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Interaction of VIP, PACAP and related peptides in normal and leukemic human monocytes and macrophages

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The activation of the cAMP signaling pathway by vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP) and related peptides was studied (i) in normal peripheral human monocytes and THP-1 leukemic human monocytes, (ii) in their derived macrophage counterparts respectively obtained after spontaneous differentiation or retinoic acid (RA) treatment, and (iii) in human bronchoalveolar macrophages. In THP-1 monocytes, PACAP increased basal adenylate cyclase activity 5.3-fold, with an affinity 50-times greater than that of VIP or helodermin ($K_a = 3.2 \times 10^{-11}$ M VIP), whereas in normal peripheral monocytes, PACAP and VIP exhibited similar affinities and only increased cAMP generation 2-fold ($EC_{50} = 10^{-9}$ M). Spontaneous and RA-induced differentiation into normal and leukemic macrophages induced a progressive loss of cAMP production and regulation of superoxide anion production by VIP and related peptides. The neoplastic transformation in THP-1 monocytes and the deficiencies in the cAMP cascade observed during the terminal differentiation of normal and leukemic human macrophages may relate to a differential genetic expression of the VIP/PACAP receptor subtypes, and alterations in the functional activity of the stimulatory and inhibitory G_s/G_i subunits of adenylate cyclase.

Pituitary adenylate cyclase-activating peptide; Vasoactive intestinal peptide; Receptor; Monocyte; Macrophage; Leukemia

1. INTRODUCTION

Agents that raise cAMP levels inhibit T-lymphocyte- and natural killer cell-mediated cytotoxicity, as well as cytolytic functions and the chemotactic response. Evidence is accumulating that the neuropeptide vasoactive intestinal peptide (VIP) regulates the proliferation and immune responses of lymphocytes and monocytes [1,2]. Previous authors reported that the THP-1 monocytic leukemia cell line could be induced to undergo morphological and functional maturation into macrophage-like cells by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and retinoic acid [3–5]. Therefore, this cell line affords a useful model for studying the functional, pharmacological and genetic expression of receptor-mediated signal transduction during monocytic differentiation. For this purpose, we investigated the interaction of VIP, pituitary adenylate cyclase activating peptide (PACAP) and related peptides (i) in THP-1 monocytes and their derived THP-1 macrophages obtained after differentiation by retinoic acid [5], (ii) in human bronchoalveolar macrophages, and (iii) in normal human peripheral monocytes and their differentiated macrophages counterparts. This study was also designed to investigate the effect of VIP-related peptides and adenylate cyclase activators on the release of superoxide anions and H_2O_2 formation in human bronchoalveolar macrophages and THP-1 macrophages. The molecular basis for the induction of the respiratory burst has not been as well characterized in macrophages as in neutrophils. In tissues and epithelia, macrophages can be activated to produce activated oxygen species such as superoxide anion radicals O_2^-, peroxide $O_2^=$ and hydroxyl radicals $^{\cdot}OH$. Hydrogen peroxide (H_2O_2) is formed by superoxide dismutation, then O_2^- and H_2O_2 interact to generate the highly reactive hydroxyl radical $^{\cdot}OH$. This respiratory burst and secretion of reactive oxygen intermediates play a fundamental role in ischemia, inflammation and host defense by killing ingested microbes and invasive cancer cells [6]. Reactive oxygen species are produced by polymorphonuclear granulocytes, monocytes, lymphocytes, mesangial cells, platelets and fibroblasts. The direct activators of PKC, phorbol esters, γ-interferon, the chemotactic agent FMLP and platelet-activating factor (PAF) are known to initiate the respiratory burst in mononuclear phagocytes. In contrast, cAMP and positive effectors of the adenylate cyclase system inhibit macrophage functions, including lysosomal enzyme release, migration, phagocytosis and cytotoxicity.

We began by comparing the action of the VIP-related peptides on cAMP generation and H_2O_2 formation in THP-1 cells with the effects produced by forskolin and

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isoproterenol. Next, the specificity of the peptidergic action on cAMP production was similarly evaluated using a series of natural peptides structurally related to VIP, including PACAP-38 and its derivative PACAP-27, secretin, helodermin, helospectin I, the peptides with NH_2-terminal histidine and C-terminal methionine (PHM-27), or with NH_2-terminal histidine and C-terminal valine (PHV), human pancreatic growth hormone-releasing factor hpGRF 1–40, gastric inhibitory peptide (GIP), pancreatic glucagon, oxyntomodulin and glucagon-like peptide-2 (GLP-2). Part of this work has been published as an abstract [7].

2. EXPERIMENTAL

2.1. *Cell culture and human alveolar macrophage collection*

THP-1 cells originally obtained from Dr. T. Breitman (National Cancer Institute, Bethesda, MD, USA) have been maintained in our laboratory since 1982. They were grown in RPMI-1640 medium supplemented with 2 mM glutamide, 100 U/ml penicillin, 100 U/ml streptomycin and 10% fetal calf serum, at 37°C in an atmosphere of 5% CO_2. The cells were routinely passaged every 3 or 4 days and seeded at a density of 2×10^5 cells/ml. Cell viability was determined by the Trypan blue exclusion method. THP-1 cells differentiated after 4 days in culture following the plating of 2×10^5 cells/ml in the presence of 5×10^{-5} M retinoic acid, added from a 10 mM stock solution in absolute ethanol. Differentiation was estimated by the percentage and absolute number of cells capable of reducing nitroblue tetrazolium (NBT) [5].

Alveolar macrophages were obtained from bronchoalveolar lavages conducted under local anesthesia in 10 patients who underwent bronchoscopy for unilateral processes. For the lavage, 300 ml of normal saline was instilled in 50 ml aliquots via a bronchoscope into the right middle lobe or lingula of the lung contralateral to the lesion [8]. Only the last five aspirations were collected. The retrieved fluid was filtered over gauze to remove mucus, then centrifuged at $400 \times g$ for 10 min at 4°C. The cells were washed 3 times by similar centrifugations and resuspensions in RPMI 1640 medium containing 30 mM $NaHCO_3$ and 25 mM HEPES neutralized to pH 7.4. One aliquot of the recovered fluid was used for microbiologic and cytologic studies, including total and differential cell counts, after cytocentrifuge preparation. Alveolar macrophages were isolated by differential adherence. The washed cells were suspended in 35 mm Petri dishes at a concentration of $3–5 \times 10^5$ cells per ml of the same RPMI medium supplemented with 50 IU/ml penicillin and 50 μg/ml streptomycin. The dishes were incubated for 2 h at 37°C in a 95% air, 5% CO_2 atmosphere to allow adherence of macrophages. Non-adherent leukocytes were removed by washing. Adherent cells were > 95% macrophages by typical morphological examination, and cell viability was > 95% as determined by Trypan blue exclusion.

2.2. *Preparation of human peripheral monocytes and macrophage activation*

Human peripheral monocytes were isolated from normal volunteers and purified by leukapheresis and counter-current centrifugation elutriation, as previously described [9,10]. Monocyte-derived macrophages were induced at 37°C from purified fractions of human monocytes cultured for 7 days in Iscove-modified Dulbecco buffer containing 3×10^{-5} M β-mercaptoethanol, 2 mM L-glutamine, 2 mM pyruvic acid, antibiotics and 2% human serum in a humidified atmosphere of 5% CO_2 in air.

2.3. *Membrane-bound adenylate cyclase preparation and assay*

THP-1 monocytes and retinoic acid-induced THP-1 macrophages were washed twice in chilled KRP buffer. The resulting cell pellet was suspended at a concentration of 20×10^6 cells/ml of 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 30 mM NaCl and 5 μM phenylmethylsulfonyl fluoride [11]. Cells were disrupted with a Polytron homogenizer (Kinematica, Luzern, Switzerland) using three bursts of 5 s each. The homogenate was centrifuged for 10 min at $600 \times g$ and 4°C in a Sorvall centrifuge RC-2 (Newton, CT). The supernatant was carefully drawn off by a Pasteur pipette and stored on ice. The pellets were resuspended by two strokes in 40 ml of homogenization buffer and centrifuged under the conditions described above. The two supernatants were then pooled and plasma membrane-enriched particles were obtained by differential centrifugation for 30 min at $20.000 \times g$ and 4°C. These fractions, used for the experiments on adenylate cyclase activity, will be subsequently referred to as plasma membranes. They were either used immediately or stored frozen at −80°C for less than 1 week.

Adenylate cyclase activity was measured as previously described [12]. Briefly, the standard incubation mixture (final volume: 250 μl) contained: 1 mM ATP, 5 mM $MgCl_2$, the ATP-regenerating system (10 mM creatine phosphate and 0.5 mg/ml creatine kinase), 0.4 mM IBMX, 4 mg/ml BSA, 1 mM EGTA, 400 μg/ml bacitracin, 20 μM GTP, and test substances in 50 mM Tris-HCl buffer (pH 7.5). The reaction was initiated by the addition of membrane-bound adenylate cyclase (0.5–1 μg membrane protein per tube) and the mixture was incubated for 15 min at 30°C. Cyclic AMP was determined by radioimmunoassay [12]. Data are expressed as nM cAMP produced per min per mg membrane protein.

2.4. *Cellular cyclic AMP production*

Cyclic AMP generation was measured in human alveolar macrophages as adherent monolayers incubated for 30 min at 20°C in 1 ml of 35 mM Tris-HCl buffer (pH 7.5) containing 1 mM IBMX as a phosphodiesterase inhibitor, 1% BSA, and 50 mM NaCl. Purified peripheral human monocytes and their activated macrophage counterparts were incubated under the same conditions, in a final volume of 500 μl. The reaction was stopped at the time indicated by adding 11 N $HClO_4$. Cyclic AMP was determined using a radioimmunoassay [11]. Each experiment was performed in triplicate.

2.5. *Measurement of H_2O_2 production*

The production of H_2O_2 was measured in human bronchoalveolar macrophages and leukemic THP-1 macrophages, using the method described by Pick and Keisari, which is based on the H_2O_2-mediated and horseradish peroxidase-dependent oxidation of Phenol red [13]. The assay solution contained 140 mM NaCl, 10 mM potassium phosphate buffer (pH 7.0), 5.5 mM glucose, 0.28 mM Phenol red and 8.5 IU/ml horseradish peroxidase. About 10^6 THP-1 macrophages were covered with 1 ml of the reaction solution containing 1 μM phorbol myristate acetate (PMA) and incubated for 60 min at 37°C in the presence or absence of the indicated effectors. Bronchoalveolar macrophages were incubated in the absence of PMA, since they exhibited a spontaneous release of superoxide anion O_2^-. At the end of incubation, the culture medium was collected and centrifuged. The cell-free fluid was made alkaline by the addition of 10 μl of 1 N NaOH Absorbance was determined at 610 nm against a blank consisting of the reaction solution supplemented with 10 μl NaOH. In control experiments, 1,250 IU/ml catalase was added to inhibit H_2O_2 formation. The cells were then scraped off their supports in Krebs–Ringer phosphate buffer and sonicated for protein determination. The results are expressed as nM H_2O_2 generated per mg cell protein.

2.6. *Chemicals*

L-Isoproterenol, prostaglandins E_2 (PGE_2), nitroblue tetrazolium (NBT), phorbol 12-myristate 13-acetate (PMA), phenolsulfonphthalein (Phenol red), horseradish peroxidase (type II, salt-free), bovine liver catalase (11,000 IU/ml), 3-isobutyl-1-methylxanthine (IBMX), cyclic adenosine 3',5'-monophosphate (cAMP) and forskolin were purchased from the Sigma Chemical Company (St. Louis, MO). Bovine serum albumin (fraction V) was from Miles Laboratories (Elkhart, IN). Synthetic ovine PACAP-38 and its derivative PACAP-27, comprising the 27 NH_2-terminal amidated residues were synthe-

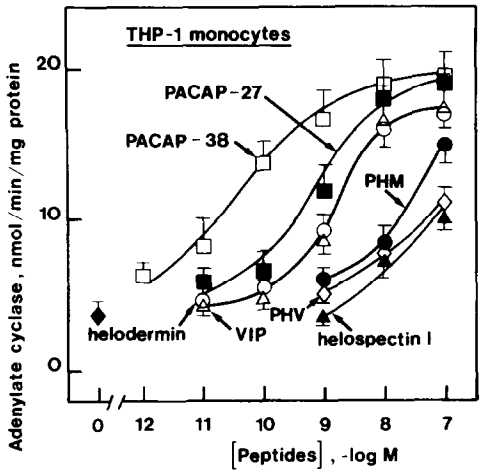

Fig. 1. Adenylate cyclase activation by PACAP-38, VIP and related peptides in THP-1 monocytes. Adenylate cyclase was tested after the addition of various concentrations of PACAP-38 (□) and -27 (■), vasoactive intestinal peptide, VIP (△), helodermin (○), the peptides with NH_2-terminal histidine and C-terminal methionine (PHM, ●), or with NH_2-terminal histidine and C-terminal valine (PHV, ◇) and helospectin I (▲). Results are means ± S.E.M. of 4–8 experiments performed in duplicate.

sized by solid-phase techniques, as previously described [14]. Highly purified natural porcine VIP and gastric inhibitory peptide (GIP) were purchased from Professor V. Mutt (Department of Biochemistry II, Karolinska Institute, Stockholm, Sweden). Crystallized porcine glucagon (Lot 421306) was from the Novo Research Institute (Bagsvaerd, Denmark). Oxyntomodulin, glucagon-like peptide-2, human secretin, helodermin, helospectin I and II, the human peptides with NH_2-terminal histidine and C-terminal methionine (PHM-27), or with NH_2-terminal histidine and C-terminal valine (PHV) were from Peninsula Laboratories (St. Helens, UK). The human pancreatic growth hormone releasing factor hpGRF 1–40 was a gift from Dr. J. Rivier (The Salk Institute for Biological Studies, San Diego, CA). RPMI-1640 medium, fetal calf serum and antibiotics were from Flow Laboratories. All-*trans*-retinoic acid (RA) was purchased from Aldrich.

3. RESULTS

3.1. *Effect of VIP, PACAP and related peptides on adenylate cyclase activity in THP-1 monocytes*

Basal adenylate cyclase activity in THP-1 monocyte membranes was 5 ± 1 nmol cAMP/min/mg protein (n = 27 experiments). In the absence or presence of sodium fluoride, PACAP or VIP, this activity was linear as a function of the membrane protein concentrations (0.3–2 μg/ml) during the incubation period considered (0–20 min). When THP-1 monocyte membranes were incubated for 15 min at 30°C, sodium fluoride (10^{-2} M), forskolin (4×10^{-5} M), and VIP (10^{-7} M) raised basal adenylate cyclase activity from 5 ± 1 nmol cAMP/min/mg protein to, respectively, 52 ± 4.5, 28 ± 1.2, and 17 ± 1.2 nmol cAMP/min/mg protein (n = 5–20 experiments).

In THP-1 monocytes, VIP and helodermin concentrations ranging from 10^{-10} to 10^{-7} M raised basal adenylate cyclase activity with a potency $K_a = 1.6 \times 10^{-9}$ M (Fig. 1). Maximal stimulation by the two peptides resulted in a 5.3-fold increase in this enzyme activity over basal levels. The neuropeptides PACAP-38 and -27 produced parallel dose–response curves to those of VIP and helodermin, and were respectively 50- and 3.2-times more potent than VIP in THP-1 membranes. Half-maximal stimulation of adenylate cyclase by PACAP-38 was observed at a K_a value of 3.2×10^{-11} M. The VIP-related peptides PHM, PHV and helospectin I produced similar rises in adenylate cyclase activity to those produced by VIP and helodermin, but at 10–40-times higher concentrations. Helospectin II was less potent than helospectin I (data not shown). At 10^{-7} and 10^{-6} M, human secretin, hpGRF, pancreatic glucagon, oxyntomodulin, and GLP-2 did not increase basal adenylate cyclase activity.

At maximally effective concentrations, paired combinations of PACAP-38, VIP and helodermin did not produce any additional increase in this activity. This result suggests that the 3 peptides activate the same pool of receptor–transducer systems, since they also raised adenylate cyclase activity to a similar degree. In contrast, paired combinations of 10^{-4} M histamine and 10^{-7} M VIP had additive effects on this activity (data not shown).

3.2. *Adenylate cyclase activation in retinoic acid-induced THP-1 macrophages*

Sodium fluoride induced similar rises in the adenylate cyclase activity of THP-1 monocytes and macrophages. In the THP-1 monocytes, this activity increased from 4.25 to 41.2 nmol cAMP/min/mg protein, i.e. 9.7-fold, and in the corresponding THP-1 macrophages, from 3.1 to 37.3 nmol cAMP/min/mg protein, i.e. 12-fold (n = 4). As shown in Fig. 2, retinoic acid-induced differentiation of THP-1 monocytes into THP-1 macrophage-like cells was associated with decreased stimulation of adenylate cyclase activity by VIP and PACAP-38 (2.1-fold), as compared to the stimulation observed in undifferentiated THP-1 monocytes, 3.7-fold. These changes in adenylate cyclase responsiveness were not accompanied by significant variations in the activation constants of the cAMP-inducing agent VIP (K_a = 1.3 and 2×10^{-9} M) in THP-1 monocytes and macrophages, respectively.

3.3. *Effects of adenylate cyclase activators on H_2O_2 formation*

Under basal conditions, H_2O_2 formation was undetectable in both THP-1 monocytes and the THP-1 macrophage-like cells induced by retinoic acid. In contrast, PMA-stimulated THP-1 macrophages produced 15.2 μmol H_2O_2/h/mg cell protein during the 60 min incubation period (n = 51). This amount of H_2O_2 was similar to that spontaneously released by normal human alveolar macrophages after 30–60 min incubation, i.e. 10.3 to 16.1 μmol H_2O_2/h/mg cell protein [8]. The PKC activator PMA was ineffective in inducing H_2O_2 production in THP-1 monocytes. The cAMP-inducing agents, fors-

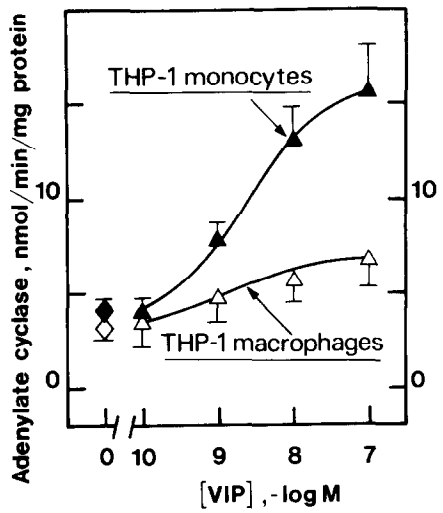

Fig. 2. Effect of the retinoic acid-induced differentiation of THP-1 monocytes into macrophages on adenylate cyclase activation by VIP. The effect of VIP on adenylate cyclase activity was tested on the same populations of THP-1 cells, cultured in the presence or absence of retinoic acid. Less than 5% of the THP-1 monocytes (closed symbols) reduced NBT, compared to 85% of the retinoic acid-induced THP-1 macrophages (open symbols). Results are means ± S.E.M. of 6 separate experiments performed in duplicate.

kolin and isoproterenol, inhibited PMA-induced H_2O_2 production in differentiated THP-1 macrophages. Isoproterenol induced 100% inhibition of oxygen metabolite formation, with a potency IC_{50} of 1.5×10^{-6} M (Fig. 3, right). However, forskolin only induced partial inhibition, and the neuropeptides VIP and helodermin had no effect on H_2O_2 production in THP-1-derived macrophages (Fig. 3, left).

Human alveolar macrophages maintained a spontaneous release of hydrogen peroxide (21 ± 3.9 μmol H_2O_2/h/mg cell protein, $n = 47$). PMA, at a concentration of 10^{-6} M, increased this release from 25.8 ± 3.8 to 51 ± 4.9 μmol H_2O_2/h/mg cell protein ($n = 16$). When bronchoalveolar macrophages were challenged with 10^{-6} M VIP, the neuropeptide had no effect on cAMP generation, and basal or PMA-induced H_2O_2 formation. In contrast, the adenylate cyclase activators, forskolin and isoproterenol, inhibited the spontaneous release of O_2^- from human alveolar macrophages by 100% (Fig. 4), and PGE_2 produced partial inhibition (75%) with a potency ($IC_{50} = 10^{-7}$ M PGE_2 (data not shown). The inhibitory potency of isoproterenol was similar on superoxide production induced by PMA in THP-1 macrophages (1.5×10^{-6} M, Fig. 3), and on the spontaneous H_2O_2 release observed in human bronchoalveolar macrophages (3.2×10^{-6} M, Fig. 4).

3.4. *Effects of VIP, PACAP and related peptides on cAMP generation in normal human peripheral monocytes and their macrophages counterparts*

Monocytes and their cultured macrophage counterparts had respective purity ranges of 90 ± 1.4% ($n = 15$) and 84 ± 3.6% ($n = 7$). In these cell fractions, basal cAMP levels were 1.7 ± 0.3 pmol cAMP/10^6 monocytes and 2.9 ± 0.5 pmol cAMP/10^6 macrophages ($n = 6$–8 experiments). In the peripheral monocytes, the neuropeptides VIP and PACAP-38 were equipotent in stimulating cAMP generation (Fig. 5). Stimulation by each of these peptides doubled cAMP production, with a potency $EC_{50} = 10^{-9}$ M. The VIP analogs we tested in purified monocytes displayed similar maximal stimulation and the following order of potency as regards their activation of cAMP production: PACAP-38, VIP > PHM, PHV > PACAP-27, hpGRF, secretin. Rat GRF was about 30-times more potent in this respect than human pancreatic GRF ($n = 3$ experiments, data not shown). VIP had no effect on cAMP generation in monocyte-derived macrophages.

4. DISCUSSION

As far as we know, the present work is the first demonstration that PACAP is a very potent peptide in stimulating adenylate cyclase activity in normal and leukemic human monocytes. In THP-1 monocytes, this enzyme's activity was stimulated by PACAP-38 concentrations as low as 10^{-12} M. Half-maximal stimulation was observed at a K_a value of 3.2×10^{-11} M PACAP-38. Thus, PACAP-38 was about 50-times more potent than VIP or helodermin in THP-1 cell membranes. Our pharmacological study further revealed that the VIP analogs tested in THP-1 monocytes stimulated adenylate cyclase activity with potencies that corresponded to their apparent affinities for the PACAP receptors previously characterized in brain and pancreas, i.e. PACAP-38 > helodermin, VIP > PHM, PHV, helospectin [15].

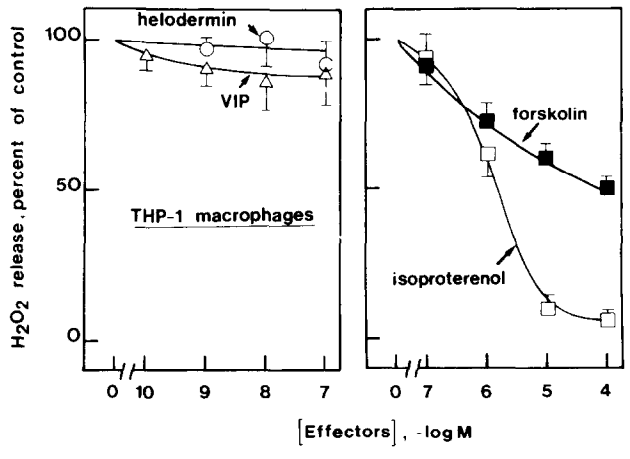

Fig. 3 Effects of VIP and helodermin on PMA-induced H_2O_2 formation by THP-1 macrophages. Comparison with the cAMP-inducing agents forskolin and isoproterenol. THP-1 macrophages were incubated for 60 min at 37°C in the presence of PMA, either alone (control activation) or combined with different concentrations of helodermin or VIP (left, $n = 6$–7 experiments), isoproterenol or forskolin (right, $n = 6$–9 experiments). Data are means ± S.E.M. All determinations were performed in triplicate.

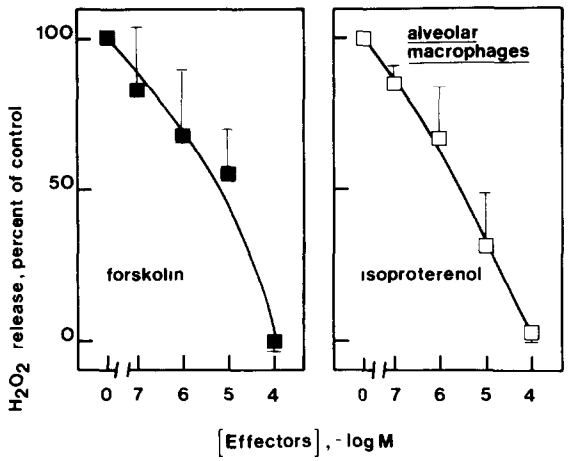

Fig. 4. Effect of the cAMP-inducing agents forskolin and isoproterenol on PMA-induced H_2O_2 formation in human bronchoalveolar macrophages. Macrophages were incubated for 60 min at 37°C in the presence of PMA alone (control activation) or combined with different concentrations of forskolin (left), or isoproterenol (right). Data are means ± S.E.M. of 4 experiments. All determinations were performed in triplicate.

Accordingly, secretin was ineffective in this system. In normal peripheral monocytes, PACAP and VIP activated cAMP production with a similar potency $EC_{50} = 10^{-9}$ M (Fig. 5), suggesting that the two peptides interact with the same receptor–effector system. However, normal peripheral monocytes showed another pharmacological response toward the VIP analogues: VIP, PACAP-38 > PHV, PHM > PACAP-27, hpGRF and secretin, suggesting that normal and leukemic monocytes may harbor different receptor subtypes encoded by distinct VIP/PACAP receptor genes, or alternatively, undergo a differential receptor processing from a common precursor. The novel neuropeptide PACAP-38 was recently isolated from ovine hypothalamus, and exhibited a 68% amino acid sequence homology with porcine VIP in the NH_2-terminal region [14]. The peptidergic neurotransmitters VIP, PACAP and PHI/PHM have been co-localized in intramural neurones of the intestine and respiratory tract [16,17], thus providing a microanatomic basis for potential neuroparacrine interactions between innervations containing VIP or PACAP and the mucosal immune cells.

Spontaneous and retinoic acid-induced differentiation of normal peripheral monocytes and leukemic THP-1 monocytes into macrophages was found to reduce the responsiveness of membrane-bound adenylate cyclase to the VIP-related peptides. We previously observed that differentiation of the human histiocytic lymphoma cell line U-937 into monocyte-like cells by retinoic acid similarly reduced the capacity of mature U-937 cells to generate cAMP after stimulation by histamine, isoproterenol or PGE_1 [18]. These reduced responses of adenylate cyclase seem to be due to the differentiation process observed in normal and leukemic leukocytes, since addition of retinoic acid to the myeloid leukemia cell lines, THP-1 and HL-60, did not reduce the amount of Gsα messenger or protein, but led to a fast decrease in the ADP-ribosylation of Gsα [19]. We therefore suggest that in THP-1 macrophages, retinoic acid treatment reduced the functional expression of the Gsα proteins which couple stimulatory hormone receptors to adenylate cyclase, or alternatively, increased the levels of functional inhibitory Giα proteins. In the present study, the adenylate cyclase activators forskolin, isoproterenol and PGE_2 were found to inhibit superoxide anion production in THP-1 macrophages and human bronchoalveolar macrophages. In contrast, VIP and helodermin failed to reduce hydrogen peroxide formation in human bronchoalveolar macrophages and activated THP-1 macrophages. Thus, in normal and leukemic human macrophages, the degree to which the cAMP-inducing agents inhibit the respiratory burst was directly related to their relative efficacy on the adenylate cyclase system.

Our results demonstrate that the VIPergic pathway is retained during the neoplastic transformation of the human monocytic lineages, as we previously reported in other peripheral tissues [19–21]. However, leukemic THP-1 monocytes display a PACAP-preferring receptor-adenylate cyclase system, while the peptidergic receptor has similar affinity for VIP and PACAP in normal peripheral human monocytes. In view of the general action of cAMP and adenylate cyclase activators on cell proliferation, migration and inflammatory responses, the present study indicates that VIP and its related peptide, PACAP, may play a physiological role and exert direct action on the immune effector cells in the bone marrow and peripheral blood. These two neuropeptides

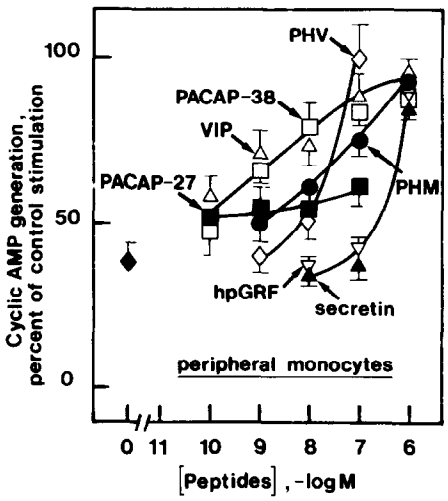

Fig. 5. Effect of VIP, PACAP and related peptides on cAMP generation in purified human peripheral monocytes. Peripheral monocytes were incubated in the presence of PACAP-38 (□) or -27 (■), VIP (△), PHM (●), PHV (◇), hpGRF (▽) or human secretin (▲). Data are means ± S.E.M. of 4–6 experiments performed in duplicate or triplicate.

may also regulate immune and inflammatory responses during the early steps of the monocyte/macrophage conversion in intestinal and respiratory mucosa [22,23], while terminal differentiation of this cell lineages altered their responses, in terms of adenylate cyclase activation and regulation of hydrogen peroxide formation.

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