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NOREPINEPHRINE AND HISTAMINE POTENTIATE THE INCREASES IN CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE ELICITED BY VASOACTIVE INTESTINAL POLYPEPTIDE IN MOUSE CEREBRAL CORTICAL SLICES: MEDIATION BY α_1-ADRENERGIC AND H_1-HISTAMINERGIC RECEPTORS[1]

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Abstract

We have examined the interactions, in eliciting cAMP accumulation, between vasoactive intestinal polypeptide (VIP) and the three monoamines norepinephrine (NE), histamine (HIS), and serotonin (5-hydroxytryptamine, 5-HT) in mouse cerebral cortical slices. We have observed that NE and HIS, but not 5-HT, act synergistically with VIP to increase cAMP levels. The rank-order of potency of several adrenergic agonists in potentiating the effect of VIP on cAMP levels is the following: epinephrine > NE > phenylephrine >> clonidine, with EC_{50} of 2.2, 5, and 10 μM, respectively (clonidine being only marginally effective). This pharmacological profile is characteristic of the activation of α_1-adrenergic receptors. This contention is substantiated by the observation that the potentiating effect of NE is antagonized by the selective α_1-adrenergic antagonist prazosin at nanomolar concentrations. The potentiating effect of HIS is mediated by H_1-histaminergic receptors since it is antagonized by the selective H_1-receptor antagonist mepyramine but not by cimetidine, a selective H_2-receptor antagonist. The synergistic interaction between VIP and NE is also observed in the presence of the adenosine antagonist theophylline, thus discarding the possibility of a mediation of the synergism by adenosine released by VIP. In view of the morphological and physiological properties of the VIP intracortical neuronal system and the noradrenergic projection to the cerebral cortex, it appears that sensory stimulation may constitute a behavioral event whereby the synergism between VIP and NE may become operative and lead to a drastic increase in the levels of cAMP within a discrete cortical volume delineated by the intersection of the tangentially organized noradrenergic fibers and a group of activated, radially oriented VIP intracortical neurons.

Vasoactive intestinal polypeptide (VIP) is a 28-amino acid polypeptide originally isolated from porcine duodenum by Said and Mutt (1970). VIP-immunoreactive nerves have been visualized throughout the gastrointestinal (Costa et al., 1980) and genitourinary (Larsson et al., 1977) tracts, and in endocrine glands such as the pancreas (Larsson et al., 1978) and the thyroid gland (Ahren et al., 1980). The presence of VIP has also been demonstrated in the PNS and CNS (Larsson et al., 1976; Loren et al., 1979). In the CNS, VIP is highly concentrated in the cerebral cortex where it is localized to a homogeneous population of intracortical, bipolar neurons oriented perpendicularly to the pial surface and arborizing only minimally (50 to 120 μm) in the horizontal plane, predominantly in layers I and IV-V (Loren et al., 1979; Morrison et al., 1984). A recent quantitative immunohistochemical study in rat visual cortex has revealed that, on the average, there is one VIP-immunoreactive neuron per cortical column of 30.6 μm diameter and that such a cortical volume partially overlaps with similar adjacent columns determined by the arborization pattern of VIP-immunoreactive neurons (Morrison et al., 1984). Thus, these morphological constraints restrict the cellular actions of VIP-containing neurons locally, to narrow and radially oriented transcortical volumes. The cellular actions of VIP in cerebral cortex include the stimulation of cAMP formation (Quick et al., 1978; Magistretti and Schorderet, 1984) and the activation of glycogenolysis (Magistretti et al., 1981). Furthermore, VIP applied by iontophoresis has been shown to excite identified corticospinal neurons (Phillis et al., 1978).

Norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) are two monoamines contained in highly divergent neuronal circuits which originate in small nuclei in the brainstem wherefrom they project to several regions of the CNS (Dahlstrom and Fuxe, 1964; Fuxe, 1965). In the cerebral cortex the noradrenergic and serotonergic fibers are organized predominantly in a plane parallel to the pial surface spanning across several functionally distinct cortical regions (Morrison et al.,

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1978, 1981; Lidov et al., 1980); therefore, in contrast to the VIP-containing intracortical neurons (Morrison and Magistretti, 1983), NE- and 5-HT-containing fibers can influence their target cells throughout a vast rostrocaudal expanse of neocortex simultaneously. Biochemical and lesion studies have revealed the existence of a third pontine monoaminergic projection which contains histamine (HIS) (Garbarg et al., 1974, 1976; Barbin et al., 1975). Like VIP, the three monoamines can increase the levels of cAMP in neural tissues (for review, see Bloom, 1975; Daly, 1975; Greengard, 1976) and promote glycogenolysis in mouse cerebral cortical slices (Quach et al., 1978, 1980, 1982).

This morphological and functional background induced us to investigate possible interactions between the VIPergic and monoaminergic cortical systems, since, although organized according to radically different morphological principles, they elicit, at least in part, identical cellular effects within the same region of the brain. The existence of such possible interactions has been substantiated by our recent observation that VIP and NE acted synergistically to increase cAMP levels in mouse cerebral cortical slices (Magistretti and Schorderet, 1984).

We report here that: (*i*) it is by activating specific α_1-adrenergic receptors that NE potentiates the effects of VIP on cAMP levels; (*ii*) HIS, via specific H_1-receptors, displays a similar synergistic interaction with VIP; and (*iii*) the synergism between NE or HIS and VIP is not a common feature of all three cortical monoaminergic afferent neuronal circuits, since 5-HT did not potentiate the effects of VIP on the levels of cAMP.

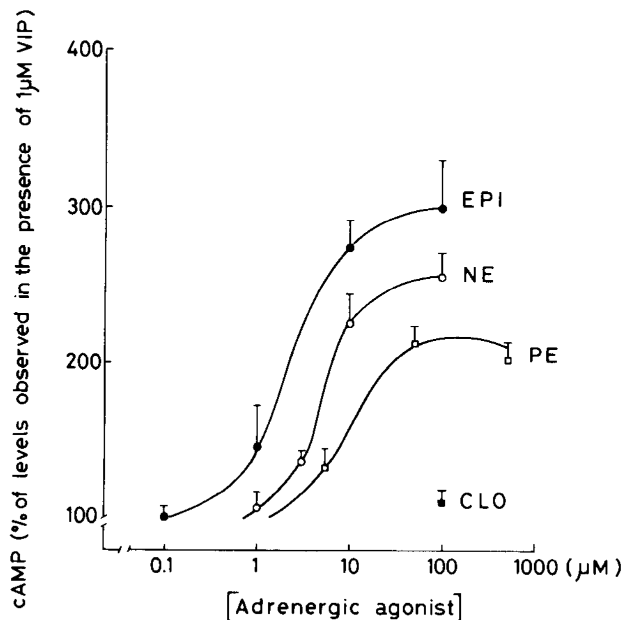

Figure 1. Concentration-response curves of the stimulatory effects of various adrenergic agents on cAMP accumulation elicited by 1 μM VIP. Mouse cerebral cortical slices were incubated in the presence of the various agents as described under "Materials and Methods." cAMP levels were determined as described by Brown et al. (1971). Results are from one experiment repeated twice, with similar results. Each *point* represents the mean ± SEM of four determinations. Basal cAMP levels ± SEM were 10.9 ± 1.63 pmol/mg of protein. cAMP levels ± SEM in the presence of 1 μM VIP were 193 ± 10.3 pmol/mg of protein. The stimulatory effect of EPI and NE was assessed in the presence of 100 μM *dl*-propranolol (see the text for discussion). cAMP (picomoles per milligram of protein) ± SEM in the presence of 100 μM EPI (*a*) was 79.8 ± 4; for 100 μM EPI plus 100 μM *dl*-propranolol (*b*) it was 20.3 ± 1.1; for 100 μM NE (*c*) it was 50.1 ± 4.6; and for 100 μM NE plus 100 μM *dl*-propranolol (*d*) it was 18.3 ± 1.5.

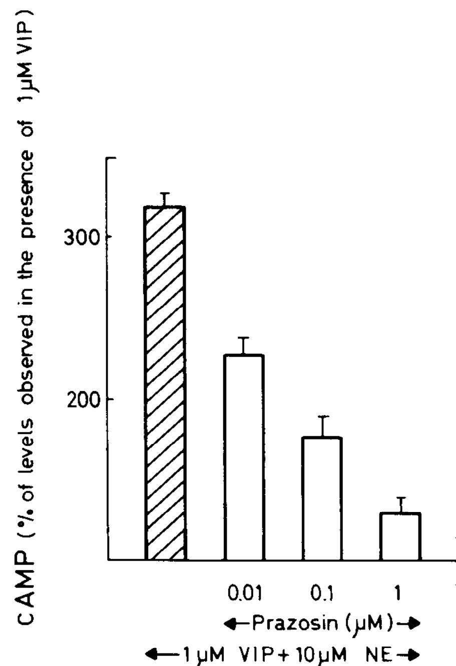

Figure 2. Effect of prazosin on the increases in cAMP elicited by 1 μM VIP in the presence of 10 μM NE. For experimental details see "Materials and Methods." Basal cAMP levels ± SEM were 5.59 ± 0.2 pmol/mg of protein. cAMP values (picomoles per milligram of protein) ± SEM in the presence of 1 μM VIP were 146.5 ± 23.9. The *error bar* at the *far right* represents SEM of VIP tested alone. Results represent the mean ± SEM of quadruplicate determinations. The antagonism by prazosin of the synergism between 1 μM VIP and 10 μM was significant at all three concentrations tested ($p < 0.001$). Statistical significance was assessed by the Student's *t* test.

Materials and Methods

Swiss male albino mice (18 to 20 gm) were used throughout this study; they were maintained in an alternating 12-hr light/12-hr dark cycle and had free access to food and water.

Cerebral cortical slices were prepared as follows (Magistretti et al., 1981). Mice were decapitated and their brains rapidly removed. The cerebral cortex was then dissected on ice and immediately placed in a modified Krebs-Ringer bicarbonate buffer, pH 7.4 (KRG), containing (in millimolar concentration): NaCl, 120; KCl, 5; $CaCl_2$, 2.6; $MgSO_4$, 0.67; KH_2PO_4, 1.2; glucose, 3; $NaHCO_3$, 27.5, previously gassed with O_2:CO_2 (95:5). The dissected cortices (usually two per experiment) were then placed on a McIlwain tissue chopper, their ventral aspect facing the chopping plate, and 250-μm slices were prepared. The plate was then rotated by 90° and the cortices were cut again. Thus, the preparation used in the experiments consisted of cortical parallelepipeds of 250 μm section and approximately 1 mm length (i.e., the cortical thickness). The slices were then resuspended in ice-cold KRG (6 ml/cortex) and incubated during 15 min at 37°C under continuous gassing (O_2:CO_2, 95:5) and vigorous shaking. The medium was then replaced and 260 μl of the slice suspension were distributed into individual polypropylene test tubes. After gassing with a stream of O_2:CO_2 (95:5), the tubes were capped and incubated at 37°C. After 30 min, drugs (20 μl) were added for 10 min. Antagonists were added 5 min prior to the agonists. The tubes were then centrifuged, and the supernatant was removed and replaced with fresh KRG (300 μl). The slice suspension was then sonicated for 5 sec, boiled for 10 min, and centrifuged again. cAMP levels were determined as follows. Aliquots of the supernatants (10 to 100 μl) were lyophilized and resuspended in 20 μl of Tris/EDTA (0.05 M/4 mM) buffer. cAMP was then determined with the saturation assay method described by Brown et al. (1971). Experimental values were calculated by computer from a standard curve ranging from 0 to 16 pmol of cAMP. Proteins were determined in the pellets as described by Lowry et al. (1951).

VIP was obtained from Prof. V. Mutt, Department of Biochemistry II, Karolinska Institutet, Stockholm, Sweden. Cimetidine, mepyramine, clonidine, *dl*-propranolol, and prazosin were generous gifts of: Dr.

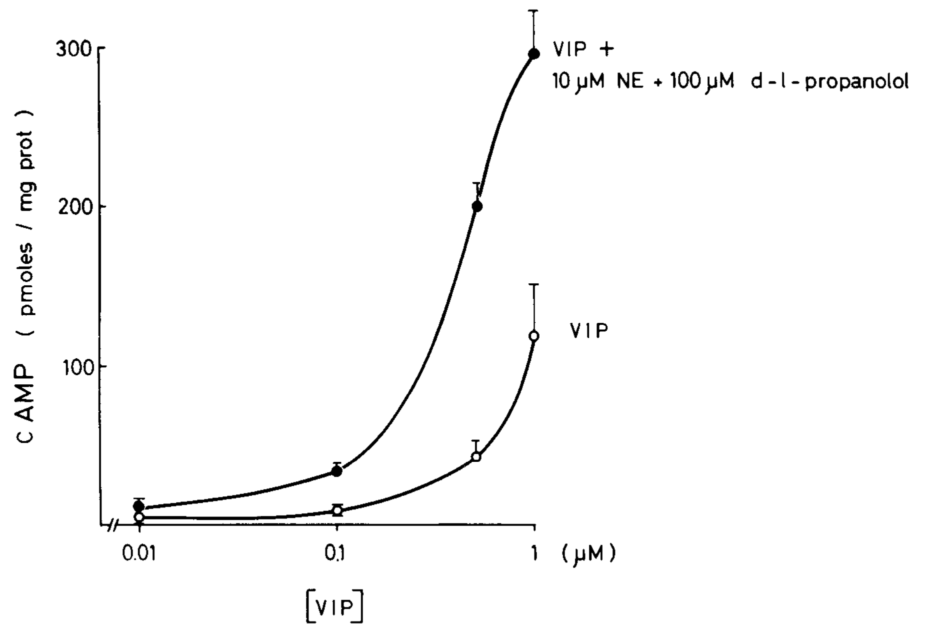

Figure 3. Concentration-response curve of the stimulatory effect of VIP on cAMP levels in the presence (●——●) and absence (○——○) of 10 μM NE. For experimental details see "Materials and Methods." The effect of 10 μM NE on the concentration-response of VIP was assessed in the presence of 100 μM *dl*-propranolol (see "Results" and legend to Fig. 1 for discussion). Each *point* on the curve represents the mean ± SEM of triplicate determinations.

TABLE I

Effect of theophylline on the synergism between VIP and NE in increasing cAMP levels

Conditions are as in Figure 1. For experimental details see "Materials and Methods." Results represent the mean ± SEM of 8 to 12 separate determinations. All values are significantly different from cAMP levels observed in the absence of added agents ($p < 0.001$). Statistical significance was assessed by the Student's *t* test.

Agent Added	Concentration (μM)	cAMP (pmol/mg of protein)
None		10.2 ± 0.43
VIP	1	160.3 ± 14
NE	10	62.6 ± 3.4
VIP plus	1	
NE	10	431 ± 29.1[a]
VIP plus	1	
NE plus	10	
Theophylline	1000	503.9 ± 25[b]

[a] Significantly different from the sum of cAMP levels observed in the presence of 1 μM VIP and 10 μM NE tested separately ($p < 0.001$).

[b] Not significantly different from 1 μM VIP and 10 μM NE.

Parsons, Smith, Kline and French, The Frythe, United Kingdom (cimetidine): Specia, Paris, France (mepyramine); Boehringer, Ingelheim am Rhein, Switzerland (clonidine); ICI, Alderley Park, United Kingdom (*dl*-propranolol); and Pfizer AG, Zurich, Switzerland (prazosin). All other chemicals were from commercial sources.

Statistical analysis was performed by the Student's *t* test.

Results

The synergism between VIP and NE in eliciting the accumulation of cAMP in mouse cerebral cortical slices is antagonized by a specific α-adrenergic antagonist, phentolamine (Magistretti and Schorderet, 1984). This observation indicates that NE, via the activation of α-adrenergic receptors, potentiates the stimulatory effects of VIP on cAMP levels. In order to define the nature of the α-adrenergic receptor subtype (α_1 or α_2) mediating this effect, we have determined the rank-order potency of several adrenergic agonists. The concentration-response curves of epinephrine (EPI), NE and phenylephrine (PE) in potentiating the effect of 1 μM VIP on cAMP levels are shown in Figure 1. The EC_{50} of the three adrenergic agonists are as follows: EPI, 2.2 μM, NE, 5 μM, and PE, 10 μM. It should be noted that, since EPI and NE per se stimulate cAMP formation via β-adrenergic receptors in mouse cerebral cortex (Schultz and Daly, 1973a; and Figure 1 legend), the potentiating effect of the two catecholamines was determined in the presence of a specific β-adrenergic antagonist, *dl*-propranolol, at 100 μM. Therefore, the EC_{50} values observed in these conditions reflect the affinity of EPI and NE for α-adrenergic receptors exclusively. As also shown in Figure 1, the selective α_2-adrenergic agonist clonidine at 100 μM did not potentiate the effects of VIP on cAMP levels. This type of adrenergic agonist profile (Langer, 1974; Aghajanian and Rogawski, 1983) indicates that NE activates specific α_1-adrenergic receptors to potentiate the increase in cAMP elicited by VIP. This contention is corroborated by the observation that the selective α_1-antagonist prazosin antagonizes in a concentration-dependent manner the potentiating effects of 10 μM NE (Fig. 2). It is worth noting that a marked antagonism of the synergism between VIP and NE was observed already at nanomolar concentrations of prazosin (Fig. 2).

We have also examined the effect of a fixed concentration of NE (10 μM) on the concentration-response curve of VIP in eliciting increases in cAMP. As shown in Figure 3, a marked leftward shift of the concentration-response curve of VIP was observed in the presence of the catecholamine. The potentiating effect of NE was assessed in the presence of the β-adrenergic antagonist *dl*-propranolol at 100 μM, thus reflecting the activation of α-adrenergic receptors exclusively.

A marked synergism in increasing cAMP levels between NE and adenosine has been reported previously by several authors (Sattin and Rall, 1970; Huang et al., 1971; Schultz and Daly, 1973b; Sattin et al., 1975). We therefore had to consider the possibility that adenosine, released by VIP, could in fact mediate the synergism observed between NE and VIP. Therefore, we assessed the effects on cAMP levels of a combination of 1 μM VIP and 10 μM NE in the presence of the adenosine

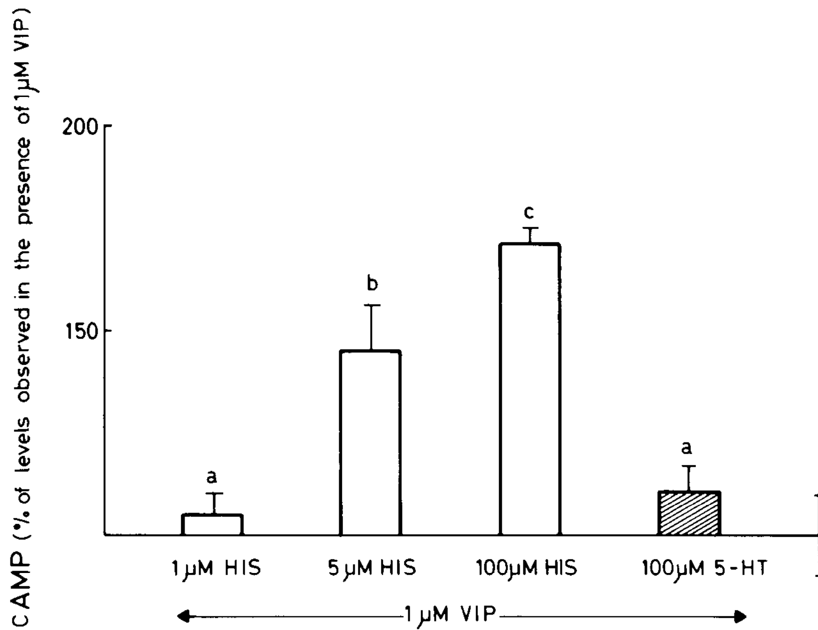

Figure 4. Effects of HIS and 5-HT on cAMP accumulation elicited by 1 μM VIP. For experimental details see "Materials and Methods." Basal cAMP levels ± SEM were 11.1 ± 0.93 pmol/mg of protein. cAMP levels (picomoles per milligram of protein) ± SEM were 145.4 ± 9.9 in the presence of 1 μM VIP, 12.5 ± 1.16 in the presence of 100 μM HIS, and 12.1 ± 2 in the presence of 100 μM 5-HT. Results are the means ± SEM from 4 to 25 separate determinations. *a*, Not significantly different from 1 μM VIP; *b*, significantly different from 1 μM VIP ($p < 0.01$); *c*, significantly different from 1 μM VIP ($p < 0.001$). Statistical significance was assessed by the Student's *t* test. The *error bar* at the *far right* represents SEM of VIP tested alone.

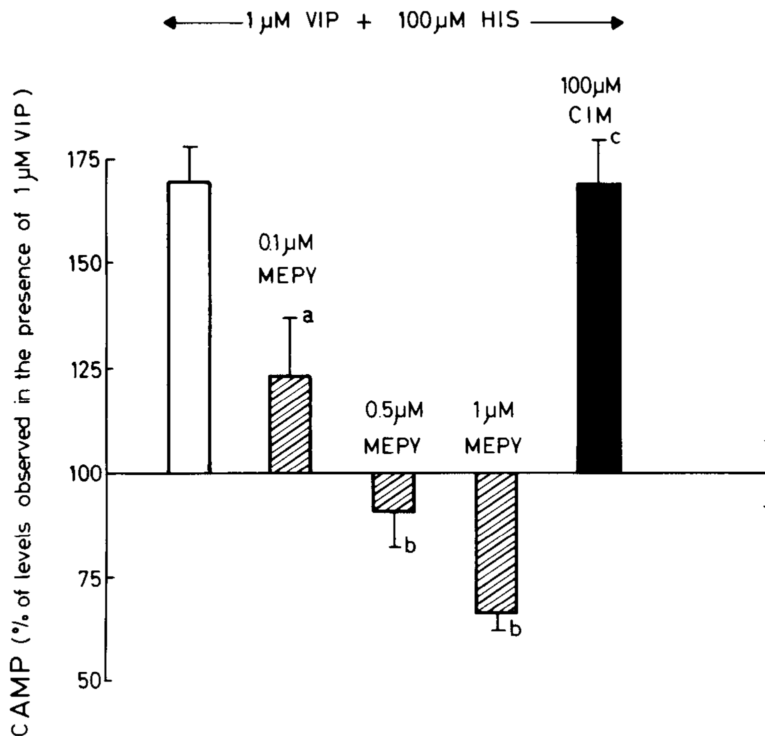

Figure 5. Effect of mepyramine (*MEPY*) and cimetidine (*CIM*) on the increases in cAMP elicited by 1 μM VIP in the presence of 100 μM HIS. For experimental details see "Materials and Methods." Basal cAMP levels ± SEM were 2.91 ± 0.5. cAMP levels (picomoles per milligram of protein) ± SEM in the presence of 1 μM VIP were 89 ± 7. The *error bar* at the *far right* represents SEM of VIP tested alone. Results represent the mean ± SEM of quadruplicate determinations. *a*, Significantly different from 1 μM VIP + 100 μM HIS ($p < 0.05$); *b*, significantly different from 1 μM VIP + 100 μM HIS ($p < 0.001$); *c*, not significantly different from 1 μM VIP + 100 μM HIS.

antagonist theophylline at 1 mM. As shown in Table I, the synergism between VIP and NE was fully expressed in the presence of theophylline. It should be noted here that, when tested alone, the adenosine antagonist, even at a concentration of 10 mM, increased only marginally cAMP levels (picomoles per milligram of protein ± SEM, $n = 4$; basal: 11.4 ± 0.45; 10 mM theophylline: 24.8 ± 1.15).

The effects of HIS and 5-HT on cAMP increases elicited by VIP were subsequently examined. As shown in Figure 4, HIS potentiated in a concentration-dependent manner the effect of 1 μM VIP in stimulating cAMP formation. The cellular actions of HIS are mediated in several cellular systems by two classes of histaminergic receptors, denominated H_1 and H_2 (Schwartz, 1979). We therefore examined which class of receptor mediated the effects of HIS on the VIP-induced accumulations of cAMP, by using specific HIS receptor antagonists such as mepyramine (H_1-antagonist) and cimetidine (H_2-antagonist). As shown in Figure 5, a concentration-dependent antagonism by mepyramine of the potentiating effect of 100 μM HIS was observed, whereas cimetidine was inactive. These observations demon-

TABLE II

Lack of antagonism by cimetidine and mepyramine of the increases in cAMP elicited by VIP

Conditions are as in Figure 1. For experimental details see "Materials and Methods." Results represent the mean ± SEM of four separate determinations. Statistical significance was assessed by the Student's *t* test.

Agent Added	Concentration (μM)	cAMP (pmol/mg of protein)
None		10.9 ± 0.87
VIP	1	129 ± 14
VIP plus	1	
Cimetidine	100	130.9 ± 14.2[a]
VIP plus	1	
Mepyramine	100	161.9 ± 36.9[a]

[a] Not signifciantly different from 1 μM VIP.

strate that the activation of specific H_1-receptors by HIS mediates the synergistic interaction between HIS and VIP. It is interesting to note that the levels of cAMP observed in the presence of 1 μM VIP + 100 μM HIS + 0.5 or 1 μM mepyramine were lower than those observed in the presence of 1 μM VIP alone (Fig. 5). As shown in Table II, this is not the consequence of an antagonism by mepyramine of the stimulatory effects of VIP on cAMP levels.

It should also be noted that 100 μM HIS did not enhance the synergism observed between 1 μM VIP and 100 μM NE (not shown).

Finally, in contrast to NE and HIS, 5-HT did not act synergistically with VIP to increase cAMP levels (Fig. 4).

Discussion

In the present investigation we have examined the interactions, on the stimulation of cAMP formation, between VIP, a *neuropeptide localized to an intracortical neuronal system*, and NE, HIS, or 5-HT, three monoamines contained in long circuits projecting from the brainstem to the cerebral cortex. We have demonstrated that activation of α_1-adrenergic and H_1-histaminergic receptors potentiates the increases in cAMP levels elicited by VIP in mouse cerebral cortex. No synergistic interaction in the formation of cAMP was observed between 5-HT and VIP.

NE has been demonstrated to promote the accumulation of cAMP in several rodent species (Bloom, 1975; Daly, 1975). In rat cerebral cortex, both α- and β-adrenergic receptors mediate

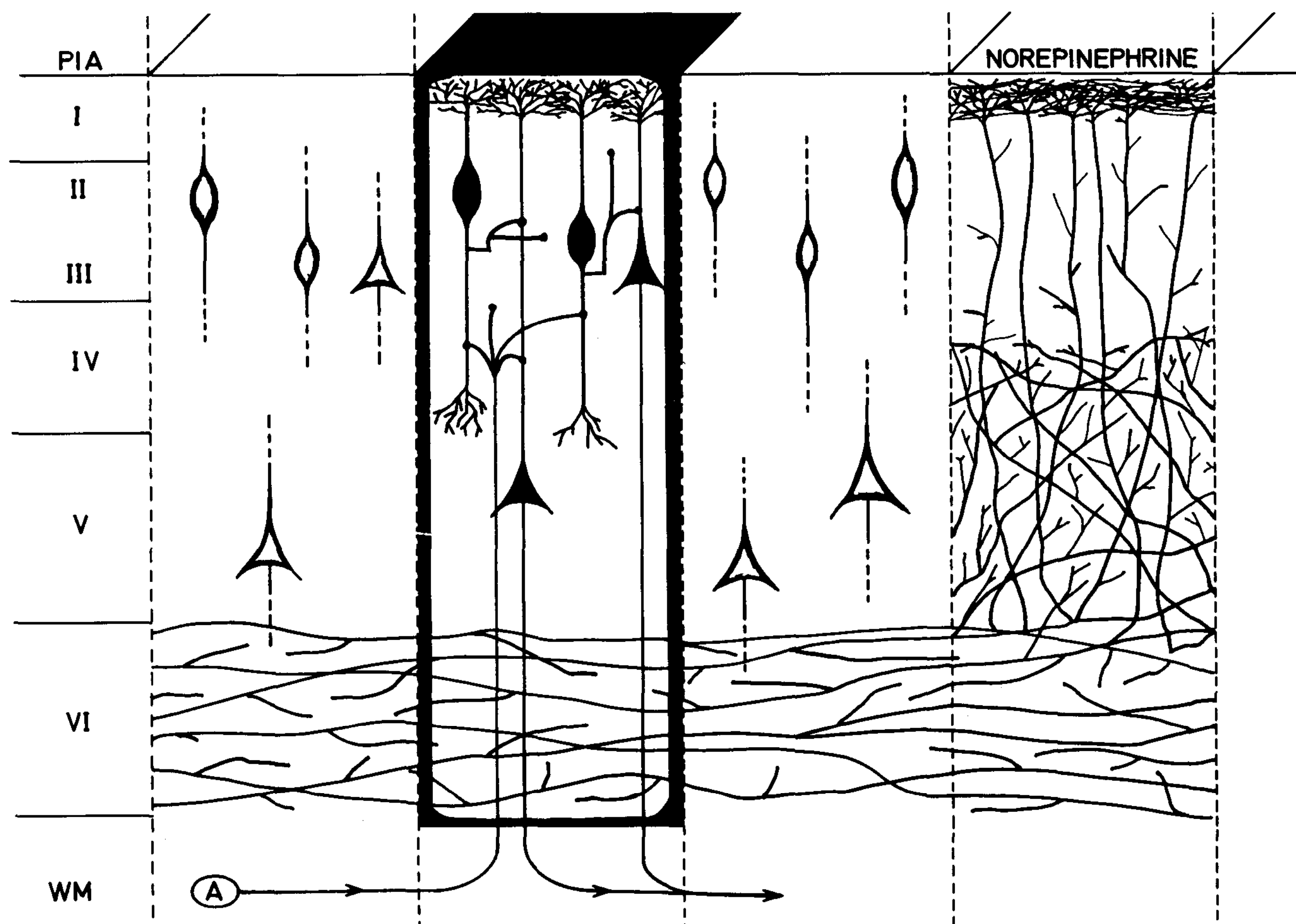

Figure 6. Diagrammatic representation of a "metabolic hot spot" resulting from the concomitant activation of noradrenergic cortical afferents and a group of VIP-containing neurons. *Bottom* (layer VI), Tangentially organized noradrenergic fibers. *Right,* Detail of the arborization pattern of noradrenergic fibers within the cortex (Morrison et al., 1978, 1981). *Ovoid-shaped cells,* VIP-intracortical neurons; *triangular-shaped cells,* pyramidal neurons. *Solid symbols* refer to activated neurons. *Far left,* Cortical layers. *WM,* white matter. The concomitant activation of noradrenergic fibers by unexpected sensory stimuli (Aston-Jones and Bloom, 1981a, b) and of a group of VIP-containing intracortical neurons by specific thalamic inputs (*A, bottom left*) would determine a drastic increase in cAMP levels within a discrete volume of somatosensory cortex (delineated in this drawing by *solid, thick lines*). See "Discussion" for details. For graphic clarity, only the VIP-containing and the pyramidal cells have been represented here. In particular, the principal target cells of the thalamocortical afferents, i.e., the small stellate cells in layer IV, have been omitted. However, any cell with the capacity of dendritic reception in layer IV may receive thalamic inputs (White, 1981).

this action (Perkins and Moore, 1973; Schultz and Daly, 1973a), whereas in mouse cerebral cortex a β-adrenergic mediation appears to predominate (Schultz and Daly, 1973a).

In contrast to NE, HIS and 5-HT per se increase only marginally cAMP levels in mouse cerebral cortex (Daly 1975; Fig. 4 legend). It is interesting to note that in guinea pig hippocampal slices, HIS stimulates the formation of cAMP predominantly through H_2-receptors and this effect is positively modulated by the simultaneous occupation of H_1-receptors (Schwartz, 1979). Furthermore, in guinea pig cerebral cortical slices, HIS and NE display greater than additive effects in stimulating cAMP formation, and this synergism appears to be mediated by the concomitant activation of α-adrenergic and H_1-histaminergic receptors (Daly, 1975). Sattin et al. (1975) have reported that the synergistic interaction between NE and adenosine in stimulating the formation of cAMP required the activation of α-adrenergic receptors by NE. Single-cell recordings from the lateral geniculate nucleus have shown that activation of α_1-adrenergic receptors increases the efficacy of excitatory synaptic inputs rather than eliciting direct excitatory effects (Rogawski and Aghajanian, 1980a, b). Thus, the results reported in this article, taken together with previous physiological and pharmacological studies of adrenergic and histaminergic receptors, suggest that activation of CNS α_1-adrenergic and H_1-histaminergic receptors may lead to the facilitation of certain cellular effects of other neurotransmitters.

A functional dimension of the synergism between VIP and NE or HIS may be proposed by relating this interaction to the morphological characteristics of the neuronal systems that contain the peptide and the two monoamines in cerebral cortex. We will focus our attention more particularly on the interaction between VIP and NE since the noradrenergic coeruleocortical projection is best characterized. Several considerations can be made. First, the existence of a functional synergism observed *in vitro* between VIP and NE on an intracellular event such as the increase in cAMP suggests that at least part of the target cells of VIPergic and noradrenergic neurons in cerebral cortex may be common to the two neurotransmitter systems. We have previously suggested that the concomitant activation of noradrenergic coeruleocortical cells and of a group of VIP-containing intracortical neurons would promote marked increases in the levels of cAMP within a discrete volume of the cerebral cortex (Magistretti and Schorderet, 1984). The spatial coordinates of such a "cortical metabolic hot spot" would be delineated by the intersection of the tangentially organized noradrenergic fibers and a group of activated, radially oriented, bipolar VIP-containing neurons (Fig. 6).

Second, Madison and Nicoll (1982) have demonstrated that NE and cAMP, by blocking a Ca^{2+}-activated K^+ conductance, profoundly increase the responses of hippocampal pyramidal cells to depolarizing stimuli. This observation has recently been confirmed for NE by Gruol and Siggins (1984). Thus, if similar cAMP-dependent intracellular mechanisms were to be operational within the neocortex, this would imply that the concomitant activation of the noradrenergic coeruleocortical projection and a group of VIP-containing intracortical neurons would lead to a sustained responsivity of neocortical pyramidal cells within a discrete cortical volume. The question then arises of when VIP- and NE-containing neuronal systems would be simultaneously active in cerebral cortex.

Electrophysiological recordings in behaving rats have demonstrated that NE-containing neurons exhibit pronounced responses to non-noxious, unexpected auditory, visual, and somatosensory stimuli (Aston-Jones and Bloom, 1981a, b). The firing pattern and activatory inputs to VIP intracortical neurons are not known. However, at the light microscopic level, the overall morphological characteristics and pattern of dendritic arborization of VIP-immunoreactive neurons resemble those of the "bipolar cell" described in Golgi-impregnated material by Peters and Kimerer (1981). These authors, by using a combined Golgi-electron microscopy technique, have demonstrated that in rat visual cortex "bipolar cells" receive direct thalamocortical inputs. These observations would therefore suggest that VIP-bipolar neurons could also be activated by sensory inputs relayed by specific thalamocortical afferents and, hence, that sensory stimulation could constitute a behavioral event whereby the synergistic interaction between VIP and NE would become operative within the terminal field of thalamocortical afferents in primary sensory cortex.

The resolution at the cellular level of the synergism between VIP and NE cannot be achieved with the experimental paradigm used in this set of investigations. Recent evidence at the ultrastructural level indicates that VIP-immunoreactive cortical neurons preferentially synapse with the shafts of small diameter dendrites and that the synapses they form are predominantly symmetric (Peters and Connor, 1983).

At present, however, the identity of the target cells where the synergistic interaction between VIP and NE (and HIS) is operational constitutes an open question. The observations reported in this article may provide a useful biochemical and pharmacological background for future electrophysiological and morphological studies aimed at the definition of the nature of the target cells of VIP-, NE-, and HIS-containing neurons within the cerebral cortex.

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