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Evidence that Endogenous Somatostatin (SRIF) Exerts an Inhibitory Control on the Function and Growth of Rat Adrenal Zona Glomerulosa. The Possible Involvement of Zona Medullaris as a Source of Endogenous SRIF

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The effect of SRIF and its antagonist cyclo(7-aminoheptanonyl-Phe-D-Trp-Lys-Thr|Bzl|)(SRIF-A) were studied in sham-operated and bilaterally adrenalectomized rats bearing ACTH- and angiotensin II (ANG-II)-responsive adrenocortical autotransplants. SRIF-A (10^{-5} M) completely annulled SRIF (10^{-6} M)-induced inhibition of ANG-II (10^{-8} M)-evoked rise in aldosterone (ALDO) secretion by both dispersed zona glomerulosa (ZG) cells and autotransplant slices. A 7-day intraperitoneal infusion with SRIF (0.3 nmol.kg^{-1}.min^{-1}) significantly lowered plasma ALDO concentration (PAC) in both groups of animals, without affecting plasma renin activity and the plasma levels of ACTH and corticosterone. This treatment caused a marked atrophy of adrenal ZG and its parenchymal cells (without inducing any significant change in the zona fasciculata morphology), as well as of ZG-like cells of autotransplants. Isolated ZG cells and autotransplant slices from SRIF-infused rats evidenced a notable decrease in both their basal and maximally ACTH- or ANG-II-stimulated ALDO production. The simultaneous infusion of rats with SRIF-A (3 nmol.kg^{-1}.min^{-1}) completely reversed all these effects of SRIF. The prolonged infusion with SRIF-A alone caused, in sham-operated rats, a marked increase in PAC and a significant hypertrophy of ZG and ZG cells; basal and maximally-stimulated ALDO secretion of dispersed ZG cells was also notably raised. Conversely, SRIF-A infusion did not evoke any appreciable effect in autotransplanted rats. These findings suggest that endogenous SRIF is specifically involved in the negative control of the secretion and growth of the rat adrenal ZG. Since regenerated adrenocortical autotransplants, which are responsive to SRIF but not to SRIF-A infusion, are completely deprived of chromaffin cells, the hypothesis is advanced that adrenal zona medullaris may be the source of endogenous SRIF regulating ZG function.

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INTRODUCTION

A large body of data indicates that exogenous SRIF exerts a direct inhibitory effect on the secretory activity and growth of adrenal zona glomerulosa (ZG). SRIF binds to specific membrane receptors of ZG cells [1–4], and probably interferes with the intracellular mechanisms mediating the adrenoglomerulotropic effect of angiotensin II (ANG-II) [5–9]. The hypothesis that endogenous SRIF may be involved in the physiologic

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Abbreviations: ACTH, adrenocorticotropic hormone; ALDO, aldosterone; ANG-II, angiotensin II; ANOVA, analysis of variance; B, corticosterone; BP, systolic blood pressure; PAC, plasma aldosterone concentration; PBC, plasma corticosterone concentration; PRA, plasma renin activity; SER, smooth endoplasmic reticulum; SRIF, somatostatin; SRIF-A, somatostatin antagonist; ZF, zona fasciculata; ZG, zona glomerulosa.

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control of the adrenal function in rats has been recently suggested by the demonstration that cysteamine, a specific depletor of SRIF in different organs of this species [10], markedly enhances the growth and secretory activity of ZG [11].

Some points, however, remain to be addressed before this hypothesis can be definitively accepted. (i) Adrenal ZG undergoes a very complex multifactorial control (see [12, 13] for review); hence, it cannot be excluded that cysteamine, apart from inducing SRIF depletion, may interfere with the mechanism of action of one or more other factors regulating ZG function. (ii) Due to the very short half-life, the level of circulating SRIF of various origin (hypothalamic, renal and intestinal) is very low (see [14, 15] for review), and surely below the minimal concentration shown to be effective *in vitro* on ZG cells ($10^{-10}/10^{-9}$ M) [5]; thus, it would be necessary to admit the existence of a local (intradrenal) source of endogenous SRIF.

Here, we report findings indicating that the prolonged administration of a specific competitive antagonist of SRIF, the cyclo(7-aminoheptanonyl-Phe-D-Trp-Lys-Thr|Bzl|) (SRIF-A) [16] enhances the function and growth of ZG in rats under basal conditions, and provide evidence that adrenal zona medullaris may be the source of endogenous SRIF regulating ZG activity.

EXPERIMENTAL

In vivo experiments

Adult male Wistar rats (200 ± 20 g body wt) were purchased from Charles-River (Como, Italy). A group of rats was bilaterally adrenalectomized, and 6 fragments of the capsular tissue of their excised adrenals were implanted in the *musculus gracilis*; the animals were employed after 4 months of regeneration of autotransplants [17]. Another group of rats was sham-adrenalectomized. The animals were kept under a 12 : 12 h light–dark cycle (illumination onset at 8:00 a.m.) at 23 ± 1°C, and maintained on a standard diet (Rat-Mouse Chow; Zoofarm, Padua, Italy) and tap water *ad libitum*.

Groups ($n = 8$) of sham-operated and autotransplanted rats were intraperitoneally infused for 7 days (Alzet osmotic pumps Mod. 2001; Alza, Palo Alto, CA) with the following peptides purchased by Sigma (St Louis, MO) and dissolved in 0.9% NaCl: SRIF (0.3 $nmol.kg^{-1}.min^{-1}$), SRIF-A (3.0 $nmol.kg^{-1}.min^{-1}$) or SRIF plus SRIF-A. The control groups were infused with the saline vehicle. The dose of SRIF was chosen according to Kasprzak *et al.* [11], and that of SRIF-A was 10-fold higher according to the *in vitro* experiment described below. By considering that peritoneal absorption is very high and the half-life of SRIF in the blood is very short, and by assuming 10–12 ml of blood/rat, it can be roughly calculated that this infusion rate produces a constant concentration of circulating SRIF about 10^{-7} M.

The systolic blood pressure (BP) was measured by tail sphygmomanometry (BP-Recorder; Basile, Comerio, Italy) 24 h before the sacrifice. The rats were decapitated between 10:00 and 11:00 a.m., their trunk blood was collected and frozen, and their adrenals or autotransplanted adrenocortical nodules were promptly removed.

Biochemical assays. Serum Na^+ and K^+ concentrations were measured with a flame photometer (LKB, Stockholm, Sweden). Plasma renin activity (PRA) was assayed by RIA of angiotensin-I generated after incubation of plasma (ANG-I RIA kit; Peninsula, Merseyside, England). ACTH was extracted from plasma [18], and its concentration was determined by RIA (ACTH-RIA kit; IRE-Sorin, Vercelli, Italy). Aldosterone (ALDO) and corticosterone (B) were extracted and purified [19], and their plasma concentrations (PAC and PBC, respectively) were measured by RIA (aldo-CTK2; IRE-Sorin, and Crtx-RIA; Eurogenetix, Milan, Italy). Intra- and interassay variations were: ANG-I, 6.0 and 8.7%; ACTH, 5.8 and 7.5%; ALDO, 5.6 and 7.1%; B, 7.0 and 8.9%.

Morphology. 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 a LKB Supernova Ultrotome (Reichert-Jung, Wien, Austria) 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, as well as the volume of nuclei, were determined on light micrographs of the paraffin and 0.5 μm thick epon sections, using conventional morphometric methods [20], as described in an earlier paper [21]. On electron micrographs of ZG cells, the volume of mitochondrial and lipid-droplet compartments, and the surface area of mitochondrial cristae and smooth endoplasmic reticulum (SER) were evaluated by the stereologic techniques described by Weibel [20], as detailed previously [21]. Adrenocortical autotransplants were sliced and processed for electron microscopy (see above). The volume and stereological parameters of subcapsular and juxta-septal ZG-like cells [17] were evaluated as described above.

In vitro experiments

Other 4 groups of sham-operated and autotransplanted rats were infused for a week as in *in-vivo* experiments. A number of rats was not infused (normal rats). The animals were decapitated, and adrenal glands and regenerated adrenocortical nodules promptly re moved. Adrenals were employed to obtain dispersed-cell preparations, while autotransplants were quartered [17].

Preparation of dispersed ZG cells. Adrenal glands were gently decapsulated to separate ZG. Dispersed capsular (ZG) cells were obtained by collagenase/DNase I digestion and mechanical disaggregation [22]. The viability of isolated cells was checked by the trypan-blue exclusion test and found to be higher than 90%. Inner-cell contamination in capsular–cell preparations, as evaluated by phase-microscopy, was always $<7\%$. Dispersed

cells obtained from 6 rats were pooled to obtain a single cell preparation, and 6 or 8 cell preparations for each incubation experiment were employed.

Incubation procedures. Dispersed cells and autotransplant quarters were put in Medium 199 (DIFCO, Detroit, MI) and potassium-free Krebs-Ringer bicarbonate buffer with 0.2% glucose (2:1, v/v), containing 5 mg.ml^{-1} human serum albumin. They were incubated as follows: (i) preparations obtained from infused rats were incubated, in replicates of 8 each, with ANG-II or $ACTH_{1-24}$ (both 10^{-8} M) (Sigma), or without any peptide. (ii) Dispersed ZG cells obtained from normal rats were incubated, in replicates of 6 each, with increasing concentrations of SRIF-A (from 10^{-10} to 10^{-4} M), in the presence or absence of ANG-II 10^{-8} M and ANG-II plus SRIF 10^{-6} M. (iii) Autotransplant quarters obtained from normal rats were incubated, in replicates of 6 each, with increasing concentrations of SRIF (from 10^{-10} to 10^{-4} M), in the presence or absence of ANG-II 10^{-8} M and SRIF-A 10^{-5} M. 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 experiments, the incubation tubes were centrifuged at 4°C, and the concentration of ALDO in the supernatants were determined as described above (intra- and interassay variations were 7.1 and 8.3%, respectively).

Statistics

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

RESULTS

SRIF 10^{-6} M provoked a marked (−60%) inhibition of ANG-II (10^{-8} M)-evoked rise (7.2-fold) in ALDO secretion by dispersed ZG cells. The acute exposure to SRIF-A did not alter either basal or ANG-II-stimulated ALDO production, but it dose-dependently counteracted the inhibitory effect of SRIF: a complete reversal was observed at a SRIF-A concentration 10^{-5} M (Fig. 1). SRIF dose-dependently decreased ANG-II (10^{-8} M)-stimulated ALDO secretion by autotransplant quarters (3.3-fold), without affecting the basal one; the maximum effect (−54%) was obtained at a concentration of 10^{-6} M. SRIF-A 10^{-5} M completely abolished the effect of SRIF (Fig. 2).

The prolonged infusion with SRIF, SRIF plus SRIF-A or SRIF-A did not cause any significant change in BP, PRA, natremia, kalaemia, ACTH plasma concentration and PBC in both sham-operated and autotransplanted rats (Table 1). SRIF infusion significantly decreased PAC in sham-operated (−23%) and autotransplanted animals (−27%), and SRIF-A reversed this effect (Table 1). The administration of SRIF-A alone did evoke a 38% rise in PAC in sham-operated rats, but not in autotransplanted animals (Table 1).

Chronic SRIF administration caused significant de-

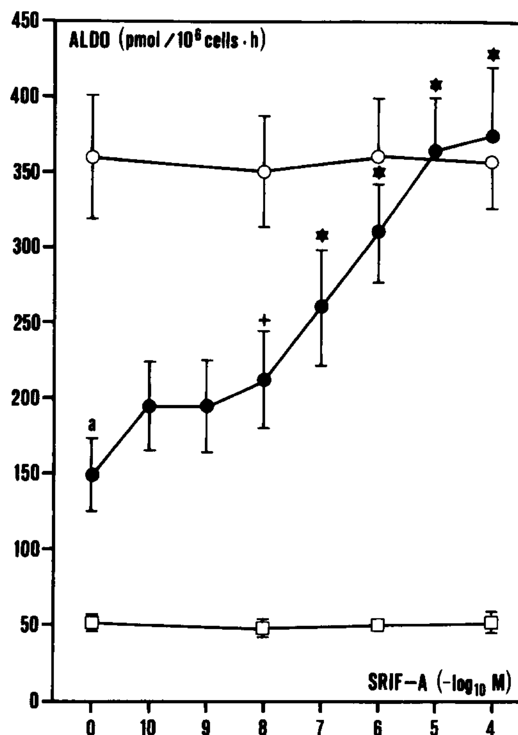

Fig. 1. Acute effect of SRIF-A on basal (□), ANG-II (10^{-8} M)-stimulated (○) and ANG-II-stimulated SRIF (10^{-6} M)-inhibited (●) ALDO secretion by dispersed rat ZG cells. Data are means ± SE (n = 6). [a]$P < 0.01$ from ANG-II-stimulated O-group; $^{+}P < 0.05$ and $^{*}P < 0.01$ from the respective O-group.

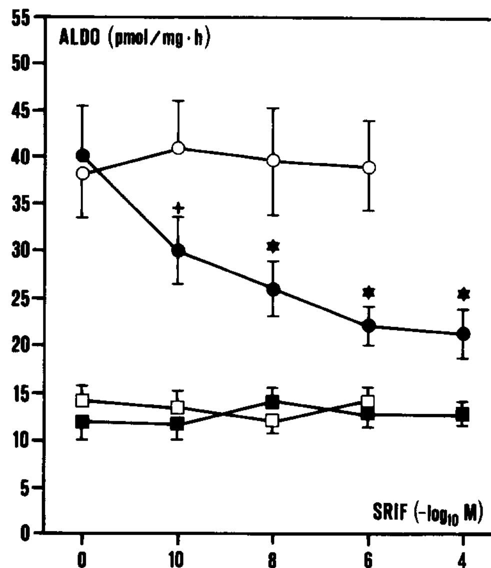

Fig. 2. Acute effect of SRIF on basal (■) and ANG-II (10^{-8} M)-stimulated (●) ALDO secretion by quarters of adrenocortical autotransplants, and the effect of the simultaneous exposure to SRIF-A (10^{-5} M) (□, ○). Data are means ± SE (n = 6). $^{+}P < 0.05$ and $^{*}P < 0.01$ from the respective O-group.

Table 1. Effects of SRIF, SRIF plus SRIF-A and SRIF-A infusions on some physical and biochemical parameters of sham-operated and autotransplanted rats

	Controls	SRIF	SRIF plus SRIF-A	SRIF-A
Sham-operated rats				
BP (mmHg)	132 ± 13	130 ± 10	135 ± 14	128 ± 11
PRA ($fmol.ml^{-1}.h^{-1}$)	5.1 ± 1.4	6.1 ± 1.6	5.2 ± 1.1	5.0 ± 1.0
Natremia ($mEq.l^{-1}$)	130.4 ± 14.6	135.5 ± 15.1	129.5 ± 13.8	131.2 ± 14.9
Kalaemia	5.0 ± 0.8	5.1 ± 0.7	4.9 ± 0.6	4.9 ± 0.8
ACTH plasma concentration (pM)	20.6 ± 7.0	21.5 ± 6.5	22.3 ± 7.2	19.8 ± 5.9
PAC (pM)	580.9 ± 182.1	438.7 ± 125.7**	604.6 ± 196.1	792.5 ± 251.9**
PBC (nM)	294.7 ± 98.0	287.1 ± 96.6	309.2 ± 100.3	300.4 ± 90.7
Autotransplanted rats				
BP (mmHg)	155 ± 19*	160 ± 25	150 ± 20	153 ± 18
PRA ($fmol.ml^{-1}.h^{-1}$)	7.9 ± 2.0*	8.1 ± 2.2	7.5 ± 1.9	8.0 ± 2.4
Natremia ($mEq.l^{-1}$)	135.3 ± 17.2	138.2 ± 16.7	130.6 ± 18.5	139.5 ± 23.1
Kalaemia ($mEq.l^{-1}$)	5.3 ± 0.9	4.9 ± 0.7	5.0 ± 1.0	5.0 ± 0.9
ACTH plasma concentration (pM)	32.1 ± 9.1*	29.7 ± 8.0	30.6 ± 9.5	34.3 ± 10.2
PAC (pM)	221.3 ± 69.7*	160.5 ± 48.3**	232.4 ± 85.0	219.1 ± 71.4
PBC (nM)	152.2 ± 45.4*	146.8 ± 47.2	149.7 ± 38.8	153.7 ± 50.1

Data are means ± SD ($n = 8$). *$P < 0.01$ from sham-operated control rats; **$P < 0.05$ from control group.

creases in the volumes of ZG (−19%) and ZG cells (−21%), without affecting the number of ZG cells and the volume of their nuclei (Table 2); also the average volume of ZG-like cells of autotransplants displayed a significant lowering (−21%) (Table 4). The SRIF-induced ZG- and ZG-like-cell atrophy was associated with significant decreases in the volume of mitochondrial compartment (−27 and −29%, respectively), and in the surface areas of mitochondrial cristae (−27 and −30%) and SER (−36 and −24%). Conversely, the volume of the lipid-droplet compartment underwent a marked rise in both types of cells (81 and 120%) (Tables 3 and 4). SRIF-A completely annulled the SRIF effects on the ZG (Tables 2 and 3) and ZG-like cells (Table 4). The chronic administration of SRIF-A alone evoked, in sham-operated rats, effects opposite to those elicited by SRIF: increases in the volumes of ZG (29%), ZG cells (26%) and mitochondrial compartment (31%), and in the surface areas of mitochondrial cristae (32%) and SER (38%); lowering in the volume of the lipid-droplet compartment (−53%) (Tables 2 and 3). Conversely, SRIF-A infusion did not alter the morphology of ZG-like cells of autotransplants (Table 4). None of the treatments evoked appreciable alterations in the ZF of sham-operated rats (Table 2).

ANG-II (10^{-8} M) and ACTH (10^{-8} M) acute exposure markedly raised ALDO output by both dispersed ZG cells (6.3- and 9.6-fold, respectively) and autotransplant quarters (3.4- and 4.0-fold) of saline infused rats (Table 5). SRIF infusion notably reduced both basal (−22 and −39%) and ANG-II- or ACTH-stimulated (−25/−26 and −25/−21%) ALDO secretion of both kinds of preparations. These effects of SRIF were annulled by the simultaneous infusion of SRIF-A (Table 5). The administration of SRIF-A alone increased basal and stimulated ALDO output by dispersed ZG cells (about 30–33%), but not by autotransplant quarters (Table 5).

DISCUSSION

According to previous investigations [5, 8], SRIF 10^{-6} M partially inhibits maximally ANG-II-stimulated ALDO secretion by rat ZG cells. SRIF-A dose-dependently counteracts SRIF effect, being a complete suppression obtained at a concentration 10-fold higher than that of SRIF.

As demonstrated in earlier investigations [6, 7], the prolonged administration of SRIF, at a dose that presumably is able to maintain a blood concentration of

Table 2. Effects of SRIF, SRIF plus SRIF-A and SRIF-A infusions on the morphometric parameters of the adrenal gland of sham-operated rats

	Controls	SRIF	SRIF plus SRIF-A	SRIF-A
ZG				
Volume of zona (mm^3)	2.952 ± 0.715	2.381 ± 0.601*	3.011 ± 0.810	3.815 ± 0.848*
Number of cells ($\times 10^3$)	3184.0 ± 617.2	3253.8 ± 710.5	3175.9 ± 698.8	3263.9 ± 750.6
Volume of cells (μm^3)	741.7 ± 178.9	585.4 ± 148.2*	758.6 ± 201.5	935.1 ± 258.7*
Volume of nuclei (μm^3)	121.5 ± 17.7	130.4 ± 19.8	125.7 ± 20.6	128.9 ± 19.5
ZF				
Volume of zona (mm^3)	16.542 ± 5.819	16.178 ± 4.159	15.987 ± 4.522	17.002 ± 5.218
Number of cells ($\times 10^3$)	8143.8 ± 2019.5	8095.7 ± 2218.5	7738.5 ± 1919.8	8047.2 ± 2153.4
Volume of cells (μm^3)	1828.1 ± 408.2	1798.5 ± 397.2	1859.3 ± 410.9	1901.5 ± 506.2
Volume of nuclei (μm^3)	160.4 ± 22.3	155.1 ± 19.8	166.3 ± 20.1	159.9 ± 22.2

Data are means ± SD ($n = 8$). *$P < 0.05$ from control group.

Table 3. Effects of SRIF, SRIF plus SRIF-A and SRIF-A infusions on the morphometric parameters of ZG cells of sham-operated rats

	Controls	SRIF	SRIF plus SRIF-A	SRIF-A
Volume of mitochondrial compartment (μm^3/cell)	130.2 ± 36.2	95.6 ± 29.7*	129.8 ± 35.1	170.2 ± 43.9*
Surface area of mitochondrial cristae (μm^2/cell)	2369.6 ± 619.5	1728.8 ± 450.2*	2401.5 ± 658.2	3114.6 ± 759.2*
Surface area of SER (μm^2/cell)	5398.8 ± 1382.6	3469.1 ± 1218.3**	5685.3 ± 1410.9	7469.3 ± 2005.2**
Volume of lipid-droplet compartment (μm^3/cell)	40.1 ± 17.2	72.7 ± 26.9**	35.8 ± 14.7	18.7 ± 8.2**

Data are means ± SD ($n = 8$). *$P < 0.05$ and **$P < 0.01$ from control group.

the peptide near the maximally effective one *in vitro* (see Experimental), profoundly depresses the growth and secretory capacity of rat adrenal ZG, without affecting ZF and the production of B, the main glucocorticoid secreted by inner adrenocortical layers in Rodentia [23]. The moderate (19%), non-significant rise in PRA may be interpreted as a negative feed-back response of the renin–angiotensin system to the lowered PAC, that in turn is counteracted by the well-known inhibitory effect of SRIF on kidney renin release [24–26]. SRIF-A, at an infusion rate 10-times higher than that of SRIF, completely annuls SRIF effects, and, when infused alone, causes a marked enhancement of the growth and steroidogenic capacity of ZG (but not of ZF).

The SRIF- and SRIF-A-induced atrophy and hypertrophy, respectively, of ZG and its parenchymal cells are coupled with decreases and increases in the volume of the mitochondrial compartment and SER. These morphologic data accord well with the notable depression or enhancement of basal and maximally agonist-stimulated ALDO secretion by dispersed ZG cells obtained from SRIF- and SRIF-A-infused rats, respectively. In fact, the enzymes of ALDO synthesis are located in both mitochondria and SER (see [27–29] for references), and the changes in the surface area per cell of mitochondrial cristae and SER tubules are tightly coupled with corresponding changes in the activity per cell of some of these enzymes [27, 30, 31]. The lowered or increased utilization of cholesterol in ALDO synthesis occurring in SRIF- and SRIF-A-infused rats, coupled with a presumably normal uptake of cholesterol from serum lipoproteins, may well account for the marked increase or decrease in the volume of lipid-droplet compartment. In fact, it is commonly agreed that cholesterol and its esters are stored in adrenocortical lipid droplets [27, 32, 33] and that lipoprotein uptake by adrenocortical cells is a receptor-mediated process mainly (if not exclusively) controlled by ACTH (see [34] for review), whose secretion is not affected by SRIF or SRIF-A treatment.

Our findings obtained in SRIF-A-infused animals strongly suggest that, under basal conditions, endogenous SRIF is involved in the negative control of the secretion and growth of ZG in rats. This contention is supported by the fact that (i) SRIF-A *per se* does not acutely affect either basal or ANG-II-stimulated ALDO secretion by isolated ZG cells, which indicates that this peptide does not disrupt rat ZG function, but only competes with SRIF receptors [16]; and (ii) the adrenoglomerulotropic action of SRIF-A is totally reversed by the simultaneous infusion with SRIF.

As pointed out in the Introduction, it seems necessary to admit the existence of a local source of endogenous SRIF, since the blood level of SRIF, under normal circumstances, is too low for this peptide to exert its modulatory effect on adrenals. Our experiments with adrenocortical autotransplanted rats appear to throw light on this topic.

Regenerated adrenocortical nodules secrete both

Table 4. Effects of SRIF, SRIF plus SRIF-A and SRIF-A infusions on the morphometric parameters of ZG-like cells of adrenocortical autotransplants

	Controls	SRIF	SRIF plus SRIF-A	SRIF-A
Volume of cells (μm^3)	670.1 ± 181.3	530.6 ± 150.1*	650.1 ± 154.8	695.7 ± 201.2
Volume of nuclei (μm^3)	92.4 ± 11.2	87.3 ± 13.2	101.5 ± 15.6	89.4 ± 13.5
Volume of mitochondrial compartment (μm^3/cell)	150.6 ± 40.8	106.3 ± 30.2**	148.9 ± 50.1	163.2 ± 48.7
Surface area of mitochondrial cristae (μm^2/cell)	2715.1 ± 681.4	1909.5 ± 418.1**	2689.4 ± 701.2	2881.0 ± 758.3
Surface area of SER (μm^2/cell)	5108.5 ± 1541.7	3858.9 ± 1213.4*	4751.5 ± 1421.8	5274.1 ± 1617.3
Volume of lipid-droplet compartment (μm^3/cell)	9.3 ± 4.0	20.7 ± 9.1**	10.2 ± 5.3	10.8 ± 3.9

Data are means ± SD ($n = 8$). *$P < 0.05$ and **$P < 0.01$ from control group.

Table 5. Effects of SRIF, SRIF plus SRIF-A and SRIF-A infusions on basal and agonist-stimulated ALDO secretion of dispersed ZG cells of sham-operated rats and adrenocortical nodules of autotransplanted rats

	Controls	SRIF	SRIF plus SRIF-A	SRIF-A
Dispersed ZG cells ($pmol/10^6 cells.h^{-1}$)				
Basal	56.8 ± 16.1	44.0 ± 12.7*	60.8 ± 18.5	75.4 ± 20.2*
ANG-II (10^{-8} M)	358.4 ± 89.2	270.5 ± 75.6*	372.5 ± 106.2	464.6 ± 152.1*
ACTH (10^{-8} M)	546.3 ± 178.1	401.7 ± 141.9*	589.8 ± 185.1	719.8 ± 235.6*
Autotransplanted quarters ($pmol.mg^{-1}.h^{-1}$)				
Basal	15.2 ± 5.0	9.3 ± 3.0*	16.8 ± 6.1	14.9 ± 3.5
ANG-II (10^{-8} M)	51.4 ± 16.8	38.7 ± 12.1*	50.7 ± 14.2	55.3 ± 15.9
ACTH (10^{-8} M)	59.6 ± 15.3	47.0 ± 12.9*	61.2 ± 18.5	56.8 ± 14.7

Data are means ± SD ($n = 8$). *$P < 0.05$ from control group.

mineralo- and glucocorticoids, and are responsive to ACTH and ANG-II; their subcapsular and juxta-septal ZG-like cells are provided with ANG-II receptors [17, 35]. However, due to the lower weight of regenerated adrenocortical tissue in comparison to that of adrenals [17], PAC and PBC are markedly lower than in sham-operated rats, which may explain why plasma ACTH concentration and PRA are relatively higher. Our present findings show that ZG-like cells of autotransplants are able to respond to SRIF as ZG cells of adrenal gland. In fact, SRIF dose-dependently inhibits maximally ANG-II-stimulated ALDO secretion; again, this effect of SRIF is completely blocked by SRIF-A (10^{-5} M), that *per se* does not affect ZG-like cell secretory activity. SRIF infusion depresses the growth and steroidogenic capacity of ZG-like cells, and the simultaneous administration of SRIF-A annuls this effect of SRIF. However, at variance with sham-operated rats, the infusion of SRIF-A alone does not exert any effect in autotransplanted animals. This finding clearly indicates that the local production of endogenous SRIF is not operative in autotransplanted rats.

Autotransplanted adrenocortical tissue obviously lost its normal vascular supply. Kidneys are able to synthesize SRIF, and direct vascular connections between kidney and adrenal have been demonstrated in the rat [36]. However, this portal rete, originating from the adrenal vein and terminating in the renal cortex, appears to be exclusively involved in the control of the kidney function by adrenal released catecholamines [36]. It has been previously shown that autotransplants, regenerated from adrenal capsular fragments, are completely deprived of chromaffin cells. This contention is based on both morphological (serial sectioning of transplants) and biochemical findings (HPLC evaluation of catecholamine content of transplants) [17]. On these grounds, it seems reasonable to hypothesize that the source of endogenous SRIF, exerting (under basal conditions) an antiadrenoglomerulotropic effect, may be zona-medullaris chromaffin cells.

Many lines of evidence indicate that adrenal zona medullaris, by secreting catecholamines and many regulatory peptides, exerts a paracrine control of the cortex function [37–45]. Our hypothesis fits well with this contention, as well as with evidence indicating that zona medullaris of some mammalian species (ox, cat and human) contains SRIF [46–50]. The mechanism by which intramedullary released SRIF may reach the cortex may be very simple, at least in the rat; in fact, a very strict interlacement between cortical and medullary tissue is commonly observed in the adrenal glands of this species [51, 52].

However, it must be recalled that, according to earlier investigations the presence of SRIF in the rat chromaffin cells is questionable [4, 50]. Studies are on course to ascertain whether rat zona medullaris, like that of other mammals, secretes sizable amounts of SRIF, that can produce effective intracortical concentrations of this peptide.

REFERENCES

1. Aguilera G., Parker D. S. and Catt K. J.: Characterization of somatostatin receptors in the rat adrenal glomerulosa zone. *Endocrinology* **111** (1982) 1376–1384.
2. Srikant C. B. and Patel Y. C.: Somatostatin receptors in the rat adrenal cortex: characterization and comparison with brain and pituitary receptors. *Endocrinology* **116** (1985) 1717–1723.
3. Maurer R. and Reubi J. C.: Somatostatin receptors in the adrenal. *Molec. Cell. Endocr.* **45** (1986) 81–90.
4. Morel G., Leroux P., Garcia-Caballero T., Beiras A. and Gossard F.: Ultrastructural distribution of somatostatin-14 and -28 in rat adrenal cells. *Cell Tiss. Res.* **261** (1990) 517–524.
5. Aguilera G., Harwood J. P. and Catt K. J.: Somatostatin modulates the effects of angiotensin II in adrenal glomerulosa zona. *Nature* **292** (1981) 262–263.
6. Rebuffat P., Robba C., Mazzocchi G. and Nussdorfer G. G.: Inhibitory effect of somatostatin on the growth and steroidogenic capacity of rat adrenal zona glomerulosa. *J. Steroid Biochem.* **21** (1984) 387–390.
7. Mazzocchi G., Robba C., Rebuffat P., Gottardo G. and Nussdorfer G. G.: Effect of somatostatin on the zona glomerulosa of rats treated with angiotensin II or captopril: stereology and plasma hormone concentrations. *J. Steroid Biochem.* **23** (1985) 353–356.
8. Hausdorff W. P., Aguilera G. and Catt K. J.: Inhibitory actions of somatostatin on cyclic AMP and aldosterone production in agonist-stimulated adrenal glomerulosa cells. *Cell. Signal.* **1** (1989) 377–386.
9. Pawlikowski M., Lewinski A., Sewerynek E., Szkudlinski M., Kunert-Radek J. and Wajs E.: Somatostatin analog (SMS 201-995) inhibits the basal and angiotensin II-stimulated ^{3}H-thymidine uptake by rat adrenal glands. *Biochem. Biophys. Res. Commun.* **166** (1990) 1171–1175.

10. Szabo S. and Reichlin S.: Somatostatin in rat tissues is depleted by cysteamine administration. *Endocrinology* **109** (1981) 2255–2257.
11. Kasprzak A., Rebuffat P., Andreis P. G., Mazzocchi G. and Nussdorfer G. G.: 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. *J. Steroid Biochem. Molec. Biol.* 38 (1991) 469–473.
12. Quinn S. J. and Williams G. H.: Regulation of aldosterone secretion. In *The Adrenal Gland* (Edited by V. H. T. James). Raven Press, New York (1992) pp. 159–189.
13. Vinson G. P., Whitehouse B. J. and Hinson J. P.: *The Adrenal Cortex.* Prentice Hall, Englewood Cliffs (1992).
14. Effendić S., Lins P. E. and Uvnäs-Wallensten K.: Extrahypophyseal effects of somatostatin. *Ann. Clin. Res.* **10** (1978) 151–156.
15. Chiodini P. G., Attanasio R., Orlandi P. and Cozzi R.: Growth hormone release inhibiting hormone. Basic and clinical aspects. In *Brain Endocrinology* (Edited by M. Motta). Raven Press, New York (1991) pp. 301–313.
16. Fries J. L., Murphy W. A., Sueiras-Diaz J. and Coy D. H.: Somatostatin antagonist analog increases GH, insulin, and glucagon release. *Peptides* 3 (1982) 811–814.
17. Belloni A. S., Neri G., Musajo F. G., Andreis P. G., Boscaro M., D'Agostino D., Rebuffat P., Boshier D. P., Gottardo G., Mazzocchi G. and Nussdorfer G. G.: Investigations on the morphology and function of adrenocortical tissue regenerated from gland capsular fragments autotransplanted in the *musculus gracilis* of the rat. *Endocrinology* **126** (1990) 3251–3262.
18. Rees L. H., Cook D. M., Kendall J. W., Allen C. F., Kramer R. M., Ratcliffe J. G. and Knight R. A.: A radioimmunoassay for rat plasma ACTH. *Endocrinology* **89** (1971) 254–261.
19. Sippell W. G., Bidlingmaier F., Becker H., Brünig T., Dörr H., Hahn H., Golder W., Hollmann G. and Knorr D.: Simultaneous radioimmunoassay of plasma aldosterone, corticosterone, 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol and cortisone. *J. Steroid Biochem.* 9 (1978) 63–74.
20. Weibel W. R.: *Stereological Methods—1. Practical Methods for Biological Morphometry.* Academic Press, London (1979).
21. Rebuffat P., Kasprzak A., Andreis P. G., Mazzocchi G., Gottardo G., Coi A. and Nussdorfer G. G.: Effects of prolonged cyclosporine-A treatment on the morphology and function of rat adrenal cortex. *Endocrinology* **125** (1989) 1407–1413.
22. Szalay K. S.: Effect of pituitary intermediate lobe extract on steroid production by the isolated zona glomerulosa and fasciculata cells. *Acta Physiol. Hung.* 57 (1981) 225–231.
23. Sandor T., Fazekas A. G. and Robinson B. H.: The biosynthesis of corticosteroids throughout the vertebrates. In *General, Comparative and Clinical Endocrinology of the Adrenal Cortex* (Edited by I. Chester-Jones and I. W. Henderson). Academic Press, London (1976) pp. 25–142.
24. Rosenthal J., Escobar-Jimenez F. and Raptis S.: Prevention by somatostatin of rise in blood pressure and plasma renin activity mediated by β-receptor stimulation. *Clin. Endocr.* **6** (1977) 455–462.
25. Izumi Y., Honda M. and Hartano M.: Effect of somatostatin on plasma renin activity. *Endocr. Jap.* **26** (1979) 389–394.
26. Sieber C., Gnädiger M., Del Pozo E., Shaw S. and Weidmann P.: Effect of a new somatostatin analogue SMS 201-995 (Sandostatin) on the renin-aldosterone axis. *Clin. Endocr.* 28 (1988) 25–32.
27. Nussdorfer G. G.: Cytophysiology of the adrenal cortex. *Int. Rev. Cytol.* **98** (1986) 1–405.
28. Miller W. L.: Molecular biology of steroid hormone synthesis. *Endocrine Rev.* **9** (1988) 295–318.
29. Hanukoglu I.: Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *J. Steroid Biochem. Molec. Biol.* 43 (1992) 779–804.
30. Nussdorfer G. G. and Mazzocchi G.: Long-term effects of ACTH on rat adrenocortical cells: a coupled stereological and enzymological study. *J. Steroid Biochem.* **19** (1983) 1753–1756.
31. Mazzocchi G., Malendowicz L. K., Rebuffat P., Robba C., Gottardo G. and Nussdorfer G. G.: Short- and long-term effects of ACTH on the adrenal zona glomerulosa of the rat: a coupled stereological and enzymological study. *Cell Tiss. Res.* **243** (1986) 303–310.
32. Moses H. L., Davis W. W., Rosenthal A. S. and Garren L. D.: Adrenal cholesterol: localization by electron-microscope autoradiography. *Science* **163** (1969) 1203–1205.
33. Mazzocchi G., Robba C., Meneghelli V. and Nussdorfer G. G.: Effects of ACTH and aminoglutethimide administration on the morphological and functional responses of rat adrenal zona fasciculata to a prolonged treatment with 4-aminopyrazolopyrimidine. *J. Anat.* 154 (1987) 55–61.
34. Gwynne T. and Strauss J. F. III: The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. *Endocrine Rev.* 3 (1982) 299–329.
35. Belloni A. S., Neri G., Andreis P. G., Musajo F. G., Boscaro M., Mazzocchi G. and Nussdorfer G. G.: Effects of prolonged sodium restriction on the morphology and function of rat adrenocortical autotransplants. *Cell Tiss. Res.* **265** (1991) 35–41.
36. Przybylski J. and Krzeski R.: Direct vascular connection between adrenal gland and kidney may play an important role in pathogenesis of hypertension. *Medical Hypotheses* 35 (1991) 351–352.
37. Bornstein S. H., Ehrhart-Bornstein M., Scherbaum W. A., Pfeiffer E. F. and Holst J. J.: Effects of splanchnic nerve stimulation on the adrenal cortex may be mediated by chromaffin cells in a paracrine manner. *Endocrinology* **127** (1990) 900–906.
38. Hinson J. P.: Paracrine control of adrenocortical function: a new role for the medulla? *J. Endocr.* 124 (1990) 7–9.
39. Andreis P. G., Neri G. and Nussdorfer G. G.: Corticotropin-releasing hormone (CRH) directly stimulates corticosterone secretion by the rat adrenal gland. *Endocrinology* 128 (1991) 1198–1200.
40. Andreis P. G., Neri G., Belloni A. S., Mazzocchi G. and Nussdorfer G. G.: Interleukin-1β enhances corticosterone secretion by acting directly on the rat adrenal gland. *Endocrinology* 129 (1991) 53–57.
41. Ehrhart-Bornstein M., Bornstein S. R., Scherbaum W. A., Pfeiffer E. F. and Holst J. J.: Role of vasoactive intestinal peptide in a neuroendocrine regulation of the adrenal cortex. *Neuroendocrinology* 54 (1991) 623–628.
42. Andreis P. G., Neri G., Mazzocchi G., Musajo F. G. and Nussdorfer G. G.: Direct secretagogue effect of corticotropin-releasing factor on the rat adrenal cortex: the involvement of the zona medullaris. *Endocrinology* 131 (1992) 69–72.
43. Bornstein S. R. and Ehrhart-Bornstein M.: Ultrastructural evidence for a paracrine regulation of the rat adrenal cortex mediated by the local release of catecholamines from chromaffin cells. *Endocrinology* 131 (1992) 3126–3128.
44. Hinson J. P., Kapas S., Orford C. D. and Vinson G. P.: Vasoactive intestinal peptide stimulation of aldosterone secretion by the rat adrenal cortex may be mediated by the local release of catecholamines. *J. Endocr.* 133 (1992) 253–258.
45. Mazzocchi G., Musajo F. G., Malendowicz L. K., Andreis P. G. and Nussdorfer G. G.: Interleukin-1β stimulates corticotropin-releasing hormone (CRH) and adrenocorticotropin (ACTH) release by rat adrenal gland *in vitro*. *Molec. Cell. Neurosci.* 4 (1993) 267–270.
46. Lundberg J. M., Hamberger B., Schultzberg M., Höckfelt T., Granberg P. O., Efendić S., Terenius L., Goldstein M. and Luft R.: Enkephalin- and somatostatin-like immunoreactivities in human adrenal medulla and pheochromocytoma. *Proc. Natn. Acad. Sci. U.S.A.* 76 (1979) 4079–4083.
47. Corder R., Mason D. F. J., Perret D., Lowry P. J., Clement-Jones V., Linton E. A., Besser G. M. and Rees L. M.: Simultaneous release of neurotensin, somatostatin, enkephalins and catecholamines from perfused cat adrenal glands. *Neuropeptides* 3 (1982) 9–17.
48. Saito H., Saito S., Ohuchi T., Oka M., Sano T. and Hosoi E.: Co-storage and co-secretion of somatostatin and catecholamines in bovine adrenal medulla. *Neurosci. Lett.* 52 (1984) 43–47.
49. Osamura R. Y., Tsutsumi Y., Yanaihara N., Imura H. and Watanabe K.: Immunohistochemical studies for multiple peptide-immunoreactivities and co-localization of met-enkephalin-Arg^6-Gly^7-Leu^8, neuropeptide Y and somatostatin in human

adrenal medulla and pheochromocytomas. *Peptides* 8 (1987) 77–87.

50. Vincent S. R., McIntosh C. H. S., Reiner P. B. and Brown J. C.: Somatostatin immunoreactivity in the cat adrenal medulla. *Histochemistry* 87 (1987) 483–486.
51. Gallo-Payet N., Pothier P. and Isler H.: On the presence of chromaffin cells in the adrenal cortex: their possible role in adrenocortical function. *Biochem. Cell. Biol.* 65 (1987) 588–592.
52. Bornstein S. R., Ehrhart-Bornstein M., Usadel H., Böckmann M. and Scherbaum W. A.: Morphological evidence for a close interaction of chromaffin cells with the cortical cells within the adrenal gland. *Cell Tiss. Res.* 265 (1991) 1–9.