the contrary, two binding sites with different binding constants have been observed for the binding of Na^+ to this channel state (*27*), and as shown in parts I and J of Fig. 3, only the ethanolamine end is accessible to Ba^{2+} interaction, indicating that the formyl end cannot be at the aqueous interface as required for the parallel double-stranded helix. Additionally, such an asymmetric binding would give rise to asymmetric single-channel current-voltage curves (*30*), whereas the dominant state of the channel exhibits single-channel current-voltage curves that are symmetric (*31*). In lecithin vesicles with an identical circular dichroism pattern, it has been shown by Weinstein *et al.* (*10*) that the formyl end is buried in the lipid and not accessible at the aqueous interface. Finally, data are available (*12*) on the thallium ion-induced chemical shifts of carbonyl carbon resonances of [(1-^{13}C)D-Val^8]gramicidin A and [(1-^{13}C)D-Leu^{14}]gramicidin A, which are included as the X's in Fig. 4B. While not helically equivalent to L-residue carbonyls, these D-residue carbonyls are helically equivalent to each other and are also susceptible to ion-induced chemical shifts, which must be consistent with the L-residue data as to location but not as to sign and magnitude. The D-residue data are not consistent with the L-residue data for the parallel double-stranded structure (Fig. 4B). Thus, the double-stranded helices are not the channel state of interest.

In Fig. 4C the data of Fig. 3, A through H, are plotted for the single-stranded β-helix. In this case, two symmetrically related binding sites are observed, as required by the symmetric single-channel current-voltage curves (*30, 31*), as required by the previous demonstration of two binding constants (the difference between the tight and weak binding constants being repulsion between ions on double occupancy) (*27*), and as required by the exposure of the ethanolamine end to the aqueous interface and by the buried formyl end [demonstrated in (*10*) and by Fig. 3, I and J].

In addition, ion-induced carbonyl carbon chemical shifts can be used to determine helical sense of the single-stranded structure. This has been done with [(1-^{13}C)D-Val^8]gramicidin A and [(1-^{13}C)D-Leu^{14}]gramicidin A (*12*). Examination of the structure of the single-stranded helix shows that for the left-handed helix the residue 14 carbonyl is in the binding-site segment of the channel, whereas the residue 8 carbonyl is well removed from the binding site segment (Fig. 4C). For the right-handed single-stranded β-helix, the reverse is the case; the residue 8 carbonyl carbon is at the center of the binding site segment, and the residue 14 carbonyl is well removed from the segment. The observed thallium ion–induced chemical shifts for residues 8 and 14 are also included as X's in Fig. 4C, where they are correct for the left-handed helical sense.

Thus, ion-induced carbonyl carbon chemical shifts have provided a complete resolution of the issue of the single- versus double-stranded helix for the channel state of gramicidin A and have determined the helical sense. It should be emphasized that this is the description of the major ion-conducting state of gramicidin in lipid bilayers and in no way counters the excellent solution and solid-state studies of polymerized L-D structures, including the gramicidins, which can occur in the double-stranded helical state.

DAN W. URRY
TINA L. TRAPANE
KARI U. PRASAD

Laboratory of Molecular Biophysics, University of Alabama School of Medicine, Birmingham 35294

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Abnormal Ion Permeation Through Cystic Fibrosis Respiratory Epithelium

Abstract. *The epithelium of nasal tissue excised from subjects with cystic fibrosis exhibited higher voltage and lower conductance than tissue from control subjects. Basal sodium ion absorption by cystic fibrosis and normal nasal epithelia equaled the short-circuit current and was amiloride-sensitive. Amiloride induced chloride ion secretion in normal but not cystic fibrosis tissue and consequently was more effective in inhibiting the short-circuit current in cystic fibrosis epithelia. Chloride ion–free solution induced a smaller hyperpolarization of cystic fibrosis tissue. The increased voltage and amiloride efficacy in cystic fibrosis reflect absorption of sodium ions across an epithelium that is relatively impermeable to chloride ions.*

Theoretical considerations (*1*) and experimental evidence (*2*) indicate that salt and water absorption are important in the regulation of the volume of liquid on the surface of proximal airways. A major driving force for volume absorption is active Na^+ transport by surface epithelial cells (*2*). This process is electrogenic [generates a transepithelial electric potential difference (PD)] and accounts for most of the basal short-circuit current (Isc) of epithelial preparations in vitro. Nasal and bronchial epithelia of subjects with cystic fibrosis (CF) are characterized in vivo by three abnormalities that reflect altered ion transport or permeability and may be linked to deranged surface liquid metabolism, thickened mucus, and recurrent pulmonary infection (*3*): (i) the basal transepithelial PD is

raised; (ii) superfusion of amiloride, an inhibitor of Na^+ transport, onto the airway surface induces a greater inhibition of PD; and (iii) superfusion of the surface with Cl^--free solution induces a smaller Cl^- diffusion PD (*4–6*). We explored the ion flows associated with these abnormalities by measuring bioelectric properties and unidirectional solute fluxes across nasal epithelia excised from CF and control subjects (*7*).

Resected specimens were placed immediately in Krebs bicarbonate Ringer solution at 37°C. Submucosal tissue was removed and tissues were mounted in flux chambers with apertures of 0.26 or 0.53 cm^2. Conductance (G), PD, and Isc were measured by standard techniques (*8*). Unidirectional flows of ^{22}Na (source, 0.3 μCi/ml), ^{36}Cl (0.3 μCi/ml), and ^{14}C-labeled mannitol (2.5 μCi/ml) were measured across specimens of short-circuited nasal epithelium. The initial sink compartment [mucosal (M) or submucosal (S)] was randomly selected. Isotope flows reached a steady state within 60 minutes and were monitored for another 45 minutes. Then the solutions were replaced with tracer-free solution. Tracer efflux from the tissue surface and electrode bridges in the source were estimated from the change in solution radioactivity during a 60-minute washout. Next, ^{22}Na and ^{36}Cl (each 6.5 μCi/ml) were added to the compartment that had served earlier as the sink. Steady-state isotope flow was measured for 45 minutes. Then amiloride was added to the mucosal solution ($10^{-8}M$) and the concentration was raised tenfold every 2 minutes (current was stabilized within 30 seconds) to $10^{-3}M$. Isotope fluxes were followed for another 45 minutes (*9*). Finally, the lumen was exposed to a Cl^--free (replaced by gluconate) solution with amiloride and the bioelectric response was monitored for 30 minutes. Tissues were fixed in the chamber and tissue morphology was evaluated by light microscopy (*8*).

The epithelium of the nasal turbinate from normal subjects was characterized by an Isc similar to that of excised human bronchi (*10*), whereas G was about two times higher (Table 1) (*11*). The difference between unidirectional Na^+ flows (net Na^+ absorption) accounted for the short-circuit current, whereas unidirectional Cl^- flows were symmetrical. The sum of Na^+ flow in the "passive" direction (S → M) and the unidirectional Cl^- flux accounted for about 85 percent of conductance.

Compared to normal turbinate, the PD of CF polyp epithelia was raised and G was lower. Isc was variable, but the mean current was not different from that of normal turbinate. The differences in PD and G appear to be a property of CF epithelia and not the result of the polypoid nature of the tissues because the PD of polyp epithelia from atopic subjects was lower (−3.2 ± 0.7 mV), and G higher (25.7 ± 1.4 mS/cm^2) than the corresponding properties of normal turbinate or CF polyp (*12*). Active (net) Na^+ absorption accounted for the basal Isc of CF tissue. Chloride ion flows were symmetrical and at least 50 percent smaller than those of normal tissue. The permeability of mannitol, a marker of solute flow through the paracellular path, tended to be smaller than that of normal turbinate, but did not parallel the much smaller G of the CF tissue.

Figure 1 shows the dose-effect relation for inhibiton of Isc by exposure of the mucosal surface to amiloride. At higher concentrations, amiloride induced significantly greater reductions in Isc (or PD) in CF tissue. The dose that inhibited Isc by 50 percent (ED_{50}) (~ $6 \times 10^{-7}M$) did not differ between groups and was typical of that measured in other airway epithelia (*2*). The amiloride-induced change in G was not significantly different between any two groups: −0.6 ± 0.6 in CF tissue, −1.6 ± 0.1 in normal turbinate, and −2.3 ± 0.9 mS/cm^2 in atopic polyp tissue. When the mucosal surface of normal turbinate was exposed to amiloride, Na^+ and Cl^- flow in the absorptive direction was inhibited (Table 2). Chloride secretion resulted (*13*). Amilor-

Table 1. Bioelectric properties and solute flows across excised CF nasal polyp and normal nasal turbinate. Bioelectric properties and ion fluxes represent the means of average values (± standard errors) for turbinates (one to two specimens per subject) from six normal subjects and polyps (two to four per subject) from three CF subjects. Mannitol permeability was estimated for tissues from five normal subjects (G = 14.7 ± 1.2 mS/cm^2) and three CF subjects. Mean Isc and G for tissues of all CF subjects (N = 4) were 2.98 ± 0.68 μEq/cm^2 per hour and 9.0 ± 2.6 mS/cm^2, respectively. M → S, unidirectional flux from mucosa to submucosa; S → M, unidirectional flux from submucosa to mucosa.

Item	Normal turbinate	CF polyp
Bioelectric properties		
PD (mV)	−4.7 ± 0.8	−11.7 ± 3.1*
Isc (μEq/cm^2-hour)	2.58 ± 0.92	2.74 ± 0.96
G (mS/cm^2)	15.6 ± 1.2	7.6 ± 3.0*
Ion fluxes (μEq/cm^2-hour)		
Na^+ (M → S)	7.24 ± 0.88	6.67 ± 1.96
Na^+ (S → M)	4.78 ± 0.82	3.16 ± 1.03
Na^+ (net)	2.46 ± 0.80†	3.54 ± 1.22†
Cl^- (M → S)	7.96 ± 0.60	3.84 ± 1.24*
Cl^- (S → M)	7.53 ± 0.59	3.02 ± 1.08*
Cl^- (net)	0.43 ± 0.62	0.82 ± 0.91
Permeability coefficient (× 10^{-7} cm/sec)		
$P_{mannitol}$	33.9 ± 6.5	25.0 ± 7.4

*Significantly different from normal turbinate ($P < 0.05$, unpaired t-test). †Significantly different from zero ($P < 0.05$, paired t-test).

Table 2. Response of nasal epithelia to exposure of the mucosal surface to amiloride and Cl^--free solution. Abbreviations: ΔJ, change in steady-state ion flow induced by amiloride; ΔPD and ΔG, change from baseline induced by replacement of Cl^- in mucosal bathing solution (all changes in PD made the mucosal solution more negative).

Condition	Normal turbinate	CF polyp	Atopic polyp
Amiloride ($10^{-4}M$)			
$\Delta J^{MS}_{Na^+}$ (μEq/cm^2-hour)	−1.50 ± 0.43*	−1.65 ± 0.46*	(*11*)
$\Delta J^{SM}_{Na^+}$ (μEq/cm^2-hour)	+0.34 ± 0.25	−0.05 ± 0.27	(*11*)
$\Delta J^{MS}_{Cl^-}$ (μEq/cm^2-hour)	−1.25 ± 0.30*†	+0.43 ± 0.22	(*11*)
$\Delta J^{SM}_{Cl^-}$ (μEq/cm^2-hour)	+0.02 ± 0.34	−0.18 ± 0.27	(*11*)
Cl^--free solution plus amiloride ($10^{-4}M$)			
ΔPD (mV)	−10.0 ± 3.0†	−4.1 ± 0.6	−8.8 ± 2.5†
ΔG (mS/cm^2)	−4.3 ± 0.8†	−1.9 ± 0.5	−8.1 ± 0.5†

*Significant change ($P < 0.05$). †Change different from that of CF tissue ($P < 0.05$).

ide affected only the absorption of Na^+ by CF tissue. Exposure of the lumen to Cl^--free solution with amiloride hyperpolarized normal turbinate and polyp and reduced conductance (Table 2). Similar trends were induced by Cl^--free solution in CF tissue, but the changes were significantly smaller.

The pattern of voltage across excised CF and normal nasal epithelia paralleled our observations in vivo (*14*). Although the somewhat greater Na^+ transport of CF tissue in vitro may contribute to the higher PD, this abnormality appears to primarily reflect the smaller conductance and Cl^- permeability of CF respiratory epithelium. These findings are consistent with the existence of a more widespread defect in epithelial Cl^- permeability. Schulz (*15*) and Quinton (*16*) postulated that the raised NaCl values in the sweat of CF patients may reflect a reduced Cl^- permeability of the sweat duct epithelium.

Although the lower conductance and smaller Cl^- fluxes of excised CF tissue (Table 1) do not pinpoint the Cl^- permeability defect of the epithelium to paths through the cell (transcellular) or around the cell (paracellular), other lines of evidence indicate that the defect is transcellular. The ratio of transepithelial Cl^- permeability to mannitol permeability of 3.4 for CF epithelium is smaller than the ratio of 5.8 for normal turbinate. Since paracellular paths across proximal airway epithelia behave like routes of free diffusion for molecules the size of Cl^- and mannitol (predicted permeability ratio, 2.9) (*17*), the ratio of P_{Cl^-} to P_{man} in CF tissue that approaches the free diffusion ratio, coupled with the smaller conductance, implies a smaller basal Cl^- flow through the cells of CF respiratory epithelium.

Because amiloride selectively affects the path of Na transport through, but not around, epithelial cells (*18*), the ability of the drug to induce asymmetries in Cl^- flows (secretion) in normal but not CF tissue is also compatible with less transcellular Cl^- permeation in CF. Since Cl^- secretion is not induced by amiloride in CF respiratory epithelia, the electrical response to the drug is dominated by inhibition of Na^+ absorption. This effect accounts for the disproportionately greater voltage response of CF nasal epithelium to amiloride exposure in vivo (*4*) and greater inhibition of Isc in vitro.

The pattern of Cl^- bi-ionic PD (Table 2) suggests that at least a portion of the lower Cl^- permeability of CF epithelial cells is attributable to the apical membrane. The hyperpolarization generated by exposure of the mucosal surface of normal tissue to Cl^--free solution in vitro and especially in vivo exceeds that predicted for nonselective paracellular paths (*5*) and hence probably reflects the contribution of the apical cell barrier.

In contrast to Cl^- permeation, the apical membrane path of Na^+ entry into CF respiratory epithelial cells does not appear to be abnormal because the change in G (Na^+ permeability) induced by amiloride in excised CF tissue was neither proportionately nor absolutely greater than that of normal nasal tissue and because the apparent affinities of amiloride (ED_{50}) for the epithelia from CF and normal subjects were similar. These studies, however, do not provide information about the driving force for Na^+ entry across these paths.

We speculated earlier that "excessive" absorption of water and Cl^- that is driven by Na^+ absorption is consistent with the desiccated airway secretions noted in CF (*4*). Decreased Cl^- permeability of the cellular path in CF could also result in excessive net volume absorption. The volume of surface liquid results from a balance between basal Na(Cl) absorption by surface epithelia and secretory counterflows from surface cells and glands. Chloride secretion by normal pulmonary epithelium may be controlled by local mediators, such as adrenergic agonists (*10*) and endogenous amiloride-like substances (*19*). The inability of Cl^--impermeable CF surface cells to secrete would shift the balance of transepithelial liquid flow away from the lumen and concentrate macromolecules (mucus) on the airway surfaces. Further, the same Cl^- secretory process may be the driving force for the net production of airway surface liquid by more distal pulmonary surfaces that are not accessible to our experimental approach. Because surface liquid flows toward the mouth (*1*), such a defect would not only contribute to desiccation of distal airway surfaces but also to dehydration of more proximal surfaces in CF.

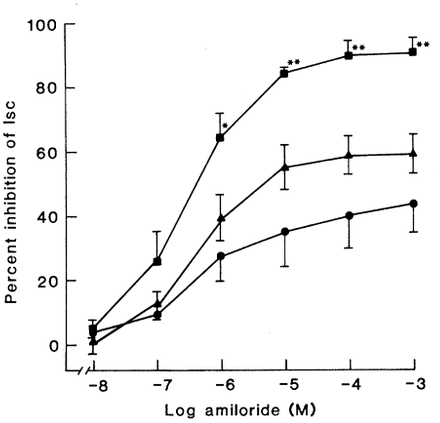

Fig. 1. Dose-effect relation for amiloride's action on the Isc of nasal polyps excised from four CF patients (■), nasal turbinates excised from six normal subjects (▲), and nasal polyps excised from five atopic subjects (●). Percent inhibition = [(Isc before exposure to drug − steady-state Isc during exposure to drug)/Isc before drug] ×100. Vertical lines denote standard errors. (*) Significantly different from atopic polyps; (**) significantly different from normal turbinate and atopic polyp (both at $P < 0.05$, *t*-test for independent means).

M. R. KNOWLES
M. J. STUTTS
A. SPOCK
N. FISCHER
J. T. GATZY
R. C. BOUCHER*

Departments of Medicine, Pharmacology, and *Surgery, University of North Carolina School of Medicine, Chapel Hill 27514,* and *Department of Pediatrics, Duke University School of Medicine, Durham, North Carolina 27710*

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7. Nasal polyps were resected from four CF subjects (ages 12 to 23) and five atopic subjects (23 to 67). Nasal turbinate was obtained from six normal subjects (24 to 48) undergoing elective septoplasties. Tissues studied were exposed to no topical medications. All procedures were approved by the University of North Carolina Human Rights Committee.
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9. The contribution of tracer from the original source to later measurements was estimated by extrapolation of a first-order (semilog) plot of the time course of the washout of radioactivity. Plots were linear and < 25 percent of any sink count was contributed by washout. Ion fluxes (J) and permeability coefficients (P) were calculated from the steady-state rate of radioisotope appearance in the sink [J. Gatzy, *Am. J. Physiol.* **228**, 1162 (1975)].
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11. Bioelectric properties represent 13 specimens from four CF subjects. Ion fluxes were measured in nine specimens from three CF subjects. Fluxes were not measured in two CF specimens and in one of nine turbinate specimens because the criterion for flux measurements (G < 20 mS/cm^2) was not met. G for all polyps from atopic subjects exceeded 20, so that only bioelectric properties were recorded. The results for tissues from the same subject were averaged.
12. Although there were some differences in the cell composition of the surface epithelium of the nasal specimens, we found no systematic correlation between morphology and bioelectric properties. As reported by E. Oppenheimer and B. Rosenstern [*Lab. Invest.* **40**, 445 (1979)], most (> 80 percent) of the exposed surface of CF and atopic polyps was lined by ciliated epithelium.
13. In airway epithelia amiloride routinely (*2*) induces active Cl^- secretion by an indirect action that involves hyperpolarization of the apical membrane. The (unidirectional) M → S flux of Cl^- (J_{Cl}^{MS}) drops because the more negative intracellular PD retards conductive Cl^- entry across the apical barrier whereas the (unidirectional) S → M flux of Cl^- (J_{Cl}^{SM}) is maintained or in-

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.
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* To whom reprint requests should be addressed at the Department of Medicine, University of North Carolina.

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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

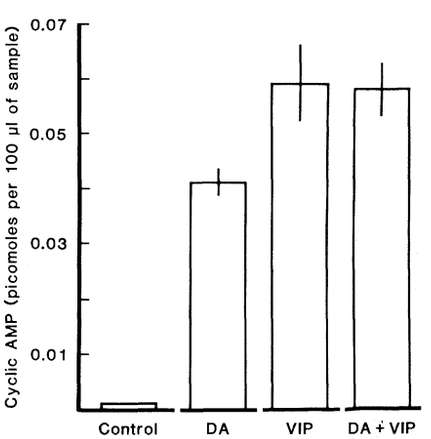

Fig. 1. Effects of dopamine (*DA*) and VIP, alone or in combination, on cyclic AMP accumulation in fractions of isolated carp horizontal cells. After a 5-minute incubation in the absence of any test substance, cells received a 10-minute incubation at 30°C in the presence of 250 μ*M* dopamine or 25 μ*M* 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.