Comparison of the Effects of VIP and PACAP on Steroid Secretion of Dispersed Rat Adrenocortical Cells

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(Received 10 March 1999; and accepted 22 April 1999)

ABSTRACT

The effects of vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP)-38 or -27, and their receptor antagonists (VIP-A, P38-A and P27-A) have been investigated on aldosterone and corticosterone secretion in dispersed rat zona glomerulosa (ZG) and zona fasciculata-reticularis (ZF/R) cells. VIP and PACAP-38 enhanced both aldosterone and corticosterone production, VIP being much more effective than PACAP-38. PACAP-27 elicited only a moderate increase in corticosterone production. The ACTH receptor antagonist corticotropin-inhibiting peptide and the β -adrenoceptor antagonist *l*-alprenolol did not affect hormonal response to the maximal effective concentration (10^{-6} M) of VIP and PACAPs. VIP-A, which is an antagonist of VPAC1 receptor subtype, counteracted only corticosterone response to PACAP-38. P38-A, which is an antagonist of PAC1 receptor and VPAC2 receptor subtypes, hampered aldosterone response to VIP and PACAP-38, and corticosterone response to VIP and PACAP-27. P27-A, whose receptor selectivity is not known. VIP-A potentiated corticosterone response to VIP, and aldosterone response to PACAP-38. These findings led us to conclude: (i) VIP and PACAPs stimulate secretion of rat adrenocortical cells, through the activation of specific receptors, being their effectiveness $VIP > PACAP-38 >> PACAP-27$; and (ii) aldosterone response of ZG cells to VIP and PACAPs is probably mediated by PAC₁ and VPAC₂ receptors, while corticosterone response of ZF/R cells involves also the VPAC₁ receptor subtype.

Vasoactive intestinal peptide (VIP) is a highly basic 28-amino acid peptide, and pituitary adenylate cyclase-activating polypeptide (PACAP) is a basic 38-amino acid C-terminally (-amidated) peptide, which were originally isolated from the gastrointestinal tract and hypothalamus, respectively. An alternative form of PACAP possessing the N-terminal 27-amino acid sequence of PACAP-38 was also isolated from

hypothalamic extracts, and named PACAP-27 (for review, see 3, 24). VIP and PACAP possess a remarkable amino-acid sequence homology, and act through common G protein-coupled VIP/PACAP receptors, three main subtypes of which are presently recognized and called PAC₁, VPAC₁ and VPAC₂ (for review, see 10).

Several investigations demonstrated the presence of a pleiad of biologically active peptides in the mammalian adrenal gland, which are localized in the medullary chromaffin cells, in the intrinsic ganglion neurons and/or in the nerve fibers of different origin. Nussdorfer (23) reviewed evidence that these peptides may affect

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adrenocortical growth and secretion, acting in a paracrine manner. VIP and PACAP belong to this group of regulatory peptides: they are contained in sizeable amounts in adrenals, adrenals are provided with VIP/PACAP binding sites, and the two peptides variously affect the *in vitro* secretory activity of adrenocortical cells (for review, see 24).

However, conflicting data are available on the direct effect of VIP and PACAP on mineralo- and glucocorticoid secretion. Therefore, the aim of this study was to compare the effects of the two peptides on aldosterone and corticosterone production of dispersed rat zona glomerulosa (ZG) and zona fasciculata-reticularis (ZF/R) cells, and to characterize the subtype(s) of VIP/PACAP receptors involved.

MATERIALS AND METHODS

VIP, PACAP-38, PACAP-27, the VIP antagonist (VIP-A)[Ac-Tyr¹, D-Phe²]-GRF (1-29) amide (29), the PACAP-38 antagonist (P38-A) PACAP(6-38)(26), and the PACAP-27 antagonist (P27-A) PACAP (6-27)(2) were obtained from Bachem (Bubendorf, Switzerland). ACTH (1-24), the ACTH-receptor antagonist corticotropin inhibiting peptide (CIP) (13), the β -adrenoceptor antagonist *l*-alprenolol (15), and other laboratory reagents were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.).

Adrenal glands, obtained from adult female Wistar rats, were gently decapsulated to separate ZG from ZF/R, and dispersed capsular (ZG) and inner (ZF/R) adrenocortical cells were obtained by collagenase digestion and mechanical disaggregation (12). Inner cell contamination of capsular-cell preparations, and the viability of dispersed cells were checked as previously detailed (17), and found to be less than 8% and higher than 92%, respectively. Dispersed cells obtained from 6-8 rats were pooled to obtain a single cell suspension, and six cell suspensions for each incubation experiment were employed.

Aliquots of each cell suspension (10^5 cells/mL in Krebs-Ringer bicarbonate buffer with 0.3% glucose and 0.2% bovine serum albumin) were incubated as follows: (i) VIP, PACAP-38 or PACAP-27 (from 10^{-12} to 10^{-6} M) alone or in the presence of 10^9 M ACTH(1-24); (ii) VIP, PACAP-38 or PACAP-27 (10^{-6} M) in the presence or absence of 10^{-5} M VIP-A, P38-A or P27-A, and (ii) CIP or *l*-alprenolol (10^{-6} M)

alone or in the presence of 10^{-6} M VIP, PACAP-38 or PACAP-27. The incubation was carried out in a shaking bath at 37°C for 60 min, in an atmosphere of 95% air-5% CO₂. At the end of the experiments, the incubation tubes were centrifuged at 4°C, and supernatants stored at -30°C.

Aldosterone and corticosterone were extracted from incubation media and purified by HPLC (22), and their concentrations estimated by specific RIA, as previously detailed (16). Intra- and interassay variation coefficients were: aldosterone, 5% and 7%; and corticosterone, 7% and 9%, respectively.

Data were expressed as means $\pm$ SEM, and their statistical comparison was done by ANOVA, followed by the multiple range test of Duncan.

RESULTS AND DISCUSSION

VIP concentration-dependently increased basal aldosterone and corticosterone secretion by dispersed ZG and ZF/R cells, respectively, maximal effective concentration being 10^{-6} M. Likewise, PACAP-38 enhanced basal production of the two hormones, but the effect became significant only at a concentration of 10^{-6} M. PACAP-27 did not affect basal secretion of aldosterone, but at a concentration of 10^{-6} M evoked a moderate rise in that of corticosterone (Fig. 1). ACTH-stimulated steroid secretion was not affected by the three peptides (data not shown).

Contrasting findings are available as to the direct effects of VIP and PACAP on mammalian adrenocortical cells. To summarize, Hinson *et al.* (11,12) did not observe any effect of VIP on dispersed rat adrenocortical cells, while Mazzocchi *et al.* (18) reported a clear-cut secretagogue action. VIP was found to stimulate cortisol production by dispersed bovine adrenocortical cells (14), but not aldosterone secretion of cultured calf ZG cells (6). This peptide also elicited a marked secretagogue action on both cultured human adrenocortical cells (7) and human adrenocortical carcinoma cell line NCI-H295 (6, 9). PACAP-38 did not affect secretion of dispersed rat and human adrenocortical cells (1, 21), but both PACAP-38 and PACAP-27 were reported to raise aldosterone secretion (and cyclic-AMP release) by bovine cultured ZG cells (6). Our present study provides evidence that VIP and PACAP are able to directly stimulate the secre-

Fig. 1 Effects of PACAPs and VIP on aldosterone (upper panels) and corticosterone secretion (lower panels) of dispersed rat ZG and ZF/R cells, respectively. Data are means $\pm$ SEM ($n=6$). $^+P<0.05$ and $^*P<0.01$ from baseline value (B).

tory activity of both ZG and ZF/R cells of the rat adrenals, their effectiveness being $VIP > PACAP-38 >> PACAP-27$. According to Arimura *et al.* (4), the concentration of PACAP-27 in the rat adrenal is about 70-fold less than that of PACAP-38, and our results are in keeping with the minor role played by PACAP-27 in the tuning of rat adrenal secretory activity.

CIP significantly increased basal secretion of aldosterone, and this effect manifested itself even in the presence of VIP and PACAPs. This result accords well with the view that CIP is able to stimulate ZG cells through a mechanism involving the activation of angiotensin-II receptors and the consequent rise in the cytosolic Ca^{2+} concentration (17). Conversely, CIP did not alter either basal or VIP- and PACAP-stimulated corticosterone production. *l*-Alprenolol was ineffective (Fig. 2).

These findings are relevant because they rule out the possibility that VIP and PACAPs act by activating ACTH receptors and β -adrenoceptors located on adrenocortical cells. This possibility stems from the observations that (i) VIP, GH-RH and dynorphin compete for a common subtype of

Fig. 2 Effects of CIP (10^{-6} M) and *l*-alprenolol (*l*-AL, 10^{-6} M) on basal and PACAP-38 (P-38), PACAP-27 (P-27) or VIP (10^{-6} M)-stimulated aldosterone (upper panels) and corticosterone secretion (lower panels) of dispersed rat ZG and ZF/R cells, respectively. Bars are means $\pm$ SEM ($n=6$). $^AP<0.01$ from the respective control value; $^+P<0.05$ and $^*P<0.01$ from the respective baseline value (B).

ACTH receptors in brain and adrenals (14), and CIP impairs *in vitro* glucocorticoid secretagogue effect of VIP and PACAP (1, 19, 20); and (ii) the β -adrenoceptor antagonists, like *l*-alprenolol or atenolol, counteract aldosterone secretagogue effect of VIP (5, 12, 18) and PACAP-38 (1). Since these last *in vitro* results were obtained using capsular-adrenal or adrenal-slice preparations, our present observations support the view that VIP and PACAP may indirectly affect aldosterone secretion by eliciting the release of catecholamines, which in turn activate β -adrenoceptor located on ZG cells in a paracrine manner (for review, see 23).

The effects of VIP/PACAP-receptor antagonists, alone or combined with maximal effective concentrations of VIP, PACAP-38 or PACAP-27 are depicted in Figs. 3 and 4. To summarize: none

of the antagonists altered *per se* basal secretion of dispersed rat adrenocortical cells, with the exception of P38-A, which evoked a moderate lowering in aldosterone production. The aldosterone and corticosterone responses to VIP were decreased by P38-A, and corticosterone response was enhanced by both VIP-A and P27-A. Aldosterone response to PACAP-38 was suppressed by P38-A and potentiated by VIP-A, which on the contrary partially reduced corticosterone response. As expected, PACAP-27 did not affect aldosterone production, but in the presence of VIP-A a slight rise was found. Corticosterone response to PACAP-27 was abolished by P38-A and unaffected by other antagonists.

As mentioned in the Introduction, VIP and PACAPs act through at least three subtypes of receptors, whose binding potency is as follows: PAC1, PACAP-38 = PACAP-27 >> VIP; VPAC1, VIP = PACAP-27 > PACAP-38; and VPAC2, VIP = PACAP-38 = PACAP-27. VIP-A and P38-A are antagonists of VPAC1 and PAC1 receptors, while selective VPAC2-receptor antagonists are not known at present (for review, see 10, 24, 25). However, PACAP(6-38), *i.e.* P38-A, has been shown to possess also high affinity for VPAC₂ receptors (8).

In light of these considerations, our results allow us to draw the following conclusions. VIP and PACAP-38 stimulate aldosterone secretion

Fig. 3 Effect of VIP and PACAP antagonists (10^{-5} M) on basal and VIP-, PACAP-38- or PACAP-27-stimulated aldosterone secretion of dispersed rat ZG cells. Data are means $\pm$ SEM ($n=6$). $^+P<0.05$ and $^*P<0.01$ from the respective baseline value (B); $^aP<0.05$ and $^AP<0.01$ from the respective control value.

Fig. 4 Effect of VIP and PACAP antagonists (10^{-5} M) on basal and VIP-, PACAP-38- or PACAP-27-stimulated corticosterone secretion of dispersed rat ZF/R cells. Data are means $\pm$ SEM ($n=6$). $^+P<0.05$ and $^*P<0.01$ from the respective baseline value (B); $^aP<0.05$ and $^AP<0.01$ from the respective control value.

acting through PAC₁ and perhaps VPAC₂ receptors, since their effect is blocked only by PACAP(6-38). The potentiation of PACAP-38 effect by VIP-A can be consequent to a higher availability of PACAP to PAC₁ and VPAC₂ receptors due to the occupancy of VPAC₁ by the antagonist, and the same mechanism could explain the moderate aldosterone secretagogue of PACAP-27 in the presence of VIP-A. VIP enhances corticosterone secretion by activating PAC₁ and perhaps VPAC₂ receptors, because its effect is exclusively counteracted by P38-A and potentiated by VIP-A and P27-A, and the same holds true for PACAP-27. In contrast, the corticosterone secretagogue action of PACAP-38 appears to be exclusively mediated by VPAC₁ receptors, inasmuch as it is exclusively counteracted by VIP-A. Collectively, these findings suggest that while rat ZG cells are almost exclusively provided with PAC₁ and VPAC₂ receptors, ZF/R cells possess all three subtypes of receptors.

Obviously, our study leaves unsettled many issues, among which are the following. VIP is much more active than PACAP-38 in eliciting both aldosterone and corticosterone response, which appears to be in contrast with its higher affinity for VPAC₁ than PAC₁ receptors. PACAP-27 is much less effective than PACAP-38, despite their binding potency to PAC₁ and VPAC₂ is similar. Perhaps, the reported occurrence of two types of PAC₁ receptors, which possess similar affinity for both PACAPs (type A) and higher affinity for PACAP-38 than PACAP-27 (type B) (27, 28), could explain this last finding. In fact, it could be hypothesized that rat adrenocortical cells are mainly provided with PAC₁ type B receptors. All the subtypes of VIP/PACAP receptors have been cloned (for review, see 10), and further molecular-biological studies are needed to elucidate the distribution and the functional role played by VIP/PACAP receptors in the adrenal gland physiology.

Acknowledgements. This work was supported by SCSR grant No. A-P05A-030-13, and performed in the frame of the Polish-Italian Agreement of Scientific and Technical Cooperation.

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