

Vasoactive intestinal polypeptide acts synergistically with norepinephrine to depress spontaneous discharge rate in cerebral cortical neurons

(transmitter interactions/iontophoresis/phenylephrine/monoamines/neuropeptide)

ANDRE FERRON*, GEORGE R. SIGGINS†, AND FLOYD E. BLOOM†

*Departement de Physiologie, Faculte de Medecine, Universite de Montreal, C.P. 6208, Succursale A, Montreal, PQ, Canada H3C 3T8; and Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

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ABSTRACT Cortical neurons are densely innervated by noradrenergic fibers and by intrinsic cortical interneurons containing vasoactive intestinal polypeptide (VIP). Biochemically, VIP and norepinephrine (NE) synergistically interact to stimulate the synthesis of cyclic AMP in cortical slices. Therefore, we sought physiological indices of this peptide-monoamine interaction by applying VIP and NE to single cortical neurons of the rat while recording their spontaneous discharge. VIP applied alone inhibited discharge of 24% and accelerated discharge in 20% of cortical neurons. NE alone had a predominantly depressant effect on the same neurons. However, when VIP was retested during the continuous application of subthreshold currents of NE, VIP exerted predominantly depressant effects. These synergistic inhibitions resulted even in cells previously showing excitations to VIP alone. If VIP alone was depressant, subthreshold NE further enhanced the VIP depression. Subthreshold amounts of phenylephrine, an α -adrenoceptor agonist, also produced comparable interactions, suggesting involvement of an α receptor, as in the biochemical studies. These results support a peptide-monoamine interaction in cortex that could have important ramifications for neuronal integration.

A neuronal messenger role for vasoactive intestinal polypeptide (VIP) in cerebral cortex is supported by its high concentration there (1, 2), its location primarily in bipolar neurons (2, 3), and its release from synaptosome fractions by a K^+ -sensitive, Ca^{2+} -dependent mechanism (4, 5). Specific binding of labeled VIP in cortex (6) and excitation of some cortical neurons by iontophoretically applied VIP (7, 8) suggest the presence of VIP receptors, as does its ability to stimulate cyclic AMP formation (9, 10) and glycogenolysis (11) in cortical slices [at lower concentrations than for norepinephrine (NE)]. Although NE- and VIP-containing neurites in cortex show different organizational patterns (12), these axons may converge on a common target neuron, the pyramidal cell (3, 13). Single unit studies of identified cortical pyramidal neurons have demonstrated comparable depressions of discharge upon iontophoresis of both NE and cyclic AMP (14). Interestingly, biochemical findings suggest that VIP and NE act synergistically to increase cyclic AMP in cerebral cortex (10). Therefore, we have applied VIP and NE iontophoretically to rat cortical neurons to determine if this interaction might be detectable at the level of the single cell. We now report that application of VIP during subthreshold NE administration results in pronounced inhibition of cellular discharge even when VIP alone had no effect or excitatory actions prior to NE.

MATERIALS AND METHODS

Sprague-Dawley rats (200–300 g body weight) were anesthetized with urethane (1.5 g/kg), placed in a stereotaxic frame and surgically prepared for single unit recording in parietal cortex (see ref. 15). Standard extracellular recording and iontophoretic methods were used with 5-barrel micropipettes (see ref. 15). The central barrel contained 3 M NaCl (5–10 M, tip resistance) for recording, as did one outer barrel used for automated current neutralization. The other outer barrels contained VIP (1 mM in 150 mM NaCl), NE·HCl (0.2 M in water) and, in some experiments, phenylephrine·HCl (0.2 M in water). All substances were applied by positive currents and retained by 5–15 nA negative currents. VIP was obtained from N. Ling (Salk Institute), J. Rivier (Salk Institute), or Peninsula Laboratories (Belmont, CA). Extracellular action potentials (300–600 V) were recorded at 1- to 10-kHz band-pass, gated by a “window” discriminator, and converted to square pulses by a Schmitt trigger. The pulses were then led to the computer for generation of histograms and also integrated over 1-sec intervals for output to a polygraph ratemeter. Recordings were taken from a cortical region 2-mm lateral from midline and 0.5- to 1.5-mm posterior to bregma. The cortical surface was covered by 2% (wt/vol) agar in saline. The bipolar stimulating electrode was placed ipsilaterally, 0.5 mm from midline at λ and 11.5–12 mm from the pial surface. Stimulus pulses were 50–150 A, 0.2 msec duration, delivered at 1–10 Hz.

RESULTS AND DISCUSSION

We initially tested the direct effects of VIP alone on the spontaneous firing rate of sensorimotor cortical neurons. Of more than 100 neurons tested with ejection currents of 50–200 nA for 30 sec, VIP depressed 24%, excited 20%, had biphasic effects on 2%, and had no apparent effect on 54% of these cells. Fourteen cells were identified as pyramidal neurons on the basis of antidromic activation from an electrode in the pyramidal tract; these cells showed the same heterogeneity of response as unidentified cells. Although a majority of cells were obtained in the deeper cortical layers (800–1500 μ m), where pyramidal neurons are concentrated, there was no apparent relationship between direction of the response and the depth from the pial surface.

After testing the effects of VIP alone, the cells were examined for their responsiveness to NE. As reported (see Ferron *et al.*, ref. 15, and ref. 16 for review), NE had a predominantly depressant action in cortex. In this study, whenever a neuron responded to NE (5–100 nA), the current was reduced until little or no effect was detectable (usually

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Abbreviations: VIP, vasoactive intestinal polypeptide; NE, norepinephrine.

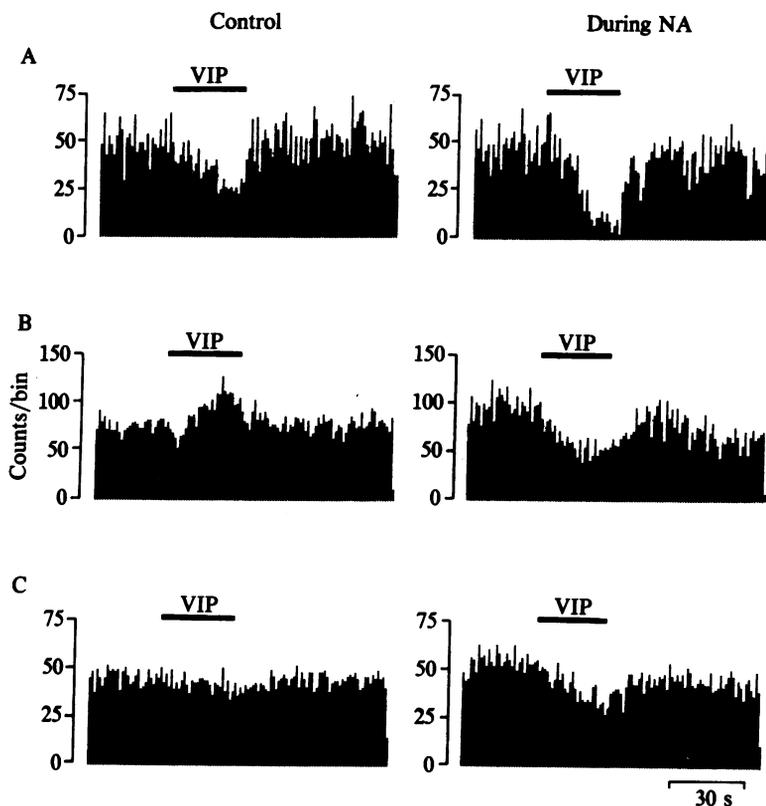


FIG. 1. Effect of VIP before and during NE application. Each panel is a peridrug cumulative frequency histogram generated by computer from 3–4 sweeps, each consisting of one 30-sec control period followed by 30 sec of VIP and 60 sec of recovery. Each bin = 1 sec. Bar above record indicates duration of VIP ejection from the micropipette. (A) Effect of NE application on the inhibitory effect of VIP. (B) Different cell in which NE changes the excitatory effect of VIP to an inhibitory response. (C) Different cell in which VIP had no apparent direct effect but had an inhibitory effect during NE application.

1–5 nA) and possible interactions between NE and VIP were investigated. The subthreshold current of NE was applied continuously for several minutes while VIP was retested with 30-sec pulses applied at 2-min intervals. Following 3 or 4 such pulses, recovery of the control response to VIP alone was always verified. Forty-seven neurons were studied in this paradigm. In over 50% of these cells, ejection of VIP during subthreshold NE administration resulted in pronounced reduction of firing rate (Fig. 1). Such synergistic inhibitions resulted whether VIP alone had previously elicited excitatory, inhibitory, or negligible effects on the test neuron (Table 1). When VIP alone had a depressant action, administration of NE markedly enhanced this depressant effect (Fig. 1). Even in cases where VIP alone was excitatory, concurrent subthreshold NE treatment reversed the VIP effect from excitation to inhibition (6 of the 9 cells; Fig. 1).

NE alone activates cyclic AMP synthesis in cortex via β -adrenergic receptors (17). However, in the studies of Magistretti and Schorderet (10), the synergism between VIP and NE in this cyclic AMP activation was blocked by phentolamine, an α -adrenoceptor antagonist, and mimicked by phenylephrine, an α -receptor agonist. Hence, the synergism of VIP-induced cyclic AMP increasingly appears to be

mediated by an α adrenoceptor. Therefore, we examined the effect of phenylephrine as well as NE pretreatment on neuronal responses to VIP. In 10 cells showing an interaction between VIP and NE, 9 revealed comparable, pronounced interactions with phenylephrine. Thus, the interaction of VIP and NE at the cellular level may also involve α -receptor activation, although confirmation of this hypothesis will require a more extensive pharmacological analysis.

It is not clear why we observe direct depressant actions of VIP on cortical neurons more frequently than reported (7, 8), despite similar conditions of iontophoresis and anesthesia. However, our results suggest that the nature of the direct response to VIP could depend upon the extent of ongoing activation of adrenoceptors by endogenous NE. An alternate explanation for the present results could be that VIP enhances NE-induced inhibitions so that its own direct excitatory effect is invariably masked by these inhibitions. Conceptually, the cortical influence of VIP-containing bipolar neurons could be viewed as bidirectionally conditional. That is, under conditions of minimal noradrenergic tonus these neurons might transmit excitatory information, whereas during periods of enhanced NE release—for example, upon behavioral arousal (16)—they could become powerfully inhibitory.

In any event, our demonstration of an interaction between VIP and NE at the cellular level is consonant with the biochemical data on cortical slices using cyclic AMP generation as the end point (10). Such parallel findings may point to the mediation by cyclic AMP of NE and VIP evoked depressions of neuronal firing in cortex, as suggested by Stone and Taylor (14) for responses of identified pyramidal cells to NE.

Our electrophysiological indications of a VIP-NE interaction at the cellular level may arise from their biochemical effects *in vitro* on cyclic AMP generation. Such parallel findings strengthen the suggestions that cyclic AMP may mediate both NE and VIP evoked depressions of neuronal firing in cortex (see ref. 14). The reported enhancement by

Table 1. VIP-NE interactions in rat cortical neurons

Response to VIP alone*	Neurons, no.		
	Change in VIP response with NE*		
	Enhanced	Converted to slowing	No change
Speeded	1	6	2
Slowed	8	—	2
No effect	—	10	18

*A response is defined as a change in spontaneous firing rate by more than 15% of control.

NE of synaptic and other transmitter responses, including inhibitory ones (see ref. 16 for review), may be related phenomena. The cyclic AMP-mediated enhancement by α receptors of noradrenergic target cell responsiveness to β -adrenergic agonists in pineal gland (18) may also be relevant. Furthermore, in rat hepatocytes, increased cyclic AMP levels induced by glucagon enhance binding of α -adrenergic agonists to these cells (19). Two modes of second messenger amplification of postsynaptic target cell mechanisms may be involved in these interactions: a cyclic AMP-mediated activation of protein kinases (as with β -adrenergic and other responses) and a calcium activation of protein kinases [as with α_1 -adrenergic and other transmitter or hormone responses (see ref. 20)]. However, it is not yet clear how these metabolic and electrophysiologic events are actually linked. If NE and VIP-containing fibers do indeed converge on the same cortical target cell, it is feasible that cyclic AMP is the intracellular mediator of their synergistic interaction.

Other similar interactions of a peptide and a monoamine at the cellular level also have been reported—for example, the depressant effect of enkephalin requires the presence of NE in the cerebral cortex (21). These examples of the interaction of a peptide and a monoamine could have important ramifications for neuronal integration in general, since several instances of the same or different nerve fibers carrying these messengers to a common target cell have been reported (3, 12, 22, 23).

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