Electrophysiological Actions of VIP in Rat Somatosensory Cortex

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SESSLER, F. M., S. M. GRADY, B. D. WATERHOUSE AND H. C. MOISES. Electrophysiological actions of VIP in rat somatosensory cortex. PEPTIDES 12(4) 715-721, 1991.—Electrophysiological and biochemical studies suggest that VIP may exert a facilitating action in the neocortical local circuitry. In the present study, we examined the actions of VIP and VIP + norepinephrine (NE) on somatosensory cortical neuron responses to direct application of the putative transmitters acetylcholine (ACH) and gamma-aminobutyric acid (GABA). Spontaneous and transmitter-induced discharges of cortical neurons from halothane-anesthetized rats were monitored before, during and after VIP, NE and VIP + NE iontophoresis. In 57 VIP-sensitive cells tested, VIP application (5-70 nA) increased (n= 18), decreased (n=36) or had biphasic actions (n=3) on background firing rate. In a group of 20 neurons tested for NE + VIP, the combined effect of both peptide and bioamine was predominantly (70%) inhibitory. On the other hand, inhibitory and excitatory responses of cortical neurons to GABA (11 of 15 cases) and ACh (10 of 18 cases), respectively, were enhanced during VIP iontophoresis. Concomitant application of VIP and NE produced additive (n = 2) or more than additive (n = 3) enhancing effects on GABA inhibition. NE administration reversed or enhanced further VIP modulatory actions on ACh-induced excitation. These findings provide electrophysiological evidence that NE and VIP afferents may exert convergent influences on cortical neuronal responses to afferent synaptic inputs such that modulatory actions are anatomically focused within the cortex.

Vasoactive intestinal polypeptide  Norepinephrine  GABA  Cortex  Acetylcholine  Neuromodulation

A number of findings suggest that vasoactive intestinal polypeptide (VIP) may play a role in neuronal transmission within the cerebral cortex (26). VIP is found in high concentration in this area (13), and is located primarily in bipolar neurons oriented perpendicular to the pial surface (13,20). The peptide can be released in a Ca²⁺-dependent manner from synaptosomal preparations (8) and rat brain slices (2). VIP binding has been observed in cerebral cortex (29) and local application of VIP to individual cortical neurons produces both inhibitory and excitatory responses (6, 9, 12, 24). Additional biochemical studies have shown that VIP can alone or by synergistic interaction with norepinephrine (NE) increase cyclic-AMP (cAMP) levels in cerebrocortical tissue (15).

VIP-containing neurons constitute an intracortical peptidergic system which is oriented perpendicular to the tangential trajectory of NE-containing axons emanating from cell bodies in the brainstem nucleus locus coeruleus. It has been suggested (18,20) that this unique anatomical arrangement may subserve a specific physiologic function whereby the potential heterosynaptic interaction of NE-VIP input on common target neurons could create conditions leading to enhancement of cAMP-mediated events. As a first step in assessing VIPergic influences on cerebrocortical synaptic transmission, we examined the interaction between microiontophoretically applied VIP and somatosensory cortical neuron responses to locally applied putative neurotransmitters, gamma-aminobutyric acid (GABA) and acetylcholine (ACh). A second goal of the study was to evaluate the effects of simultaneous application of NE and VIP on spontaneous and evoked cortical neuronal activity.

The results indicate that VIP produces predominantly facilitating effects on transmitter-induced responses of somatosensory cortical neurons. Simultaneous application of NE and VIP produces either additive or more than additive facilitating effects on somatosensory cortical neuronal responsiveness to GABA application. By contrast, NE administration reverses or enhances VIP modulatory effects on ACh-induced excitation. A preliminary report of this work has appeared previously (32).

METHOD

Male Long-Evans hooded rats, 250–350 g, were anesthetized with halothane (0.8–1.5% in oxygen), intubated and allowed to breathe spontaneously. Animals were fixed in a stereotaxic apparatus and body temperature was maintained at 37°C with a heating lamp. Portions of the calvarium and dura were removed to obtain access to the somatosensory cortex. The exposed brain tissue was covered with 2% agar in balanced salt solution. The somatosensory cortex recording electrode was aimed: A-P=0 to 2 mm; L=3 to 5 mm, and 0 to 1.8 mm deep to pial surface (Paxinos et al., 1982). Five-barrel micropipets with 4–8
μm tips were used for drug application and single-unit recording. The center recording barrel of the pipet was filled with 4 M NaCl. One side barrel was filled with 3 M NaCl and used for automatic current balancing. The remaining side barrels contained drug solution as follows: 0.5 M dl-norepinephrine HCl, pH 4.5 (Sigma); 0.5 M GABA, pH 3.8 (Sigma); 1 M acetylcholine chloride, pH 4.5 (Sigma); 0.5–1 mM VIP (in 0.1 M NaCl or distilled water). pH 5.5 (a gift of Dr. S. I. Said of the University of Oklahoma Health Sciences Center, Oklahoma City). Control procedures for assessing potential current artifacts and vehicle (NaCl) effects were carried out routinely. Extracellularly recorded action potentials were monitored using conventional methods.

Regularly spaced iontophoretic pulses of GABA or ACh (10–20 s duration; 5–50 nA) were applied to individual cells until stable agonist responses were obtained. Perievent histograms were then collected before, during and after VIP or VIP + NE iontophoresis. Agonist ejection currents were selected to induce approximately 40–50% of the response maxima. Putative transmitter responses were quantitated by comparing the average discharge rate of the neuron during transmitter application with the mean firing rate between drug pulses and expressing the difference as a percentage inhibition or excitation of baseline firing frequency.

Drug interactions were frequently conducted using iontophoretic doses of VIP which were subthreshold for causing changes in the baseline firing rate of recorded neurons. This procedure reduced the possibility that changes in cell responsiveness observed during VIP administration were due simply to an algebraic summation of ongoing excitatory or inhibitory VIP effects. All interactions between neurotransmitters were judged against criteria based on quantification of responses from histogram records (27,31). Changes in responses to excitatory or inhibitory stimuli by VIP were assessed by comparing the discharge rate in identical epochs of baseline and drug-induced activity taken from control and VIP histograms. VIP was declared to have no significant differential effect on inhibitory or excitatory responses when the percentage of change in evoked versus spontaneous discharge was equivalent. A difference of at least 15% between percent of VIP-induced changes in evoked versus spontaneous activity was established as the criterion for declaring a differential effect (i.e., “augmentation,” “enhancement” or “antagonism”) on evoked versus spontaneous discharge. “Augmentation” of an inhibitory response was declared when firing rate during the response epoch was depressed more by VIP application than was spontaneous discharge. Conversely, the interaction was defined as “antagonistic” when spontaneous discharge was depressed more by VIP than activity occurring during the inhibitory response period.

“Enhancement” of excitatory responses was declared when evoked spiking was increased above control levels relative to no change or suppression of background firing or when activity during evoked excitation was suppressed by VIP but to a lesser extent than was background firing. The interaction was termed “antagonistic” when the excitatory response was more suppressed than spontaneous firing during VIP iontophoresis. These criteria for quantitative evaluation of VIP interactions with neuronal responses to synaptic stimuli have been employed previously to assess NE actions in cerebral cortex (30), cerebellum (19,28) and hypothalamus (4,27).

RESULTS

Of 140 neurons recorded from the somatosensory cortex, 65 (46%) were sensitive to VIP application. The majority of these

| TABLE 1 |
| VIP AND VIP + NE EFFECTS ON SPONTANEOUS FIRING ACTIVITY OF SOMATOSENSORY CORTICAL NEURONS |
|-----------------|-----------------|-----------------|
| N | Inhibition | Biphasic | Excitation |
|-----------------|-----------------|-----------------|
| VIP effects on spontaneous activity (SA) | Enhanced | 57% | 63% |
| Enhanced Inhibition (or reversal) | Enhanced Excitation (or reversal) |
| NE effects on VIP-induced inhibition | 11 | 73% |
| NE effects on VIP-induced excitation | 9 | 67% |

(n = 56, 86%) were found in cortical layers between 700–1600 μm deep from the pial surface. VIP-induced changes in cortical unit responsiveness to ACh and GABA application were observed in cells within this same region.

Effects of VIP on Cortical Neuron Spontaneous Discharge

Only spontaneously active VIP-responsive somatosensory cortical neurons (N = 57) were used in this study. Their frequency of discharge ranged from 0.5 to 26.7 Hz (mean ± 7.1 ± 0.7 Hz; mean ± SE). No significant relation could be established between cell depth, spontaneous firing frequency and response to VIP.

In 36 of 57 cells exhibiting spontaneous discharge, continuous iontophoretic application of VIP (5–70 nA) reduced firing rate by 27.1 ± 3.2% (mean ± SEM) from control. In 18 other cells, spontaneous activity was increased an average of 42.5 ± 10.1% during VIP application. In three remaining cells, VIP induced biphasic effects on baseline activity (see Table 1).

The combined effects of NE + VIP application were examined in 20 VIP-sensitive cells (9 excited and 11 inhibited by VIP). In 70% of these cases (n = 14), NE + VIP application produced depression of somatosensory cortical neuron discharge even though VIP alone evoked excitation in 6 of these cells. In most of these cases, NE was applied at doses that alone produced little or no change in spontaneous firing. In 6 other cases, the combination of NE + VIP produced: 1) excitation like that observed with VIP alone (n = 3) or 2) reversal of VIP-induced suppression of spontaneous activity (n = 3) (see Table 1).

Overall, these results show that VIP alone has facilitatory and inhibitory actions on background firing; however, the combined effect of NE + VIP is predominantly inhibitory. Thus, as previously reported by Ferron et al. (6), NE can convert VIP excitation into inhibition.

Effects of VIP on Cortical Neuronal Responses to GABA

Pulsatile applications of GABA (10-s duration, every 40 s) produced consistent decreases in somatosensory cortical cell discharge. Interactions between VIP and neuronal responses to microiontophoretically applied GABA were analyzed in 15 cells. In the majority of the cases (n = 11, 73%), inhibitory responses to GABA were markedly enhanced during VIP iontophoresis (see Table 2). An example of this effect is shown in Fig. 1A. In this neuron, administration of the peptide (20 nA) produced a reduction in discharge rate during the GABA response period from 4.26 (control) to 1.42 (VIP) spikes/s, but little change in the background firing rate of the cell. This differential action of
FIG. 1. VIP-mediated enhancement of GABA-induced inhibition and interaction with NE-mediated augmentation of GABA inhibition of cortical neurons. Continuous ratemeter (top: A1, B1, C1) and histogram (bottom, A2, B2, C2) show the responses of a cortical neuron to iontophoretic pulses (30 nA; every 40 s) of GABA (solid bars) before, during and after continuous microiontophoresis (broken bars) application of: VIP 20 nA (A); NE 15 nA (B); and VIP 20 nA + NE 15 nA (C). Histograms sum activity during 5 consecutive GABA applications. (A2) Constant application of VIP markedly enhanced the GABA-mediated inhibition from -35% to -79%, without affecting the background activity of the cell, yielding a 126% increase of the inhibition relative to control. (B2) Administration of NE 15 nA augmented the GABA-mediated inhibition from -49% to -63%, yielding a 28% increase of the inhibition relative to control. (C2) Simultaneous administration of VIP and NE augmented the GABA inhibition from -30% to -92%, yielding a 206% increase of the inhibition relative to control period. Calibration: vertical, 5 spikes; horizontal, 20 s (top), 10 s (bottom).

VIP on transmitter-induced versus spontaneous activity yielded a 125% enhancement (from 35% to 79%) of the GABA response relative to the control condition. Recovery to the control level of response was observed a few minutes after cessation of VIP administration.

A summary of the results of similar quantitative analyses for all 15 neurons examined (open squares) is presented in Fig. 2. The majority (73%) of data points lie well above the 45 degree "equivalence" line, indicating cases in which firing rate during the GABA-induced inhibitory period was depressed more during
VIP iontophoresis than was spontaneous discharge. In 4 of 15 cases (27%), spontaneous activity and discharge rate during the GABA response period were affected to an equivalent extent during VIP administration (points plotted closest to the 45 degree "equivalence" line). Overall, this distribution of points indicates a trend toward a VIPergic facilitation of GABA inhibitory action.

Effects of VIP on Cortical Neuronal Responses to ACh

Pulsatile iontophoretic application of ACh (10–20-s duration, every 40–50 s) produced consistent increases in somatosensory cortical cell discharge as illustrated in Fig. 3. Interactions between VIP and cortical neuronal responses to microiontophoretically applied ACh were analyzed in 18 cells (see Table 2). In 10 of these cells (56%), VIP application enhanced ACh-induced discharge relative to spontaneous firing. Figure 3 illustrates one neuron whose responses to ACh administration were increased above control levels during an iontophoretic dose of VIP (30–50 nA) which caused little change in the background activity of the cell. Quantitative assessment of the VIP-induced changes in evoked versus spontaneous firing frequency revealed an absolute increase (38%) in the ACh response from 8.2 (control) to 11.3 (VIP) spikes/s, whereas spontaneous activity was decreased from 1.1 (control) to 0.9 (VIP) spikes/s. Recovery was observed a few minutes after cessation of VIP iontophoresis.

In 4 cells, ACh-induced excitation was antagonized during local administration of VIP, and in 4 other cases spontaneous and ACh-evoked discharge were equally affected by VIP, yielding no net differential change in cholinergic response (see Table 2).

VIP + NE Effects on Cortical Neuronal Responses to ACh and GABA

The effects of VIP + NE on cortical neuronal responses to GABA (n = 6) or ACh (n = 14) were examined in 20 cells. Simultaneous application of VIP and NE enhanced GABA-induced inhibition in 5 of 6 cells tested. In 2 of these cases, VIP + NE-mediated augmentation of GABA responses was equivalent to algebraic summation of the actions of VIP and NE alone on GABA responses. In the 3 other neurons tested, the resulting augmentation was equivalent to a more than additive effect of the monoamine and peptide on GABA depressant actions (see Table 3). Figure 1 illustrates one such case where VIP alone, NE alone and VIP + NE produced 125% (from 35% to 79%), 28% (from 49% to 63%) and 206% (from 30% to 92%) augmentation of GABA responses, respectively.

By contrast, continuous iontophoretic administration of VIP + NE during pulsatile ACh application (n = 14) produced varied effects which were not predictable on the basis of an algebraic summation of the actions of NE and VIP alone on ACh-induced excitation. For example, Fig. 4 illustrates a case where VIP alone and NE alone produced 156% (41% to 105%) and 122% (from 37% to 82%) increases in ACh-evoked responses, respectively. However, in the same cell simultaneous application of VIP + NE produced a 41% (from 37% to 22%) reduction in ACh-induced excitation relative to background firing (see Table 3).

Overall, we observed that NE application further augmented VIP-mediated enhancement of GABA-induced suppression of cortical cell discharge, yet VIP actions on ACh-induced excitation were either enhanced or reversed by NE administration.

DISCUSSION

Although the anatomic organization of VIP-containing neurons in the neocortex has been well studied (16, 17, 21), the effects of VIP on cortical neuron excitability have not yet been examined in great detail. In this study, we employed several experimental strategies to characterize the effects of VIP on neuronal responsiveness within the rat somatosensory cortex. Iontophoretic application of VIP produced both excitatory and inhibitory effects on the firing rate of spontaneously active cortical neurons. The direction of these effects was independent of cortical depth and spontaneous firing frequency of the neurons tested. Concomitant application of NE increased VIP's inhibitory action or, conversely, reversed VIP's excitatory effect on spontaneous discharge. Synergistic interactions between this monoamine and VIP have been reported previously by Ferron et al. (6). In addition, low doses of iontophoretically applied VIP were capable of modulating both excitatory and inhibitory responses.
VIP ACTIONS ON CORTICAL NEURONS

VIP Enhancement of ACh-Induced Excitation

Fig. 3. VIP enhancement of ACh-induced excitation. Continuous ratemeter (top) and corresponding histogram (bottom) records illustrate the response of a somatosensory cortical neuron to iontophoretic pulses (50 nA; every 40 s) of ACh before, during and after continuous microiontophoretic application of VIP (30-50 nA). VIP administration increased the ACh-induced excitation by 72%, from 646% to 1116%. Calibration: Horizontal, 10 s; vertical, 10 (top), 5 (bottom) spikes.

VIP enhancement of cortical neuronal responses to iontophoretic application of ACh and GABA, respectively. We also observed that NE in combination with VIP could enhance peptide-induced changes in neuronal excitability.

It is worth noting that many cells responding to VIP in the present study were located in deep cortical layers (700-1600 μm). VIP-responsive cells have been identified by others as pyramidal neurons (6, 12, 24). Such cells have long apical dendrites which extend from layer V or III to layer I and thus are well positioned to receive convergent synaptic inputs from VIPergic and noradrenergic afferents (18). In addition, because of this unique arrangement (13, 18, 20, 21), VIP-containing neurons and NE-containing cortical afferents may directly influence and modulate the cortico-cortical and corticofugal outputs from targeted regions of the cortex.

VIP Enhancement of Cortical Neuronal Responses to GABA

The results presented here demonstrate that iontophoretically applied VIP can facilitate GABA-induced suppression of cortical neuronal discharge. An important characteristic of this potentiating action is that this effect does not appear to result from a simple summation of hyperpolarizing influences. As shown in Fig. 1, changes in neuronal responsiveness to GABA were observed at ejection currents of VIP that had little direct effect on spontaneous discharge. Thus amplification of GABA responses by VIP can occur independently of direct inhibitory effects on spontaneous firing mechanisms. We have recently observed in pilot experiments that iontophoretically applied VIP can also potentiate (7 of 8 cases) responses of cortical cells to synaptically mediated inhibition.

In terms of what is known of VIP’s cellular actions, it seems reasonable to suggest a link between the potentiating effect of VIP on inhibitory neuronal responses and its ability to stimulate intracellular production of cAMP. VIP has been shown to activate adenylate cyclase in each of the tissues that possess high-affinity VIP receptors (23, 26). Furthermore, cyclic AMP-mediated phosphorylation of a subunit of the GABA-A receptor has been proposed as a possible means of regulating the efficacy of GABA at central synapses (11). In this regard, VIP effects on GABA-induced neuronal responses are similar to those observed with other agents which elevate intracellular levels of cyclic AMP such as isoproterenol (beta agonist), 8-bromo cyclic AMP (membrane permeant analog of cyclic AMP), forskolin (direct activator of adenyl cyclase) and IBMX (phosphodiesterase inhibitor) (28).

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<th>TABLE 3</th>
<th>VIP + NE EFFECTS ON GABA- AND ACh-INDUCED NEURON RESPONSES</th>
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<td>GABA-induced inhibition</td>
<td>6 50%</td>
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<td>ACh-induced excitation</td>
<td>14 29%</td>
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When VIP and NE were applied simultaneously, they produced additive and in some cases more than additive enhancement of GABA-evoked inhibition. These alterations in GABA-responsiveness may be related to a combined action of VIP and NE affecting intracellular levels of cAMP. For example, the interaction between VIP and other drugs known to stimulate production of cAMP (e.g., dopamine, norepinephrine, isoproterenol, prostaglandin E1, adenosine) can produce additive (25) and in some case more than additive (15) increases of cAMP in brain tissue (25).

VIP Modulation of Cortical Neuronal Excitatory Responses

Iontophoretically applied VIP produced predominantly facilitatory (56%) and in some cases antagonistic (22%) effects on ACh-evoked discharges of cortical neurons. Results from other studies have shown that such VIPergic influences on ACh-evoked responses are specific for cholinergic mechanisms (12). More recent experimental evidence suggests that VIP may also exert its facilitating action on ACh-induced excitation through a cAMP-dependent intracellular mechanism. In the hippocampus (14) and neocortex (7), intracellular increase of cAMP, or direct application of a membrane permeant analog of cAMP (14) has been shown to block accommodation of pyramidal cell discharge, thus resulting in a net increase in neuronal response to depolarizing stimuli. Since mechanisms responsible for blockade of accommodation are also sensitive to ACh (22), they could be a target for converging VIP + ACh influences on neuronal excitability.

The likelihood of a physiologically relevant VIP-ACh interaction is further supported by a report describing an intracortical cholinergic system with the same morphological characteristics as VIP neurons (5). Over 80% of the choline acetyltransferase (ChAT)-positive neurons described in that study also contained VIP. The fact that chronic atropine treatment causes a 75% increase in VIP receptors in cerebral cortex (1) also suggests a functional VIP-ACh interaction.

In many cases, application of NE potentiated VIP's actions on ACh-evoked excitatory responses. These effects were observed even if NE and VIP alone had opposite actions on cholinergic responses. In other cases, NE application antagonized VIP's effects on ACh responses. This antagonistic interaction was observed even when application of NE or VIP alone had facilitating effects on ACh responses (Fig. 4). While the present studies made no attempt to identify the mechanisms responsible for these mixed effects, the data suggest a unique mode of modulatory interaction between monoamine and peptide in cortical circuits.

In summary, these findings provide electrophysiological evidence that VIP can alter inhibitory and excitatory somatosensory cortical neuronal responses to iontophoretically applied putative transmitters. This action of VIP could be of importance in understanding the precise function of this neuropeptide in cortical circuits and its involvement in various pathological conditions (3,10). Our observations further suggest that VIP and NE can act together to alter the efficacy of local excitatory and inhibitory inputs to neocortical neurons. These findings support the hypothesis that the essentially perpendicular orientation of NE
fibers and VIPergic neurons in the cerebral cortex may subserve a precise physiological function (15,18). Specifically, NE and VIP afferents may exert convergent influences on cortical neuronal responses to afferent synaptic inputs such that modulatory actions are anatomically focused within the cortex. VIP neurons could then be part of a local mechanism whose function would be to regulate an otherwise global effect of NE on signal processing within neocortical circuits.

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