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EFFECT OF SOMATOSTATIN ON THE ZONA GLOMERULOSA OF RATS TREATED WITH ANGIOTENSIN II OR CAPTOPRIL: STEREOLOGY AND PLASMA HORMONE CONCENTRATIONS

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Summary—Chronic somatostatin (SRIF) administration induced atrophy of zona glomerulosa cells of the rat adrenal cortex and a noticeable fall in the plasma concentration of aldosterone. The effects of SRIF were comparable with those of captopril, a specific inhibitor of the angiotensin-converting enzyme. SRIF completely abrogated the adrenoglomerulotrophic effects of angiotensin II (AII); the inhibitory actions of SRIF and captopril were not additive. The slight but significant enhancement of zona fasciculata cell growth and plasma corticosterone levels caused by chronic AII administration were not reversed by SRIF. We interpret these data to indicate that SRIF specifically modulates the stimulatory effects of AII on the growth and steroidogenic capacity of rat zona glomerulosa.

INTRODUCTION

In a previous investigation [1], we have demonstrated that chronic somatostatin (SRIF) administration directly inhibits the growth and the steroidogenic capacity of the rat adrenal zona glomerulosa. Many lines of *in vitro* evidence indicate that SRIF does not act *per se*, but by interfering with the stimulatory effect of angiotensin II (AII) on aldosterone biosynthesis [2,3]. Since AII is well known to participate in the maintenance of rat zona glomerulosa growth [4,5], it appeared worthwhile to ascertain whether the mechanism underlying the long-term effects of SRIF involves the blockade of this action of AII.

EXPERIMENTAL

Thirty-six adult male rats of the Wistar strain, b wt 200g, were divided into 6 equal experimental groups. Group 1 served as a control. Groups 2 and 3 received i.p. injections of 0.5 mg/kg angiotensin II (Human form, synthetic [Asp–Arg–Val–Tyr–Ile–His–Pro–Phe]), Sigma Chemical Company, St Louis, U.S.A.) or 50 mg/kg captopril (Capoten, Squib 14225), both dissolved in 0.5 ml saline, twice a day for 5 consecutive days. Group 4 was given s.c. injections of 100 μg/kg somatostatin (Sigma) dissolved in 0.2 ml corn oil every 8 h for 5 consecutive days [1]. Groups 5 and 6 were treated as groups 2 and 3, but in addition received SRIF at the above dosage. The rats were maintained on Purina rat–mouse chow and tap water *ad libitum* and sacrificed at 10.00 a.m. (8 h after the last injection of SRIF) by cervical dislocation.

For each rat the blood was collected by heart puncture, and aldosterone and corticosterone were extracted, simultaneously separated and purified according to Sippell[6]. Corticosterone was assayed by competitive protein binding [7] and plasma concentration of aldosterone was determined by radioimmunoassay, using standard kits (Aldosterone-[^{3}H] RIA Pak, New England Nuclear, Frankfurt/M, W. Germany). Each sample was assayed in triplicate and counted in a Beckman 100C liquid scintillation spectrometer.

Sliced pieces of the left adrenal gland from each rat were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% OsO_4 in 0.1 M phosphate buffer and embedded in an epoxy resin. Thick sections were made with LKB III ultramicrotomes for light microscopy to select representative zona glomerulosa and zona fasciculata areas. Thin sections were counterstained with lead hydroxide and examined in a Hitachi HS-9 electron microscope.

For morphometric assessments the sampling procedure used was that described elsewhere [8,9]. The average volume of zona glomerulosa and zona fasciculata cells and the absolute amount of the various organelles per cell were determined according to Nussdorfer[10], employing conventional stereological procedures [11].

Biochemical and morphometric data obtained from each rat were averaged per experimental group, and the standard deviation of the mean was calculated. Student's t-test was used for the statistical comparison of the data. All the statistical procedures were performed using an IBM Personal Computer.

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Table 1. Effects of somatostatin on the morphometric parameters of the zona glomerulosa of normal, angiotensin-treated or captopril-administered rats

Experimental groups (6)	Volume of cells (μm^3)	Volume of nuclei (μm^3)	Volume of mitochondrial compartment (μm^3/cell)	Surface of mitochondrial cristae (μm^2/cell)	Surface of SER membranes (μm^2/cell)	Volume of lipid droplet compartment (μm^3/cell)
1. Control rats	701.6 ± 78.1	103.2 ± 11.0	168.3 ± 18.5	1528.3 ± 169.2	4722.6 ± 538.9	60.7 ± 7.0
2. AII-treated rats	823.2 ± 92.1 $P_1 < 0.01$	120.4 ± 13.8 $P_1 < 0.02$	195.8 ± 21.7 $P_1 < 0.02$	1801.4 ± 200.3 $P_1 < 0.01$	5901.7 ± 658.5 $P_1 < 0.01$	42.3 ± 5.2 $P_1 < 0.01$
3. Captopril-treated rats	512.4 ± 60.6 $P_1 < 0.01$	81.2 ± 9.1 $P_1 < 0.01$	118.7 ± 12.0 $P_1 < 0.01$	1022.4 ± 119.5 $P_1 < 0.01$	3003.7 ± 366.4 $P_1 < 0.01$	74.3 ± 8.4 $P_1 < 0.02$
4. SRIF-treated rats	530.3 ± 62.8 $P_1 < 0.01$ P_3 NS	82.5 ± 9.0 $P_1 < 0.01$ P_3 NS	124.4 ± 13.6 $P_1 < 0.01$ P_3 NS	1119.6 ± 127.8 $P_1 < 0.01$ P_3 NS	3166.3 ± 394.1 $P_1 < 0.01$ P_3 NS	70.2 ± 7.9 $P_1 < 0.05$ P_3 NS
5. AII/SRIF-treated rats	585.7 ± 62.4 $P_1 < 0.01$ $P_{3,4}$ NS	90.6 ± 10.2 $P_1 < 0.05$ $P_{3,4}$ NS	135.6 ± 15.2 $P_1 < 0.01$ $P_{3,4}$ NS	1220.4 ± 142.1 $P_1 < 0.01$ $P_{3,4}$ NS	3336.7 ± 428.2 $P_1 < 0.01$ $P_{3,4}$ NS	71.1 ± 8.0 $P_1 < 0.02$ $P_{3,4}$ NS
6. Captopril/SRIF-treated rats	522.4 ± 62.5 $P_1 < 0.01$ P_{3-5} NS	88.5 ± 9.2 $P_1 < 0.01$ P_{3-5} NS	119.1 ± 13.5 $P_1 < 0.01$ P_{3-5} NS	1068.8 ± 120.6 $P_1 < 0.01$ P_{3-5} NS	3103.9 ± 372.2 $P_1 < 0.01$ P_{3-5} NS	72.5 ± 8.1 $P_1 < 0.02$ P_{3-5} NS

Values are group means ± SD. The number of rats in the experimental groups is indicated in parentheses. The degree of variability in the intra-animal determinations as compared to the intra-group means was evaluated by the analysis of variance and found to be not significant ($P > 0.6$–0.7). P, level of significance of the difference from the group indicated by the subscript. NS, not significant.

RESULTS

Chronic administration of AII provoked a marked rise in the volume of both zona glomerulosa and zona fasciculata cells (Tables 1 and 2). Cell hypertrophy was associated to a significant increase in the volume of the mitochondrial compartment, and in the surface area per cell of mitochondrial cristae and smooth endoplasmic reticulum (SER) membranes. The volume of the lipid droplet compartment was notably reduced (Tables 1 and 2). Long-term AII administration induced a significant rise in plasma concentration of both aldosterone and corticosterone (Table 3).

Chronic captopril or SRIF administration caused a striking decrease in the volume of zona glomerulosa cells (Table 1). Cell atrophy was coupled with a significant decrease in the volume of the mitochondrial compartment and in the surface area per cell of mitochondrial cristae and SER profiles. The volume of the lipid droplet compartment was slightly but significantly increased (Table 1). No obvious changes were found in zona fasciculata cells (Table 2). Both treatments caused a significant lowering in the plasma concentration of aldosterone, while not apparently affecting that of corticosterone (Table 3).

SRIF administration not only completely abrogated the adrenoglomerulotrophic effects of AII, but also provoked a marked atrophy of zona glomerulosa cells. In fact, the morphometric parameters of group 5 rats did not differ significantly from those of groups 3 and 4 (Table 1). Conversely, SRIF did not exert any inhibitory action on the AII-induced hypertrophy of zona fasciculata cells (Table 2). SRIF administration did not cause any significant change in the plasma concentration of corticosterone in AII-treated rats, whereas it markedly reduced that of aldosterone (Table 3).

SRIF did not add to the effects of captopril on either zona glomerulosa morphology (Table 1) or on aldosterone blood levels (Table 3).

DISCUSSION

As shown previously [12], chronic administration of AII is able to provoke a marked rise in the volume

Table 2. Effects of somatostatin on the morphometric parameters of the zona fasciculata of normal, angiotensin-treated or captopril-administered rats

Experimental groups (6)	Volume of cells (μm^3)	Volume of nuclei (μm^3)	Volume of mitochondrial compartment (μm^3/cell)	Surface of mitochondrial cristae (μm^2/cell)	Surface of SER membranes (μm^2/cell)	Volume of lipid droplet compartment (μm^3/cell)
1. Control rats	1741.2 ± 184.7	120.7 ± 13.2	539.8 ± 60.3	9611.1 ± 1054.2	10969.5 ± 1120.4	128.2 ± 14.2
2. AII-treated rats	1957.7 ± 206.1 $P_1 < 0.05$	125.1 ± 13.9 $P_1 <$ NS	606.9 ± 68.7 $P_1 < 0.05$	10863.5 ± 1123.2 $P_1 < 0.05$	12329.1 ± 1350.1 $P_1 < 0.05$	69.7 ± 8.5 $P_1 < 0.01$
3. Captopril-treated rats	1770.5 ± 183.2 P_1 NS	122.1 ± 13.0 P_1 NS	552.7 ± 63.1 P_1 NS	9684.9 ± 1037.6 P_1 NS	11028.3 ± 1197.4 P_1 NS	132.5 ± 14.3 P_1 NS
4. SRIF-treated rats	1780.8 ± 186.3 P_1 NS	118.5 ± 12.4 P_1 NS	536.2 ± 59.4 P_1 NS	9640.6 ± 1048.3 P_1 NS	11219.0 ± 1214.2 P_1 NS	123.2 ± 14.0 P_1 NS
5. ALL/SRIF-treated rats	1928.6 ± 191.6 $P_1 < 0.05$ P_2 NS	130.2 ± 15.1 P_1 NS P_2 NS	601.7 ± 66.1 $P_1 < 0.05$ P_2 NS	10728.4 ± 1140.5 $P_1 < 0.05$ P_2 NS	12242.8 ± 1306.5 $P_1 < 0.05$ P_2 NS	72.5 ± 9.1 $P_1 < 0.01$ P_2 NS
6. Captopril/SRIF-treated rats	1756.1 ± 179.2 P_1 NS	119.4 ± 12.8 P_1 NS	523.4 ± 59.7 P_1 NS	9579.8 ± 1063.2 P_1 NS	10843.5 ± 1122.3 P_1 NS	130.4 ± 14.6 P_1 NS

Values are group means ± SD. The number of rats in the experimental groups is indicated in parentheses. The degree of variability in the intra-animal determinations as compared to the intra-group means was evaluated by the analysis of variance and found to be not significant ($P > 0.5$–0.7). P, level of significance of the difference from the group indicated by the subscript. NS, not significant.

Table 3. Effects of somatostatin on the hormonal plasma concentrations of normal, angiotensin-treated or captopril-administered rats

Experimental groups (6)	Plasma aldosterone concentration (ng/100 ml)	Plasma corticosterone concentration (μg/100 ml)
1. Control rats	25.4 ± 3.6	32.4 ± 4.0
2. AII-treated rats	34.9 ± 4.2	38.1 ± 4.1
	$P_1 < 0.01$	$P_1 < 0.02$
3. Captopril-treated rats	15.7 ± 2.2	31.3 ± 4.0
	$P_1 < 0.01$	P_1 NS
4. SRIF-treated rats	16.8 ± 2.0	30.2 ± 3.8
	$P_1 < 0.01$	P_1 NS
	P_3 NS	P_3 NS
5. AII/SRIF-treated rats	15.2 ± 1.7	39.2 ± 4.3
	$P_1 < 0.01$	$P_1 < 0.01$
	$P_1 < 0.01$	P_1 NS
	$P_{3,4}$ NS	$P_{3,4} < 0.01$
6. Captopril/SRIF-treated rats	14.9 ± 2.0	32.5 ± 4.5
	$P_1 < 0.01$	P_1 NS
	$P_{3,4}$ NS	$P_{3,4}$ NS
	P_5 NS	$P_5 < 0.01$

Values are group means $\pm$ SD. The number of rats in the experimental groups is indicated in parentheses. The degree of variability in the intra-animal determinations as compared to the intra-group means was evaluated by the analysis of variance and found to be not significant ($P > 0.6$–0.8). P, level of significance of the difference from the group indicated by the subscript. NS, not significant.

of rat zona glomerulosa cells, principally due to the increase in the volume of the mitochondrial compartment and SER proliferation. Zona glomerulosa cell hypertrophy is coupled with a marked rise in the plasma concentration of aldosterone, the principal mineralocorticoid secreted by the rat adrenal cortex [13]. In complete agreement with earlier findings [1,14], the opposite effects can be observed after chronic treatment with captopril, a specific inhibitor of the angiotensin-converting enzyme [15], or SRIF.

These observations fit well (i) with the abundant biochemical evidence that the enzymes involved in aldosterone synthesis are located in both mitochondrial cristae and SER membranes [16,17], and (ii) with the demonstration that the changes in the surface area per cell of mitochondrial cristae and SER tubules are associated with corresponding changes in the activity of some of these enzymes [18]. On these grounds, the opposing effects of AII on the one hand and captopril or SRIF on the other, in the term of the volume of the lipid droplet compartment, can be easily explained. Lipid droplets contain cholesterol and cholesterol esters [19, 20], the main precursors in aldosterone synthesis, and the uptake of cholesterol from serum lipoproteins is a process primarily controlled by ACTH [21]. Thus, lipid droplet depletion or accumulation may be considered the morphologic expression of accelerated or depressed aldosterone synthesis, in hypertrophic or atrophic zona glomerulosa cells respectively.

SRIF not only exerts inhibitory effects, comparable to those of captopril, on the growth and steroidogenic capacity of rat zona glomerulosa cells, but also completely abrogated the adrenoglomerulotrophic action of AII. Furthermore, our present findings seem to suggest that the inhibitory effects of SRIF and captopril are not additive. In the light of these considerations, it appears reasonable to suggest that the mechanism underlying the action of SRIF on the rat zona glomerulosa involves blockade of the effects of AII.

Some lines of evidence indicate that AII is able to enhance the secretory activity of zona fasciculata cell preparations maintained *in vitro* [22–24]. Moreover, long-term AII administration was also found to stimulate the growth of the rat zona fasciculata *in vivo*, and the possibility that this effect of AII could be due to the activation of the hypothalamo–hypophyseal axis was excluded, since the animals were treated with dexamethasone and maintenance doses of ACTH [25]. Our present data strengthen this contention, showing that chronic administration of AII elicits hypertrophy of zona fasciculata cells, and increases the plasma concentration of corticosterone, the main glucocorticoid hormone produced by the rodent adrenal gland [13]. However, this effect of AII is of moderate dimension and doubtful physiological relevance, inasmuch as captopril does not appear to affect zona fasciculata growth and steroidogenic capacity. SRIF does not counteract the stimulatory effects of AII on the rat zona fasciculata.

This last finding is hard to explain, but it excludes the possibility that SRIF acts by competitively inhibiting the binding of AII to its receptors. In fact, like zona glomerulosa cells, zona fasciculata cells have also been found to possess specific AII-receptors [26,27]. According to Aguilera and associates [2,28], SRIF acts on the zona glomerulosa by binding to specific high affinity receptors and interfering with the second messenger system mediating the stimulatory effects of AII. It is not unreasonable to presume that the lack of SRIF effects on the zona fasciculata cells of AII-treated rats reflects the fact that this cell type lacks specific receptors for SRIF.

In conclusion, the present data lend support to the view that SRIF is specifically involved in the modu-

lation of the long-term effects of the renin–angiotensin system on the growth and steroidogenic capacity of rat zona glomerulosa.

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