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Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: A possible regulatory mechanism for the local control of energy metabolism

(cerebral cortex/peptides/brain energy metabolism/glycogen/norepinephrine)

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ABSTRACT **Mouse cerebral cortex slices will synthesize [^{3}H]glycogen *in vitro*. Vasoactive intestinal polypeptide (VIP) stimulates the enzymatic breakdown of this [^{3}H]glycogen. The concentration giving 50% of maximum effectiveness (EC_{50}) is 26 nM. Under the same experimental conditions norepinephrine also induces a concentration-dependent [^{3}H]glycogen hydrolysis with an EC_{50} of 500 nM. The effect of VIP is not mediated by the release of norepinephrine because it is not blocked by the noradrenergic antagonist *d*-1-propranolol and is still present in mice in which an 85% depletion of norepinephrine was induced by intracisternal 6-hydroxydopamine injections. Other cortical putative neurotransmitters such as γ-aminobutyric acid, aspartic acid, glutamic acid, somatostatin, and acetylcholine (tested with the agonist carbamylcholine) do not induce a breakdown of [^{3}H]glycogen. This glycogenolytic effect of VIP and norepinephrine, presumed to be mediated by cyclic AMP formation, should result, at the cellular level, in an increased glucose availability for the generation of phosphate-bound energy. Given the narrow radial pattern of arborization of the intracortical VIP neuron and the tangential intracortical trajectory of the noradrenergic fibers, these two systems may function in a complementary fashion: VIP regulating energy metabolism locally, within individual columnar modules, and norepinephrine exerting a more global effect that spans adjacent columns.**

Vasoactive intestinal peptide (VIP) is a 28-amino acid polypeptide first isolated from porcine intestine by Said and Mutt (1). It shares structural homologies with other gastrointestinal peptides, such as glucagon, secretin, and gastric inhibitory peptide (2). Its spectrum of biological activities includes systemic vasodilation, increased cardiac output, and hyperglycemia (1); smooth muscle relaxation (3); some differential effects on secretory processes in the gastrointestinal tract (4–6); and glycogenolysis in liver slices (7). VIP-immunoreactive material has been identified in the nervous system (8–11), the highest concentration being in cerebral cortex (9, 11); neurons with VIP-like immunoreactivity have been visualized in the central nervous system (10–13). In addition to its presence in neurons, other criteria for attributing a possible neurotransmitter function to VIP include its localization in (14) and potassium-induced release from (14, 15) synaptosomal preparations. Furthermore, specific recognition sites for radiolabeled VIP have been demonstrated in rat (16) and guinea pig (17) brain membranes, and a VIP-stimulated adenylate cyclase has been identified in various areas of the central nervous system (18, 19).

Glycogen is the single largest energy reserve in the brain (20). It can be visualized by light and electron microscopy both in glial cells and in neurons (21, 22). A large number of studies have been published with regard to the various aspects of glycogen metabolism in glial and neuronal elements (23–28). Furthermore, in a series of elegant biochemical and autoradiographic studies, Wolfe and Nicholls (29) have shown that a mechanism for [^{14}C]glucose uptake and the subsequent synthesis of [^{14}C]glycogen existed in glial cells and neurons of the leech central nervous system. They also showed that this uptake and synthesis process could take place in neurons deprived of their glial environment (29). More recently, Quach *et al.* (30) have developed a sensitive method by which the effects of putative neurotransmitters on [^{3}H]glycogen levels newly synthesized from [^{3}H]glucose can be monitored in brain slices.

Here we report that VIP induces a concentration-dependent hydrolysis of newly synthesized [^{3}H]glycogen in mouse cortical slices. This effect is independent of and 20 times more potent than the glycogenolytic action of norepinephrine (30), confirmed by us in this study. The presence of two independent transmitters mediating glycogenolytic responses in cerebral cortex, similar to their action in the periphery, may indicate that hormones regulating energy metabolism systemically also may regulate this function within precise anatomical domains of the central nervous system.

MATERIALS AND METHODS

Animals. Young adult male Swiss albino mice (18–20g) were used throughout the study. They were housed six per cage and maintained in an alternating light/dark cycle (12:12 hr) with free access to food and water.

Tissue Preparation and Incubations. The method described by Quach *et al.* (30) was used: mice were killed by decapitation, the brain quickly was removed, the cerebral cortex was dissected on ice and placed in a modified Krebs–Ringer bicarbonate buffer (120 mM NaCl/5 mM KCl/2.6 mM $CaCl_2$/0.67 mM $MgSO_4$/1.2 mM KH_2PO_4/3 mM glucose/27.5 mM $NaHCO_3$) previously gassed with O_2/CO_2 (95:5) to maintain pH 7.4. The cortex was then placed, with its ventral surface facing down, on a McIlwain tissue slicer (Mickle Laboratory Engineering, Gomshall, Surrey, England); 250 × 250 μm slices were prepared and resuspended in ice-cold Krebs-Ringer bicarbonate buffer (6 ml per cortex). After replacing the supernatant with fresh medium, the slices were incubated in a shaking water bath at 37°C under continuous gassing (O_2/CO_2, 95:5). After 15 min, the supernatant was again replaced with fresh medium, and 270-μl aliquots (approximately 2 mg of protein per ml) were distributed into individual tubes. [^{3}H]Glucose (20 nmol) was added, and

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Abbreviations: VIP, vasoactive intestinal polypeptide; EC_{50}, concentration giving 50% of maximal effect.

the tubes were gassed with a stream of O_2/CO_2 (95:5), capped, and incubated for 30 min at 37°C. At the end of that period, 20 μl of drug solutions or vehicle was added, and the tubes were regassed, capped, and incubated again for 20 min. In time course experiments, the duration of the incubations was varied. At the end of the incubation, the tubes were rapidly centrifuged, the supernatants were replaced with fresh medium, and the tubes were sonicated for 5 sec. Aliquots of the resulting homogenates were placed into a boiling water bath for 10 min and subsequently centrifuged. The supernatants were sampled for [^{3}H]glycogen assays. Proteins were determined in another aliquot of the homogenates (31).

[^{3}H]Glycogen Assay. [^{3}H]Glycogen was isolated from the supernatants by the convenient ethanol precipitation technique described by Solling and Essman (32). Aliquots (150 μl) of the deproteinized supernatants were pipetted on Whatman 31-ET filter paper discs (24-mm diameter) and immersed into an ice-cold 60% (vol/vol) ethanol/10% (wt/vol) trichloracetic acid solution (10 ml per filter) for 10 min. The filters were then placed into 66% ethanol solutions (10 ml per filter) at room temperature for six successive 10-min periods. After a final 5 min in acetone, filters were dried, placed into scintillation counting vials, and 2 ml of boiling distilled water was added. After cooling, 15 ml of Aquasol (New England Nuclear) was added, and the vials were assayed for ^{3}H in a liquid scintillation counter (Beckman LS-3150-T) with an efficiency of 34%. By using this isolation technique, the contamination by glucose was <0.5% and the glycogen recovery was >99%. In control experiments, aliquots of the deproteinized supernatants were incubated for 30 min at 37°C with or without 4.2 units of amyloglucosidase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3), an enzyme hydrolyzing glycogen to glucose units (33, 34), before submitting the aliquots to the glycogen extraction procedure. The radioactivity recovered from the enzymatically treated samples was approximately 10% of that recovered from the untreated samples. This amyloglucosidase-insensitive radioactivity may represent glucose-containing ganglioside and cerebroside molecules (33).

6-Hydroxydopamine Lesions. Mice, under ether anesthesia received a 5-μl intracisternal injection of a 5 mg/ml solution of 6-hyroxydopamine (total dose, 25 μg per mouse). The injection was repeated 48 hr later, and the animals were killed 16 days after the first injection. By using this procedure, an 85% depletion of the cortical norepinephrine content was obtained. The catecholamine levels were determined by high-performance liquid chromatography (35).

Chemicals. [6-^{3}H]Glucose (0.5 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained from Amersham. Somatostatin, VIP, and secretin were gifts of Nicholas Ling (The Salk Institute, San Diego, CA). Norepinephrine, γ-aminobutyric acid, aspartic acid, carbamoylcholine, *d*-1-propranolol, 6-hydroxydopamine, glucagon, and glycogen were purchased from Sigma. Aquasol was obtained from New England Nuclear, and amyloglucosidase was from Boehringer Mannheim.

Results were analyzed for statistical significance by one-way analysis of variance, followed by paired comparisons with the Newman–Keul test.

RESULTS

Effects of VIP on [^{3}H]Glycogen Levels. The [^{3}H]glycogen content of the slices increased linearly during 30 min of incubation, at which time the rate of synthesis decreased and a plateau was eventually reached (Fig. 1). Addition of 1.0 μM VIP after 30 min of incubation induced a rapid fall in the [^{3}H]glycogen content (Fig. 1). This glycogenolytic action of VIP was concentration-dependent (Fig. 2). The Eadie–Hofstee plot of the dose–response curve shown in Fig. 2 yields 26 mM as the concentration giving 50% maximal effectiveness (EC_{50}) and a maximal glycogenolytic effect of 77% of basal levels (correlation coefficient of regression line = 0.992).

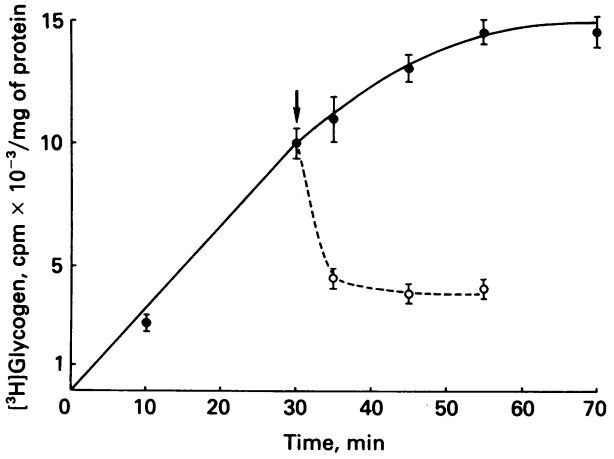

FIG. 1. Time course of the synthesis of [^{3}H]glycogen and of VIP-induced glycogenolysis. After 30 min of incubation, Krebs-Ringer buffer or 1 μM VIP were added (arrow) to different sets of test tubes in quadruplicate for each time point. Error bars represent SEM.

Effects of Other Substances on [^{3}H]Glycogen Levels. Norepinephrine also displayed a glycogenolytic action (Table 1). An Eadie–Hofstee plot of the dose–response curve (not shown) gives an EC_{50} of 500 nM and a maximal glycogenolytic effect of 70.5% of basal levels (correlation coefficient of regression line = 0.972). Secretin at 0.5 μM also decreased the [^{3}H]glycogen content of the slices to 40.6% of basal levels (Table 2). Glucagon, another peptide structurally related to VIP (2), had no glycogenolytic effect. Other cortical putative neurotransmitters (38),

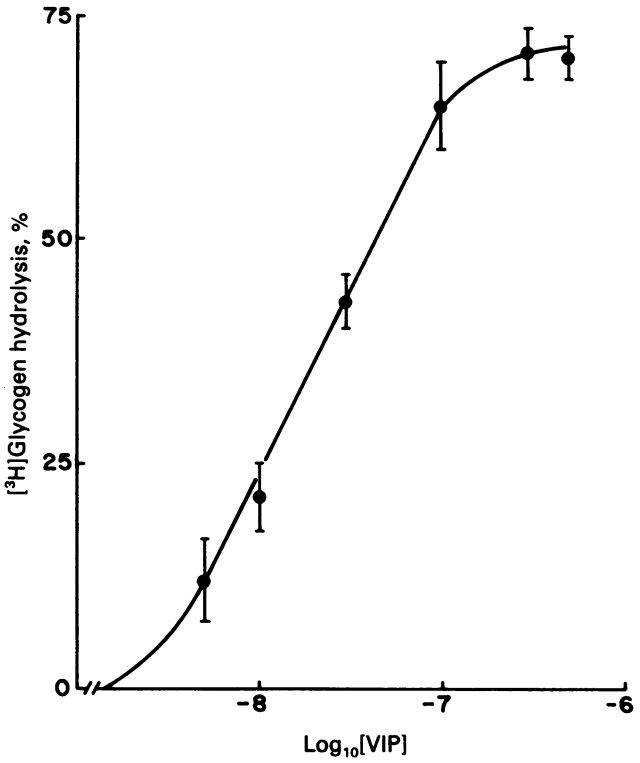

FIG. 2. Dose–response curve of the [^{3}H]glycogen hydrolysis induced by VIP. Each concentration was tested in quadruplicate. Error bars represent SEM.

Table 1. Interactions between VIP and noradrenergic drugs on [^{3}H]glycogen levels

Drug tested	μM	cpm/mg of protein*	Glycogenolysis, %
None		15,923 ± 846	—
Propranolol	10	16,120 ± 1,603	−1.2
NE	1	9,029 ± 480†	43.3
NE plus propranolol	1 10	13,860 ± 860	12.9
VIP	0.5	5,328 ± 140	66.5
VIP plus propranolol	0.5 10	5,664 ± 625	64.4
NE	5	6,212 ± 158	60.9
NE plus VIP	5 0.5	5,653 ± 1,191‡	64.4

NE, norepinephrine. Results are the mean ± SEM from quadruplicate assays from one series. Similar results were obtained from 3–4 replicate series. Data were analyzed by one-way analysis of variance, followed by paired comparisons with the Newman–Keul test.

* Statistical significance of values when compared to [^{3}H]glycogen levels in the absence of added drugs was $P < 0.01$ except for additions of 10 μM propranolol and of 1 μM NE plus 10 μM propranolol, which gave statistically not significant value.

† Significantly different from NE (μM) plus propranolol (10 μM), $P < 0.01$.

‡ Not significantly different from NE (5 μM) and VIP (0.5 μM).

such as γ-aminobutyric acid, glutamic acid, aspartic acid, and somatostatin did not decrease the [$_3$H]glycogen levels in the slices (Table 2). Carbamylcholine, a cholinergic agonist, was similarly inactive (Table 2). Preliminary results indicate that somatostatin does not antagonize the glycogenolytic effect induced by VIP (not shown).

Interactions Between VIP and Norepinephrine. The decrease in [^{3}H]glycogen levels induced by μM norepinephrine was effectively blocked ($P < 0.01$) by 10 μM *d*-1-propranolol, a β-adrenergic antagonist (Table 1). In contrast to this result, *d*-1-propranolol did not antagonize the glycogenolytic action of 0.1 μM VIP (Table 1). The β-adrenergic blocker did not affect the [^{3}H]glycogen levels when tested alone (Table 1).

No significant difference in the glycogenolytic effect of 0.1 μM VIP was apparent between mice in which an 85% depletion in cortical norepinephrine was induced by intracisternal 6-hyroxydopamine injections and control mice (Fig. 3).

Table 2. Effects of various pharmacological agents on [^{3}H]glycogen levels

Agent	μM	cpm/mg of protein*
None		22,333 ± 1,515
GABA	10	22,627 ± 1,944
Glutamic acid	10	19,316 ± 670
Aspartic acid	10	25,375 ± 1,118
Carbamylcholine	10	24,288 ± 2,888
Somatostatin	0.5	25,128 ± 1,645
Glucagon	0.5	24,359 ± 1,336
Secretin	0.05	22,155 ± 1,864
	0.5	13,381 ± 861†

Results are the mean ± SEM from quadruplicate assays from one series. Similar results were obtained from 2–3 replicate series. Data were analysed by one-way analysis of variance, followed by paired comparison with Newman–Keul test.

* There was no statistical significance of values when compared to [^{3}H]glycogen levels in the absence of added drugs except in the case of secretin at 0.5 μM ($P < 0.01$).

† Corresponds to 40.6% of [^{3}H]glycogen hydrolysis.

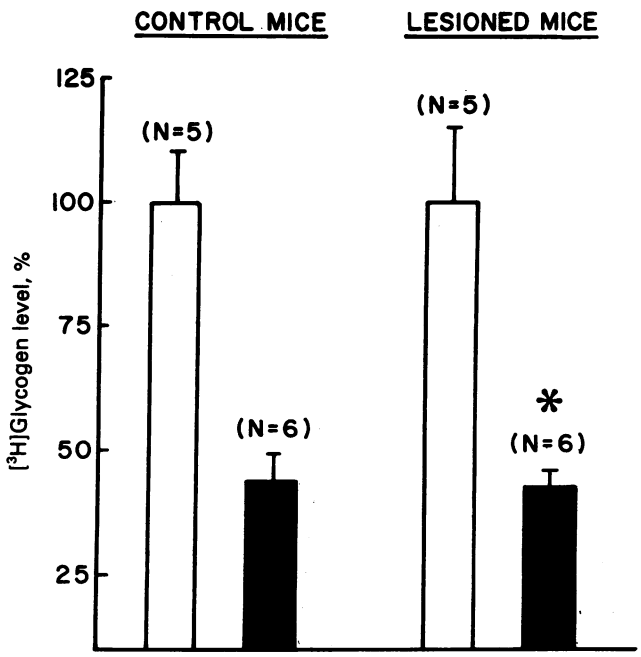

FIG. 3. Effects of 6-hydroxydopamine lesions on the VIP-induced [^{3}H]glycogen hydrolysis. Mice received two 25-μg intracisternal injections of 6-hydroxydopamine 48 hr apart. The effect of 0.1 μM VIP on newly synthesized [^{3}H]glycogen levels was tested in each animal separately. Results are expressed as percent ± SEM of [^{3}H]glycogen levels in the absence of added VIP (□). N, number of mice. *, Comparison of the effect of 0.1 μM VIP between control and lesioned mice: $P > 0.1$, by Student's *t*-test.

Finally, VIP and norepinephrine tested together at supramaximal concentrations showed no additive effects (Table 1).

DISCUSSION

We have studied the effects of various cortical putative neurotransmitters on the levels of [^{3}H]glycogen newly synthesized from [^{3}H]glucose *in vitro* by mouse cortical slices. The identity with [^{3}H]glycogen of the ^{3}H-labeled product isolated by ethanol precipitation is demonstrated by its solubility properties and by its sensitivity to amyloglucosidase.

Among the various putative neurotransmitters tested, only VIP and norepinephrine had an effect on [^{3}H]glycogen levels: they induced a concentration-dependent hydrolysis of the newly synthesized ^{3}H-labeled polysaccharide. The EC_{50} for this effect was 26 nM for VIP and 500 nM for norepinephrine. The maximal [^{3}H]glycogen hydrolysis induced by both neurotransmitters was 75–80% of basal levels. Secretin, a structural homolog of VIP, at 500 nM induced a significant decrease in [^{3}H]glycogen levels.

The preparation used consists of cortical slices 250 μm in section and approximately 1500 μm in length (depth of cortex). Therefore, in these slices a number of cell bodies and processes are intact and some local contacts may be maintained. At this level of neuronal organization, therefore, an effect observed with one neurotransmitter could be mediated by the release of another. Because norepinephrine displays a glycogenolytic action (ref. 30; this report), we tested the possibility that the effect of VIP on [^{3}H]glycogen levels was mediated by norepinephrine release. Two sets of experiments demonstrated that this could not account for the VIP effect. First, *d*-1-propranolol, a β-adrenergic antagonist, effectively antagonized the action of norepinephrine but not that of VIP (Table 1). Second, in mice whose noradrenergic cortical innervation was reduced by 85%

by 6-hydroxydopamine, VIP had the same glycogenolytic effect as in control mice.

Other putative neurotransmitters present in cortex (36) were also tested. Excitatory neurotransmitters such as glutamate, aspartate and the inhibitory neurotransmitter γ-aminobutyric acid (37) did not induce any [^{3}H]glycogen hydrolysis. Acetylcholine (tested with the stable potent agonist carbamylcholine) and somatostatin were also inactive. The effects of dopamine on [^{3}H]glycogen levels were not tested. The extensive studies on the electrophysiological actions of norepinephrine have demonstrated an enabling effect of the monoamine (38–40) rather than a clear excitatory or inhibitory action. The general effects of VIP on neuronal excitability are currently unkown, but preliminary reports indicate excitatory effects on cortical (41) or hippocampal (42) neurons.

Secretin is structurally related to VIP (2); therefore, its glycogenolytic action, observed only at rather high concentrations for a peptide (Table 2), may be a consequence of an interaction with VIP receptors. However, a direct interaction of secretin with specific secretin receptors cannot be excluded, particularly in view of the fact that this peptide has been demonstrated to stimulate cyclic AMP formation in cultured mouse brain cells through receptors separate from those for VIP (43). Although a secretin-like bioactive material has been extracted from pig brain (44), a clear neurotransmitter function has not yet been attributed to this unidentified peptide.

It should be noted here that Quach *et al.* have reported the glycogenolytic action of two other monoamines—namely histamine and serotonin (30). Interestingly, in analogy to the coeruleo-cortical noradrenergic projection (45–47), both the serotonergic (48, 49) and possibly histaminergic (50) ascending projections are highly divergent circuits, originating from relatively small nuclei in the brain stem and projecting widely throughout the cerebral cortex.

A common feature that distinguishes norepinephrine and VIP from other cortical neurotransmitters is their ability to stimulate the membrane-bound enzyme adenylate cyclase (18, 19, 51). This action suggests a possible molecular mechanism responsible for the glycogenolytic action of the two substances. Quach *et al.* have demonstrated that the effect of norepinephrine on [^{3}H]glycogen levels was potentiated by the phosphodiesterase inhibitor isobutylmethylxanthine and mimicked by dibutyryl-cyclic AMP (30). In our studies, the effect of VIP also was potentiated by isobutylmethylxanthine (not shown), suggesting that the glycogenolytic action of the peptide was mediated by cyclic AMP. In peripheral tissues (52), brain (23, 24), neuroblastoma, and astrocytoma cell lines (25, 26), cyclic AMP activates phosphorylase through a cascade of enzymatic phosphorylation steps, thus initiating glycogen degradation. This process, along with the inhibition of glycogen synthetase (26), ultimately results in a decrease in the cellular glycogen content. However, a calcium-mediated phosphorylase activation mechanism exists in muscle (53), and cyclic AMP-independent glycogenolysis also has been observed in astrocytoma and neuroblastoma cell lines (26). The effects of calcium on the glycogenolytic action of VIP may be difficult to determine, given the important role of this cation in adenylate cyclase activity regulation (54).

Glycogen hydrolysis induced by the two cortical neurotransmitters, will result in an increased glucose availability for the generation of phosphate-bound energy in those cellular elements receiving terminals from VIP and norepinephrine neurons. This similar action of the two neurotransmitters at the cellular level is particularly interesting, given the neuronal organization of the cortical noradrenergic and VIP systems. A major feature of the intracortical trajectory of the ceruleo-cortical noradrenergic projection is its tangential organization (45–47). This tangential pattern of organization stands in striking contrast with the strictly radial orientation of the intracortical VIP neuron (11, 55). Recently, we have extended (55) the studies of other investigators (10–13) on the immunohistochemical characterization of the VIP neurons within the cerebral cortex. Our observations indicate that the processes of an individual VIP neuron extend across the entire vertical thickness of the cerebral cortex, arborizing in a narrow radial column with minimal branching in the horizontal plane. This orthogonal pattern of organization, confers upon the VIP neuron the capacity to regulate energy metabolism locally, within individual columnar modules. This anatomical profile contrasts with the coeruleo-cortical noradrenergic projection, which has the capacity to exert its metabolic actions more globally, throughout a vast expanse of cortex.

A final observation can be made: VIP and norepinephrine display similar glycogenolytic actions in peripheral tissues. This action may indicate that certain substances with specific hormonal roles in several cell systems may exert similar homeostatic functions at the cellular level within the central nervous system, which are constrained by the spatiotemporal functional precision inherent to neural transmission.

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