G protein on their surfaces can, in the absence of other virus proteins, be used to investigate the ability of VSV G protein to cause membrane fusion. Fusion was observed at low *p*H and in the absence of any other virus-specific proteins. The formation of polykaryons required that G protein be anchored at the cell surface and was specifically inhibited by monoclonal antibodies to G protein.

R. Z. FLORKIEWICZ
J. K. ROSE

Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California 92138

References and Notes

1. J. White, M. Kielian, A. Helenius, *Q. Rev. Biol. Phys.* **16** (No. 2), 151 (1983).
2. M. Homma and M. Ohuchi, *J. Virol.* **12**, 1457 (1973).
3. M. Marsh *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **156**, 835 (1982).
4. A. Helenius, J. Kartenbeck, K. Simons, E. Fries, *J. Cell Biol.* **84**, 404 (1980).
5. J. E. Dahlberg, *Virology* **58**, 250 (1974).
6. D. P. Fan and B. M. Sefton, *Cell* **15**, 985 (1978).
7. C. Kondor-Koch, B. Burke, H. Garoff, *J. Cell Biol.* **97**, 644 (1983).
8. J. White, A. Helenius, M.-J. Gething, *Nature (London)* **300**, 658 (1982).
9. H. Garoff, A.-M. Frischauf, K. Simons, H. Lehrach, H. Delius, *ibid.* **288**, 236 (1980).
10. J. K. Rose and J. E. Bergmann, *Cell* **30**, 753 (1982).
11. R. Z. Florkiewicz, A. Smith, J. Bergmann, J. K. Rose, *J. Cell Biol.* **97**, 1381 (1983).
12. H. Fraenkel-Conrat and R. R. Wagner, *Comprehensive Virology* (Plenum, New York, 1975), pp. 1–93.
13. R. C. Mulligan and P. Berg, *Science* **209**, 1422 (1980).
14. D. R. Lowy *et al.*, *Nature (London)* **287**, 72 (1980).
15. L. Lefrancois and D. Lyles, *Virology* **121**, 157 (1982).
16. J. J. Skehel and M. D. Waterfield, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 93 (1975).
17. J. J. Skehel, P. M. Bayley, E. B. Brown, S. R. Martin, M. D. Waterfield, J. M. White, I. A. Wilson, D. C. Wiley, *ibid.* **79**, 968 (1982).
18. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
19. F. Chany-Fournier, C. Chany, F. Lafay, *J. Gen. Virol.* **34**, 305 (1977).
20. K. Handa, F. Chany-Fournier, S. Rousset, C. Chany, *Biol. Cell* **44**, 261 (1982).
21. We thank J. White for providing advice on the protocol for fusion: D. Lyles for monoclonal antibodies; R. Doolittle for performing the computer analysis of the VSV G protein; and L. Zokas, C. Machamer, B. Sefton, and T. Hunter for helpful suggestions concerning the manuscript. Supported by grants from the Public Health Service (AI15481) and the National Cancer Institute (CA 14195) and by a Public Health Service fellowship (1 F32 AI06911-01) to R.Z.F.

17 February 1984; accepted 6 June 1984

Immunologically Induced Alterations of Airway Smooth Muscle Cell Membrane

Abstract. *Active and passive sensitization, both in vivo and in vitro, caused significant hyperpolarization of airway smooth muscle cell preparations isolated from guinea pigs. An increase in the contribution of the electrogenic Na^+ pump to the resting membrane potential was responsible for this change. Hyperpolarization, as induced by passive sensitization, was not prevented by agents that inhibit specific mediators of anaphylaxis but was abolished when serum from sensitized animals was heated. The heat-sensitive serum factor, presumably reaginic antibodies, appears to be responsible for the membrane hyperpolarization of airway smooth muscle cells after sensitization.*

A number of respiratory diseases, including bronchial asthma, are characterized by an increased bronchoconstrictive response to numerous stimuli such as histamine or methacholine inhalation. The physiological factors underlying this so-called airway hyperreactivity are poorly understood. One idea is that fundamental changes may occur in the excitability and contractile properties of the airway smooth muscle itself (*1*). We showed that, in guinea pigs, sensitization with ovalbumin is associated with hyperpolarization of airway smooth muscle and that this hyperpolarization is, in turn, related to an increase in the contribution of the electrogenic Na^+ pump to the resting membrane potential. Further, hyperpolarization of the airway smooth muscle could be produced by passive sensitization in vitro and was not prevented by agents that inhibit mediators of anaphylaxis. However, heating serum obtained from sensitized animals prevented the change in resting membrane potential. These findings suggest that the airway response induced by sensitization to antigen involves a direct interaction between specific serum antibodies and the airway smooth muscle cell membrane. Thus, in addition to the role of the vagal reflex (*2*), release of mediators from mast cells (*3*), and possible alteration of specific membrane receptors (*4*), changes in airway smooth muscle membrane can be responsible for the phenomenon of airway hyperreactivity.

Segments of the middle portion of trachea isolated from male guinea pigs of the Camm-Hartley strain were studied in a temperature-controlled chamber as described (*5*). Single smooth muscle cells of tracheal muscle were impaled with glass microelectrodes made of borosilicate glass filled with 3*M* KCl and having a tip potential less than 10 mV and resistance of 80 to 90 megaohms. The tip potential and the resistance of each electrode were measured after each impalement. Successful impalement of a cell was indicated by a prompt negative deflection of the oscilloscope trace and maintenance of a steady potential (within 5 mV) for at least 10 seconds (*6*). Simultaneously with the measurement of resting membrane potential (E_m), the isometric force developed by tracheal segments was measured by means of a special

Table 1. The effect of active sensitization, active sensitization and resensitization, passive in vivo and in vitro sensitization on the resting membrane potential of guinea pig airway smooth muscle cells, and the response of airway smooth muscle preparations to ovalbumin, ouabain ($10^{-5}M$) and K^+-free solution. Values are means ± standard error; N.R., no response; N.D., not done.

Condition	E_m (mV)	Peak response to ovalbumin		E_m (mV)	
		E_m (mV)	Peak isometric force (g)	After $10^{-5}M$ ouabain	After K^+-free solution
Controls	−61.3 ± 0.5	N.R.	N.R.	−49.3 ± 0.6	−50.7 ± 0.4
Active sensitization	−72.7 ± 0.6*	−56.3 ± 0.3	3.8 ± 0.3	−51.8 ± 0.5	−51.9 ± 0.6
Active sensitization and resensitization	−78.1 ± 0.5*	−53.7 ± 0.8	7.8 ± 0.4	−49.9 ± 0.9	−51.4 ± 0.5
Controls	−60.5 ± 0.4	N.R.	N.R.	N.D.	N.D.
Passive sensitization in vivo	−69.5 ± 0.3*	−51.2 ± 1.3	5.3 ± 1.1	−50.5 ± 0.8	N.D.
Controls	−60.7 ± 0.6	N.R.	N.R.	−49.5 ± 5	N.D.
Passive sensitization in vitro	−68.5 ± 0.4*	−53.0 ± 0.9	4.7 ± 0.7	−50.2 ± 0.6	N.D.

*$P < 0.001$ compared to control.

Table 2. The effect of passive sensitization on the resting membrane potential of guinea pig airway smooth muscle cells in the absence and presence of specific mediator inhibitors of antigen-antibody reaction and disodium cromoglycate (DSCG). Response of tracheal preparations to 0.1 percent ovalbumin. ETYA is 5,8,11,14-eicosatetraynoic acid (Roche); FPL 55712 is sodium 7-3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy-4-oxo-8-propyl-4H-1-benzopyren-2-carboxylate (Fisons). Values are means ± standard error; N.R., no response.

Condition	E_m (mV)	Peak response to ovalbumin	
		E_m (mV)	Isometric force (g)
Controls	−60.7 ± 0.3	N.R.	N.R.
Passive sensitization in vitro	−68.5 ± 0.7	15.5 ± 0.8	5.7 ± 0.6
With diphenhydramine ($10^{-5}M$)	−68.7 ± 0.2	15.3 ± 0.2	5.0 ± 0.2
With methysergide ($10^{-6}M$)	−68.2 ± 0.3	14.2 ± 0.5	3.7 ± 0.6
With indomethacin ($10^{-6}M$)	−69.4 ± 0.5	18.4 ± 0.2	7.7 ± 0.4
With ETYA ($10^{-5}M$)	−68.1 ± 0.4	14.3 ± 0.7	4.1 ± 0.6
With FPL 55712 ($10^{-6}M$)	−67.9 ± 1.3	5.9 ± 0.2	1.9 ± 0.4
With DSCG (2 mg/100 ml)	−68.3 ± 0.8	12.7 ± 0.5	3.8 ± 0.4

metal holder on which a pair of microstrain gauges was attached (*5*). In the first part of these studies, four experimental groups were investigated: (i) animals sensitized to ovalbumin and pertussis vaccine (*5*); (ii) animals sensitized to ovalbumin and resensitized for 2 weeks (*5*); (iii) animals passively sensitized in vivo (*7*); and (iv) airway smooth muscle preparations passively sensitized in vitro (*7, 8*). Table 1 shows the value of E_m for each group studied and the corresponding control groups. The E_m of normal airway smooth muscle cells was −61.3 ± 0.5 mV (standard error) (464 cells). Fourteen days after sensitization of animals to ovalbumin and pertussis vaccine, the value of E_m increased to a mean of −72.7 ± 0.6 mV (583 cells; $P < 0.001$). In a separate series of experiments in which the guinea pigs were sensitized with ovalbumin only, we found a similar increase in E_m (−72.4 ± 0.4 mV; 380 cells). In the group of resensitized animals (after the third resensitization), E_m increased to −78.1 ± 0.5 mV (328 cells). Passive sensitization in vivo was induced with two intraperitoneal injections of serum obtained from resensitized animals given within a period of 48 hours. Control animals received normal serum in a similar period. As compared to controls, which had a mean E_m of −60.5 ± 0.4 mV, the E_m of the passively sensitized airway smooth muscle increased to −69.5 ± 0.3 (350 cells; $P < 0.005$). Passive sensitization in vitro of airway smooth muscle preparations from both guinea pigs and rabbits was done with an Austen technique (*8*). Preparations of airway smooth muscle were incubated with serum (final dilution, 1:10) obtained from resensitized animals (*9*). After 2 hours of incubation at 37°C and repeated washing of the chamber, the E_m of these passively sensitized airway smooth muscle cells was −68.5 ± 0.4 mV (250 cells) for guinea pigs and −68.2 ± 0.8 mV (200 cells) for rabbits. Control cells (exposed similarly to normal serum) had an E_m of −60.7 ± 0.6 mV for guinea pigs (200 cells) and −60.1 ± 0.9 mV for rabbits (150 cells). After ovalbumin, all actively and passively sensitized tissues developed a contractile response and were depolarized (see Table 1).

To test whether specific antibodies were involved in passive sensitization, we exposed airway smooth muscle preparations from normal guinea pigs and rabbits to serum from sensitized guinea pigs and rabbits, respectively, in which reaginic antibodies were inactivated (incubation of serum for 2 hours at 60°C) (*7*). Passive in vitro sensitization with this treated serum did not increase the E_m of rabbit airway smooth muscle cells, and these tissues did not show contractile responses to ovalbumin. In the airway smooth muscle preparations from guinea pigs, a passive in vitro sensitization with a heated serum partially inhibited both an increase in the E_m and the contractile response to ovalbumin ($P < 0.001$). In guinea pigs, sensitization usually produces two types of reaginic antibodies, immunoglobulins G and E (IgG and IgE), whereas in rabbits, only IgE is detected (*10*). Of these two types of antibodies, only IgE is heat sensitive (*10*). Thus, our data suggest that in guinea pigs both IgE and IgG may cause an increase in E_m, whereas in rabbits, changes in E_m are related to the presence of IgE only.

Since E_m depends on both active and passive ion transport, we performed experiments to determine the contribution of the electrogenic Na^+ pump to E_m in normal and sensitized airway smooth muscle preparations. This was done by measuring the membrane potential before and after the pump was inhibited with ouabain ($10^{-5}M$) or K^+-free solution (*11*). All tissue from actively sensitized animals and from actively sensitized and resensitized animals, as well as tissue sensitized passively in vivo and in vitro, showed marked potentiation of the contribution of the electrogenic Na^+ pump to the E_m of airway smooth muscle cells (Table 1). Thus, the increase in E_m after sensitization appears to be due to an increase in the contribution of the electrogenic Na^+ pump.

It is possible that sensitization-induced hyperpolarization is produced by mediators released from mast cells (*3, 7*). Alternatively, a direct interaction of specific antibodies and airway smooth muscle cell membrane may result in membrane hyperpolarization. To test these possibilities, passive sensitization in vitro was done in the presence of specific inhibitors of mediators of anaphylaxis before E_m was measured. Selection of doses of these specific antagonists was based on the data of Coleman (*12*), who showed the efficacy of these concentrations in mediator release in guinea pigs. The addition of diphenhydramine (a histamine H_1 receptor antagonist), methysergide (an antagonist of serotonin), indomethacin, and ETYA (5,8,11,14-eicosatetraynoic acid), both prostaglandin and leukotriene synthesis inhibitors, FPL 55712 (an antagonist of the slow-reacting substance) and disodium cromoglycate (an agent that stabilizes the membrane of mast cells), to the incubation medium did not inhibit the increase in the resting membrane potential of airway smooth muscle cells (Table 2). This fact suggests that the release of one or more mediators of anaphylaxis is not responsible for the hyperpolarization of the cells that follows passive sensitization in vitro. However, treatment of airway smooth muscle preparations with these compounds modified both the degree of depolarization and the contraction induced by ovalbumin. Treatment of the preparations with methysergide, ETYA, FPL 55712, and disodium cromoglycate decreased the magnitude of the contractile response, suggesting the possible participation of serotonin, prostaglandins, leukotrienes, or other mediators released from mast cells in this response. In contrast, treatment with indomethacin potentiated both contractile and electrical responses, a finding reported earlier by others (*13*).

These experiments suggest that the membrane hyperpolarization observed in sensitized airway smooth muscle is probably not due to the release of mediators from mast cells or other inflammatory cells. Most of the mediators of anaphylaxis would be expected to cause a depolarization rather than hyperpolarization of smooth muscle cells (*14*). Fur-

thermore, the presence of sodium cromoglycate, which stabilized the membrane of mast cells, did not influence the observed hyperpolarization. However, the role of mediators such as vasoactive intestinal peptide or substance P was not tested.

Stimulation of the electrogenic Na^+ pump could occur through binding of antibodies or circulating immunocomplexes to the cell membrane. This could increase membrane permeability for sodium with a consequent stimulation of the electrogenic Na^+ pump. This possibility is consistent with the observation that a receptor-ligand type of interaction between immunocomplexes and polymorphonuclear leukocytes also leads to hyperpolarization (*15*). In macrophages, the Fc receptor is a ligand-dependent sodium channel (*16*). After binding of specific reaginic antibodies on Fc receptors, an increase in Na^+ influx was observed, followed by an increase in Na^+- and K^+-dependent adenosinetriphosphatase activity and consequent increases in the resting membrane potential of macrophages (*16*). Thus, the alteration in the Na^+ gradient which occurs as a result of membrane permeability for Na^+ may lead to the increase in the calcium influx through the Na^+-Ca^{2+} exchange. This may cause the airway smooth muscle to be more responsive to stimulation. Furthermore, the binding of specific antibodies on the membrane may result in an increase in Ca^{2+}-activated K^+ conductance (*16*).

Calcium flux across rat mast cell membrane is promoted by cross-linking IgE receptors (*17*). In addition, the interaction of circulating immunocomplexes with complement during sensitization could activate complement (*18*) with the possible formation of the anaphylatoxin component of complement, C5a. It has been indeed shown (*19*) that C5a and the synthetic bacterial factor analog fMet-Leu-Phe can induce a hyperpolarization of macrophages or polymorphonuclear leukocytes primarily by stimulation of the electrogenic Na^+ pump. Finally, the alteration in function of some receptors, such as beta receptors, that occurs after sensitization may increase the electrogenic Na^+ pump activity. Antibodies to beta receptors may be present in the serum of patients with bronchial asthma (*4*).

It can be argued that alteration of the resting membrane potential is related to the use of pertussis vaccine. However, in a separate series of experiments, guinea pigs were sensitized with ovalbumin only, and we observed a similar increase in the resting membrane potential. Furthermore, in our studies, rabbits were sensitized with ovalbumin only.

In addition to the other factors that are involved in the mechanism of airway hyperreactivity—including the activation of the vagal reflex (*2*), the release of mediators from mast cells and other cells (*3, 8*), and alteration of specific receptors—this study showed that sensitization caused a direct alteration of airway smooth muscle membrane. It is of interest that an alteration of the cell membrane, including the activation of the electrogenic Na^+ pump, was shown recently to exist in vascular smooth muscle of animals with systemic hypertension (*20*).

M. SOUHRADA
J. F. SOUHRADA
John B. Pierce Foundation Laboratory, Yale University, New Haven, Connecticut 06519

References and Notes

1. J. A. Nadel, in *Bronchial Asthma: Mechanisms and Therapeutics*, E. B. Weiss and M. S. Segal, Eds. (Little, Brown, Boston, 1976), pp. 155–162; B. G. Simonsson. *Eur. J. Respir. Dis.* **61** (Suppl. 108), 21 (1980).
2. W. M. Gold, G. F. Kessler, D. Y. C. Yu, O. L. Frick, *J. Appl. Physiol.* **33**, 496 (1972).
3. G. K. Adams III and L. W. Lichtenstein, *J. Immunol.* **122**, 555 (1979); R. A. Lewis and K. F. Austen, *Nature (London)* **293**, 103 (1981).
4. J. C. Venter, C. M. Fraser, L. C. Harrison, *Science* **207**, 1361 (1980).
5. M. Souhrada and J. F. Souhrada. *Respir. Physiol.* **46**, 17 (1981).
6. D. M. Taylor, D. M. Paton, E. E. Daniel, *Life Sci.* **8**, 769 (1969).
7. J. L. Mongar and H. O. Schild. *J. Physiol. (London)* **150**, 546 (1980).
8. K. F. Austen, in *Asthma: Physiology, Immunopharmacology, and Treatment*, K. F. Austen and L. W. Lichtenstein, Eds. (Academic Press, New York, 1973), pp. 109–137.
9. After the second resensitization, the serum dilution titer for the guinea pig was 1:2560. In sensitized rabbits, the titer of antiserum as measured by the dilution technique was 1:1280.
10. Z. Ovary, B. Kaplan, S. Kojima, *Int. Arch. Allergy Appl. Immunol.* **51**, 416 (1976).
11. W. W. Fleming, *Annu. Rev. Pharmacol. Toxicol.* **20**, 129 (1980).
12. R. A. Coleman, *Br. J. Pharmacol.* **69**, 359 (1980); ——— and G. P. Levy, *ibid.* **52**, 167 (1974).
13. J. F. Burka and A. M. Paterson, *Eur. J. Pharmacol.* **70**, 489 (1981).
14. C. T. Kirkpatrick, *J. Physiol. (London)* **244**, 263 (1975); R. F. Coburn and T. Yamaguchi, *J. Pharmacol. Expt. Ther.* **201**, 276 (1977).
15. H. M. Korchak and G. Weisman, *Proc. Natl. Acad. Sci. U.S.A.* **15**, 3818 (1978).
16. J. D. Young *et al.*, *ibid.* **80**, 1357 (1983).
17. T. Ishizaka, J. C. Foreman, A. R. Sterk, K. Ishizaka, *ibid.* **76**, 5858 (1978).
18. T. E. Hugli and H. J. Muller-Eberhard, *Adv. Immunol.* **26**, 1 (1978).
19. E. K. Gallin and J. I. Gallin, *J. Cell Biol.* **75**, 277 (1977); P. H. Naccache, H. J. Showell, E. J. Becker, R. I. Sha'afi, *ibid.* **73**, 428 (1977); E. L. Becker, J. V. Talley, H. J. Showell, P. H. Nacchache, R. I. Sha'afi, *ibid.* **77**, 329 (1978).
20. K. Hersmsmeyer. *Circ. Res. Suppl.* **11**, 1153 (1976); H. G. P. Swarts, S. C. Bonting, J. Joep, DePont, F. Schuurmans-Stekhoven, T. A. Thien, A. Van't Laar, *Hypertension* **3**, 641 (1981).
21. For the generous gift of ETYA, we thank W. E. Scott, Hoffman–La Roche, Nutley, N.J., and for FPL 55712 we thank P. Sheard, Fisons Pharmaceuticals, Leicestershire, United Kingdom. We thank A. DuBois, Yale University, and H. Showell, Pfizer for editorial help. The serum dilution titer was measured by R. Carr, Halifax Hospital, Newfoundland. This work is supported by NIH grant HL-28063.

4 November 1983; accepted 13 January 1984

Neural Mechanisms of Sound Localization in an Echolocating Bat

Abstract. *The mustache bat emits a three-harmonic echolocation pulse. At the external ear, large interaural intensity differences are generated only when a sound originates within a limited area of two-dimensional space, and this area is different for each pulse harmonic. As a consequence, the external ear generates pronounced binaural spectral cues containing two-dimensional spatial information. This information is encoded in the inferior colliculus by neurons tuned to one of the harmonics and sensitive to interaural intensity differences.*

Many mammals, particularly nocturnal species that rely heavily on audition, can resolve the horizontal and vertical spatial coordinates of a sound source with precision. It is generally accepted that horizontal sound location is determined through a binaural comparison of arrival time, phase, and intensity disparities (*1*). Less is known of the mechanisms underlying vertical sound localization, but psychoacoustic studies in humans suggest that spectral cues may play an important role (*2*). For example, we tend to associate higher frequencies with an elevated sound source. Such studies suggest that sound frequency contains inherent spatial information (*3*).

Spectral cues are generated by the frequency-dependent directional properties of the external ear (*4–6*). Maximum acoustic energy reaches the tympanum when a sound originates along the "acoustic axis" of the external ear (*7*). The angles of the acoustic axis, relative to the tympanum, change with frequency (*6*). Different frequencies are therefore perceived as loudest when they originate at different points in space, hence their spatial attributes. It follows, then, that the ear will modify the power spectrum of a broadband sound as a function of sound location. The perceived power spectrum will also differ at the two ears as a function of location, creating binaural spectral cues. Such cues permit a simultaneous comparison of interaural