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INTERACTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) WITH A MOUSE ADRENAL CELL LINE (Y-1) : SPECIFIC BINDING AND BIOLOGICAL EFFECTS

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SUMMARY

Y-1 cells specifically bind radiolabelled vasoactive intestinal peptide (VIP) with a dissociation constant of about 10^{-9} M. [^{125}I]-VIP bound was not displaced by ACTH. VIP stimulates both steroid and cAMP production, with half-maximal stimulation at 10^{-9} and 10^{-8} M, respectively. At maximal concentration VIP produces the same stimulation of steroidogenesis as ACTH, but induced three times lower production of cAMP than ACTH. Y-1 DNA synthesis is inhibited by VIP in a dose-dependent manner with half-maximal inhibition at 10^{-8} M. At submaximal concentrations the effects of VIP and ACTH on cAMP and steroid production and on inhibition of DNA synthesis are additive. Similar additive effects on cAMP production and on inhibition of DNA synthesis were observed at submaximal ACTH and maximal VIP concentration, but the phenomenon was no longer seen at maximal concentrations of both peptides. These data suggest that in Y-1 cells VIP stimulates, through its own distinct receptors, only a part of the pool of adenylate cyclase sensitive to ACTH.

Specific binding sites for vasoactive intestinal polypeptide (VIP) have been described in several tissues of the digestive tract, including liver (1-3), exocrine pancreas (4, 5) and enterocytes (6-9), fat cells (1, 2) and brain (10). In all these tissues VIP stimulates adenylate cyclase activity of plasma membranes and/or cAMP production by intact cells. Recently it has been reported (9) that VIP stimulates steroid production by a mouse adrenal cell line (Y-1). Since in this adrenal cell line ACTH, in addition to stimulating cAMP and steroid production (12), inhibits DNA synthesis (13-15), the effects of VIP on these three parameters were studied. Moreover the binding of radiolabeled VIP to Y-1 cells was investigated and compared to its biological effects.

MATERIALS AND METHODS

Y-1 cells were routinely grown in Ham's F-10 medium complemented with 10 % heat-inactivated horse serum, 2.5 % heat-inactivated fetal calf serum (FCS), penicillline (50 U/ml) and streptomycine (25 µg/ml) at 37°, in an atmosphere of 95 % air/5 % CO_2. Before each experiment the cells were

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harvested with phosphate buffer Ca^{2+} and Mg^{2+} free, pH 7.4, containing 1 mM EDTA and 0.1 % trypsin, plated on petri dishes and incubated for 24-48 hrs with the above medium. Then the medium was removed and the cells were synchronized by incubation in serum-deprivated medium (0.1 % fetal calf serum) for 72 hrs. In experiments in which the effects of several compounds on cAMP and steroid products and on DNA synthesis were investigated, the medium was replaced by Ham's F-10 medium 0.1 mM 3-isobutyl-1-methylxanthine (MIX), 20 mM N-2-hydroxyethylpiperazine-N'-3-ethanesulfonic acid (Hepes) pH 7.4, and the FCS concentrations indicated in the legends to Figures and Table . Two hrs later aliquots of the medium were removed for measurements of cAMP and 20α-dihydroprogesterone (20 OHP). [^{3}H]thymidine (2 µCi/ml) was added 10 hours later and the incubation continued for 8 additional hours. Then the medium was removed and the cells washed twice with cold phosphate buffer and twice with trichloroacetic acid 5 %. The cells were removed from the culture dish with 0.5 ml of 0.5 N NaOH containing 0.4 % deoxycholate and counted in 5 ml of scintillation liquid.

Binding of ^{125}I-VIP (SA 200 µCi/µg) was performed by incubating the attached cells with Ham's F-10 medium 0.1 mM MIX, 20 mM Hepes, pH 7.4 containing 1 % FCS and ^{125}I-VIP in the absence or presence of unlabeled VIP at 37°C for the time indicated in the figures. At the end of the incubation the medium was removed and the cells washed five times with 3 ml of the above medium at 0°. Then the cells were removed with 0.5 ml of 0.5 N NaOH, 0.4 % deoxycholated and assayed for radioactivity in a gamma counter.

cAMP and 20 OHP were measured in triplicate by radioimmunoassay as described (14). Porcine VIP was graciously supplied by Dr. V. Mutt. Lysine-vasopressine was gift from Dr. S. Jard.

Porcine VIP was radioiodinated with carrier-free ^{125}I (IMS 300, 600-800 mCi/ml from Amersham) as previously described (2) with modifications (17). In a typical experiment the following were added sequentially at room temperature to a glass tube containing 5 µg of lyophilized VIP : 20 µl of 0.3 M sodium phosphate buffer (SPB) pH 7.5, 2.5 µl (1.5 mCi) of ^{125}I-solution and 5 µl of chloramine-T (5 µg freshly diluted in 0.3 M SPB). After a 30 sec. incubation, the reaction was terminated by adding 5 µl of sodium metabisulfite (10 µg in 0.3 M SPB) and 400 µl of 0.3 M SPB containing 2.5 % (w/v) bovine serum albumin and 0.04 % (w/v) bacitracin. The percentage of ^{125}I incorporated into VIP was monitored by adsorption of VIP on talc powder (17) and by precipitating a small aliquot of the reaction mixture with 10 % (v/v) trichloracetic acid, the two methods giving identical results. In the present experiment, this percentage was 70 % resulting in a specific activity of 200 Ci/g i.e. a mean of 0.3 atom of ^{125}I per molecule. We have shown that this ^{125}I-labeled VIP and native VIP have identical affinity for VIP binding sites (8).

RESULTS

Binding of ^{125}I VIP to Y-1 cells. The time course of ^{125}I-VIP binding to adrenal cells is shown in Fig. 1. Maximal values for total and specific binding were reached at 20 min, and remained in plateau for about 20 min. Thereafter both values declined slightly. On the contrary non-specific binding reached maximal value at 5 min and remained constant for at least 40 min. The kinetic of specific binding at 20° was slower, steady state was reached at 2 h (data not shown). However, since the biological effects of VIP on Y-1 cells were studied at 37°, all the following binding studies were carried out at 37° for 30 min.

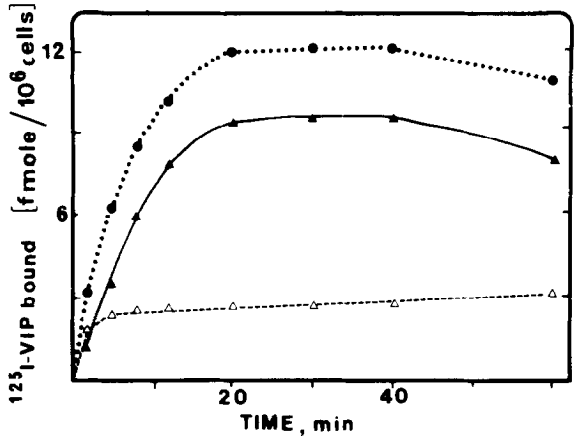

Figure 1 : Time course of binding of ^{125}I-VIP to Y-1 cells. ^{125}I-VIP (2×10^{-10} M) was incubated with 10^6 Y-1 cells in the absence (●) or presence (△) of 10^{-7} M VIP at 37°. The difference between the two curves (▲) represents the specific binding. The incubation medium was Ham's F-10 containing 1 % FCS, 0.1 M MIX and 20 mM Hepes. Each value is the mean of triplicate determinations.

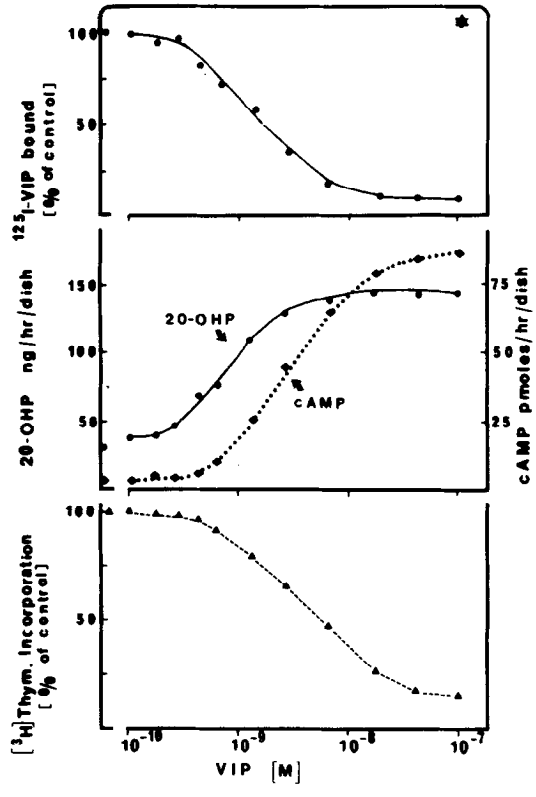

Figure 2. Upper panel : Inhibition of ^{125}I-VIP (2×10^{-11} M) binding to Y-1 cells (6×10^5) by unlabeled VIP (●) and 10^{-5} M of either glucagon, or ACTH or insulin (★). The incubation was carried out at 37°C for 30 min.

Binding of ^{125}I-VIP to Y-1 cells was inhibited by unlabeled VIP : 50 % inhibition was observed at 2 x 10^{-9} M (mean of 3 experiments) and more than 80 % with 2 x 10^{-8} M (Fig. 2, upper panel). The specificity of the binding was inferred by the fact that neither glucagon, ACTH nor insulin at 10^{-5} M had any effect (Fig. 2).

Effects of VIP on cAMP and steroid productions by Y-1 cells. Half-maximal and maximal steroidogenesis were observed at about 10^{-9} M and 10^{-8} M VIP, respectively. These concentrations are similar to those required to produce half-maximal and maximal inhibition of the binding of ^{125}I-VIP (Fig. 2, upper and middle panels). However, the concentrations required to induce half-maximal and maximal cAMP production are about one order of magnitude higher. The specificity of both biological effects of VIP on Y-1 cells is inferred by the fact that the parent hormone glucagon, and other peptides such as lysine-vasopressine and angiotensin II had no effect (Table 1). At high concentrations the steroidogenic potency of VIP is similar to that of ACTH, but at maximal concentration VIP induced production on of cAMP is three times lower than that induced by ACTH (Table 1).

Additive effects of ACTH and VIP on steroids and cAMP productions are observed when both hormones are used at submaximal concentrations, but the additive effect is not seen at maximal concentrations (Table 1). It is noteworthy that cAMP production under cholera toxin stimulation was almost twice that induced by ACTH alone or ACTH plus VIP, while steroidogenesis was similar (Table 1).

Effects of VIP on DNA synthesis. DNA synthesis and cell proliferation of Y-1 cells are inhibited by ACTH and these effects seem to be mediated by cAMP since this nucleotide and its active derivatives produce the same effects (11-13). Therefore the effects of VIP on Y-1 DNA synthesis and cell multiplication were investigated. Half-maximal and maximal inhibition of DNA synthesis were observed at about 10^{-8} and 10^{-7} M VIP (Fig. 2, lower panel), concentrations that are close to those required to produce half-maximal and maxi-

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Middle and lower panels : Dose-response of VIP on cAMP (◆) and 20α-dihydroprogesterone (20 OHP) (●) production and on [^{3}H]thymidine incorporation into DNA (▲). Y-1 cells were preincubated for 72 hrs in low serum medium (0.1 %). Then the medium was replaced by medium containing 1 % FCS, 0.1 M MIX and 20 mM Hepes and the indicated concentration of VIP. Two hrs later an aliquot was removed for measurement of 20 OHP and cAMP. [^{3}H]-thymidine incorporation into DNA was measured as indicated in Methods. The results are the mean of quadriplicate determinations. The range of values was within ≃ 8 % of the mean.

TABLE 1 : Effects of several compounds on cAMP and 20 OHP production and on [^{3}H] thymidine incorporation into DNA by Y-1 cells. Cells (2 x 10^5) were incubated in low serum containing medium (0.1 % fetal calf serum) for 72 hrs. The medium was removed and replaced by medium containing 5% FCS, 0.1 mM MIX and 20 mM Hepes without or with the compounds indicated. cAMP and 20 OHP were measured in an aliquot taken two hours later and [^{3}H] thymidine incorporation into DNA was measured as indicated in Methods.

	cAMP	20 OHP	[^{3}H] thymidine incorporation
	pmoles/2 hrs/dish	ng/2 hrs/dish	cpm/dish
Control	4.6 ± 0.6[a]	24 ± 4	52,500 ± 4200
ACTH 10^{-10} M	62 ± 6	94 ± 6	28,400 ± 2100
ACTH 10^{-7} M	280 ± 19	150 ± 8	6500 ± 400
VIP 10^{-9} M	44 ± 4	46 ± 5	42,400 ± 3800
VIP 10^{-7} M	90 ± 9	142 ± 10	12,600 ± 900
ACTH 10^{-10} + VIP 10^{-9}	100 ± 12	128 ± 12	21,200 ± 2000
ACTH 10^{-10} + VIP 10^{-7}	160 ± 12	148 ± 11	9400 ± 1100
ACTH 10^{-7} + VIP 10^{-7}	285 ± 22	154 ± 9	6300 ± 600
Glucagon 10^{-7} M	4.2 ± 0.5	21 ± 3	54,800 ± 5600
Lysine-vasopressine 10^{-7} M	4.8 ± 0.4	22 ± 6	53,900 ± 5200
Angiotensin II 10^{-7} M	4.1 ± 0.5	20 ± 6	51,400 ± 4800
Cholera toxin 10 µg/ml	420 ± 0.5	162 ± 12	2100 ± 300
DbcAMP 10^{-3} M	-	160 ± 9	2400 ± 200

a = mean ± SD of four dishes

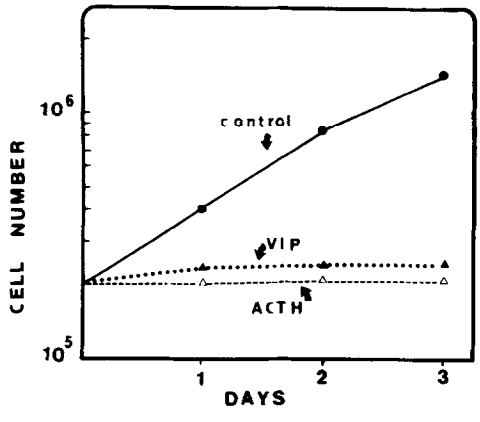

Figure 3 : Effects of VIP or ACTH on Y-1 cells in exponential growth. Cells were synchronized by incubation in low serum medium (0.1 % FCS) for 72 hrs. Then the medium was replaced by fresh medium containing 10 % FCS without (●) or with either 10^{-7} M VIP (▲) or 10^{-8} M ACTH (△). Medium was changed every day. Cell numbers are the average of triplicate plates at each time point.

mal cAMP production. At submaximal concentrations, the inhibitory effects of ACTH and VIP on DNA synthesis are additive, but not at maximal concentrations (Table 1).

The inhibitory effect of VIP on Y-1 cell multiplication is shown in Fig. 3 and compared to that of $ACTH_{1-24}$. It is clear that both compounds inhibited the mitogenic effect of 10 % FCS.

DISCUSSION

Specific binding sites for VIP have been described in many tissues (1-8) and in most of them VIP produces an increase of cAMP production. However its final biological effects have been less well defined. Our results demonstrated that Y-1 cells have VIP specific binding sites, and these sites appear to be functional, since the peptide, like ACTH, is capable of eliciting increases in both cAMP and steroid production and inhibition of DNA synthesis. Despite of the fact that maximal steroidogenic response is similar with VIP and with ACTH (11 and Table 1), several arguments suggest that the receptors for the two peptides are different : First, bound ^{125}I-VIP is not displaced by ACTH, and likewise VIP does not inhibit binding of ^{125}I-$ACTH_{1-24}$ (data not shown) ; second, at maximal concentration VIP-induced production of cAMP is three times lower than that induced by ACTH.

The binding affinity of ^{125}I-VIP for Y-1 cells is similar to that reported for several tissues (1-4, 6-8). Good agreement was found between the binding affinity of VIP for Y-1 cells, and the concentration producing half-maximal steroidogenic response. However, the concentrations of VIP required to produce half-maximal cAMP production are an order of magnitude higher. Similar discrepancy has been also reported for the dose-effect of ACTH on cAMP and steroid production (18-20). Since the dose-response of ACTH on steroidogenesis and on activation of the protein kinase cAMP dependent are parallel (20) it has been suggested that very small increases of cAMP are able to activate the protein kinase and therefore steroidogenesis. It is likely that the same explanation could account for the discrepancy observed with VIP.

In vitro ACTH inhibits DNA synthesis and cell multiplication of Y-1 (13-15). This inhibitory effect seems to be mediated by cAMP, since this nucleotide and its derivatives have the same action (14, 15). VIP also inhibits Y-1 DNA synthesis, and this effect seems to be correlated with cAMP production since the dose-response of VIP for cAMP production and inhibition of DNA synthesis are parallel.

At submaximal concentrations the effects of VIP and ACTH are additive, on both cAMP and steroid production and inhibition of DNA synthesis. This additive phenomenon is no longer seen when both hormones are used at maximal concentrations. However, when VIP and ACTH are added to the culture at maximal VIP and submaximal ACTH concentrations, the additive effect was observed on cAMP production and inhibition of DNA synthesis but not on steroidogenesis (Table 1). Taken together, the above data suggested that VIP through a specific receptor, stimulates a fraction of the ACTH sensitive pool of adenylate cyclase. The fact that cholera toxin induces a higher cAMP production than that produced by ACTH plus VIP at maximal concentrations indicate that other cAMP production exists and favours indirectly the hypothesis.

While one cannot conclude from our study whether or not VIP has a physiological role in the regulation of adrenal function. It is noteworthy that rat adrenal plasma membranes have specific VIP binding sites and that rat adrenal cortex extracts contain measurable amounts of VIP (21).

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