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Stimulatory Effect of Vasoactive Intestinal Peptide (VIP) on the Secretory Activity of Dispersed Rat Adrenocortical Cells. Evidence for the Interaction of VIP with ACTH Receptors

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VIP dose-dependently increased basal, but not submaximally ACTH (10^{-10} M)-stimulated, aldosterone (ALDO) and corticosterone (B) secretion of dispersed rat capsular and inner adrenocortical cells, respectively. The maximal stimulatory effect (60–70% rise) was obtained with a VIP concentration of 10^{-8} M. [4-Cl-D-Phe^6,Leu^{17}]-VIP, a VIP-receptor antagonist (VIP-A), and corticotropin-inhibiting peptide (CIP), an ACTH receptor antagonist (both 10^{-6} M), completely annulled VIP (10^{-8} M)-evoked rises in basal ALDO and corticosterone secretions. The ACTH (10^{-10} M)-enhanced (about 5-fold) production of both hormones was completely reversed by CIP (10^{-6} M) and only partially reduced (about −30%) by VIP-A (10^{-6} M). The hypothesis is advanced that the weak secretagogue effect of VIP on dispersed rat capsular and inner adrenocortical cells may be due to its positive interaction with ACTH receptors.

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INTRODUCTION

Compelling evidence indicates that VIP exerts an acute secretagogue effect on the mammalian adrenal cortex *in vivo* [1, 2] and *in vitro* [3–5]. However, *in vitro* findings were obtained by employing perifusion of adrenal-capsular tissue or perifusion of the entire gland. According to Hinson *et al.* [5], the integrity of the adrenal tissue is needed so that VIP may exert its stimulatory effect, since VIP would mainly act indirectly by increasing the flow rate of the perfusion medium and adrenalin release by the islets of chromaffin cells located in the cortex.

The studies dealing with VIP effect on dispersed adrenocortical cells gave controversial results. Enyedi *et al.* [6] and Hinson *et al.* [5] reported no effect, while Morera *et al.* [7] and Li *et al.* [8] described a weak but significant stimulatory effect of VIP on the mouse adrenal Y-1 cell line and rat inner adrenocortical cells, respectively. It, therefore, seemed worthwhile to investigate the effect of VIP on the secretory activity of dispersed rat capsular (zona glomerulosa, ZG) and inner (zona fasciculata and zona reticularis, ZF/ZR) cells.

EXPERIMENTAL

All chemicals were obtained from Peninsula (Merseyside, England), with the following exceptions: collagenase (type I) and DNase I from Sigma (St Louis, MO), and tissue culture Medium 199 from DIFCO (Detroit, MI). The animals used were male Wistar rats (300 ± 30 g body wt), purchased from Morini (Reggio Emilia, Italy). The rats were killed by cervical dislocation, and their adrenals were promptly removed and freed of pericapsular fat.

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Abbreviations: ACTH, adrenocorticotropic hormone; ALDO, aldosterone; ANOVA, analysis of variance; B, corticosterone; CIP, corticotropin-inhibiting peptide; HPLC, high performance liquid chromatography; RIA, radioimmunoassay; VIP, vasoactive intestinal peptide; VIP-A, vasoactive intestinal peptide antagonist; ZF, zona fasciculata; ZG, zona glomerulosa; ZR, zona reticularis.

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Preparation of dispersed adrenocortical cells

Adrenal glands were gently decapsulated to separate ZG from ZF/ZR, and then bisected; decapsulated adrenal halves were enucleated to remove zona medullaris. Dispersed capsular and inner adrenocortical cells were obtained by collagenase/DNase I digestion and mechanical disaggregation [9]. 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 <6–7%. Dispersed cells obtained from the adrenal pairs of 4 rats were pooled to obtain a single cell suspension, and 6 or 8 cell preparations for each incubation experiment were employed.

Incubation procedures

Dispersed cells were put in Medium 199 and potassium-free Krebs–Ringer bicarbonate buffer with 0.2% glucose (2:1 v/v), containing 5 mg/ml human serum albumin. Dispersed capsular and inner cells were incubated (3×10^5 cells/ml) as follows: (i) increasing concentrations of VIP (human, porcine, rat) (from 10^{-12} to 10^{-6} M), in the presence or absence of ACTH (rat) 10^{-10} M; (ii) VIP receptor antagonist (VIP-A; [4-Cl-D-Phe^6,Leu^{17}]-VIP) 10^{-6} M or corticotropin-inhibiting peptide (CIP; $ACTH_{7-38}$) 10^{-6} M, in the presence or absence of VIP 10^{-8} M; and (iii) VIP-A or CIP (both 10^{-6} M), in the presence or absence of ACTH 10^{-10} M. The incubation was carried out for 90 min in a shaking bath at 37°C in an atmosphere of 95% O_2 and 5% CO_2.

Hormonal assays

The production of aldosterone (ALDO) by capsular cells, and corticosterone (B) by inner cells was assayed by measuring the concentrations of the two hormones in the incubation media. Hormones were extracted and purified by HPLC, as detailed previously [10], and their concentrations were measured by RIA, using the following kits. ALDO-CTK2 (IRE-Sorin, Vercelli, Italy); sensitivity: 5 pg/ml; cross-reactivity: ALDO 100%, 17-iso-ALDO and other steroids <0.1%; intra- and interassay variations: 7.5 and 9.3%, respectively. CORTX-RIA kit (Eurogenetix, Milan, Italy); sensitivity: 25 pg/ml; cross-reactivity: B and cortisol 100%, 11-deoxycorticosterone and progesterone 2%, other steroids <0.001%; intra- and interassay variations: 5.3 and 6.9%, respectively.

Statistics

The data were averaged per experimental group, and their statistical comparison was done by Student's *t*-test or by one-way ANOVA followed by the Multiple Range Test of Duncan. A P value <0.05 was considered significant.

RESULTS AND DISCUSSION

VIP dose-dependently enhanced basal ALDO secretion by capsular cells [$F(5,25) = 2.95$, $P < 0.01$]

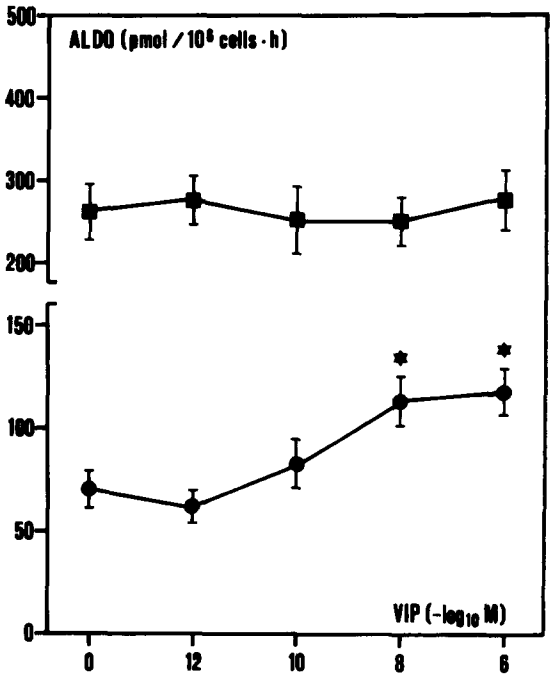

Fig. 1. Acute effect of VIP on basal (●) and ACTH (10^{-10} M)-stimulated (■) ALDO secretion by dispersed rat capsular cells. Values are means ± SE ($n = 6$). *$P < 0.01$ from control group.

(Fig. 1), and basal B secretion by inner cells [$F(5,25) = 4.08$, $P < 0.01$] (Fig. 2). The maximal increase (ALDO, 70%; B, 60%) was obtained with a VIP concentration 10^{-8} M. These data clearly show a weak direct secretagogue effect of VIP on rat adrenocortical

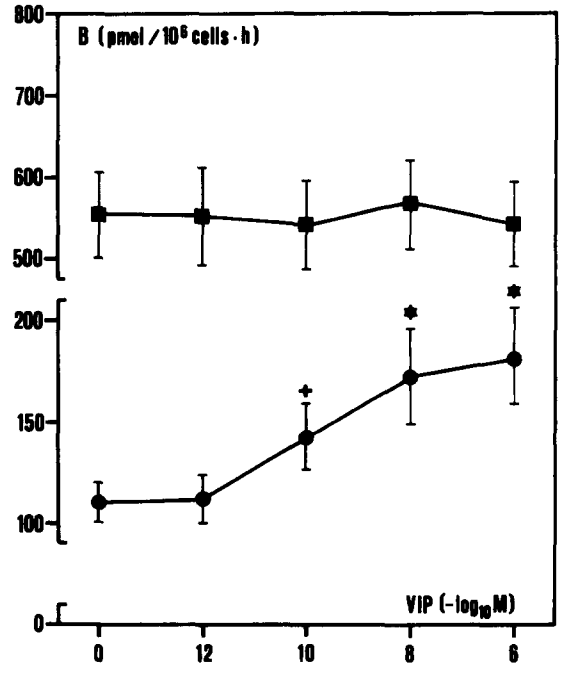

Fig. 2. Acute effect of VIP on basal (●) and ACTH (10^{-10} M)-stimulated (■) B secretion by dispersed rat inner cells. Values are means ± SE ($n = 6$). $^+P < 0.05$ and *$P < 0.01$ from control group.

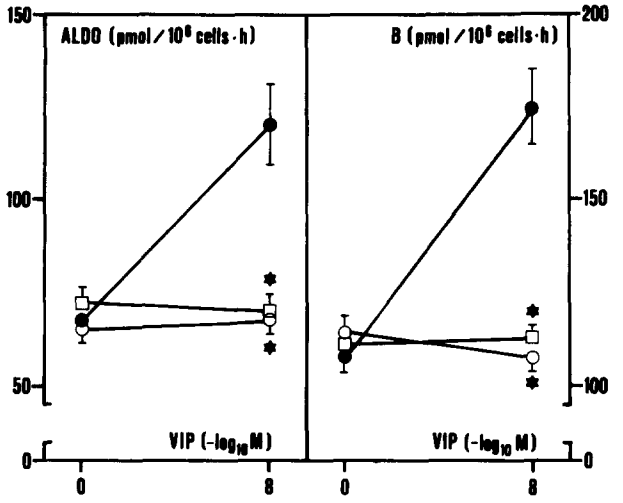

Fig. 3. Stimulation by VIP (10^{-8} M) of ALDO and B secretions by dispersed rat adrenocortical cells (●), and its inhibition by VIP-A 10^{-6} M (○) and CIP 10^{-6} M (□). Values are means ± SE ($n = 8$). *$P < 0.01$ from the respective control group.

cells, and accord well with the autoradiographic demonstration of the existence of a specific VIP binding in adrenocortical cells [11].

However, VIP did not affect hormonal secretion of both kinds of cell preparations submaximally stimulated (4.5- to 5.4-fold) by ACTH 10^{-10} M (Figs 1 and 2), the most potent stimulator of *in-vitro* secretion of both ZG and ZF/ZR cells [12]. This finding could be reasonably explained by assuming that the weak stimulatory effect of VIP is masked by that of ACTH and that both agonists share the same mechanism of action. In fact, evidence is available that, like ACTH, VIP enhances adenylyl-cyclase activity in some cell types, including Y-1 cell line [7] and adrenal chromaffin cells [13, 14]. An alternative possibility stems from the

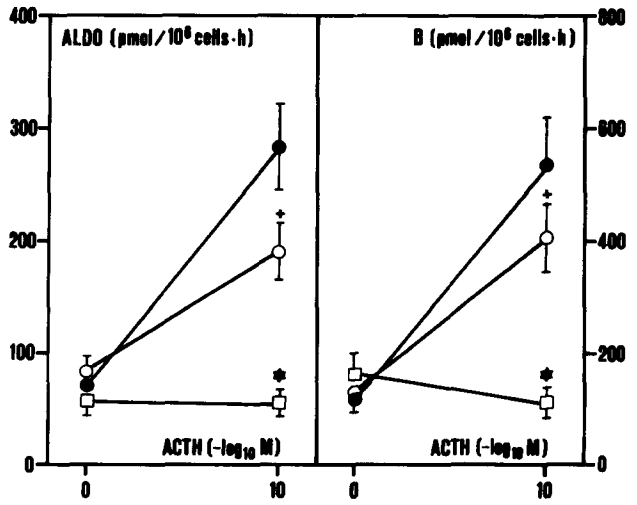

Fig. 4. Stimulation by ACTH (10^{-10} M) of ALDO and B secretions by dispersed rat adrenocortical cells (●), and its inhibition by VIP-A 10^{-6} M (○) and CIP 10^{-6} M (□). Values are means ± SE ($n = 8$). $^{+}P < 0.05$ and *$P < 0.01$ from the respective control group.

investigations of Li *et al.* [8], who reported convincing findings that VIP agonistically competes with ACTH receptors in brain and adrenals.

We checked this last possibility by the use of VIP-A and CIP, two competitive inhibitors of VIP and ACTH binding, respectively [15, 16]. VIP-A (10^{-6} M) and CIP (10^{-6} M) completely annulled a VIP (10^{-8} M)-evoked rise in the basal secretions of ALDO and B by capsular and inner cells, respectively (Fig. 3). The ACTH (10^{-10} M)-induced increases in ALDO and B secretions by capsular and inner cells were, in turn, completely reversed by CIP (10^{-6} M) and significantly reduced (−29/−33%) by VIP-A (10^{-6} M) (Fig. 4).

In light of these results the hypothesis is advanced that the weak secretagogue effect of VIP on ZG and ZF/ZR cells may be due to its positive interaction with ACTH receptors. Li *et al.* [8] showed that VIP competes only with a subtype of ACTH receptors (i.e. those recognizing a $ACTH_{11-24}$ sequence), and this would explain why VIP-A only partially hampers the secretagogue effect of the entire ACTH molecule.

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