three groups as above. The significance of the difference between the groups was calculated by Student's *t*-test. These data demonstrated a clear increase in the amount of poly(A) mRNA in relation to total RNA in the hearts perfused with hypertrophying extract, in comparison with hearts perfused with control extract or hearts perfused with Krebs solution only. There is no significant difference between the latter two groups (Table 1).

Although our assay system, involving treatment of intact beating hearts, is cumbersome, it provides clear repeatable results. We have tried simpler systems based on tissue slices, but the results were erratic. In tissue slices, cell contact arrangements were distorted, heart cells were not contracting, the vascular endothelium was not intact, and molecules were not bound or metabolized by the endothelium before the molecules could enter parenchymal cells (*8*). We believe that essentially normal anatomic and physiologic arrangements of an organ are required in order to obtain reliable biological responses. Such intact organ preparations are imperative when attempting to demonstrate the presence or absence of growth-initiating factors derived from tissue extracts.

The discrete early spread of translation curves that plateau at approximately 30 minutes suggests that increased amounts of mRNA account for the increased translation. An alternative, refuted by our experiments, is an increase in translational activity of preexisting mRNA. An increase in mRNA is substantiated by the increased proportion of poly(A) RNA, as determined by poly(U) hybridization, in the experimental groups. The molecules that provoke hypertrophy are probably basic to cell metabolism and appear to have been strongly conserved during evolution. Our results indicate that the controlling molecules exert their cellular effects by increasing transcription; that is, by activating genes.

GRAEME L. HAMMOND
YIU-KAY LAI
Department of Surgery, Yale Medical School, Yale University, New Haven, Connecticut 06520
CLEMENT L. MARKERT
Department of Biology, Yale University

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Relaxation of Isolated Gastric Smooth Muscle Cells by Vasoactive Intestinal Peptide

Abstract. *Vasoactive intestinal peptide caused a prompt, dose-dependent relaxation of isolated gastric smooth muscle cells of the guinea pig and a significant increase in intracellular adenosine 3′,5′-monophosphate coincidentally with optimum relaxation. Relaxation was augmented by a threshold concentration of isobutyl methylxanthine. The direct relaxant effect of vasoactive intestinal peptide and the distribution of nerves containing this peptide to circular smooth muscle support the view that vasoactive intestinal peptide is the neuromuscular transmitter of enteric inhibitory nerves.*

Neurons that show positive reactions with antibody to vasoactive intestinal peptide (VIP) are present in the myenteric and submucosal plexuses of the gut; their axons innervate all cell types including other neurons, smooth muscle cells, and mucosal cells (*1*, *2*). Axons arising from VIP neurons in the myenteric plexus project in a caudal direction forming varicose rings around VIP-reactive and nonreactive neurons. Other axons project into the circular muscle layer and run parallel with smooth muscle cells. The topography of VIP neurons and nerve fibers and the known relaxant effect of VIP on circular smooth muscle (*3*–*5*) are consistent with its being the transmitter of the enteric inhibitory nerves. These nerves mediate a series of descending inhibitory reflexes which facilitate the passage of material traveling down the digestive tract (*4*).

Evidence favoring VIP as the principal transmitter of this pathway includes release of VIP into the venous effluent concomitantly with neurally induced gastric relaxation (*6*) and partial blockade of neurally induced relaxation of the lower esophageal sphincter by VIP antiserum (*7*). The design of the experiments that gave these results left open the possibility that VIP was chiefly an interneuronal transmitter. In more recent studies, Angel *et al.* (*8*) showed that neurally induced relaxation of the gastric muscularis mucosae and concomitant VIP release could be abolished by the axonal blocking agent tetrodotoxin; relaxation was also abolished by antiserum to VIP.

Decisive evidence that VIP acts directly on smooth muscle cells rather than indirectly by way of local neural pathways would be demonstration of an effect of VIP on isolated smooth muscle cells devoid of neural elements. We have developed a procedure for isolation of smooth muscle cells from the stomach of several mammalian species, including humans, and devised an image-splitting technique for measurement of contraction and relaxation (*9*). In the present study, we demonstrate the presence of high-affinity VIP receptors that are capable of mediating prompt relaxation of isolated gastric smooth muscle cells of the guinea pig; this relaxation occurs concomitantly with an increase in intracellular levels of adenosine 3′,5′-monophosphate (cyclic AMP).

Cells were isolated from the stomach of guinea pigs as described (*9*) (see legend to Fig. 1). Isolated cells are in a state of optimum relaxation; therefore, the

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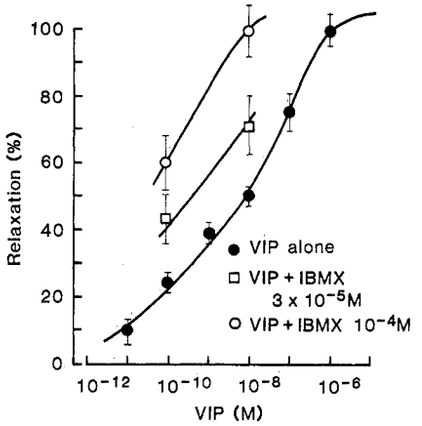

Fig. 1. Dose-response curves for the effect of VIP on the relaxation of isolated gastric smooth muscle cells. Each point is the mean (± standard error) of four to seven experiments. Cells were isolated from the circular muscle layer of the stomach of guinea pigs by incubating the tissue for 45-minute periods in 0.1 percent collagenase as described (*9*). The cells were harvested in enzyme-free Krebs buffer by filtration through 500 μm Nitex. The cells were incubated for 30 seconds with VIP alone or in combination with IBMX and then incubated for 30 seconds with $10^{-9}M$ CCK-OP. Relaxation was expressed as the percentage inhibition of the contractile response to CCK-OP.

effect of VIP was measured against a background of maximum contraction caused by $10^{-9}M$ cholecystokinin-octapeptide (CCK-OP). The cells (25×10^3 cells in 0.5 ml) were incubated for periods ranging from 5 to 60 seconds with VIP (10^{-12} to $10^{-6}M$) or isobutyl methylxanthine (IBMX 3×10^{-5} to $3 \times 10^{-4}M$), alone or in combination with each other. CCK-OP was then added for 30 seconds and the reaction interrupted with acrolein. The contractile response to CCK-OP alone was expressed as the mean decrease in cell length from control. The relaxant response was expressed as the percentage inhibition of the contractile response to CCK-OP. The mean length of freshly prepared cells was 116.7 ± 1.9 μm; the length decreased to 76.7 ± 1.7 μm upon exposure to $10^{-9}M$ CCK-OP (mean ± standard error; $N = 32$ experiments).

The kinetics of relaxation were uniform at all doses of VIP: the full extent of relaxation was attained by prior incubation of the cells with VIP for 30 seconds and was not exceeded by prior incubation for 60 seconds. At high doses of VIP (10^{-7} to $10^{-6}M$), a significant relaxation was evident when the cells had been incubated with the peptide for only 5 seconds. The kinetics of relaxation induced by IBMX were similar to those for VIP. The threshold and maximally effective doses of IBMX were $3 \times 10^{-5}M$ and $3 \times 10^{-4}M$, respectively.

The relaxant effect of VIP was dose-dependent (Fig. 1). A significant relaxation (9.4 ± 1.3 percent; $N = 7$; $P < .01$) was observed at $10^{-11}M$ and maximum relaxation (99.4 ± 4.7 percent; $N = 6$; $P < .001$); that is, abolition of the CCK-induced contraction and reversal to control cell length was observed at $10^{-6}M$ VIP. A half-maximum relaxation dose (D_{50}) was observed at $10^{-8}M$ VIP. Addition of a threshold dose of IBMX ($3 \times 10^{-5}M$) shifted the dose-response curve of VIP to the left and caused a tenfold decrease in the D_{50} of VIP. Addition of a higher but submaximum dose of IBMX shifted the dose-response curve further to the left and caused more than a 200-fold decrease in the D_{50} of VIP (Fig. 1).

The effect of IBMX by itself and its potentiatory effect at a threshold dose are consistent with relaxation being mediated by an increase in intracellular concentrations of cyclic AMP. In support of this notion, prior incubation of the cells for 30 seconds with $10^{-4}M$ dibutyryl cyclic AMP alone caused 75 percent relaxation; incubation with $10^{-3}M$ dibutyryl cyclic AMP caused 93 percent relaxation. Further support was sought by measuring the change in cyclic AMP content of isolated smooth muscle cells caused by VIP or IBMX. The experimental conditions were the same as those used for measuring relaxation, except that ethyl alcohol was used in place of acrolein to interrupt the reaction. Incubation with IBMX alone for 30 seconds caused a significant (51 ± 12 percent; $P < .01$; $N = 6$) increase in intracellular cyclic AMP (cyclic AMP in control cells was 7.8 ± 0.8 pmole per 10^6 cells). VIP alone had no effect; however, a combination of VIP and IBMX caused a potentiated increase in cyclic AMP of 173 percent ($P < .03$) after 30 seconds and of 408 percent ($P < .01$) after 60 seconds of incubation, that is, coincidentally with the time of optimum relaxation (Fig. 2). Much greater increases (up to 13 times above basal levels) occurred after prolonged incubation (10 minutes); these increases, however, were probably not coupled to relaxation. The need for IBMX to demonstrate VIP-induced stimulation of cyclic AMP suggests a high level of phosphodiesterase activity in isolated gastric smooth muscle cells. The increase in cyclic AMP caused by VIP fits a pattern for the effect of this peptide in several tissues (exocrine pancreas, intestinal mucosa, liver, and visceral smooth muscle) (*5*). Honeyman *et al.* (*10*) reported that an increase in cyclic AMP occurred during relaxation of amphibian gastric muscle cells caused by

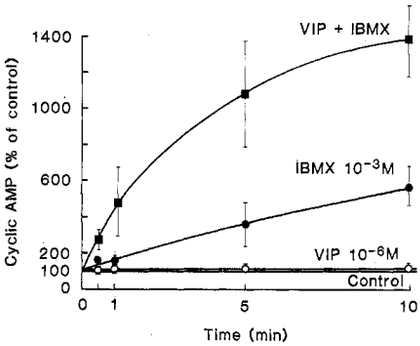

Fig. 2. Effect of VIP or IBMX on the concentrations of cyclic AMP in isolated gastric smooth muscle cells. The *x*-axis shows the time of incubation with IBMX or VIP. Control levels of cyclic AMP at various periods ranged from 6.5 ± 0.9 to 9.7 ± 1.8 pmole per 10^6 cells. The increase in cyclic AMP was significant after 0.5 and 1 minute of incubation with IBMX or with IBMX plus VIP, respectively, and was thus coincident with the time of optimum relaxation. The data are the means (± standard error) of six experiments.

the β-adrenergic agonist, isoproterenol. Although an increase in cyclic AMP is a consistent effect of relaxant agents (*11*), its exact relation to the process of relaxation is not clear.

The relaxant effect of VIP demonstrated in this study has been duplicated in studies on smooth muscle cells isolated from the human gastric antrum (*12*). In related studies, secretin, a close homolog of VIP, caused relaxation of gastric muscle cells of the guinea pig, but its potency relative to VIP was 40 times less.

The demonstration of receptors for VIP capable of causing direct relaxation of gastric smooth muscle cells does not preclude additional interactions between VIP-immunoreactive nerves and adjacent nerves in the circular muscle layer. However, this is unlikely because the effect of exogenous VIP on circular muscle strips that retain intrinsic innervation is not blocked by tetrodotoxin. Furthermore, VIP is the only peptide found in these nerves that is capable of causing relaxation; methionine- and leucine-enkephalin, bombesin, and CCK-OP all cause contraction of isolated smooth muscle cells (*9, 13*).

In recent years, Burnstock (*14*) has argued persuasively that the neurotransmitter of the nonadrenergic inhibitory pathway in the gut is the nucleotide adenosine triphosphate (ATP): in consequence, the pathway has been named "purinergic." The arguments in favor of a purinergic neurotransmitter can be adduced with equal cogency in favor of a peptidergic neurotransmitter, VIP. Unlike ATP, which is ubiquitous, VIP is

located precisely in nerves supplying circular smooth muscle. Like ATP, VIP is present in large dense granules of the terminals of p-type nerves, is released by electrical stimulation of nonadrenergic inhibitory nerves, and can cause relaxation of circular smooth muscle (*3–8*). The profile of neurally stimulated relaxation is more closely mimicked by exogenous ATP than by VIP, but this is probably because of slower diffusion of VIP across muscle layers. The probability of neurotransmission by VIP need not preclude a role for ATP. The topographical and functional parallelism between VIP and ATP may reflect a modulatory, perhaps synergistic, interplay between these two substances.

K. N. BITAR
G. M. MAKHLOUF
Departments of Medicine and *Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298*

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Current Speed and Filtration Rate Link Caddisfly Phylogeny and Distributional Patterns on a Stream Gradient

Abstract. *Patterns of body size and net construction suggest that current speed and food-particle concentration (not size) influence the distribution of suspension-feeding caddisflies on a downstream gradient. Large ancestral taxa with high filtration rates occur in resource-poor upstream habitats; more derived members of the phylogeny enter successively in downstream reaches with slower current and greater concentrations of particulate food.*

Larvae of the net-spinning caddisflies (Trichoptera: Hydropsychidae) capture suspended particulate food from stream water passing over their retreats (*1, 2*). Their abundance and worldwide distribution suggest that filtering net spinners play an important role in stream ecosystems. In Rocky Mountain and Appalachian streams, particulate concentration increases downstream on long altitudinal gradients; both the density of individuals and the number of net-spinning species show a correlated increase. In all thoroughly studied drainages, these community changes have a similar pattern; large ancestral hydropsychid species inhabit the most nutrient-poor upstream reaches, and more derived taxa of smaller body size are added to the community sequentially at lower elevation (*3, 4*).

Studies of distributional ecology and community structure have focused most often on two aspects of hydropsychid biology. First, the observation that coexisting species show differences in the mesh dimension of catch nets suggested that interspecific partitioning of food particle sizes is an important feature of community organization (*5*). According to this view, increasing particle availability influences the number of coexisting species by extending the range of potential particle size specialties or by permitting changes in the specialization or overlap of the taxa. This partitioning hypothesis is weakened by the occurrence of several instar classes and net sizes in each species and by recent quantitative studies demonstrating extremely broad overlap and little specialization in the particle sizes ingested by taxa with very different nets (*4, 6*). The second major focus of hydropsychid ecology has been microhabitat selection. Evidence suggests that the current speed requirements of net spinners are very precise (*7, 8*). Artificial diversions cause insects to abandon retreats and relocate (*9*), and active selection of current speed occurs in the laboratory (*10, 11*). Interspecific partitioning of microhabitat space has been demonstrated in the field with a nearest-neighbor analysis (*4*), and the patterns are associated with current speed preferences. Flow rate differences are related to the functional morphology of large and small catch nets, but current alone cannot explain the pattern of distributions on a stream gradient. For example, sheltered slow currents are available in even the most precipitous upstream reaches of a Rocky Mountain stream, yet species characteristic of this microhabitat at lower elevations are absent.

Data showing (i) a correlation of insect abundance and species diversity with increasing particle concentration, (ii) little specialization in the kinds of food items taken, and (iii) interspecific variation in current speed requirements are all consistent with a model of hydropsychid distributions based on resource availability and filtration rate. Particle capture must depend on the concentration of material in suspension and the volume of water filtered. Caddisfly taxa may compensate for low particle availability by adaptations that permit large amounts of water to pass through the net. A filtration rate model of hydropsychid distributions predicts that species found in the most resource-poor sites at high elevation will have large net structures and occupy fast-current microhabitats. Taxa with lower filtration rates should be restricted to downstream reaches with a higher concentration of particulate resources. Several types of data support this prediction.

1) Net structures must reflect the current speeds in which they function. Silk strands need sufficient thickness to withstand the current's force, and mesh cells must vary in size to adjust drag in different flow rates. Among most hydropsychids, these net properties are related to the insect's size and the dimension of mouthparts involved in the net spinning process. A distributional model based on filtration rate predicts that body size, silk strand thickness, and mesh dimension should all decline with downstream entry sequence on the resource concentration gradient. Rocky Mountain hydropsychids illustrate this pattern clearly (*4*). Average catch-net diameters also decline (Table 1). Thus net structures are consistent with the hypothesis that upstream taxa accommodate a low resource concentration by filtering more water than downstream species do.

2) Direct measurements of current speed around caddisfly nets and retreats decline in the same predicted sequence (*12*). Data taken by timing the dissolution of a salt pill show that large arctopsychines from resource-poor headwaters occupy the fastest microhabitats, and currents associated with species that enter downstream are successively slower

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