creased because Cl^- flow across the basolateral barrier reflects in part neutral NaCl entry that is driven by the Na^+ and Cl^- chemical gradients.
14. Absolute in vivo voltages of the exposed surface of polyps of three CF subjects averaged −15 mV compared to −60 mV of the protected inferior surface of the turbinate. The morphology of normal turbinate resembled that of a transitional metaplastic region of the medial surface of the turbinate, which typically exhibits an in vivo PD of −13 mV compared to −30 mV in the more protected recess of the nose. Consequently, all specimens were representative of epithelia from less protected regions of the nose.
15. I. Schulz, *J. Clin. Invest.* **48**, 1470 (1969).
16. P. Quinton, *Nature (London)* **301**, 421 (1983).
17. J. Gatzy and R. Boucher, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1244 (1982).
18. A. Cuthbert, in *Drugs and Transport Processes*, A. B. Callingham, Ed. (Univ. of Paris Press, Paris, 1974), p. 172.
19. G. Legris, P. Will, U. Hopfer, *Ann. N.Y. Acad. Sci.* **372**, 345 (1981).
20. We wish to thank Dr. Mary Rose and the University of Michigan Cystic Fibrosis Clinic for assistance in studying one of their patients and Dr. V. Ranga for histological studies. This work was supported by grants HL22624 (R.C.B.), HL00787 (M.R.K.), HL16674 (J.T.G.) and a Cystic Fibrosis Foundation Research Resource Development Program. R.C.B. is an Established Investigator of the American Heart Association.
* To whom reprint requests should be addressed at the Department of Medicine, University of North Carolina.

22 December 1982; revised 4 April 1983

Vasoactive Intestinal Peptide Alters Membrane Potential and Cyclic Nucleotide Levels in Retinal Horizontal Cells

Abstract. *Vasoactive intestinal peptide stimulated the synthesis of adenosine 3′,5′-monophosphate in fractions of isolated carp horizontal cells. When applied extracellularly to isolated and cultured horizontal cells, the peptide also induced a slow depolarization (30 to 40 millivolts) accompanied by a decrease in membrane resistance. However, analogs of adenosine 3′,5′-monophosphate applied extracellularly or intracellularly, and forscolin applied extracellularly, had no effect on the membrane potential of cultured horizontal cells, indicating that the induced depolarization was not related to the accumulation of adenosine 3′,5′-monophosphate in these cells.*

Horizontal cells are second-order neurons believed to play an important role in the processing of information in the outer plexiform layer of the retina (*1*). In the cyprinid fish retina, the H_1 (or luminosity type) horizontal cells are known to receive two synaptic inputs, one from the cone photoreceptors (*2*), which may use glutamate as their neurotransmitter (*3–5*), and the other from a class of interplexiform cells, which is known to use dopamine as its neurotransmitter (*6*). A dopamine-sensitive adenylate cyclase has been identified in isolated horizontal cells separated from the enzymatically dissociated carp retina (*7*), suggesting that increased intracellular levels of adenosine 3′,5′-monophosphate (cyclic AMP) mediate the physiological effects of dopamine in carp horizontal cells. Isolated carp horizontal cells maintained in culture are also amenable to intracellular recording techniques, and recent experiments have demonstrated that L-glutamate and its agonists quisqualate and kainate induce large, long-lasting depolarizing responses when applied to these cells (*4*). Taken together, these data imply the presence of both dopamine receptors and glutamate receptors on carp horizontal cells (*8*).

Vasoactive intestinal peptide (VIP), a single-chain polypeptide containing 28 amino acids, is able to stimulate cyclic AMP accumulation both in the brain (*9–11*) and in the retina (*12, 13*), including the retina of the carp (*14*). Surprisingly, we have observed that the effects of VIP on cyclic AMP accumulation in intact pieces of carp retina are not additive with those of dopamine (*14*). This suggests the possible co-localization of VIP- and dopamine-stimulated adenylate cyclase systems on the same populations of neurons within the carp retina. To investigate the possible presence of a third class of neurotransmitter receptor on carp horizontal cells, we examined the effects of VIP on cyclic AMP accumulation and membrane potential in these neurons. We report that VIP can both induce cyclic AMP accumulation and alter membrane potential in isolated horizontal cells. However, the membrane potential change does not appear to be linked to the increase in intracellular cyclic AMP levels.

For experiments involving an investigation of VIP-dependent cyclic AMP accumulation, fractions of horizontal cells were isolated from the enzymatically dissociated carp retina using previously described techniques (*7, 15*). Briefly, retinas were dissected from dark-adapted carp (*Cyprinus carpio*) and incubated in an appropriate Ringer solution containing 0.08 percent trypsin for 90 minutes. Following dissociation of the cells, the resulting cell suspension was applied to the surface of gradients containing 0.8 to 4 percent Ficoll. Cells were left to sediment out at unit gravity for 4 hours at 9°C. Fractions containing horizontal cells were then collected and pooled. The horizontal cells were harvested by centrifugation, resuspended in Ringer (final volume, 500 μl), and examined for their ability to accumulate cyclic AMP in the presence of VIP or dopamine or both.

The effects of 250 μ*M* dopamine or 25 μ*M* VIP or both on cyclic AMP accumulation in fractions of isolated carp horizontal cells are illustrated in Fig. 1. In agreement with previous studies (*7, 15*), 250 μ*M* dopamine induced a large increase in cyclic AMP accumulation during a 10-minute incubation. A similar large response was observed in the presence of 25 μ*M* VIP. In contrast, a number of other peptides, including substance P, α-melanocyte stimulating hormone, and cholecystokinin octapeptide-(26–33), were ineffective at stimulating cyclic AMP accumulation in fractions of horizontal cells. While it is possible that these effects of VIP may be mediated through an interaction with dopamine

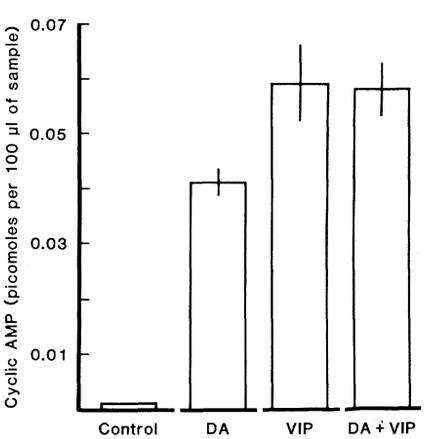

Fig. 1. Effects of dopamine (*DA*) and VIP, alone or in combination, on cyclic AMP accumulation in fractions of isolated carp horizontal cells. After a 5-minute incubation in the absence of any test substance, cells received a 10-minute incubation at 30°C in the presence of 250 μ*M* dopamine or 25 μ*M* VIP or both. As a routine measure, 2 m*M* IBMX, a phosphodiesterase inhibitor, was added to the incubation Ringer to prevent the enzymatic breakdown of cyclic AMP during incubation. Incubations were terminated by boiling for 3 minutes, after which triplicate 100-μl samples from each reaction tube were assayed for cyclic AMP content by using an acetylated radioimmunoassay (Collaborative Research, Inc.). Due to the small number of cells present in each reaction tube, it was not possible to express data in picomoles of cyclic AMP per milligram of protein. Final data were expressed, therefore, as picomoles of cyclic AMP per 100 μl of assay sample. In the absence of added dopamine or VIP, cyclic AMP levels were below the level of detection (0.0025 pmole per 100 μl of sample). Each histogram is the mean ± standard error for 9 to 12 determinations.

receptors, the presence of specific VIP receptors coupled to adenylate cyclase is more likely since experiments performed on intact pieces of carp retina have shown that dopamine antagonists are totally ineffective at blocking the VIP-induced cyclic AMP accumulation (*14*). Furthermore, in preliminary experiments we have found that VIP in the presence of haloperidol, a dopamine antagonist, stimulates significant accumulations of cyclic AMP in isolated horizontal cells. We have also observed that when horizontal cells were incubated simultaneously with 250 μM dopamine and 25 μM VIP, the resulting cyclic AMP response was not additive (Fig. 1), suggesting that the dopamine and VIP receptors are on the same horizontal cells.

For electrophysiological experiments, membrane potentials of isolated horizontal cells maintained in tissue culture for 2 to 5 days were recorded using previously described techniques (*4*). Drugs were delivered via pressure ejection through two independently positioned triple barrel micropipettes. Test agents were dissolved in Ringer and applied as 0.5- to 1.0-second pulses of 0.3 μl.

The effects of an application of 10 μM VIP (*16*) on an isolated, cultured horizontal cell are illustrated in Fig. 2A. In response to VIP the cell depolarized relatively slowly by about 40 mV, from a resting level of around −80 mV. A decrease in input resistance during the potential change suggested that the VIP exerted its effect by increasing membrane conductance to some ions. We recorded similar responses on 37 occasions from 19 cells (*17*). On one cell we applied 10 μM VIP four times; each application resulted in a similar depolarization of 35 to 45 mV. A number of other peptides, including substance P, somatostatin, and cholecystokinin octapeptide-(26–33), were also applied to cultured horizontal cells. None of these induced consistent membrane potential changes.

For comparison, a response of a horizontal cell to 25 μM kainate is illustrated in Fig. 2B. Kainate, like L-glutamate and quisqualate, induces a very rapid, approximately 80-mV depolarization that lasts about 60 seconds. This characteristic response to glutamate and its analogs is distinctly different from that induced by VIP and appears to consist of at least two components, an underlying graded depolarization due possibly to a decreased K^+ conductance and a prolonged regenerative potential due mainly to Ca^{2+} (*4*).

An important question is whether the changes in membrane potential brought about by VIP are linked to the peptide's ability to activate adenylate cyclase in horizontal cells. Our evidence indicates that they are not. For example, the extracellular application of 500 μM 8-bromo cyclic AMP or 500 μM dibutyryl cyclic AMP, analogs of cyclic AMP that cross the plasma membrane, had no consistent effects on the membrane potentials of cultured horizontal cells (Fig. 2C). Furthermore, when iontophoresed intracellularly into isolated horizontal cells or horizontal cells in the intact retina, neither of these analogs, nor cyclic AMP itself, changed the membrane potentials of the neurons. This was true even in the presence of 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor. Finally, the nonspecific adenylate cyclase activator forscolin (*18*), applied to single isolated horizontal cells at a concentration of 10 μM, also had no effect on membrane potential.

That cyclic AMP does not affect the membrane potential of isolated horizontal cells was further substantiated by applying dopamine or the rigid dopamine agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to these neurons (Fig. 2C). Both of these agents activate adenylate cyclase in fractions of carp horizontal cells (Fig. 1) (*19*). However, when applied to isolated, cultured horizontal cells, they do not alter membrane potential at concentrations (10 to 300 μM) that promote large increases in cyclic AMP accumulation in fractions of horizontal cells (*7*). In earlier reports (*4, 20*), we noted that higher concentrations of dopamine (0.5 to 10 mM) induce relatively small membrane potential changes (1 to 15 mV) of either polarity in a small percentage (15 to 30 percent) of cultured horizontal cells. We think it likely that these effects, like the effect of VIP on horizontal cell membrane potential, are not linked to cyclic AMP accumulation in the cell.

Our results indicate, therefore, that there are at least three classes of neurotransmitter receptors on carp horizontal cells. One receptor type interacts with L-glutamate and its analogs, a second with dopamine and its analogs, and a third with VIP. The latter two receptors appear to be linked to adenylate cyclase and promote the accumulation of cyclic

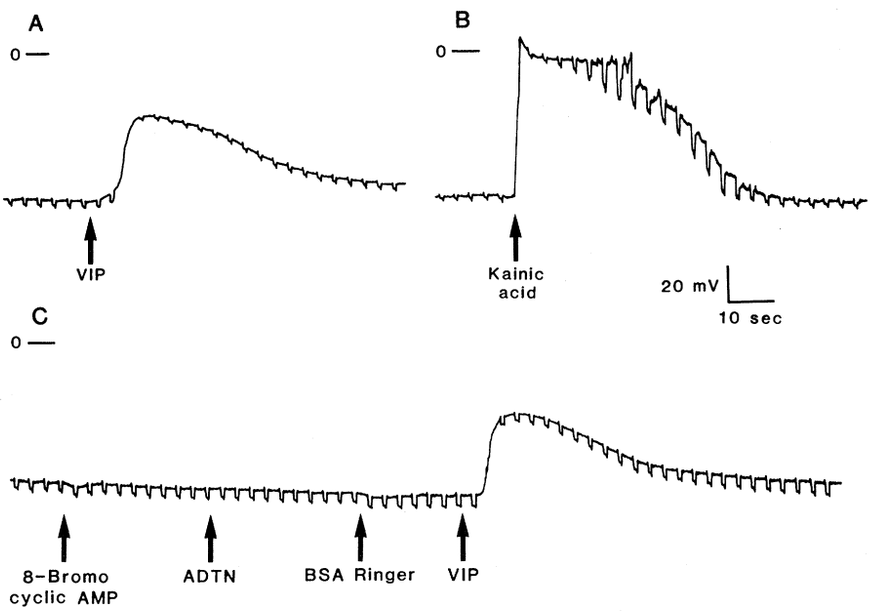

Fig. 2. (A) Response of an isolated, cultured carp horizontal cell to 10 μM of applied VIP. The response had a slow time course; it took about 7 seconds for the response to reach a peak. In addition, there was an increase in membrane conductance after VIP application, as seen by a decrease in the amplitude of the constant current pulses (10 pA) used to monitor input resistance. Resting input resistance varied by as much as two- to threefold between cells. However, regardless of the initial input resistance, the decrease induced by VIP was always about 50 percent of the resting value (48.8 ± 2.5 percent; $N = 11$). (B) The response of a horizontal cell to 25 μM kainic acid. This response is more complex than and dramatically different from the response of horizontal cells to VIP. The depolarization is rapid and reaches a peak in ≤ 1 second. There is a prominent plateau and the fall to resting levels is accompanied by an increase in membrane resistance. The irregularities of the test pulses during the recovery phase of the response are due to the nonohmic properties of the horizontal cell membrane (*4, 21*). (C) Another VIP response and the lack of responses in a cultured horizontal cell to 500 μM 8-bromo cyclic AMP, 500 μM ADTN, and 0.2 percent bovine serum albumin (*BSA*) in carp Ringer (the vehicle in which VIP was applied). Bovine serum albumin was added to the carp Ringer to minimize binding of the VIP to the delivery pipette.

AMP in horizontal cells. When applied at low concentrations, VIP, but not dopamine, consistently depolarizes carp horizontal cells. This depolarization does not appear to be related to cyclic AMP accumulation in horizontal cells. Rather, the VIP seems to act directly on the membrane to increase conductance to one or more ions. Thus VIP appears to exert two distinct effects on horizontal cells: a depolarization of resting membrane potential and a promotion of the synthesis of cyclic AMP. An intriguing question is whether one receptor mediates these two effects, that is, is linked to both adenylate cyclase and a membrane channel, or whether there are two subtypes of VIP receptors on carp horizontal cells. In either case, VIP may play a dual role in retinal function: its ability to alter membrane potential may mediate a relatively rapid transfer of information, while its ability to promote cyclic AMP accumulation could allow for the modulation of neuronal function over a longer period of time.

Note added in proof: In recent experiments it was found that 1 μ*M* VIP produced responses similar to those shown here, indicating that the 10 μ*M* concentration used is well above threshold.

ERIC M. LASATER
KEITH J. WATLING*
JOHN E. DOWLING

Department of Cellular and Developmental Biology, Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. F. S. Werblin and J. E. Dowling, *J. Neurophysiol.* **32**, 339 (1969).
2. W. K. Stell and D. O. Lightfoot, *J. Comp. Neurol.* **159**, 473 (1974).
3. A. T. Ishida and G. L. Fain, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5890 (1981).
4. E. M. Lasater and J. E. Dowling, *ibid.* **79**, 936 (1982).
5. J. W. Rowe and K. H. Ruddock, *Neurosci. Lett.* **30**, 57 (1982).
6. J. E. Dowling and B. Ehinger, *Proc. R. Soc. London Ser. B* **201**, 7 (1978); W. L. Hedden and J. E. Dowling, *ibid.*, p. 27.
7. R. Van Buskirk and J. E. Dowling, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7825 (1981).
8. Four types of horizontal cells have been identified in the cyprinid retina; three are driven exclusively by cones and one by rods (*2*). The most common of the horizontal cells is the cone-driven H_1 cell, which appears to use γ-aminobutyric acid (GABA) as its transmitter [R. E. Marc, W. K. Stell, D. Bok, D. M. K. Lam, *J. Comp. Neurol.* **282**, 221 (1978)]. After isolation, approximately 75 percent of the horizontal cells take up GABA (D. O'Brien and E. M. Lasater, unpublished observations). We infer from this that results obtained from isolated horizontal cells reflect mainly the properties of the H_1 cells.
9. M. Quik, L. L. Iversen, S. R. Bloom, *Biochem. Pharmacol.* **27**, 2209 (1978).
10. C. Borghi *et al.*, *Life Sci.* **24**, 65 (1979).
11. R. W. Kerwin, S. Pay, K. D. Bhoda, C. J. Pycock, *J. Pharm. Pharmacol.* **32**, 561 (1980).
12. M. Schorderet, J.-Y. Sovilla, P. J. Magistretti, *Eur. J. Pharmacol.* **71**, 131 (1981).
13. M. Longshore and M. H. Makman, *ibid.* **70**, 237 (1981).
14. K. J. Watling and J. E. Dowling, *J. Neurochem.*, in press.
15. R. Van Buskirk and J. E. Dowling, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3350 (1982).
16. The concentration of VIP in the ejection drug pipette was usually 10 μ*M*. The dilution between drug pipette and cell was determined by applying known concentrations of KCl and comparing the observed depolarization with that found when isolated horizontal cells were bathed in various concentrations of KCl (*21*). From this we estimate the maximum dilution was 20 percent and thus the concentration of VIP at the cell surface was at least 8 μ*M*.
17. Not in every preparation did we observe responses to VIP. However, in those experiments that gave positive results, every cell tested (N = 3 to 9 per culture dish) gave responses. In the negative experiments, none of the cells (N = 1 to 3) responded. We think it likely that in the latter experiments VIP was not ejected from the delivery pipette owing to binding to the micropipette glass (see legend to Fig. 2).
18. K. B. Seamon, W. Padgett, J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3363 (1981).
19. K. J. Watling and M. Williams, *Eur. J. Pharmacol.* **77**, 321 (1982).
20. J. E. Dowling, E. M. Lasater, R. Van Buskirk, K. J. Watling, *Vision Res.* **23**, 421 (1983).
21. M. Tachibana, *J. Physiol. (London)* **321**, 141 (1981).
22. Supported in part by NIH grants EY-00811 (J.E.D.), EY-00824 (J.E.D.), and EY-05476 (E.M.L.) and by an award from the Retina Research Foundation, Houston. K.J.W. was supported in part by a travelling fellowship from the Medical Research Council, England, and an award from Fight-for-Sight, Inc., New York City.
* Present address: Neuroscience Research Centre, Merck Sharp & Dohme Ltd., Hertford Road, Hoddesdon Herts, EN11 9BU, England.

28 February 1983; revised 25 April 1983

Cerebral Cortices of East African Early Hominids

Abstract. *An endocast of the frontal lobe of a reconstructed skull, which is approximately 2 million years old, from the Koobi Fora region of Kenya appears to represent the oldest human-like cortical sulcal pattern in the fossil record, while the endocast from another skull from the same region produces an endocast that appears apelike in its frontal lobe and similar to endocasts from earlier South African australopithecines. New analysis of paleoanatomical evidence thus indicates that at least two taxa of early hominids coexisted in East Africa.*

The oldest human-like brains in the hominid fossil record were considered represented by endocranial casts from South African australopithecines (*1, 2*), which are between 2.5 to 3.0 million years old and on the average of ~ 440 cm^3 (*3*). The sulcal pattern of the cerebral cortex of South African australopithecines was considered to be human-like largely because of a relatively caudal position of the lunate sulcus (*2*), which delineates the rostral border of visual cortex in anthropoid primates (*4*). However, when the complete sulcal patterns of seven South African natural endocasts were compared with sulcal patterns of human, gorilla, and chimpanzee brains (*5*), it was concluded that South African australopithecines appeared ape-like in their entire sulcal patterns, including the position of the lunate sulcus, as suggested earlier by observations about the lunate sulcus in the Taung endocast (*6*).

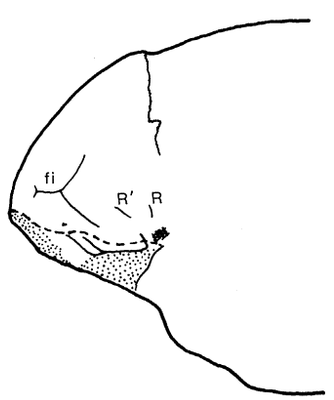

Fig. 1. Endocast of left frontal lobe of KNM-ER 1470, attributed to *Homo* and dated at less than 2 million years. Dots are reconstructed portion of frontal lobe, and hatching represents damaged area. Abbreviations: *fi*, inferior frontal sulcus; *R'*, horizontal branch of Sylvian sulcus; *R*, ascending branch of Sylvian sulcus.

I now report my studies of fossil hominid cranial remains from the Koobi Fora region of Kenya that are between 1 and 2 million years old (*7*). Endocranial surfaces of fragments from numerous individuals were studied, and whole endocasts were prepared (*8*) from reconstructed skulls KNM-ER 1470 and KNM-ER 1805.

Figure 1 illustrates the sulcal pattern near the orbital edge of the left frontal lobe of the endocast from KNM-ER 1470, a specimen that was found in area 131, 35 m below the KBS (Kay Behrensmeyer site) Tuff of the Koobi Fora region, and later reconstructed (*9–11*). A branch of the inferior frontal sulcus is parallel to the orbital edge of the frontal lobe, which is formed in part by a gyrus delimited medially by this branch. This condition is derived and represents the typical configuration found in extant humans (*4, 5*). The sulci identified as the horizontal and ascending branches of the Sylvian sulcus (R′ and R, Fig. 1) form the anterior and posterior boundaries of Brodmann's area 45 (pars triangularis) in extant humans (*4*). Broca's speech area in humans is formed by part of area 45 and area 44 directly caudal to it in left hemispheres (*12*). The frontal lobe of KNM-ER 1470 lacks a fronto-orbital sulcus that characterizes all extant ape brains (*4*) as well as australopithecines