Pergamon

0960-0760(94)00106-5

J. Steroid Biochem. Molec. Biol. Vol. 51, No. 1/2, pp. 81–88, 1994
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0960-0760/94 $7.00 + 0.00

Evidence that Endogenous Vasoactive Intestinal Peptide (VIP) Plays a Role in the Maintenance of the Growth and Steroidogenic Capacity of Rat Adrenal Zona Glomerulosa

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The effects of a 7-day intraperitoneal infusion with VIP (0.03 nmol.kg^{-1}.min^{-1}) and its antagonist [4-Cl-D-Phe^{6},Leu^{17}]-VIP (VIP-A; 3 nmol.kg^{-1}.min^{-1}) were studied in sham and bilaterally adrenalectomized rats bearing ACTH and angiotensin II (ANG-II)-responsive adrenocortical autotransplants. VIP significantly increased plasma aldosterone (ALDO) concentration (PAC) and lowered plasma renin activity (PRA) in both groups of animals, without affecting plasma levels of ACTH and corticosterone. This treatment caused a marked hypertrophy of adrenal zona glomerulosa (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 quarters obtained from VIP-infused rats evidenced a notable increase in both their basal and maximally ACTH- or ANG-II-stimulated ALDO secretion. The simultaneous infusion of rats with VIP-A completely reversed all these effects of VIP. The infusion with VIP-A alone caused, in sham-operated rats, a net decrease in PAC, coupled with a rise in PRA, and a marked atrophy of ZG and ZG cells; basal and maximally stimulated ALDO secretion of dispersed ZG cells was also significantly lowered. Conversely, VIP-A did not evoke any appreciable effect in autotransplanted rats. These findings suggest that endogenous VIP is specifically involved in the maintenance of the growth and secretory capacity of rat adrenal ZG. Since regenerated adrenocortical autotransplants, which are responsive to VIP but not to VIP-A infusion, are completely deprived of chromaffin cells, the hypothesis is advanced that adrenal medulla may be the source of endogenous VIP regulating ZG function.

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INTRODUCTION

Compelling evidence indicates that VIP acutely stimulates rat adrenal steroidogenesis *in vivo* [1, 2] and *in vitro* [3–7]. Furthermore, it has been shown that prolonged (7-day) infusion with VIP specifically enhances the growth and steroidogenic capacity of adrenal zona glomerulosa (ZG) in rats [8]. A specific binding of VIP has been autoradiographically demonstrated in the rat adrenal cortex [9], and high levels of VIP immunoreactivity are present in the rat adrenals, especially in the medulla [10–13]. On these grounds, the hypothesis that endogenous VIP may be

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Abbreviations: ACTH, adrenocorticotropic hormone; ALDO, aldosterone; ANG, angiotensin; ANOVA, analysis of variance; B, corticosterone; BP, systolic blood pressure; CRH, corticotropin-releasing hormone; HPLC, high pressure liquid chromatography; PAC, plasma aldosterone concentration; PBC, plasma corticosterone concentration; PRA, plasma renin activity; RIA, radio-immunoassay; SER, smooth endoplasmic reticulum; VIP, vasoactive intestinal peptide; VIP-A, vasoactive-intestinal-peptide antagonist; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis.
Received 21 Apr. 1994; accepted 30 May 1994.

involved in the physiologic control of adrenal functions in rats has been suggested [4, 5].

Here, we report findings indicating that prolonged administration of a specific competitive antagonist of VIP, the [4-Cl-D-Phe^6, Leu^{17}]-VIP (VIP-A)[14] exerts a specific inhibitory effect on the function and growth of rat ZG under basal conditions, and provide evidence that adrenal chromaffin cells may be the source of endogenous VIP regulating the activity of ZG.

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 from the excised adrenals were implanted in the *musculus gracilis*; the animals were used after 4 months of regeneration of the autotransplants [15]. Another group of rats was sham-adrenalectomized. The rats were kept under a 12:12 h light–dark cycle (light on at 8:00 a.m.) at 22 ± 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 Peninsula (Merseyside, England) and dissolved in 200 μl 0.9% NaCl: VIP (0.03 nmol.kg^{-1}.min^{-1}), VIP-A (3 nmol.kg^{-1}.min^{-1}) or VIP plus VIP-A. The control groups were infused with the saline vehicle. The dose of the peptides were chosen from previous studies [6] showing that VIP exerts its maximal secretagogue effect on rat-adrenal capsular preparations at a concentration 10^{-8} M, and that VIP-A completely suppresses it at a concentration two orders of magnitude higher. In fact, given that the peritoneal absorption rate in rats is very high and VIP half-life in blood is very short, and assuming 10–12 ml of blood per rat, it can be calculated that the infusion rates used produce blood concentrations of VIP and VIP-A of about 10^{-8} and 10^{-6} M, respectively.

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

Biochemical assays. Plasma 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). ACTH was extracted from plasma [16], and its concentration was determined by RIA (ACTH–RIA kit; IRE-Sorin, Vercelli, Italy). Aldosterone (ALDO) and corticosterone (B) were extracted and purified by HPLC, as described previously [17], and their plasma concentrations (PAC and PBC, respectively) measured by RIA (Aldo-CTK2; IRE-Sorin, and CTRX-RIA; Eurogenetix, Milan, Italy). Intra- and interassay variations were: ANG-I, 6.4 and 8.2%; ACTH, 6.0 and 7.6%; ALDO 5.4 and 6.8%; and B, 7.5 and 9.1%.

Morphology. The left adrenals were fixed in Bouin's solution, embedded in paraffin and serially cut at a thickness of 6–7 μm. Sliced pieces of the right glands were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in epon. Thick (0.5μm) and thin (60–70 nm) sections were cut with an 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, by conventional morphometric methods [18] as described in an earlier paper [19]. 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 stereological techniques described by Weibel [18], as described previously [19]. Adrenocortical autotransplants were sliced and processed for electron microscopy (see above). The volume and stereological parameters of subcapsular and juxta-septal ZG-like cells [15, 20] were evaluated as described above.

In vitro experiments

Four other groups of sham-operated and autotransplanted rats were infused for a week as in the *in vivo* experiments. The animals were decapitated, and adrenal glands and regenerated adrenocortical nodules were promptly removed. Adrenals were employed to obtain dispersed-cell preparations, while autotransplants were quartered [15].

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 [21]. 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 less than 7%. Dispersed cells obtained from 6 rats were pooled to obtain a single cell preparation, and 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

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

	Controls	VIP	VIP plus VIP-A	VIP-A
Sham-operated rats				
BP (mmHg)	130 ± 13	118 ± 10[a]	125 ± 10	135 ± 15
PRA (fmol/ml.h)	5.1 ± 1.4	4.1 ± 0.9[a]	4.8 ± 1.1	6.6 ± 2.2[a]
Plasma Na^+ concentration (mEq/l)	128.5 ± 14.2	134.4 ± 16.0	125.9 ± 14.8	130.4 ± 15.6
Plasma K^+ concentration (mEq/l)	5.1 ± 0.9	4.8 ± 0.8	4.6 ± 0.8	5.0 ± 1.1
ACTH plasma concentration (pM)	21.4 ± 6.8	19.8 ± 6.2	20.8 ± 7.1	23.5 ± 8.0
Autotransplanted rats				
BP (mmHg)	165 ± 18[A]	125 ± 15[b]	161 ± 18	170 ± 13
PRA (fmol/ml.h)	7.5 ± 1.8[A]	5.4 ± 1.1[a]	7.9 ± 2.0	8.0 ± 1.9
Plasma Na^+ concentration (mEq/l)	134. ± 13.7	130.6 ± 14.2	129.7 ± 13.5	132.5 ± 15.2
Plasma K^+ concentration (mEq/l)	5.8 ± 0.8	4.9 ± 0.8	4.7 ± 0.9	5.1 ± 1.0
ACTH plasma concentration (pM)	30.6 ± 8.9[A]	28.7 ± 8.0	31.0 ± 10.2	29.5 ± 8.7

Data are means ± SD ($n = 8$). [A]$P < 0.01$ compared with sham-operated rats; [a]$P < 0.05$, and [b]$P < 0.01$ compared with the respective control rats.

incubated ($n = 8$) with 10^{-8}M ANG-II or $ACTH_{1-24}$ (Sigma, St Louis, MO), or without any peptide. 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 concentration of ALDO in the supernatants was determined as described above (intra- and interassay variations were 7.4 and 8.2%, respectively).

Statistics

The data obtained were averaged per experimental group, and SD or SE was calculated. Statistical analysis was by ANOVA, followed by the Duncan's Multiple Range Test.

RESULTS

Prolonged infusion with VIP induced a moderate decrease in BP (−10%) and PRA (−20%) in sham-operated rats; VIP-A infusion completely reversed these effects of VIP; although the infusion of VIP-A alone did not alter BP, it caused a net rise in PRA (30%). Plasma Na^+, K^+ and ACTH concentrations were not affected by any treatment (Table 1). Autotransplanted rats displayed higher BP (27%), PRA (47%) and ACTH plasma concentration (42%) than sham-operated animals. VIP infusion provoked a marked lowering of BP(−25%) and PRA (−28%), reversed by the simultaneous infusion of VIP-A; the administration of VIP-A alone did not affect either BP or PRA. As in the case of sham-operated rats, none of these treatments altered other biochemical parameters (Table 1).

PAC and PBC were markedly lower in autotransplanted than in sham-operated animals (−60 and −40%, respectively) (compare Figs 1 and 2). VIP infusion significantly raised PAC in both sham-operated (34%) and autotransplanted rats (40%), and VIP-A reversed this effect (Figs 1 and 2); the infusion of VIP-A alone produced a 28% decrease in PAC in sham-operated rats (Fig. 1), but not in autotransplanted animals (Fig. 2). None of these treatments appeared to alter PBC (Figs 1 and 2).

Chronic VIP administration caused significant increases in the volume of the ZG (28%) and ZG cells (29%), without affecting ZG cell number or nuclear volume (Table 2); the average volume of ZG-like cells in the autotransplants also showed a significant rise (24%) (Table 4). ZG- and ZG-like-cell hypertrophy was associated with significant increases in the volume of mitochondrial compartment (27 and 23%, respectively), and in the surface areas per cell of mitochondrial cristae (30 and 25%) and SER membranes (39 and 32%). Conversely, the volume of the lipid-droplet

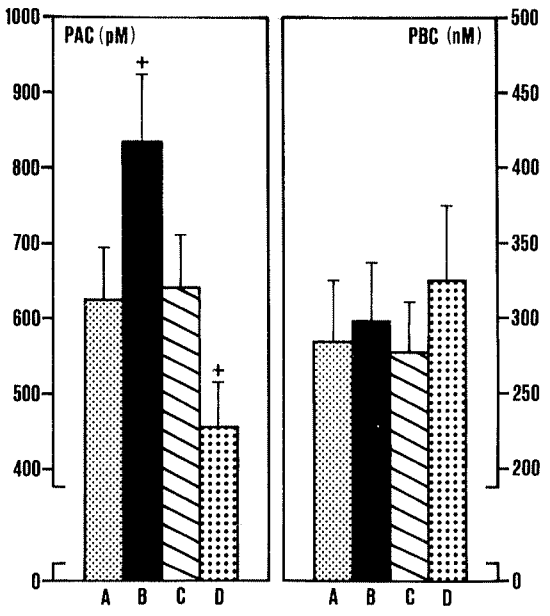

Fig. 1. Effects of VIP, VIP plus VIP-A and VIP-A infusions on basal PAC (left panel) and PBC (right panel) in sham-operated rats. A, Controls; B, VIP; C, VIP plus VIP-A; D, VIP-A. Bars represent group means ± SE ($n = 8$). $^+P < 0.05$ from control rats.

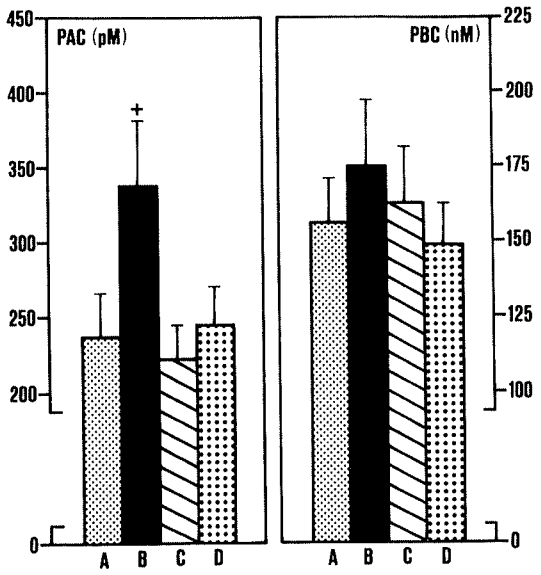

Fig. 2. Effects of VIP, VIP plus VIP-A and VIP-A infusions on basal PAC (left panel) and PBC (right panel) in rats bearing adrenocortical autotransplants. A, Controls; B, VIP; C, VIP plus VIP-A; D, VIP-A. Bars represent group means ± SE ($n = 8$). $^{+}P < 0.05$ from control rats. Control group of autotransplanted rats differs from control group of sham-operated rats: PAC, −60% ($P < 0.01$); PBC, −40% ($P < 0.01$).

compartment underwent a marked reduction in both types of cells (−47 and −45%) (Tables 3 and 4). VIP-A completely annulled the VIP effects on both ZG (Tables 2 and 3) and ZG-like cells (Table 4). The chronic infusion of VIP-A alone evoked, in sham-operated rats, effects opposite to those elicited by VIP: decreases in the volume of ZG (−24%), ZG cells (−25%) and mitochondrial compartment (−26%), and in the surface areas per cell of mitochondrial cristae (−26%) and SER membranes (−41%); in addition, rats infused with VIP-A alone showed a conspicuous increase in the volume of lipid-droplet compartment (about 2-fold) (Tables 2 and 3). On the contrary, VIP-A infusion did not alter the morphology of ZG-like cells of autotransplants (Table 4). None of these treatments evoked appreciable alterations in the ZF of sham-operated rats (Table 2).

ANG-II (10^{-8}M) and ACTH (10^{-8}M) markedly raised ALDO output by both dispersed ZG cells (6.9- and 8.8-fold, respectively) and autotransplant quarters (2.1- and 2.3-fold) of saline-infused rats (Table 5). VIP infusion enhanced by about one-third both basal and stimulated ALDO secretion from both preparations, and this effect of VIP was annulled by the simultaneous administration of VIP-A (Table 5). The infusion of VIP-A alone decreased to about 70% basal and stimulated ALDO production by dispersed ZG cells of sham-operated rats, but not by autotransplant quarters (Table 5).

DISCUSSION

According to previous studies [8], the prolonged administration of VIP, at a dose that presumably is able to maintain a blood concentration of the peptide near the maximal effective one *in vitro* (see Experimental), markedly enhances the growth and secretory capacity of rat adrenal ZG. The moderate lowering in PRA may be easily interpreted as the negative feedback response of the renin–angiotensin system to the increased PAC; it may account, along with the well-known vasodilatatory effect of VIP [22], for the small decrease in BP. VIP-A, at an infusion rate 100-times higher than that of VIP, completely blocks the VIP adrenoglomerulotrophic effect, and, when infused alone, caused a significant inhibition of the growth and steroidogenic capacity of ZG.

The VIP- and VIP-A-induced hypertrophy and atrophy, respectively, of ZG and its parenchymal cells are coupled with increases and decreases in the volume of the mitochondrial compartment and SER. These morphologic data accord well with the notable enhancement or depression of basal and maximally agonist-stimulated ALDO secretion by dispersed ZG cells obtained from VIP- and VIP-A-infused rats, respectively. In fact, the enzymes of ALDO synthesis are located in both mitochondria and SER (see [23–25] 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 [26, 27]. The increased

Table 2. Effects of VIP, VIP plus VIP-A and VIP-A infusions on the morphometric parameters of rat adrenal gland

	Controls	VIP	VIP plus VIP-A	VIP-A
ZG				
Volume of zona (mm^3)	2.841 ± 0.701	3.649 ± 1.128[a]	2.798 ± 0.790	2.172 ± 0.506[a]
Number of cells ($\times 10^3$)	3003 ± 684.7	3084 ± 651.8	2898 ± 716.3	3058 ± 705.2
Volume of cells (μm^3)	756.8 ± 195.2	972.7 ± 301.4[a]	769.5 ± 215.2	568.2 ± 153.8[a]
Volume of nuclei (μm^3)	118.2 ± 19.4	130.4 ± 18.2	120.8 ± 13.4	125.7 ± 16.2
ZF				
Volume of zona (mm^3)	15.029 ± 3.542	15.587 ± 4.102	16.008 ± 4.512	14.932 ± 3.198
Number of cells ($\times 10^3$)	7539 ± 1612	7574 ± 1578	7924 ± 1756	7661 ± 1552
Volume of cells (μm^3)	1794 ± 501.9	1852 ± 612.9	1818 ± 611.4	1754 ± 490.7
Volume of nuclei (μm^3)	165.8 ± 24.7	164.5 ± 27.1	175.5 ± 25.1	170.2 ± 20.8

Data are means SD ($n = 8$). [a] $P < 0.05$ compared with control rats.

Table 3. Effects of VIP, VIP plus VIP-A and VIP infusions on the stereological parameters of rat ZG cells

	Controls	VIP	VIP plus VIP-A	VIP-A
Volume of mitochondrial compartment (μm^3/cell)	128.1 ± 35.6	162.8 ± 43.8[a]	135.7 ± 33.9	94.5 ± 30.1[a]
Surface area of mitochondrial cristae (μm^2/cell)	2357 ± 661.5	3065 ± 995.8[a]	2471 ± 709.5	1738 ± 490.2[a]
Surface area of SER (μm^2/cell)	6018 ± 1418	8374 ± 2019[b]	5821 ± 1502	3519 ± 910.5[b]
Volume of lipid-droplet compartment (μm^3/cell)	38.7 ± 16.9	20.6 ± 13.8[b]	42.3 ± 18.5	75.4 ± 30.2[b]

Data are means SD ($n = 8$). [a]$P < 0.05$ and [b]$P < 0.01$ compared with control rats

or lowered utilization of cholesterol in ALDO synthesis occurring in VIP- and VIP-A-infused rats, coupled with a presumably normal uptake of cholesterol from serum lipoproteins, may well account for the marked decrease or increase in the volume of the lipid-droplet compartment. In fact, it is commonly agreed that cholesterol and its esters are stored in adrenocortical lipid droplets [23, 28, 29], and that lipoprotein uptake by adrenocortical cells is a receptor-mediated process mainly (if not exclusively) controlled by ACTH (see [30] for review), secretion of which is not affected by VIP-A treatment.

Our findings obtained in VIP-A-infused animals strongly suggest that, under basal conditions, endogenous VIP is involved in the positive control of the secretion and growth of ZG in rats. This contention is supported by the fact that (i) VIP-A does not acutely affect either basal or agonist-stimulated ALDO secretion by isolated ZG cells [6, 7], a finding indicating that VIP-A does not affect rat ZG function per se, but only competes with VIP receptors; and (ii) the anti-adrenoglomerulotrophic action of VIP-A is totally reversed by the simultaneous infusion of VIP.

Under normal circumstancies, the blood level of VIP is too low for this peptide to exert its modulatory effect on adrenals; consistently with this, the existence of a possible local (intra-adrenal) source of endogenous VIP has been demonstrated in rats [10–13]. Our experiments with autotransplanted rats confirm the existence of this source and suggest its localization in the zona medullaris.

Regenerated adrenocortical nodules secrete both mineralo- and glucocorticoids, and are responsive to ACTH and ANG-II; their subcapsular and juxta-septal ZG-like cells have ANG-II receptors [15, 20] and display a clearcut ALDO response to acute *in vitro* exposure to VIP [6]. However, due to the lower weight of regenerated adrenocortical tissue in comparison to that of normal adrenals [15, 20], PAC and PBC are markedly lower than in sham-operated rats, which may explain why PRA and plasma ACTH concentration are relatively higher. Higher PRA and the consequent raised production of ANG-II may account for the hypertension seen in autotransplanted animals. Autotransplants from adrenal capsular fragments are completely devoid of chromaffin cells, a contention based on both morphological (serial sectioning) and biochemical findings (HPLC evaluation of catecholamine content)[15].

Autotransplanted rats responded to VIP infusion as sham-operated animals: PAC is raised, PRA and BP are lowered, and growth and steroidogenic capacity of ZG-like cells are notably enhanced. Parenthetically, it must be noted that VIP-induced decrease in BP is markedly higher in autotransplanted than in sham-operated rats (−25 vs −10%). A tentative explanation of this finding may be that in sham-operated (but obviously not in autotransplanted) rats the hypotensive

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

	Controls	VIP	VIP plus VIP-A	VIP-A
Volume of cells (μm^3/cell)	658.2 ± 170.3	814.1 ± 201.5[a]	619.8 ± 148.6	700.8 ± 183.4
Volume of nuclei (μm^3/cell)	89.1 ± 10.4	93.6 ± 11.2	90.4 ± 10.6	85.7 ± 9.9
Volume of mitochondrial compartment (μm^3/cell)	142.3 ± 39.8	176.8 ± 41.8[a]	135.7 ± 35.6	151.2 ± 50.6
Surface area of mitochondrial cristae (μm^2/cell)	2561 ± 612.7	3196 ± 712.9[a]	2483 ± 721.2	2733 ± 784.1
Surface area of SER (μm^2/cell)	5017 ± 1617	6628 ± 1811[a]	4653 ± 1508	5529 ± 1715
Volume of lipid-droplet compartment (μm^3/cell)	8.7 ± 4.6	4.8 ± 2.1[a]	9.1 ± 3.7	6.9 ± 4.2

Data are means SD ($n = 8$). [a]$P < 0.05$ compared with control rats.

Table 5. Effects of VIP, VIP plus VIP-A and VIP-A infusions on basal and stimulated ALDO secretion of isolated ZG cells and autotransplant quarters

	Controls	VIP	VIP plus VIP-A	VIP-A
Isolated ZG cells (pmol/10^6 cells.h)				
Basal	54.2 ± 15.8	72.3 ± 24.9[a]	58.1 ± 14.5	39.4 ± 12.5[a]
ANG-II (10^{-8} M)	308.1 ± 84.5	411.5 ± 138.1[a]	321.4 ± 95.2	221.7 ± 60.2[b]
ACTH (10^{-8} M)	481.6 ± 162.7	645.6 ± 207.2[a]	458.2 ± 145.6	332.9 ± 115.8[b]
Autotransplant quarters (pmol/mg.h)				
Basal	12.5 ± 4.2	18.8 ± 6.8[b]	11.7 ± 3.5	12.1 ± 3.9
ANG-II (10^{-8} M)	25.7 ± 6.4	34.5 ± 11.0[a]	24.9 ± 5.4	27.8 ± 8.5
ACTH (10^{-8} M)	28.9 ± 7.5	36.4 ± 10.9[a]	29.0 ± 8.1	26.5 ± 7.6

Data are means SD ($n = 8$). [a]$P < 0.05$, and [b]$P < 0.01$ compared with control group.

effect of VIP is counteracted by the well-known stimulatory action of this peptide on catecholamine release by chromaffin cells [31, 32]. The simultaneous administration of VIP-A annuls all the effects of VIP. However, at variance with sham-operated rats, the infusion of VIP-A alone does not exert any effect in autotransplanted animals. This last result clearly indicates that the local production of endogenous VIP is not operative in autotransplanted rats. On these grounds, it seems reasonable to hypothesize that the source of endogenous VIP, exerting (under basal conditions) an adrenoglomerulotrophic effect, is the adrenal medulla. Many lines of evidence indicate that rat-adrenal medulla, by secreting catecholamines and many regulatory peptides, exerts a paracrine control of the cortical function [33–39], the morphologic reflection of which may be the presence of several islets of chromaffin cells scattered in the adrenal cortex of this species [40, 41]. Our present results lend support to this view.

Evidence indicates that catecholamines are able to enhance steroidogenesis *in vitro*, being ZG and ALDO secretion their main target in rodents [42–47], and that VIP is a neurotransmitter in adrenal medulla, being a potent activator of adenylyl-cyclase and catecholamine release [31, 32, 48, 49]. Hinson *et al.* [5] and Mazzocchi *et al.* [6] suggested that the acute mineralocorticoid secretagogue effect of VIP may be indirectly mediated, at least in part, by the enhanced release of catecholamines, since the β-blocker (−)alprenolol strongly attenuates the *in vitro* VIP-induced rise in ALDO yield by adrenal slices. However, this indirect mechanism does not appear to underlie the effect of VIP on the maintenance and stimulation of rat adrenal ZG growth and secretory capacity, inasmuch as this action of VIP can be observed also in rats bearing adrenocortical autotransplants lacking chromaffin cells. This contention fits wells with the presence of VIP specific receptors in rat ZG cells [9].

Before concluding, comment should be made on the absence of effect of prolonged VIP infusion on ACTH secretion and ZF function in rats, a finding that appears to be in disagreement with the following two sets of evidence. First, VIP has been reported to acutely enhance pituitary ACTH release *in vitro* [50–52], an effect that *in vivo* is probably mediated by CRH [53–55] and which accords well both with the presence of a significant number of VIP- and VIP mRNA-containing perikarya in the parvocellular part of the hypothalamic paraventricular nucleus [56, 57], and with demonstrations of VIP immunoreactivity in anterior–pituitary cells [58–60]. Secondly, Li *et al.* [61] reported convincing findings that VIP can occupy ACTH receptors in brain and adrenals; we have shown that VIP stimulates *in vitro* glucocorticoid secretion by rat adrenals, an effect attenuated not only by VIP-A, but also by corticotropin-inhibiting peptide [6, 7], a competitive antagonist of ACTH [62].

We tentatively explain these discrepancies by assuming that during prolonged VIP infusion, the negative feedback mechanisms controlling the hypothalamo–pituitary CRH/ACTH system have dampened the acute agonistic effect of the peptide on ACTH release. In this connection, it is interesting to recall that a marked desensitization of the hypothalamo–pituitary–adrenal axis has been shown to occur after prolonged administration of CRH or vasopressin [63]. In addition, the nonspecific weak activation by VIP of ACTH receptors is masked *in vivo* by the basal tonic one by circulating ACTH. This would explain why PBC significantly increases after exogenous administration of VIP [1] in hypophysectomized animals, but not in animals with an intact pituitary gland.

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