0270-6474/83/0312-2487$02.00/0

Printed in U.S.A.

The Journal of Neuroscience
Vol. 3, No. 12, pp. 2487-2493
December 1983

ACTIVATORS OF CYCLIC ADENOSINE 3′:5′-MONOPHOSPHATE ACCUMULATION IN RAT HIPPOCAMPAL SLICES: ACTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP)[1]

ANNE M. ETGEN*,[2] AND EDWARD T. BROWNING‡

* *Department of Biological Sciences, Rutgers University, Livingston Campus, New Brunswick, New Jersey 08903 and* ‡*Department of Pharmacology, Rutgers Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854*

Received March 7, 1981; Revised June 20, 1983; Accepted June 29, 1983

Abstract

The present experiments tested the ability of putative neurotransmitters and neuromodulators to regulate cyclic adenosine 3′:5′-monophosphate (cAMP) levels in rat hippocampal slices. Slices from ovariectomized adult female rats were equilibrated for 1 hr and incubated for 20 min with various test compounds, and cAMP was extracted and quantified using a competitive protein-binding assay. Norepinephrine, adenosine, histamine, and prostaglandins E_1 and $E_{2\alpha}$, induced moderate (1.5- to 5-fold) increases in cellular cAMP, whereas dopamine, serotonin, prostaglandin $F_{2\alpha}$, and glutamate were relatively ineffective. Most striking was the observation that vasoactive intestinal peptide (VIP) produced marked elevation (approximately 80-fold at 6 μM) of hippocampal slice cAMP content. In contrast, other peptides produced only 2-fold increases (glucagon, somatostatin) or no change in cellular cAMP levels (enkephalins, LHRH, ACTH analogue, arginine vasopressin). Significant elevations in cAMP were seen with VIP concentrations as low as 20 nM; the cAMP response was half-maximal at 1 μM VIP and maximized between 10 and 20 μM. At maximally effective concentrations, VIP was 86% as effective in increasing cAMP as maximal concentrations of forskolin, a compound which activates adenylate cyclase in most cell types. The cAMP response to 10 μM VIP was pronounced after a 1-min incubation (16-fold elevations) and was maximal at 30 min (140-fold elevation). When slices from other brain areas were compared, it was found that regions known to contain high levels of VIP (cerebral cortex) also responded to VIP treatment with 30- to 50-fold elevations in cAMP. Hypothalamus-preoptic area, midbrain, and cerebellum, whose endogenous VIP levels decrease in that order, showed reduced cAMP responses to VIP treatment (9-, 7-, and 1.2-fold elevations, respectively). Further experiments demonstrated that removal of calcium from the bathing medium severely attenuated the ability of VIP to stimulate cAMP accumulation in hippocampal slices. Moreover, VIP-induced cAMP elevations were not dependent on neural firing since co-incubation of slices with VIP and 2 μM tetrodotoxin did not alter the cAMP response to VIP. These data suggest that VIP may be an important physiological regulator of hippocampal cAMP, probably via direct interactions of the peptide with VIP-specific receptors.

Neurotransmitters and neuromodulators may regulate neuronal function in several instances by initiating a cascade of events involving cyclic adenosine 3′:5′-monophosphate (cAMP), calcium, and specific protein phosphorylations (see Greengard, 1981). In preliminary studies, we have reported (Etgen and Browning, 1982) that treatment of rat hippocampal slices with forskolin, a drug which directly activates adenylate cyclases (Seamon et al., 1981), both elevates cAMP content and increases the phosphorylation of one or more proteins in the slices. Since hippocampal slices maintained *in vitro* retain many of the metabolic and electrophysiological properties demonstrated by the structure *in vivo* (see Lynch et al., 1975), slices of this limbic structure may be used as a model system to study the physiology, biochemistry, and pharmacology of higher brain function. The experiments described in the present paper were designed to identify which putative neurotransmitters and neuromodulators

[1] We express our appreciation for excellent technical assistance to Ms. Beverly Poelstra. This work was supported by United States Public Health Service Grants MH 36041 and BRSG PHS RR 07058 and National Science Foundation Grant BNS 81-10564.

[2] To whom correspondence should be addressed.

might be endogenous regulators of cAMP in our hippocampal slices. Future studies then can evaluate the role of endogenous regulators of cAMP as stimulants to protein phosphorylation in selected target cells of the hippocampus.

Most of the compounds chosen for initial study have been identified previously as potential neurotransmitter candidates capable of altering cyclic nucleotide levels in the cerebral cortex and/or hippocampus, e.g., norepinephrine (Segal, 1981; Segal et al., 1981), histamine (Haas et al., 1978; Segal et al., 1981), adenosine, serotonin, glutamate (Nimit et al., 1981; Segal et al., 1981), dopamine, prostaglandins (Palmer et al., 1973; Wellman and Schwabe, 1973), and vasoactive intestinal peptide (VIP) (Quik et al., 1978; Seamon et al., 1981; Daly et al., 1982). The neuropeptide VIP is particularly interesting because (*a*) the highest endogenous concentrations of VIP are found in the cerebral cortex and certain limbic structures including the hippocampus (Bryant et al., 1976; Larsson et al., 1976; Said and Rosenberg, 1976; Fuxe et al., 1977; Besson et al., 1979; Loren et al., 1979); (*b*) VIP has been reported to stimulate adenylate cyclase in mammalian brain (Deschodt-Lanckman et al., 1977; Quik et al., 1978; 1979; Borghi et al., 1979; Kerwin et al., 1980; Huang and Rorstad, 1983); (*c*) VIP is localized in synaptic vesicles and can be released from synaptosomes and from brain slices by various depolarizing agents (Giachetti et al, 1977; Emson et al., 1978; Pelletier et al., 1981; Besson et al., 1982); and (*d*) it has been reported that hippocampal VIP levels are regulated by corticosterone (Rotsztejn et al., 1980), whose major neural target is the hippocampus (see McEwen, 1982).

Materials and Methods

Preparation and incubation of tissue slices. Adult female Sprague-Dawley rats were ovariectomized at least 1 week before use. Animals were sacrificed by decapitation and the brains were quickly removed and placed into ice-cold incubation medium (NaCl, 124 mM; KCl, 5 mM; KH_2PO_4, 1.24 mM; $MgSO_4$, 1.3 mM; $CaCl_2$, 2.4 mM; $NaHCO_3$, 26 mM; glucose, 10 mM; Yamamoto, 1972). Hippocampi were removed bilaterally and cut into 350-μm transverse slices with a McIlwain tissue chopper. Slices were randomly placed (1 or 2/well) into wells of Linbro tissue culture trays (3.5-ml capacity) containing 300 to 350 μl of freshly oxygenated incubation medium and were maintained at 33 to 35°C with shaking (80 oscillations/min) in an O_2/CO_2 (95/5) saturated environment. In one series of experiments, slices of cerebellum, hypothalamus-preoptic area, cerebral cortex, and midbrain were prepared in a similar manner. Hypothalamic and cerebellar slices were cut in the sagittal plane; all other slices were cut in the transverse plane. In all experiments, slices were allowed to equilibrate for 1 hr before addition of drugs. Unless indicated, incubations with drugs were for 20 min. Drugs other than forskolin were added directly to the incubation wells as concentrated solutions in 3 to 3.5 μl of incubation medium. Forskolin was initially dissolved in ethanol at 10 mM and diluted to 200 μM with incubation fluid just before addition. Addition of ethanol to incubation samples in amounts equivalent to that added along with forskolin produced no change in cAMP content of slices. Experiments were terminated by transferring slices to 400 μl of ice-cold 5% (w/v) trichloroacetic acid (TCA) and mixing immediately.

Slice extraction. After standing on ice for approximately 30 min with occasional shaking, the slices were disrupted by sonication and centrifuged 4 min at 8000 × *g*. The supernatant material was removed, made 0.1 M in HCl, and extracted five times with 2 vol of water-saturated ether to remove TCA. The resulting aqueous extracts were concentrated by lyophilization and were analyzed for cAMP. The TCA-insoluble cellular residues were dissolved in 1 M NaOH and analyzed for protein by the method of Lowry et al. (1951).

cAMP assay. cAMP was assayed using a binding assay essentially as described by Brostrom et al. (Brostrom and Kon, 1974; Brostrom et al., 1979).

Statistical analysis. The cAMP data were expressed as picomoles per milligram of cellular protein, subjected to a log transformation, and analyzed by one-way analysis of variance. When significant overall differences were found, planned post hoc pairwise comparisons were made using the Newman-Keuls procedure at a significance level of $p < 0.05$ (Winer, 1962).

Materials. Sprague-Dawley rats were purchased from Blue Spruce Farms, Inc. (Altamont, NY). VIP was purchased from either Sigma Chemical Co. (St. Louis, MO). or Bachem (Torrance, CA) or was a gift of Dr. S. I. Said. Samples from all three sources produced large increases in cAMP of the rat hippocampal slices. Substance P, Met-enkephalin, and D-Ala^2,Met^5-enkephalinamide were obtained from Bachem. Forskolin was obtained from Calbiochem-Behring Corp. (La Jolla, CA). All other biochemicals were obtained from Sigma.

Results

Effect of forskolin, putative neurotransmitters, and neuropeptides on cAMP levels. Forskolin, an activator of adenylate cyclase in most types of animal cells, was included in the present study to test for the responsiveness of the cAMP synthetic system of the slice preparation. Data in Table I show that 20 μM forskolin produced a 60-fold elevation in cellular cAMP content after 20 min of incubation. This concentration of forskolin produces a maximum increase in the cAMP content of the hippocampal slice preparation (A. M. Etgen and E. T. Browning, unpublished observations). Norepinephrine (10 μM) and adenosine (100 μM) produced much smaller but statistically significant rises in cellular cAMP (4.7- and 2-fold, respectively), while histamine (100 μM) and prostaglandins E_1 and E_2 (10 μM) produced still smaller increases which were not significant statistically. Dopamine (100 μM), serotonin (100 μM), and prostaglandin $F_{2\alpha}$ (10 μM) were without effect, and glutamate (1 mM) tended to lower cAMP concentrations. In contrast, VIP (6 μM) was a potent stimulator of cAMP accumulation, producing a 76-fold increase in cAMP.

Because of the large increase in cAMP produced by VIP, this effect was explored further. First, VIP was compared to other peptides to assess the specificity of

the action of VIP. Table II demonstrates that the hippocampal cAMP response is specific to VIP. It can be seen that at equimolor concentrations (0.5 μM), VIP induced a significant 55-fold elevation of cAMP whereas the structurally related peptide glucagon and the neurohormone somatostatin produced only 2-fold increases which were not significant statistically. Enkephalins, LHRH, and an ACTH analogue had no effect. Arginine vasopressin at a physiological concentration (0.1 nM) produced a small (35%) elevation of cellular cAMP which was not significant statistically.

Concentration and time dependence of VIP-induced cAMP accumulation. Figure 1*A* shows that VIP-induced cAMP elevation is concentration dependent. VIP concentrations as low as 20 nM elevated the cAMP content of rat hippocampal slices 2-fold in 20 min. The response was 50% maximal at approximately 1 μM VIP and maximized between 10 and 20 μM. At these high VIP concentrations, the cAMP content of the slices was increased as much as 84-fold above basal levels. With 30-min incubations, maximal concentrations of VIP produced a cAMP increase that was 86% as large as that produced by maximal concentrations of forskolin, a general activator of adenylate cyclase (Table III).

Figure 1*B* demonstrates that the slices' accumulation of cAMP in response to 10 μM VIP is rapid and time dependent. The concentration of cAMP in hippocampal slices increased 16-fold after a 1-min incubation, reached a maximal value of 140-fold after 30 min, and was declining somewhat at 45 and 60 min. It is not known whether the decline observed at the later times is due to breakdown of VIP by endogenous proteases (Keltz et al., 1980) or to desensitization of the cAMP system following prolonged stimulation.

Regional specificity. Both radioimmunochemical and immunocytochemical methods have shown that VIP concentrations vary widely in different brain regions. Therefore, the regional specificity of VIP-induced cAMP accumulation was investigated. Slices from brain areas containing high (frontal and temporal-parietal cortex, hippocampus), intermediate (hypothalamus-preoptic area, midbrain), and low (cerebellum) endogenous VIP levels were prepared from the same animal and were incubated for 20 min in 1 μM VIP. In Table IV, it can be seen that basal cAMP levels vary considerably among the brain regions examined and that the magnitude of VIP-induced elevations of cAMP content is region specific. As in previous experiments, 1 μM VIP dramatically (71-fold) increased hippocampal cAMP levels. Similarly, slices of frontal and temporal-parietal cortex, which contain the highest endogenous levels of VIP, showed 30- to 46-fold increases in cellular cAMP. The hypothalamus-preoptic area, midbrain, and cerebellum, whose endogenous VIP levels decrease in that order, showed correspondingly reduced cAMP elevations in response to VIP treatment (8.8-, 7.1-, and 1.2-fold increases, respectively).

Calcium dependence. VIP stimulation of cAMP levels in rat brain slices from cerebral cortex and hypothalamus has been reported to be calcium dependent (Quik et al., 1978), and VIP activation of adenylate cyclase in cultured GH3 cells is augmented in the presence of calcium (Brostrom et al., 1983). Therefore, we determined whether changes in the calcium ion concentration altered the cAMP response to VIP in rat hippocampal slices. Data in Table V show that cAMP elevation in response to 1 μM VIP is highly calcium dependent. Maximal increases in cellular cAMP from 6.9 to 330 pmol/mg were seen when slices were incubated in 2.4 mM $CaCl_2$; at 0.8 mM $CaCl_2$, basal cAMP levels were slightly higher (11.7 pmol/mg) and the VIP-stimulated levels were slightly lower (288 pmol/mg). When hippocampal slices were incubated without added calcium, unstimulated cAMP levels remained near 11 pmol/mg of protein but the VIP elevated levels were markedly lower (66 pmol/mg of protein versus 330 pmol/mg of protein). A further de-

TABLE I

Effect of 20 min incubation with putative neurotransmitters on the cAMP content of rat hippocampal slices

Tissue slices were prepared, incubated, and analyzed for cAMP and protein as described under "Materials and Methods." Data presented are mean values ± SEM with the number of replicates indicated in parentheses. The data are a compilation of the results of several different experiments. There was a significant overall effect of treatment ($F(11, 48) = 47.18, p < 0.0001$).

Agent	Concentration	cAMP Content
	μM	*pmol/mg protein*
Control		6.4 ± 1.4 (8)
Forskolin	20	381.0 ± 41.0 (7)[a]
Norepinephrine	10	28.0 ± 9.0 (4)[b]
Dopamine	100	5.5 ± 0.9 (7)
Histamine	100	8.1 ± 1.5 (7)
Serotonin	100	4.8 ± 1.9 (4)
VIP	6	488.0 ± 15.0 (3)[a]
Prostaglandin E_1	10	9.5 ± 2.6 (4)
Prostaglandin E_2	10	10.0 ± 2.3 (4)
Prostaglandin $F_{2\alpha}$	10	5.8 ± 1.9 (3)
Adenosine	100	12.6 ± 1.9 (4)[c]
Glutamate	1000	3.2 ± 1.6 (5)

[a] Significantly different from control, $p < 0.001$.
[b] Significantly different from control, $p < 0.01$.
[c] Significantly different from control, $p < 0.05$.

TABLE II

Effect of 20 min incubation with peptide hormones on the cAMP content of rat hippocampal slices

Data presented are mean values ± SEM with the number of replicates indicated in parentheses. The data are a compilation of the results of several different experiments. There was a significant overall effect of treatment ($F(9, 33) = 54.88, p < 0.0001$).

Agent	Concentration	cAMP Content
	μM	*pmol/mg protein*
Control		5.1 ± 0.9 (6)
VIP	0.5	197.0 ± 12.0 (6)[a]
Glucagon	0.5	10.7 ± 2.7 (5)
Somatostatin	0.5	10.8 ± 4.0 (5)
Substance P	0.5	5.9 ± 0.4 (4)
LHRH	0.5	5.0 ± 0.6 (3)
ACTH analogue	0.5	5.2 ± 0.3 (3)
Met-enkephalin	0.5	5.5 ± 0.4 (4)
D-Ala^2,Met^5-enkephalinamide	0.5	6.0 ± 0.6 (4)
Arginine vasopressin	0.0001	6.9 ± 0.6 (4)

[a] Significantly different from control, $p < 0.0001$.

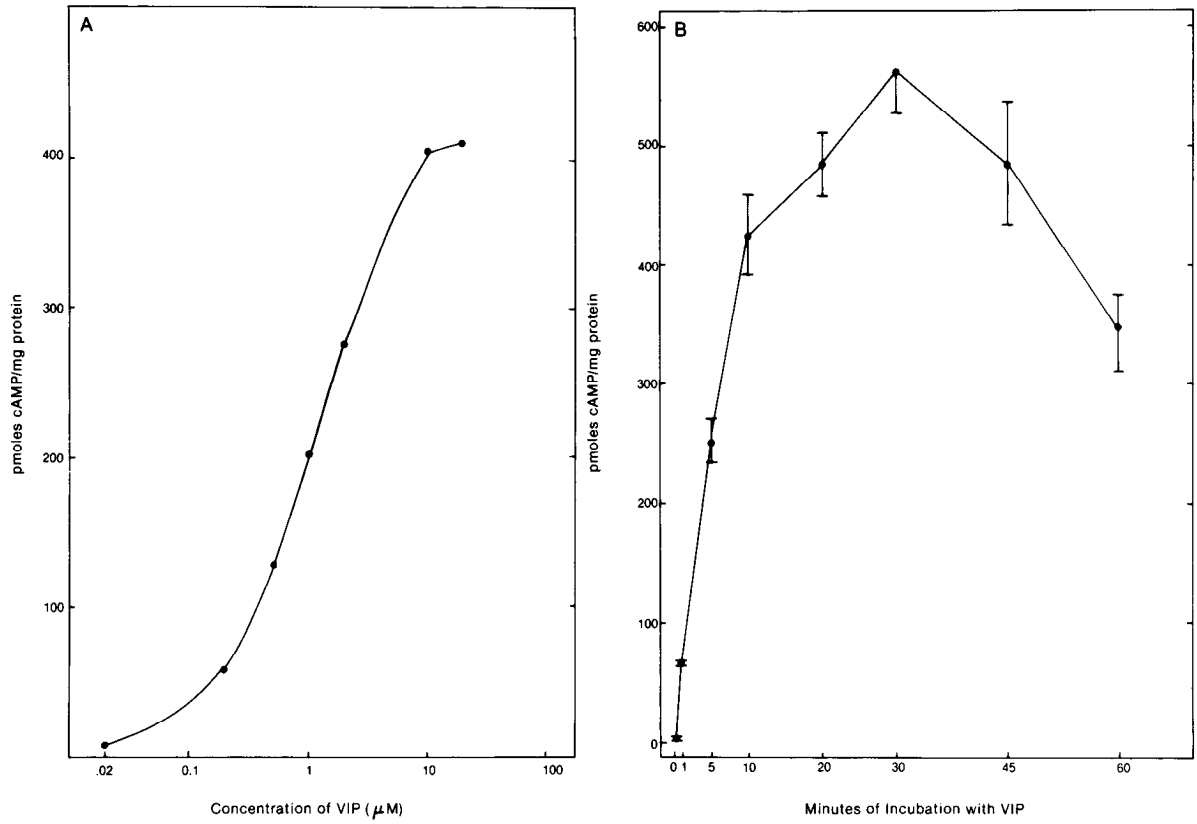

Figure 1. Dose and time dependence of the effect of VIP on the cAMP content of hippocampal slices. *A*, Dose response curve; *B*, time course.

TABLE III

Comparison of VIP and forskolin stimulation of cAMP accumulation in hippocampal slices

In these experiments the incubation time with drugs was 30 min. Data presented are mean values ± SEM with the number of replicates indicated in parentheses.

Agent	Concentration	cAMP Content
	μM	*pmol/mg protein*
Control		5.6 ± 0.4 (6)
Forskolin	20	710.0 ± 40.0 (6)
Forskolin	50	721.0 ± 73.0 (6)
VIP	10	616.0 ± 50.0 (6)
VIP	20	610.0 ± 58.0 (5)

TABLE IV

Effect of VIP on the cAMP content of slices from different rat brain regions

Data presented are mean values ± SEM with the number of replicates indicated in parentheses.

Brain Region	VIP (1 μM)	cAMP Content
		pmol/mg protein
Hippocampus	−	5.1 ± 0.7 (4)
	+	363.0 ± 57.0 (4)
Cerebral cortex (frontal)	−	8.8 ± 0.7 (4)
	+	264.0 ± 43.0 (4)
Cerebral cortex (temporal-parietal)	−	7.8 ± 0.7 (4)
	+	357.0 ± 67.0 (4)
Hypothalamus-preoptic area	−	27.5 ± 7.6 (4)
	+	241.0 ± 57.0 (4)
Midbrain	−	8.6 ± 2.9 (4)
	+	61.2 ± 11.9 (4)
Cerebellum	−	208.0 ± 64.0 (4)
	+	248.0 ± 55.0 (4)

crease in VIP responsiveness (4.8 to 21.4 pmol/mg) was observed with the addition of 100 μM EGTA.

Tetrodotoxin sensitivity. A final experiment assessed whether the VIP-induced elevation of hippocampal cAMP was secondary to VIP stimulation of neuronal firing (Table VI). Equilibrated slices were incubated for 30 min with 2 μM tetrodotoxin (TTX) plus 10 or 20 μM VIP, or with VIP alone. TTX alone had no effect on cAMP levels of the slices. Likewise, TTX did not decrease the approximately 110-fold increase in cAMP content produced by 10 and 20 μM VIP in these experiments. In fact, the cAMP level in slices incubated with TTX plus 20 μM VIP (858 pmol/mg of protein) was significantly greater than in slices treated with 20 μM VIP alone (610 pmol/mg of protein).

Discussion

The present results are in agreement with previous observations (Quik et al., 1978; Nimit et al., 1981; Seamon et al., 1981; Segal, 1981; Segal et al., 1981; Daly et al., 1982) that the putative neurotransmitters norepinephrine, adenosine, and VIP significantly increase cAMP concentrations in rat brain slices. Therefore, our slice preparation appears to be comparable to those used in several other laboratories. More notably, the neuroac-

TABLE V

Calcium dependence of the VIP effect on cAMP content of rat hippocampal slices

Data presented are mean values ± SEM with the number of replicates indicated in parentheses.

Calcium Concentration	EGTA (100 μM)	VIP (1 μM)	cAMP Content
mM			*pmol/mg protein*
2.4	−	−	6.9 ± 0.24 (6)
2.4	−	+	330.0 ± 12.0 (6)
0.8	−	−	11.7 ± 1.1 (6)
0.8	−	+	288.0 ± 30.0 (6)
0	−	−	11.4 ± 3.3 (6)
0	−	+	66.8 ± 20.1 (6)
0	+	−	4.8 ± 1.4 (6)
0	+	+	21.4 ± 5.9 (6)

TABLE VI

Effect of tetrodotoxin on VIP-stimulated cAMP accumulation of rat hippocampal slices

Data presented are mean values ± SEM with the number of replicates indicated in parentheses. There was significant overall effect of VIP treatment ($F(5, 29) = 33.03, p < 0.0001$).

Agent	cAMP Content
	pmol/mg protein
Control	5.6 ± 0.4 (6)
2 μM TTX	7.7 ± 1.9 (6)
10 μM VIP	616.0 ± 50.0 (6)[a]
20 μM VIP	610.0 ± 58.0 (5)[a]
10 μM VIP + 2 μM TTX	600.0 ± 62.0 (6)[a]
20 μM VIP + 2 μM TTX	858.0 ± 116.0 (6)[a, b]

[a] Different from control, $p < 0.0001$.

[b] Different from VIP alone, $p < 0.01$.

tive peptide VIP produced a dramatic stimulation of cAMP accumulation by the hippocampal slices. A number of the characteristics of the cAMP response to VIP suggest that this peptide may be an important physiological regulator of cyclic nucleotide levels in the hippocampus. First, the greater than 100-fold elevation of cAMP content stimulated by maximal VIP concentrations (10 to 20 μM) was 86% as great as that produced by maximal concentrations of forskolin, a drug which should directly stimulate adenylate cyclase in most if not all hippocampal cell types (Seamon et al., 1981). Thus, it seems likely that many or perhaps all hippocampal cells are responsive to VIP. Second, VIP concentrations within the physiological range (20 nM) reliably produced a doubling in slice cellular cAMP content, and increased cAMP levels could be detected after 1 min of incubation with VIP, the earliest time interval at which measurements were made. Indeed, the present data may underestimate the potency of VIP in hippocampal slices in that diffusion distances are relatively long and VIP hydrolysis in brain extracts has been demonstrated to proceed at rates which could significantly decrease the VIP concentration within the slice (Straus et al., 1982). Moreover, when a series of neuroactive peptides was incubated with hippocampal slices, only VIP was found to stimulate cAMP accumulation. These observations suggest that the cAMP response is mediated by interaction with VIP-specific receptors (Robberecht et al., 1978; Taylor and Pert, 1979) rather than with glucagon and/or somatostatin receptors. This notion is supported further by our finding that a VIP fragment (VIP 10-28) does not significantly elevate hippocampal cAMP content under identical conditions (A. M. Etgen and E. T. Browning, unpublished observations).

The demonstration that there is a good correlation between endogenous VIP levels in various brain regions and the magnitude of VIP-induced cAMP accumulation is in accord with the observations of regional differences in VIP activation of adenylate cyclase in brain homogenates by Kerwin et al. (1980). Slices from brain regions which, like the hippocampus, contain high endogenous levels of VIP (i.e., frontal and temporal-parietal cortex) also showed 30- to 50-fold increases in cellular cAMP levels when incubated with 1 μM VIP. The hypothalamus-preoptic area and midbrain, which have intermediate concentrations of the peptide, had correspondingly reduced elevations in cAMP (9- and 7-fold, respectively) under the same conditions. Furthermore, cerebellum, which has only 1% of the cerebral cortical VIP concentration, showed very little change in cAMP concentration following a 20-min treatment with 1 μM VIP. These data are also consistent with the hypothesis that VIP plays a significant role in hippocampal physiology.

Quik et al. (1978) have reported a 41% increase in cAMP concentration in hippocampal slices following a 10-min incubation with 0.5 μM VIP. The reason for the more than two orders of magnitude greater response in the present studies is not clear; sex differences in the source of hippocampal tissue are unlikely to account for the discrepancy since we have observed comparable cAMP responses to VIP in slices from intact males, castrated males, and ovariectomized females (A. M. Etgen and E. T. Browning, unpublished observations). However, many other methodological differences exist between the two studies. For example, the 0.5 μM VIP and the 10-min incubation time used by Quik et al. (1978) clearly produced submaximal responses in the present studies (see Fig. 1). Furthermore, data concerning VIP-stimulated cAMP in the brain (Quik et al., 1978) and pituitary (Brostrom et al., 1983) suggest that the cAMP response may be calcium dependent. Our experiments utilized 2.4 mM calcium, whereas those of Quik et al. (1978) utilized 0.8 mM calcium. It seemed possible that our larger cAMP responses might have resulted from our use of the higher calcium concentrations. Indeed when we lowered the calcium concentration of our medium to 0.8 mM, the increase in slice cAMP induced by 1 μM VIP decreased from 48-fold to 25-fold. However, it is still apparent that, under the same concentration, time, and calcium conditions, our slices showed a much greater VIP-induced elevation of cellular cAMP than has been reported previously.

An additional factor which might underlie the VIP sensitivity of our slices is that differences in tissue preparation might render our slices more viable and thus more responsive to VIP stimulation. Quik et al. (1978, 1979) cross-chopped their brain tissue at 260 μm × 260 μm intervals, whereas we used 350-μm transverse slices, a procedure which maintains many intact synaptic contacts and allows one to record both spontaneous and

evoked electrical activity in the slices. Similarly, much greater elevations in retinal cAMP are found when intact retinae (Schorderet et al., 1981) rather than retinal homogenates (Longshore and Makman, 1981) are treated with VIP.

The reason for the extreme calcium dependence of the cAMP response to VIP in the present experiments is unknown. One explanation is that incubation of slices in the absence of calcium for 60 min could produce seizures, resulting in the release of adenosine or other transmitters which desensitize adenylate cyclase (Schwabe and Daly, 1977). Alternately, previous reports that VIP excites CA1 pyramidal cells of rat hippocampal slices (Dodd et al., 1979) and corticospinal neurons (Phillis et al., 1978) suggested that the VIP-induced cAMP elevation might be secondary to the release of neurotransmitters following VIP excitation of neuronal firing. This hypothesis is clearly eliminated by our demonstration that VIP stimulates similar levels of cAMP accumulation in the presence and absence of 2 μM TTX, a concentration which should completely eliminate neural firing. These data provide further support for the idea that the cAMP response is mediated by a direct interaction of the peptide with VIP-specific receptors. The finding that 20 μM VIP induces significantly higher cAMP levels in the presence than in the absence of TTX might even suggest that TTX blocked the release of a neurotransmitter which would normally attenuate the cAMP response to VIP by interacting with receptors (e.g., α_2-adrenergic; see Timmermans and Van Zwieten, 1981) that inhibit activation of adenylate cyclase. In this regard, it is interesting to note that van Calker et al. (1981) reported that stimulation of α_2-adrenergic receptors attenuated VIP-induced increases in cAMP in glial cell cultures from neonatal mouse brain.

These results all argue strongly for the identification of VIP as a significant physiological modulator of cyclic nucleotide metabolism in the hippocampus, probably via direct interactions with VIP receptors. The potency and specificity of VIP in stimulating adenylate cyclase may make it a valuable tool for manipulating cAMP in hippocampal cells. Of particular interest will be studies on the role of VIP in hippocampal protein phosphorylation, the regulation of which may be involved in neural plasticity (e.g., effects of repetitive synaptic stimulation; Browning et al., 1979, 1981) in this brain structure. In addition, the observations that hippocampal VIP levels are regulated by glucocorticoids (Rotsztejn et al., 1980) and that glucocorticoids inhibit VIP-induced cAMP accumulation in cultured pituitary cells (Rotsztejn et al., 1981) suggest the existence of an important neuorendocrine interface. If such an interaction also occurs in the hippocampus, it would provide a mechanism whereby the steroid hormone could indirectly affect cyclic nucleotide metabolism and thereby modify neuronal function.

References

Besson, J., W. Rotsztejn, M. Laburthe, J. Epelbaum, A. Beaudet, C. Kordon, and G. Rosselin (1979) Vasoactive intestinal peptide (VIP): Brain distribution, subcellular localization and effect of deafferentation of the hypothalamus in male rats. Brain Res. *165:* 79–85.

Besson, J., W. Rotsztejn, B. Poussin, A. M. Lhiaubet, and G. Rosselin (1982) Release of vasoactive intestinal peptide from rat brain slices by various depolarizing agents. Neurosci. Lett. *28:* 281–285.

Borghi, C., S. Nicosia, A. Giachetti, and S. I. Said (1979) Vasoactive intestinal peptide (VIP) stimulates adenylate cyclase in selected areas of rat brain. Life Sci. *24:* 65–70.

Brostrom, C. O., and C. Kon (1974) An improved protein binding assay for cyclic AMP. Anal. Biochem. *58:* 459–468.

Brostrom, M. A., C. O. Brostrom, and D. J. Wolff (1979) Calcium dependence of hormone-stimulated cAMP accumulation in intact glial tumor cells. J. Biol. Chem. *254:* 7548–7557.

Brostrom, M. A., C. O. Brostrom, L. A. Brotman, and S. S. Green (1983) Regulation of Ca^{2+}-dependent cyclic AMP accumulation and Ca^{2+} metabolism in intact pituitary tumor cells by modulators of prolactin production. Mol. Pharmacol. *23:* 399–408.

Browning, M., T. Dunwiddie, W. Bennett, W. Gispen, and G. Lynch (1979) Synaptic phosphoproteins: Specific changes after repetitive stimulation of the hippocampal slice. Science *203:* 60–62.

Browning, M., W. F. Bennett, P. Kelly, and G. Lynch (1981) Evidence that the 40,000 M_r phosphoprotein influenced by high frequency synaptic stimulation is the alpha subunit of pyruvate dehydrogenase. Brain Res. *218:* 255–266.

Bryant, M. G., J. M. Polak, I. Modlin, S. R. Bloom, R. H. Albuquerque, and A. G. E. Pearse (1976) Possible dual role for vasoactive intestinal peptide as gastrointestinal hormone and neurotransmitter substance. Lancet *1:* 991–993.

Daly, J. W., W. Padgett, and K. B. Seamon (1982) Activation of cyclic AMP-generating systems in brain membranes and slices by the diterpene forskolin: Augmentation of receptor-mediated responses. J. Neurochem. *38:* 532–544.

Deschodt-Lanckman, M., P. Robberecht, and J. Christophe (1977) Characterization of VIP-sensitive adenylate cyclase in guinea-pig brain. FEBS Lett. *83:* 76–80.

Dodd, J., J. S. Kelly, and S. I. Said (1979) Excitation of CA1 neurones of the rat hippocampus by the octacosapeptide, vasoactive intestinal polypeptide (VIP). Br. J. Pharmacol. *66:* 125P.

Emson, P. C., J. Fahrenkrug, O. B. Schaffalitzky de Muckadell, T. M. Jessel, and L. L. Iversen (1978) Vasoactive intestinal polypeptide (VIP). Vesicular localization and potassium evoked release from rat hypothalamus. Brain Res. *143:* 174–178.

Etgen, A. M., and E. T. Browning (1982) Analysis of glucocorticoid and cyclic AMP effects on protein expression and phosphorylation in hippocampal slices using two-dimensional gel electrophoresis. Soc. Neurosci. Abstr. *8:* 565.

Fuxe, K., T. Hokfelt, S. I. Said, and V. Mutt (1977) Vasoactive intestinal polypeptide and the nervous system: Immunohistochemical evidence for localization in central and peripheral neurons, particularly intracortical neurons of the cerebral cortex. Neurosci. Lett. *5:* 241–246.

Giachetti, A., S. I. Said, R. C. Reynolds, and F. C. Koniges (1977) Vasoactive intestinal polypeptide in brain: Localization in and release from isolated nerve terminals. Proc. Natl. Acad. Sci. U. S. A. *74:* 3424–3428.

Greengard, P. (1981) Intracellular signals in the brain. Harvey Lect. *75:* 277–331.

Haas, H. L., P. Wolf, J. M. Palacios, M. Garbarg, G. Barbin, and J. C. Schwartz (1978) Hypersensitivity to histamine in the guinea-pig brain: Microiontophoretic and biochemical studies. Brain Res. *156:* 275–291.

Huang, M., and O. P. Rorstad (1983) Effects of vasoactive intestinal polypeptide, monoamines, prostaglandins, and 2-chloroadenosine on adenylate cyclase in rat cerebral micro-

vessels. J. Neurochem. *40:* 719–726.

Keltz, T. N., E. Straus, and R. S. Yalow (1980) Degradation of vasoactive intestinal polypeptide by tissue homogenates. Biochem. Biophys. Res. Commun. *92:* 669–674.

Kerwin, R. W., S. Pay, K. D. Bhoola, and C. J. Pycock (1980) Vasoactive intestinal polypeptide (VIP)-sensitive adenylate cyclase in rat brain: Regional distribution and localization on hypothalamic neurons. J. Pharm. Pharmacol. *32:* 561–566.

Larsson, L. -I., L. Edvinsson, J. Fahrenkrug, R. Hakanson, C. H. Owman, O. Schaffalitzky de Muckadell, and F. Sundler (1976) Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Res. *113:* 400–404.

Longshore, M. A., and M. H. Makman (1981) Stimulation of retinal adenylate cyclase by vasoactive intestinal peptide (VIP). Eur. J. Pharmacol. *70:* 237–240.

Loren, I., P. C. Emson, J. Fahrenkrug, A. Bjorklund, J. Alumets, R. Hakanson, and F. Sundler (1979) Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. Neuroscience *4:* 1953–1976.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Lynch, G., R. L. Smith, M. D. Browning, and S. Deadwyler (1975) Evidence for bidirectional dendritic transport of horseradish peroxidase. Adv. Neurol. *12:* 297–313.

McEwen, B. S. (1982) Glucocorticoids and the hippocampus: Receptors in search of a function. In *Adrenal Actions on Brain*, D. Ganten and D. Pfaff, eds., pp. 1–22, Springer-Verlag, New York.

Nimit, Y., P. Skolnick, and J. W. Daly (1981) Adenosine and cyclic AMP in rat cerebral cortical slices: Effects of adenosine uptake inhibitors and adenosine deaminase inhibitors. J. Neurochem. *36:* 908–912.

Palmer, G. C., F. Sulser, and G. A. Robison (1973) Effects of neurohumoral and adrenergic agents on cyclic AMP levels in various areas of the rat brain *in vitro*. Neuropharmacology *12:* 327–337.

Pelletier, G., R. Leclerc, R. Puviani, and J. M. Polak (1981) Electron immunocytochemistry in vasoactive intestinal peptide (VIP) in the rat brain. Brain Res. *210:* 356–360.

Phillis, J. W., J. R. Kirkpatrick, and S. I. Said (1978) Vasoactive intestinal polypeptide excitation of central neurons. Can. J. Physiol. Pharmacol. *56:* 337–340.

Quik, M., L. L. Iversen, and S. R. Bloom (1978) Effect of vasoactive intestinal peptide (VIP) and other peptides on cAMP accumulation in rat brain. Biochem. Pharmacol. *27:* 2209–2213.

Quik, M., P. C. Emson, J. Fahrenkrug, and L. L. Iversen (1979) Effect of kainic acid injections and other brain lesions on vasoactive intestinal peptide (VIP)-stimulated formation of cAMP in rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. *306:* 281–286.

Robberecht, P., P. De Neef, M. Lammens, M. Deschodt-Lanckman, and J. -P. Christophe (1978) Specific binding of vasoactive intestinal peptide to brain membranes from the guinea pig. Eur. J. Biochem. *90:* 147–154.

Rotsztejn, W. H., J. Besson, B. Briaud, L. Gagnant, G. Rosselin, and C. Kordon (1980) Effect of steroids on vasoactive intestinal peptide in discrete brain regions and peripheral tissues. Neuroendocrinology *31:* 287–291.

Rotsztejn, W. H., M. Dussaillant, F. Nobou, and G. Rosselin (1981) Rapid glucocorticoid inhibition of vasoactive intestinal peptide-induced cyclic AMP accumulation and prolactin release in rat pituitary cells in culture. Proc. Natl. Acad. Sci. U. S. A. *78:* 7584–7588.

Said, S. I., and R. N. Rosenberg (1976) Vasoactive intestinal polypeptide: Abundant immunoreactivity in neural cell lines and normal nervous tissue. Science *192:* 907–908.

Schorderet, M., J. -Y. Sovilla, and P. J. Magistretti (1981) VIP- and glucagon-induced formation of cyclic AMP in intact retinae *in vitro*. Eur. J. Pharamcol. *71:* 131–134.

Schwabe, U., and J. W. Daly (1977) The role of calcium ions in accumulations of cyclic adenosine monophosphate elicited by alpha and beta adrenergic agonists in rat brain slices. J. Pharamcol. Exp. Ther. *202:* 134–143.

Seamon, K. B., W. Padgett, and J. W. Daly (1981) Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc. Natl. Acad. Sci. U. S. A. *78:* 3363–3367.

Segal, M. (1981) The action of norepinephrine in the rat hippocampus: Intracellular studies in the slice preparation. Brain Res. *206:* 107–128.

Segal, M., V. Greenberger, and R. Hofstein (1981) Cyclic AMP-generating systems in rat hippocampal slices. Brain Res. *213:* 351–364.

Straus, E., T. N. Keltz, and R. S. Yalow (1982) Enzymatic degradation of VIP. In *Vasoactive Intestinal Peptide*, S. I. Said, ed., pp. 333–338, Raven Press, New York.

Taylor, D. P., and C. B. Pert (1979) Vasoactive intestinal polypeptide: Specific binding to rat brain membranes. Proc. Natl. Acad. Sci. U. S. A. *76:* 660–664.

Timmermans, P. B. M. W. M., and P. A. Van Zwieten (1981) The postsynaptic alpha$_2$-adrenoreceptor. J. Auton. Pharmacol. *1:* 171–183.

van Calker, D., M. Muller, and B. Hamprecht (1980) Regulation by secretin, vasoactive intestinal peptide, and somatostatin of cyclic AMP accumulation in cultured brain cells. Proc. Natl. Acad. Sci. U. S. A. *77:* 6907–6911.

Wellmann, W., and U. Schwabe (1973) Effects of prostaglandins E_1, E_2 and F_{2A} on cyclic AMP levels in brain *in vivo*. Brain Res. *59:* 371–378.

Winer, B. J. (1962) *Statistical Principles in Experimental Design*, McGraw-Hill Book Co., New York.

Yamamoto, C. (1972) Activation of hippocampal neurons by mossy fiber stimulation in thin brain sections *in vitro*. Exp. Brain Res. *14:* 423–435.