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EFFECTS OF PROLONGED CYSTEAMINE ADMINISTRATION ON THE RAT ADRENAL CORTEX: EVIDENCE THAT ENDOGENOUS SOMATOSTATIN IS INVOLVED IN THE CONTROL OF THE GROWTH AND STEROIDOGENIC CAPACITY OF ZONA GLOMERULOSA

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Summary—A week daily administration of cysteamine (CYS, 300 mg kg^{-1}) lowered plasma aldosterone concentration in rats, without affecting PRA, kalaemia and the plasma levels of ACTH and corticosterone. Prolonged CYS treatment caused a notable hypertrophy of adrenal zona glomerulosa (ZG) and its parenchymal cells, without inducing any apparent change in zona fasciculata morphology. Isolated ZG cells from CYS-treated rats evidenced a notable enhancement in their basal and maximally-stimulated productions of aldosterone and corticosterone. All these effects of chronic CYS administration were completely reversed by the simultaneous infusion of rats with somatostatin (SRIF, 12 $\mu g\ kg^{-1}\ h^{-1}$). CYS exposure was not found to directly affect the secretory activity of isolated ZG cells from normal rats. Since CYS is known to be a specific depletor of SRIF in different organs of rats, these findings suggest that endogenous SRIF may be involved in the modulation of ZG function.

INTRODUCTION

Many lines of evidence indicate that exogenous somatostatin (SRIF) plays a direct negative modulatory role on zona glomerulosa (ZG) secretory activity [1–3], by binding to specific receptors [4–6]. We have previously reported that the chronic administration of high doses of SRIF markedly inhibits the growth and steroidogenic capacity of rat adrenal ZG [7], probably by interfering with the adreno-glomerulotrophic effect of angiotensin II (ANG-II) [8]. The possibility, however, cannot be ruled out that this long-term effect of SRIF is only a pharmacologic one. To address this issue, we have investigated the effects of a prolonged treatment with cysteamine (CYS) on the rat adrenal cortex. In fact, CYS is known to be a quite specific depletor of SRIF content in different organs of rats [9, 10].

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EXPERIMENTAL

Animal treatment

Adult male Wistar rats (300 ± 30 g body wt) were used, and divided into 3 equal groups ($n = 8$). The control group was subcutaneously infused for 7 days (Alzet osmotic pumps Mod. 2001; Alza, Palo Alto, Calif.) with 0.9% NaCl and was given daily subcutaneous injections of 1 ml saline for 7 days. The second group was subcutaneously infused with the saline vehicle and received 7 daily subcutaneous injections of CYS (2-mercaptoethanylamine; 300 mg kg^{-1}, dissolved in 1 ml 0.9% NaCl, pH 7.4; Sigma, St Louis, Mo.). The third group was treated as the second one, but had SRIF in the infusion vehicle (12 $\mu g\ kg^{-1}\ h^{-1}$; Sigma). The doses of CYS and SRIF were chosen according to Sewerynek *et al.* [11] and Rebuffat *et al.* [7], respectively. The animals were decapitated between 10:00 and 11:00 a.m., 24 h after the last CYS injection, and their trunk blood was collected and frozen.

Biochemical assays

Serum Na^+ and K^+ concentrations were measured with a flame photometer (LKB,

Stockholm, Sweden). PRA was assayed by RIA of angiotensin I generated after incubation of plasma (ANG-I RIA-Kit; Peninsula, Merseyside, U.K.). ACTH was extracted from plasma [12], and its concentration was determined by RIA (ACTH-RIA kit; IRE-Sorin, Vercelli, Italy). Aldosterone and corticosterone were extracted and purified [13], and their concentrations were measured by RIA (Aldo CTK2; IRE-Sorin. Cortex-RIA kit; Eurogenetix, Milan, Italy). Intra- and interassay variations were: angiotensin I, 7 and 9%; ACTH, 5 and 8%; aldosterone, 4 and 6%; corticosterone, 6 and 8%.

Morphology

The adrenal glands were promptly removed, freed of adherent fat, and weighed. The left adrenals were fixed in Bouin's solution, embedded in paraffin and serially cut at 6–7 μm. Sliced pieces of the right glands were fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in epon. Thick (0.5 μm) and thin (60–70 nm) sections were cut with LKB III ultramicrotomes at the level of the ZG and zona fasciculata (ZF). Thin sections were counterstained with lead-hydroxide, and examined and photographed in a Hitachi H-300 electron microscope.

The volume of ZG and ZF, and the number and volume of their parenchymal cells were determined on light micrographs of the paraffin and 0.5-μm thick sections, using conventional morphometric methods [14], as described in an earlier paper [15]. On electron micrographs of thin sections, the volume of nuclei and mitochondrial and lipid-droplet compartments, as well as the surface area of mitochondrial cristae and smooth endoplasmic reticulum (SER), were evaluated by the sterologic techniques described by Weibel [14], as detailed previously [15].

Table 1. Effects of CYS exposure on the secretory activity of isolated rat ZG cells

	Aldosterone production ($pM/10^6$ cells h^{-1})	Corticosterone production ($pM/10^6$ cells h^{-1})
Basal	86.8 ± 33.4	65.7 ± 22.2
CYS 0.2 mg	79.1 ± 20.6	67.5 ± 30.5
CYS 2 mg	80.2 ± 31.5	59.8 ± 20.7
CYS 20 mg	58.4 ± 19.5†	38.6 ± 15.9*

Values are group means ± SD ($n = 6$).
*$P < 0.01$ and †$P < 0.05$.

Preparation and treatments of dispersed adrenocortical cells

Other rats were divided into 3 equal groups ($n = 24$), which were treated as described above. Dispersed capsular (ZG) adrenocortical cells were prepared by collagenase–DNAse digestion [16] from the 3 groups of rats. Viability of isolated cells was checked by trypan-blue exclusion test. Inner-cell contamination in capsular-cell preparations, as evaluated by phase microscopy, was always less than 6–7%.

Isolated cells were suspended in medium 199 (DIFCO, Detroit, Mich.) and potassium-free Krebs–Ringer bicarbonate buffer (2:1, v/v), containing 5 $g\,l^{-1}$ human serum albumin. Aliquots of capsular-cell suspensions (2×10^5 cells ml^{-1}) were incubated, in replicates of 8 each, with ACTH 10^{-8} M (Sigma), ANG-II 10^{-8} M (Sigma), or potassium 10 mM. Aliquots of isolated cells were incubated without any stimulator. Other isolated capsular cells, obtained from normal-rat adrenals, were incubated, in replicates of 6 each, with increasing concentrations of CYS (0.1–10 mg ml^{-1}).

The incubation was carried out in a shaking bath at 37°C for 90 min, in an atmosphere of 95% O_2 and 5% CO_2. At the end of the experiment, the incubation tubes were centrifuged at 4°C, and the concentrations of aldosterone and corticosterone in the supernatants were determined as described above.

Table 2. Effects of CYS and SRIF on some biochemical parameters of rats

Parameters	Control rats	CYS-treated rats	CYS and SRIF-treated rats
Plasma ACTH concentration (pg ml^{-1})	118.5 ± 36.1	135.6 ± 43.5	110.7 ± 25.8
PRA (pg ml^{-1} h^{-1})	5.2 ± 1.3	5.9 ± 2.0	5.0 ± 1.8
Natremia (mEquiv l^{-1})	137.1 ± 17.5	135.2 ± 21.4	139.5 ± 30.6
Kalaemia (mEquiv l^{-1})	4.4 ± 0.7	4.5 ± 1.0	4.8 ± 1.1
Plasma aldosterone concentration (ng dl^{-1})	26.2 ± 6.1	39.7 ± 8.9*	25.4 ± 7.0
Plasma corticosterone concentration (μg dl^{-1})	11.4 ± 2.7	13.5 ± 3.6	12.6 ± 3.1

Values are means ± SD ($n = 8$).
*$P < 0.01$.

Statistics

The data obtained were averaged per experimental group and the SD of the mean was calculated. The statistical comparison of the data was done by ANOVA followed by the Multiple Range Test of Duncan.

RESULTS

CYS, at relatively low concentrations, did not alter basal aldosterone and corticosterone secretion by isolated rat ZG cells. However, the exposure to high concentration of CYS provoked a slight but significant inhibitory effect (Table 1).

Prolonged CYS administration did not cause any significant change in the plasma levels of ACTH and corticosterone, nor did it affect PRA, natremia and kalaemia. Conversely, CYS treatment evoked a significant rise in the plasma concentration of aldosterone (52%). This last effect of CYS was completely reversed by SRIF infusion (Table 2).

Chronic CYS treatment caused a significant increase in the volume of ZG (25%) and ZG cells (39%) and nuclei (23%), without affecting the number of ZG cells. Stereology showed that CYS-induced ZG-cell hypertrophy was associated with significant increases in the volume of the mitochondrial compartment (43%) and in the surface area per cell of mitochondrial cristae (41%) and SER tubules (47%). The volume of the lipid-droplet compartment displayed a conspicuous drop (−53%). The morphometric parameters of the ZF were not significantly changed. SRIF infusion completely annulled the effects of CYS on the ZG morphology (Table 3).

ANG-II, potassium and ACTH significantly enhanced aldosterone (6.6-, 6.3- and 9-fold) and corticosterone production (5-, 5.8- and 11-fold) by isolated ZG cells of control rats. CYS administration significantly raised basal secretion of aldosterone and corticosterone by capsular cells (about 70%), and notably increased their responses to ANG-II, potassium and ACTH (aldosterone response from 45 to 67%; corticosterone response from 46 to 78%). SRIF infusion totally abolished all these effects of CYS (Table 4).

Table 3. Effects of CYS and SRIF on the morphometric parameters of rat adrenal gland

	Zona glomerulosa			Zona fasciculata		
Parameters	Control rats	CYS-treated rats	CYS and SRIF-treated rats	Control rats	CYS-treated rats	CYS and SRIF-treated rats
Volume of zona (mm^3)	2.415 ± 0.608	3.018 ± 0.722†	2.319 ± 0.482	14.501 ± 4.198	14.911 ± 3.914	13.998 ± 4.282
Number of cells ($\times 10^3$)	2635.8 ± 591.4	2367.6 ± 554.2	2640.4 ± 611.8	7590.4 ± 1751.3	7221.6 ± 1705.2	6978.8 ± 1803.3
Volume of cells (μm^3)	708.5 ± 202.7	985.5 ± 314.5†	658.7 ± 198.5	1719.5 ± 518.2	1858.3 ± 485.4	1805.2 ± 509.6
Volume of nuclei (μm^3)	127.1 ± 31.2	161.3 ± 46.2†	130.4 ± 29.7	170.2 ± 50.1	161.5 ± 49.2	172.3 ± 51.6
Volume of mitochondrial compartment (μm^3/cell)	152.6 ± 34.5	218.4 ± 62.1*	146.7 ± 36.5	591.5 ± 132.4	624.5 ± 160.8	609.5 ± 154.3
Surface area of mitochondrial cristae (μm^2/cell)	2304.3 ± 552.1	3252.7 ± 931.5*	2229.8 ± 491.7	11,711.7 ± 2632.4	11,803 ± 3039.1	11,214.8 ± 2813.5
Surface area of SER (μm^2/cell)	4788.9 ± 1101.5	7030.8 ± 1586.3*	4111.5 ± 984.6	9950.3 ± 2008.2	11,076.5 ± 3405.9	10,709.2 ± 3019.6
Volume of lipid-droplet compartment (μm^3/cell)	42.4 ± 17.2	20.0 ± 11.2*	44.5 ± 20.6	128.6 ± 42.7	141.5 ± 40.2	145.6 ± 51.3

Values are group means ± SD ($n = 8$).
*$P < 0.01$ and †$P < 0.05$.

DISCUSSION

Our present findings provide clear-cut evidence that the prolonged administration of CYS

Table 4. Effects of CYS and SRIF pre-treatments on the basal and stimulated secretory activity of isolated rat ZG cells

	Aldosterone production (pM/10^6 cells h^{-1})			Corticosterone production (pM/10^6 cells h^{-1})		
	Control rats	CYS-treated rats	CYS and SRIF-treated rats	Control rats	CYS-treated rats	CYS and SRIF-treated rats
Basal	79.9 ± 28.1	128.7 ± 46.1*	68.5 ± 19.7	54.5 ± 19.6	93.5 ± 31.3*	61.4 ± 22.4
ANG-II (10^{-8} M)	495.1 ± 181.4	718.3 ± 251.3†	504.2 ± 180.4	279.2 ± 111.5	496.1 ± 191.3*	302.4 ± 115.0
Potassium (10 mM)	478.2 ± 204.6	801.5 ± 300.6*	413.4 ± 135.3	315.2 ± 121.2	525.4 ± 184.5*	289.9 ± 119.8
ACTH (10^{-8} M)	689.5 ± 215.7	1092.2 ± 380.1*	701.5 ± 212.7	601.9 ± 197.1	871.5 ± 321.5†	580.5 ± 222.7

Values are group means ± SD ($n = 8$).
*$P < 0.01$ and †$P < 0.05$.

is able to enhance the growth and steroidogenic capacity of rat ZG, without affecting ZF and its production of corticosterone, the main hormone secreted by inner adrenocortical layers in rats [17].

The CYS-induced hypertrophy of ZG cells is mainly due to the increase in the volume of the mitochondrial compartment and to the proliferation of SER. These morphologic data accord well with the CYS-evoked enhancement in basal and maximally-stimulated secretory activity of ZG cells, since the enzymes of steroid synthesis are located in both mitochondria and SER [see 17, 18, for references], and the changes in the surface area per cell of mitochondrial cristae and SER tubules are closely coupled with corresponding changes in the activity per cell of some of these enzymes [19, 20]. The accelerated utilization of cholesterol in aldosterone and corticosterone synthesis, the two main hormones secreted by ZG cells *in vitro* [21], associated with a presumably normal uptake of cholesterol from serum lipoproteins, may easily explain the striking decrease in the volume of the lipid-droplet compartment in ZG cells of chronically CYS-administered rats. In fact, it is commonly agreed that cholesterol and cholesterol esters are stored in adrenocortical lipid droplets [17, 22], and that lipoprotein uptake by adrenocortical cells is a receptor-mediated process mainly controlled by ACTH [23].

CYS is a depletor of SRIF [9, 10], which, when exogenously administered, is well known to exert a strong antiadrenoglomerulotrophic effect by interfering with ANG-II [8]. Therefore, our data suggest that CYS effects may be due to the release of ZG from the inhibitory action of endogenous SRIF. This contention appears to be the most convincing one, inasmuch as (i) CYS exposure *per se* does not stimulate ZG cells *in vitro*, and (ii) the adrenoglomerulotrophic effect of CYS is reversed by SRIF infusion. The possibility that CYS treatment may stimulate one or more of the three main adrenoglomerulotrophic factors can be excluded. A chronic activation of the hypothalamo–hypophyseal–adrenal axis or the renin–angiotensin system are not at play, since neither plasma levels of ACTH and ZF growth are changed nor PRA is significantly affected; kalaemia and natremia do not display any appreciable alteration. Parenthetically, these last findings indicate that the renin–angiotensin system and electrolyte balance undergo a very fine and complex regulation *in vivo*, inasmuch as in the presence of a high level of circulating aldosterone it would be reasonable to observe a lowered PRA and alterations in plasma electrolytes: they may be tentatively and partly explained by assuming that CYS has released kidney renin-secretion by the well-known inhibitory effect of SRIF [24–26]. Some studies have shown that doses of CYS over 150 mg kg^{-1} may also lower the levels of prolactin [27, 28]. However, this effect does not conceivably seem to be involved in the mechanism underlying the adrenoglomerulotrophic action of CYS, since prolactin is a stimulator of the ZG growth in rats [29].

In conclusion, our study supports the view that endogenous SRIF is somehow involved in the physiologic modulation of ZG function in rats, a contention which is also in keeping with the presence of SRIF receptors in ZG cells [4–6]. The possible source of SRIF involved in such an adrenocortical effect is not known at present. However, we want to mention that SRIF is co-stored with catecholamines in the chromaffin granules of adrenal medulla [30–34], and that evidence is accumulating that zona medullaris exerts a paracrine control of ZG function [see 35, for review]. Investigations are on course to ascertain whether prolonged CYS administration depletes SRIF content not only in the brain and gastrointestinal tract, but also in the adrenal medulla.

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