Differential Effects of Peptide Histidine Isoleucine (PHI) and Related Peptides on Stimulation and Suppression of Neuroblastoma Cell Proliferation

A NOVEL VIP-INDEPENDENT ACTION OF PHI VIA MAP KINASE*

Vincent Lelièvre‡, Nicolas Pineau§, Joanna Du‡, Chia-Hui Wen‡, Thinh Nguyen‡, Thierry Janets§, Jean-Marc Muller§, and James A. Waschek‡¶

From the ‡Department of Psychiatry, Mental Retardation Research Center, UCLA, Neuropsychiatric Institute, Los Angeles, CA 90024 and the §CNRS UMR 6558, Laboratoire de Biologie des Interactions Cellulaires, UFR Sciences, Université de Poitiers, 40 avenue du Recteur PINEAU, 86022 Poitiers Cedex, France

The vasoactive intestinal peptide (VIP)1 neuropeptide family includes VIP, pituitary adenylate cyclase-activating peptide 27 (PACAP-27), the carboxyl-terminal extended isoform PACAP-38, peptide histidine/isoleucine (PHI), and its human homologue peptide histidine/methionine (PHM), secretin, glucagon, growth hormone releasing factor (GRF), and also analogues isolated from reptile venom (1). Expression of the VIP and PACAP genes results in the biosynthesis of peptide precursors, which also give rise to PHI/PHM and PACAP-related peptide (PRP), respectively (2, 3). The neuropeptides VIP, PHI/PHM, and PACAP are commonly expressed and secreted in neuroblastoma and certain neuroendocrine tumors (4). Moreover the finding of functional VIP and PACAP receptors expressed in neuroblastoma cell lines and freshly excised tumor resections suggests that these neuropeptides may be involved in autocrine/paracrine loops (5, 6). Although these peptides are well known to act in the processes of neurotransmission (1, 7, 8), they have been shown to regulate the proliferation rate and differentiation of numerous cell lines (9). These include those derived from neuroblastoma (10–15), glioblastoma (16), and pheochromocytoma (17) and from a variety of epithelial tumors including breast, intestine, lung, pancreas, and prostate (9). In contrast, much less information is available concerning physiological actions of PHI and PRP (18–21) on either neurotransmission or proliferation.

High affinity receptors for VIP-related peptides have been identified in numerous tissues from several species. At least three different receptors have been characterized in terms of sequence, peptide affinity profiles, tissue expression, and signaling pathways. These belong to the seven-transmembrane-spanning domain, G-protein-coupled receptor family. Two of these recognize VIP and PACAP with equal high affinity, VPAC1 and VPAC2 (22, 23), and the third is a PACAP-prefering receptor, PAC1 (24).2 In addition, at least seven splice variants have been reported for the PACAP receptor (25–28). Cloned and expressed forms of these variants have been reported to differ in their ability to bind PACAP analogs and in their coupling to signaling pathways. In contrast, no splice variant has yet been described for VIP receptors.

* Supported in part by National Institutes of Health Grants HD04612, HD06576, and HD34475, by the UCLA Jonsson Cancer Center (to V. L.), and the French Region Poitou-Charentes (to N. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: 68–225 NPI, UCLA, 760 Westwood Plaza, Los Angeles, CA 90024. Tel.: 310-825-0179; Fax: 310-206-5061; E-mail: jwaschek@mednet.ucla.edu.

1 The abbreviations used are: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating peptide; PHI, peptide histidine isoleucine; PHM, peptide histidine/methionine; PRP, PACAP-related peptide; PAC1, PACAP-prefering receptor; VPAC1, and VPAC2, polyvalent VIP/PACAP receptor types 1 and 2, respectively; PKA, protein kinase A; PKC, protein kinase C; MAP kinase, mitogen-activated protein kinase; FBS, fetal bovine serum; IBMX, isobutylmethylxanthine; ANP, atrial natriuretic peptide.

2 The nomenclature used is that recommended by IUPHAR (42).
VPAC1 and VPAC2, and PAC1 receptors are commonly expressed alone or in combination in neuroblastomas (6, 29). The cAMP-dependent pathway mediates many of the actions of VIP and PACAP, but many recent reports indicate the involvement of nitric oxide, phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3-kinase), and MAP kinase pathways (7, 8, 18, 25, 30–32). In this study, we studied the ability of VIP, PACAP-27, PACAP-38, and PHI to modulate proliferation of the mouse neuroblastoma cell line Neuro2a. We also determined if changes in cAMP content and MAP Kinase activity were associated with these proliferative actions, and if these actions were sensitive to specific pathway inhibitors (33–35). Finally, we analyzed receptor expression by Northern analysis and by radiolabeled ligand displacement.

MATERIALS AND METHODS

Cultures—Neuro2a cells were maintained in 75 cm² flasks (Falcon) in 20 ml of Dulbecco’s modified Eagle’s medium culture medium (Mediatech, Inc.) supplemented with 8% fetal bovine serum (FBS) (Life Technologies, Inc.) and antibiotics (penicillin/streptomycin) under a 5% CO₂, 95% air controlled atmosphere at 37 °C. Medium was changed every 3 days. Passages were performed after trypsinization (0.05% trypsin, 0.53 mM EDTA, Life Technologies, Inc.).

Proliferation Studies—The actions of VIP-related peptides (purchased from Sigma) on cell proliferation were initially investigated using a tetrazolium-derived (MTS) colorimetric assay (cell titer96 from Promega) as described previously (17). In brief, subcultures were performed in 96-well plates seeded with 8,000 cells/well. 24 h after seeding, vehicle or peptide were added from 10× stock solutions. After 48 h of peptide treatment, the MTS proliferation assay was initiated by incubation of the cells in 100 μl of fresh medium for 1 h at 37 °C, followed by addition of 20 μl of reagent solution to each well. After incubation at 37 °C for 90 min, absorbency at 490 nm was determined on a 96-well plate reader (Bio-Rad).

For H-thymidine incorporation assay, cells were seeded in 24-well plates (80,000 cells/well) in 1 ml of medium and cultured for 24 h. Medium was then replaced with fresh serum-free medium. After 1.5 h at 37 °C, cells were precipitated for an additional hour with vehicle or signal transduction inhibitors (H89 and GF109203x from Calbiochem, and PD98059 from New England Biolabs). Peptides were then added, and 1 h later, 1 μCi/well ³H-thymidine was also added. Four hours later, ³H-thymidine incorporation was determined as described previously (29).

cAMP Measurements—Cells (80,000/well) were cultured for 3 days in 24-well plates. Medium was replaced with serum-free medium with or without 1 μM isobutylmethylxanthine (IBMX). After 15 min incubation at 37 °C, peptide were added and incubated for 15 min. Cell lysis and cAMP radioimmunoassay (NEN Life Science Products) were performed as described (17).

Northern Analysis—Neuro2a cells were cultured in eight flasks (75 cm²) and harvested at 90% confluency with 0.05% trypsin, 0.02% EDTA. Trypsin was neutralized with the tissue culture medium containing 8% serum, and then cells were centrifuged and rinsed with phosphate-buffered saline. Subsequent isolation of poly(A)-selected mRNA was carried out as described (36). Receptor probes and hybridization conditions were exactly as described previously (29).

Radioiodination of PHI—Because ¹²⁵I-PHI is not commercially available, PHI was radioiodinated with the chloramine-T technique as described previously (37). Iodinated peptide was separated by reverse-phase HPLC (Spectraphysics) using a 5 μM VYDAC C18 column (Interchrom, France). A gradient of acetonitrile, H₂O, 0.1% trifluoroacetic acid was required for elution. Fractions containing ¹²⁵I-PHI were evaporated under nitrogen and stored at -20 °C.

Binding Studies—Cells were seeded at 80,000/ml/well in 24-well dishes and cultured for 3 days. Fifteen min before the initiation of the binding, cells were incubated in fresh medium at 37 °C. Culture medium was removed and replaced with 270 μl of cold binding medium containing 30,000 cpm of radiotracer (¹²⁵I-VIP and ¹²⁵I-PACAP, 2200 cpm/mmol, from NEN Life Science Products) and 30 μl of specified concentrations of unlabeled peptides (for more details, see Ref. 13). Radioiodinated PHI binding experiments were performed on intact cells in suspension, using a slightly different procedure to increase the sensitivity. In brief, nonconfluent cultures were harvested by incubation for 5 min at 37 °C in a phosphate buffer (0.05 M, pH 7.4) containing 0.53 mM EDTA.

Cells were counted and aliquoted into Eppendorf tubes at a concentration of 1.2 million cells per tube. Cells were then quickly centrifuged, pellets were reconstituted in the binding buffer containing 5,000 cpm of ¹²⁵I-PHI and specified concentrations of the unlabeled ligands, and then cells were incubated under shaking. The incubation times were determined by time course experiments conducted at 4 °C and then fixed at 120, 150, and 210 min for VIP, PACAP, and PHI displacements, respectively. Incubation was stopped with a quick rinse with 2 ml of cold phosphate-buffered saline containing 0.1% bovine serum albumin, followed by cell lysis in 0.5 M NaOH solution. Extracts were transferred into 5-ml tubes, and radioactivity was counted using a γ-counter (Wallac).

MAP Kinase Assays—Cells were cultured for 24 h in 24-well plates (150,000 cells/well). After a quick rinse, cells were incubated for 12 h in the presence of culture medium containing 0.2% fetal bovine serum. Cultures were then rinsed twice with serum-free medium and incubated in this medium for 120 min. PD98059 (30 μM) or vehicle was added for another hour and then peptides for 10 min at 37 °C. The incubation was stopped by replacement of the stimulation buffer with 100 μl of lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EGTA, 2 mM diethiothreitol, 1 mM orthovanadate supplemented with 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, and leupeptin, aprotonin, and antipain (10 μg/ml each). Sigma). ERK activity was determined by the Biotrack p42/p44 MAP kinase enzyme assay kit according to the recommendation of the manufacturer (Amersham Pharmacia Biotech). Protein was determined by the Bradford assay with Bio-Rad reagents and bovine serum albumin as a standard.

RESULTS

Cell Proliferation—Both MTS (Fig. 1) and thymidine incorporation assay (Fig. 2) demonstrated that VIP and PHI were potent inhibitors of Neuro2a cell proliferation. The MTS assay, carried out at the end of a 2-day period of drug stimulation, indicated that maximal inhibition by VIP was reached at about 10 nM, whereas PHI action reached near maximal values at concentrations in the subnanomolar range (Fig. 1). PACAP-27 and PACAP-38 induced a biphasic response: a significant stimulation at very low concentrations (0.1 and 1 pM, respectively) and then an inhibition at the highest doses of peptides. In subsequent experiments, thymidine incorporation over a 5-h period after initiation of drug treatment was used to assay the effect of peptides on DNA synthesis. This assay produced qualitatively similar results, although the effects were generally shifted to higher concentrations (Fig. 2A).

Various inhibitors were added to characterize the possible signal transduction pathways mediating the proliferative actions. The PKA inhibitor H89 (20 μM) (33) specifically abolished the inhibition of DNA synthesis by 10 nM and 1 μM PACAP-27 and PACAP-38, resulting in a stimulation of H-thymidine incorporation at all concentrations 0.1 nM and above (Fig. 2, compare panel A versus panel B). This suggests that in control cultures, activation of PKA by higher concentrations of PACAP masked
a positive proliferative response that could otherwise only be seen with lower concentrations of PACAP. H89 also blocked the antiproliferative action of VIP but clearly did not block the inhibitory action of PHI. Nearly identical effects on neuropeptide responses were seen when cultures were treated with another PKA inhibitor, RT-cAMP (200 μM, data not shown). In contrast to the effects of PKA inhibitors, treatment with the MEK1/2 inhibitor PD98059 (30 μM) (34) completely suppressed the PACAP-induced stimulation (Fig. 2A versus 2C). Paradoxically PD98059 inhibited the antiproliferative action of PHI but appeared not to affect the antiproliferative actions of VIP or high concentrations of PACAP. The blockade of PHI action by PD98059 implies that inhibitory action of this peptide required a basal level of MAP kinase activity. DNA synthesis was in fact inhibited by PD98059 in control cultures (Fig. 2C, second column) implying that a MAP kinase pathway coupled to growth stimulation was indeed active in these cells under basal conditions. The PKC inhibitor GF109203X (10 μM) (35) also strongly inhibited 3H-thymidine incorporation in control cultures, but did not appear to interfere with the neuropeptide modulation of DNA synthesis (Fig. 2A versus 2D).

cAMP Response—Intracellular cAMP content was specifically and dose-dependently increased by addition of PACAP-38, PACAP-27, and VIP, either in the absence (Fig. 3A) or presence (Fig. 3B) of the phosphodiesterase inhibitor IBMX (1 μM). No elevation of the cAMP levels was detected when cells were stimulated by PHI alone (Fig. 3A). Indeed, only a slight increase in cAMP content was observed in the presence of IBMX at the highest concentrations of PHI.

MAP Kinase Activity—Changes in ERK1/2 MAP kinase activity were measured at the selected peptide concentrations shown in Fig. 4. For PACAP-27, the peak of activation was reached at 1 pM, whereas peak activation by PACAP-38 was shifted to about 0.1 nM. In contrast, VIP did not modify MAP kinase activity at any concentration from 1 pM to 1 μM. PHI at 1 nM, on the other hand, significantly inhibited MAP kinase activity by about 25%. The MEK1/2 inhibitor PD98059 reduced ERK1/2 activity in control cells (Fig. 4, second bar), indicating that some basal activity of the MAP kinase pathway existed in these cells, perhaps from active compounds in serum (medium contained 0.2% FBS, see “Materials and Methods”). Consistent with this possibility, the PHI action was more pronounced (78% inhibition) when experiments were carried out in medium containing 2% FBS (data not shown).

Expression of VIP and PACAP Receptor Genes—Northern analysis indicated that the PACAP-preferring PAC1 receptor gene was expressed in Neuro2a cells, whereas neither VPAC1 nor VPAC2 receptor gene expression could be detected under these conditions (Fig. 5, lane 1). The specificity and integrity of hybridization was tested using internal positive and negative

---

**Peptide Concentrations**

**Fig. 2.** Thymidine incorporation assays in Neuro2a cells stimulated by increasing concentrations of neuropeptides. Cells were stimulated with peptides for 5 h, and assays were performed as described under “Materials and Methods.” A, effects of neuropeptides on 3H-thymidine incorporation in the absence of inhibitors. B, action of H89 (20 μM) on the modulation of thymidine incorporation induced by VIP, PACAP, and PHI. C, action of PD98059 (30 μM) on the modulation of thymidine incorporation induced by VIP, PACAP, and PHI. D, action of GF109203X (10 μM) on the modulation of thymidine incorporation induced by VIP, PACAP, and PHI. Basal incorporation is indicated as □ and as ■ in the presence of the specified inhibitor. Peptide order is: VIP □, PACAP-27 ■, PACAP-38 □, and PHI ■. Data are the mean ± S.E. of a representative of four independent experiments, each performed in triplicate. Statistical analysis of the data (analysis of variance) has been performed. *, p < 0.05; **, p < 0.01; ***, p < 0.005.
controls given by Fig. 5, lanes 2 though 4 (36): rat pancreatic tumor cell line AR4–2J (positive for PAC1), human breast tumor cell line T47D (positive for VPAC1), and mouse olfactory bulb (positive for PAC, and VPAC2). Radioligand Binding—Binding sites for VIP and PACAP in Neuro2a cells were assessed, using commercially available radiotracers, and for PHI, using chloramine T-labeled radioiodinated peptide (see “Materials and Methods”). Preliminary studies of time-course binding demonstrated that the optimized incubation times at 4 °C were 120, 150, and 210 min for 125I-labeled VIP, PACAP, and PHI, respectively. Preliminary saturation experiments and Scatchard analyses were also performed and suggested the presence of one binding site for VIP \( (K_p, 0.35 \text{ nM}; R_{\text{max}} 1450 \text{ sites/cell}) \) and at least two different sites for PACAP: high affinity sites \( (K_p 2.1 \text{ pM}; R_{\text{max}} 980 \text{ sites/cell}) \) and low affinity sites \( (K_p 60 \text{ nM}; R_{\text{max}} 18,200 \text{ sites/cell}) \).

Increasing concentrations of different analogs (VIP, PACAP-27, PACAP-38, and PHI) displaced the binding of radioiodinated VIP (Fig. 6A). Curves were fit to the sigmoidal equation, revealing Hill values close to 1 for all the tested analogues. Moreover VIP, PACAP-27, and PACAP-38 displaced 125I-VIP binding with a high affinity (VIP, IC50 1.2 ± 0.35 nM; PACAP-27, IC50 0.42 ± 0.6 nM; PACAP-38, IC50 3.6 ± 0.5 nM), whereas PHI exhibited a very low affinity (IC50 1.2 ± 0.8 nM).

Data from 125I-PACAP binding displacement experiments fit well to a two-site equation, suggesting the presence of high-(IC50 about 1 pM; 77 ± 0.8% binding total) and low-affinity sites (IC50 > 1 nM; 23 ± 0.8% of the total binding) (Fig. 6B). PACAP-38 (IC50 3.7 ± 0.4 pM/5.7 ± 1.8 nM) appeared to be more potent than PACAP-27 (IC50 20 ± 0.3 pM/52 ± 2.3 nM) to displace the radioligand binding. VIP and PHI only partially displaced the total 125I-PACAP binding (VIP, 25 ± 2.5%; PHI, 37 ± 2.0%), with an IC50 of 25 ± 0.6 and 62 ± 0.8 pm, respectively. Hill slopes from displacement curves by VIP and PHI were 1.02 ± 0.3 and 0.97 ± 0.17, respectively.

Displacement curves of 125I-PHI binding by different analogues (native PHI, VIP, and PACAP isoforms) also suggested a multiple site system in Neuro2a cells. Three different PHI binding sites appeared to be present based on the capacity of these analogues to displace the binding of 125I-PHI (Fig. 6C). Displacement by native PHI resulted in curve characterized by a Hill slope only 0.33 (IC50, 0.2 ± 0.45 nM). VIP displaced only 55% of total 125I-PHI binding with IC50 51 ± 1.65 pm (Hill slope: 0.71). PACAP-27 and PACAP-38 displaced almost completely (87%) the PHI binding. Data fit to a two-site model indicated high affinity sites (IC50 PACAP27, 4.7 ± 2.1 pm; PACAP38, 40 ± 2.36; representativity: 56%) and low affinity binding sites (IC50 PACAP27, 61 ± 5.21 nM; PACAP-38, 60 ± 6.54 nM; representativity: 44%). Finally about 13% of radioiodinated PHI was displaced only by native PHI.

**DISCUSSION**

We demonstrate here that VIP, PHI, and PACAP potently regulate the proliferation of mouse neuroblastoma Neuro2a cells. VIP and PHI inhibited proliferation, PHI being considerably more potent. PACAP, on the other hand, stimulated proliferation at low concentrations and inhibited proliferation at higher concentrations. These observations lead us to hypothesize that multiple receptors were present in these cells, coupling to different growth-related signaling pathways. An analysis of proliferation and intracellular responses to peptides in the presence and absence of specific signaling pathway inhibitors, and an assessment of binding sites using radiolabeled peptides strongly supported this hypothesis.

Of particular interest was the anti-proliferative action of...
PHI. Although PHI acts as a moderate to weak agonist on all well known forms of VIP receptors, its anti-proliferative action in the present system was more than 100-fold more potent than VIP. One explanation for this finding is that PHI acted on a unique receptor that preferred PHI to VIP. Consistent with this possibility was the fact that these peptides appeared to act by different signaling pathways. The action of VIP (but not PHI) was associated with an increase in cAMP levels and was selectively blocked by inhibitors of PKA. In contrast, PHI action was uniquely associated with ERK1/2 inhibition and was selectively blocked by a MAP kinase pathway inhibitor. Finally, binding studies using 125I-PHI as a tracer indicated the presence of up to two classes of high affinity receptors that selectively bound PHI over VIP.

We also found the action of PACAP to be intriguing. Very low concentrations of this peptide (up to 0.1 nM) stimulated MAP kinase activity and proliferation, whereas higher PACAP concentrations, like VIP, stimulated cAMP production and inhibited proliferation. MAP kinase and PKA pathways were very likely involved in these opposing proliferative responses. Whereas the MEK1/2 inhibitor blocked the stimulation of proliferation observed at low PACAP doses (Fig. 2B), blockade of PKA resulted in stimulation of proliferation at all PACAP doses (Fig. 2C). Biphasic responses to peptides in this family have been reported in other systems. For example, VIP at 50 pM stimulated c-src activity and the proliferation of cultured retinal pigmented epithelial cells, whereas higher concentrations stimulated cAMP production and inhibited proliferation (38). In addition, biphasic action of VIP on cell growth has been reported for Lo-Vo colonic carcinoma cells (39) and T98G glioblastoma cells (17).

The Northern analysis revealed expression of only the PACAP-prefering PAC1 receptor gene, whereas binding studies suggested the presence of multiple receptors that bind these peptides. One possible explanation is that receptors arising from alternatively spliced forms of PAC1, differentially bind and respond to these peptides. Several forms have been characterized (25–28), including one displaying a 21-amino acid deletion in the amino-terminal part of the receptor believed to be important for ligand binding. A cloned form of this latter receptor has been shown to differ slightly from the original receptor with respect to the relative binding affinities for PACAP-27 versus PACAP-38 (27). However, the ability of this variant to bind VIP, PHI, or related peptides has not been reported. We tried to detect alternative transcripts corresponding to this region of the PAC1 receptor by reverse transcription-polymerase chain reaction (27). Only the original form of receptor was identified in these experiments (data not shown). Alternatively, a low level of VPAC1 receptor expression (not detectable by Northern analysis) could potentially explain some of the actions of VIP and/or PHI because the rat VPAC1 binds PHI with an affinity only 3-fold lower than VIP. However, the considerably more potent action of PHI on growth (Fig. 1) and its selective coupling to the MAP kinase pathway suggest the presence of a novel high affinity receptor that preferentially binds PHI versus VIP.

Because very high affinity PHI receptors are not well described in the literature, we radiodinated PHI to investigate the pharmacological properties of the PHI binding sites. Displacement studies were consistent with the presence of polyclonal PACAP/VIP/PHI sites (estimated to be 40% of the total) and up to two other receptors, PACAP/PHI (50%) and possibly PHI-prefering (10%), whose molecular characteristics have not been determined. The presence of a VPAC2 receptor seems unlikely because the VPAC2-selective agonist helodermin (23) was unable to displace the binding of 125I-VIP or 125I-PACAP (data not shown). In the context of our data suggesting the existence of novel receptors, a recent report indicating that VIP family peptides interact with “clearance receptors” for the seemingly unrelated atrial natriuretic peptide (ANP) family of neuropeptides is of interest (40). These receptors were named “clearance” because early attempts to characterize these ANP binding sites did not reveal any signaling function, suggesting that they might be involved in removal of the peptide. Interestingly, ANP interaction with these single transmembrane domain receptors in smooth muscle cells triggered inhibition of MAP kinase activation and growth inhibition (41). Because PHI also inhibits MAP kinase pathways and growth in Neuro2a cells, one could speculate that a “clearance-like” receptor could mediate the activity induced by PHI in Neuro2a cells.

In conclusion, we demonstrated here that at least three closely related peptides of the neuropeptide VIP family regulated the proliferation of the mouse neuroblastoma Neuro2a cells.
cells. These actions appeared to occur through interaction with at least three different functional receptors that were differentially coupled, positively or negatively, to the PKA and MAP kinase signaling pathways. Moreover, the combined actions of these neuropeptides on proliferation may be relevant to the specific behavior of neuroblastoma tumor since an autocrine/paracrine action for these neuropeptides has been proposed (5, 6, 10, 11, 29).

Acknowledgments—We thank the following investigators at UCLA for useful discussions and suggestions: Dr. Lutz Birnbaumer, Dr. Harvey Herschman, and Dr. Shalani Kumar.

REFERENCES

1. Said, S. I. (1991) Trends Endocrinol. Metab. 2, 107–112
2. Ish, N., Obata, K., Yanaihara, N., and Okamoto, H. (1983) Nature 304, 547–549
3. Ohkubo S., Kimura C., Ogi K., Okasaki K., Hosoya M., Onda H., Miyata A., Arimura A., and Fujino M. (1992) DNA Cell Biol. 1, 21–30
4. Yamaguchi, K., Abe, K., and Yanahaira N. (1992) Biomed. Res. 13, 279–283
5. Muller, J.-M., Lolait, S. J., Yu, V. C., Sadee, W., and Waschek, J. A. (1989) J. Biol. Chem. 264, 3647–3650
6. Vertongen, P., Devalck, C., Sariban, E., De Laet, M-H., Martelli, H., Paraf, F., Helardot, P., and Robberecht, P. (1996) J. Cell. Physiol. 167, 36–46
7. Chistophe J. (1993) Biochim. Biophys. Acta 1154, 183–199
8. Rawling, S. R., and Hezareh, M. (1996) Endoex. Rev. 17, 4–29
9. Muller, J.M., Lelievre, V., Bocq-Giraudon, L., and Meunier, A.C. (1995) Mol. Neurobiol. 10, 115–134
10. O’Dorisio, M. S., Fleshman, D. J., Qualman, S. J., and O’Dorisio, T. M. (1992) Regul. Pept. 37, 213–226
11. Pence, J. C., and Shorter, N. A. (1993) J. Cell. Physiol. 157, 272, 133–137
12. Wollman, Y., Lilling, G., Goldstein, M. N., Fridkin, M., and Gozes, I. (1993) J. Biol. Chem. 268, 17456–17462
13. Lelievre, V., Becq-Giraudon, L., Meunier, A-C., and Muller, J-M. (1996) J. Neurosci. Res. 43, 5267–5272
14. Chajiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshiooka, T., and Hidaka H. (1990) J. Biol. Chem. 265, 5267–5272
15. Chattiwee, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshiooka, T., and Hidaka H. (1990) J. Biol. Chem. 265, 5267–5272
16. Alesci, D. R., Cuenda, A., Cohen, P., Dudley, D. T., and Saltiel A. R. (1995) J. Biol. Chem. 270, 2749–27494
17. Toulec, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E., Llorielle, P., Duhamel, L., Charon, D., and Kirilovsky, J. (1991) J. Biol. Chem. 266, 15771–15781
18. Waschek, J. A., Richards, M. L., and Bravo, D. T. (1995) Cancer Lett. 92, 143–149
19. Prine, B. A., Weber, M. J., Hu, R. M., Pedram, A., Daniels, M., and Levin, E. R. (1996) J. Biol. Chem. 271, 14356–14363
20. Harmar, A. J., Arimura, A., Gozes, I., Journot, L., Laburthe, M., Piseign, J. R., Rawlings, S. R., Robberecht, P., Said, S. I., Sreedharan, S. P., Wank, S. A., and Waschek, J. A. (1998) Pharmacol. Rev., in press
21. Rais, D. R., Trowbridge, C., and Kuroki, Y. (1995) Neuropeptides 28, 167–173
22. Wray, V., Nokihara, K., Naruse, S., Ando, E., Rakoschelke, C., and Wei, M. (1995) Biomed. Pept. Proteins Nucleic Acids 4, 77–82
23. Hannibal, J., Mikkelsen, J. D., Clausen, H., Holst, J. J., Wulff, B. S., and Fahrenkrug, J. (1995) Regul. Pept. 55, 133–148
24. Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K., and Nagata, S. (1992) Neuro 8, 811–819
25. Lutz, E. M., Shewad, W. J., West, J. M., Morrow, J. A., Fink, G., and Harmar, A. J. (1993) FEBS Lett. 354, 1–8
26. Piseign, J. R., and Wank, S. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6345–6349
27. Spengler, D., Waebber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P. H., and Journot, L. (1993) Nature 365, 170–175
28. Journot, L., Waebber, C., Pantaloni, C., Holsboer, F., Seeburg, P. H., Bockaert, J., and Spengler, D. (1995) Biochem. Soc. Trans. 23, 133–137
29. Pantaloni, C., Brabet, P., Bilanges, B., Dumas, A., Houssami, S., Spengler, D., Bockaert, J., and Journot, L. (1996) J. Biol. Chem. 271, 22146–22151
30. Chatterjee, T. K., Sharma, R. V., and Fisher R. A. (1996) J. Biol. Chem. 271, 32226–32232