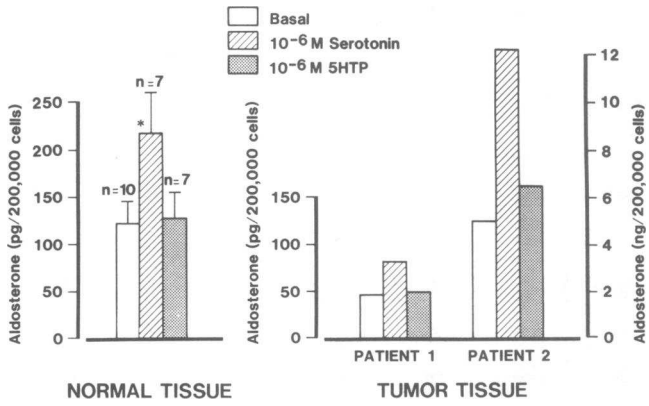

Figure 7. Aldosterone levels after incubation of human glomerulosa cells in vitro with 5HTP or serotonin, both 10^{-6} M. Normal tissue was taken from three separate patients. *n*, Total number of tubes. * $P < 0.05$ compared with basal using two-tailed t test with Bonferroni protection.

levels of serotonin and its metabolites in the cerebrospinal fluid achieved by this combination have been shown to be higher than those achieved by administration of 5HTP alone (38).

Although Magnussen and Van Woert (39) have reported that doses of carboxydopa up to 200 mg daily do not completely inhibit peripheral decarboxylation of 5HTP, it is unlikely that peripheral serotonin was responsible for the stimulation of aldosterone secretion after carboxydopa pretreatment. No significant increase in plasma levels of serotonin occurred after 5HTP, no correlation was seen between plasma serotonin and plasma aldosterone levels, and the time course of changes in the two variables was dissimilar. Direct peripheral stimulation of aldosterone by 5HTP also appears unlikely, since this agent was ineffective in stimulating aldosterone secretion from rat or human glomerulosa cells in vitro. The results of this study are most compatible with a central serotonergic mechanism as the mediator of the aldosterone stimulation by 5HTP. A direct central effect of 5HTP is also possible, and the two possibilities cannot be distinguished by the present studies.

A clinical disorder in which hypersecretion of aldosterone might be the result of central serotonergic stimulation is the syndrome of idiopathic aldosteronism. Cyproheptadine, a serotonin antagonist, has been shown to decrease aldosterone levels in these patients (6). Idiopathic aldosteronism is present in ~½–1% of hypertensive patients. It is characterized by hypertension, hypokalemia, low PRA, and normal ACTH levels (40–42). Increased adrenal sensitivity to angiotensin II has also been demonstrated (43, 44). Central serotonergic stimulation of the pituitary to release an aldosterone secretogogue could account for many of these features.

Multiple pituitary factors have been found to stimulate aldosterone. These include: β-endorphin (7), β-lipotropin (8), α-MSH (9, 10), γ-MSH (11, 12), and aldosterone-stimulating factor (13, 14). Aldosterone-stimulating factor and γ-MSH have both been reported to be elevated in patients with idiopathic aldosteronism (45, 46). A patient with idiopathic aldosteronism has been reported to have hyperplasia of the pars intermedia of the pituitary (47). These results suggest a role for a pituitary-derived aldosterone secretogogue other than ACTH in the normal and pathologic regulation of aldosterone secretion.

Serotonin has been shown to be involved in the stimulation of several types of the pituitary hormones, including ACTH in rats (14, 15) and in man (16), β-endorphin in rats (17), and prolactin in rats and man (18). Serotonin has also been found to be involved in the stimulation of the hypothalamic-pituitary-adrenal axis induced by insulin hypoglycemia (48). It has been postulated that serotonin plays a role in the regulation of ACTH secretion by pituitary intermediate lobe adenomas, causing Cushing's syndrome (49).

That all four patients with idiopathic aldosteronism responded to 5HTP stimulation is consistent with the suggestion that serotonergic stimulation might play a role in the etiology of this condition. The variable response in patients with adrenal adenoma implies a loss of responsiveness of some of these tumors to central stimulation. Hypokalemia in patients with adenoma may also have contributed to the blunted response.

The aldosterone-stimulating property of 5HTP is enhanced by inhibition of peripheral tryptophan decarboxylase activity. Since 5HTP has no demonstrable direct effect on aldosterone secretion, these results are most consistent with a central nervous system site of action for serotonin or 5HTP. We propose that serotonin may be an important neural regulator of the secretion of pituitary-derived aldosterone stimulators. Furthermore, central serotonergic stimulation may be responsible for the hyperaldosteronism of idiopathic aldosteronism.

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