which would lead to a decrease in peptide binding capacity.

Although self protein would be the major source of Ia-binding peptides in vivo, we could not exclude the possibility that some of the Ia-binding peptides were derived from the fetal calf serum that was used to grow the I-A^d–expressing B cell lymphoma A20-1.11 in vitro. To address this point, we purified I-A^k from the lymphoma AKTB-1b, a tumor that is propagated in vivo. The low molecular weight I-A^k binding material that was eluted had activity similar to that obtained from the A20-1.11 cells grown in fetal calf serum (Fig. 3). Thus, we can conclude that antigen presenting cells can process self proteins of cellular or extracellular origin and that the peptide site on Ia contains such processed self peptides constitutively.

If the self peptides we have characterized are representative of naturally processed antigen, then processing would appear to generate peptides no larger than M_r 12,000, with most of the processed peptides being in the range of 2000 to 4000. The I-A^d–bound self peptides were somewhat larger than those bound to I-E^d, suggesting somewhat different structural requirements for binding to the two Ia isotypes. The fraction of Ia occupied by self peptides is unknown, but a rough estimate could be obtained from these experiments. If it is assumed that the eluted material consists of peptides with an affinity for I-A^d similar to that of the control peptide Ova(323-336), it is possible to calculate how many Ova peptide equivalents were eluted. From 3.3 nmol of I-A^d, 1.7 to 2.2 nmol equivalents (50% to 70% yield) of Ova(323-336) were obtained. This suggests that most of the Ia molecules in vivo are occupied by self peptides. Indeed, on the basis of our previous observation that only 5% to 10% of affinity-purified Ia was capable of binding exogenously added peptide, the fraction of occupied Ia may exceed 90%. This high degree of occupancy by chemically heterogeneous material suggests that Ia would appear to a T cell as very heterogeneous. As a corollary to this, T cell responses against peptides generated from foreign proteins would have to be induced by the small fraction of the total Ia available for presentation of newly processed exogenous antigen.

The existence of self peptide–Ia complexes could explain the high frequency of alloresponsive T cells since, if such T cells recognize allo-Ia plus peptide, the heterogeneity of the peptides bound to Ia would greatly expand the T cell clonotypes capable of recognizing allo-Ia. This concept is a variation of a previously proposed model for allorecognition (*12, 13*). Also, the generation of the T cell repertoire during ontogeny may be influenced by the nature of the Ia-associated self peptides in the thymus, both with respect to positive and negative selection of T cells. Finally, the binding of self peptides to MHC proteins in peripheral lymphoid tissue might provide a mechanism for immune surveillance, since neoantigens would be presented by the class I MHC of a tumor cell for recognition by killer T lymphocytes.

REFERENCES AND NOTES

1. K. H. Ziegler and E. R. Unanue, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 175 (1982).
2. R. Shimonkevitz, J. Kappler, R. Marrack, H. Grey, *J. Exp. Med.* **158**, 303 (1983).
3. S. Buus *et al.*, *Cell* **47**, 1071 (1986).
4. B. P. Babbitt, P. M. Allen, G. Matsueda, E. Haber, E. R. Unanue, *Nature* **317**, 359 (1985).
5. S. Buus *et al.*, *Science* **235**, 1353 (1987).
6. J. Guillet *et al.*, *Nature* **324**, 260 (1986).
7. A. Sette *et al.*, *ibid.* **328**, 395 (1987).
8. B. P. Babbitt, G. Matsueda, E. Haber, E. R. Unanue, P. M. Allen, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4509 (1986).
9. P. Björkman *et al.*, *Nature* **329**, 506 (1987).
10. P. Björkman, *ibid.*, p. 512.
11. S. Buus *et al.*, *Immunol. Rev.* **98**, 115 (1987).
12. P. Matzinger and M. J. Bevan, *Cell. Immunol.* **29**, 1 (1977).
13. P. Marrack and J. Kappler, *Nature* **332**, 840 (1988).

14. The expert technical assistance of B. Palmer, and secretarial assistance of K. Crumrine and J. Joseph are gratefully acknowledged. Supported by NIH grants AI18634 and AI09758.

21 June 1988; accepted 12 September 1988

A Bitter Substance Induces a Rise in Intracellular Calcium in a Subpopulation of Rat Taste Cells

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The sense of taste permits animals to discriminate between foods that are safe and those that are toxic. Because most poisonous plant alkaloids are intensely bitter, bitter taste warns animals of potentially hazardous foods. To investigate the mechanism of bitter taste transduction, a preparation of dissociated rat taste cells was developed that can be studied with techniques designed for single-cell measurements. Denatonium, a very bitter substance, caused a rise in the intracellular calcium concentration due to release from internal stores in a small subpopulation of taste cells. Thus, the transduction of bitter taste may occur via a receptor–second messenger mechanism leading to neurotransmitter release and may not involve depolarization-mediated calcium entry.

ALTHOUGH THE FUNCTION OF THE taste buds has been inferred for over 100 years, very little is known about the physiology of the taste cells or the molecular mechanisms involved in the gustatory transduction process (*1*). This lack of knowledge is due primarily to the small number of taste cells and their relative inaccessibility in situ. Recently, several patch clamp studies of isolated amphibian taste cells have identified a variety of ion channels in the taste cells, but their relation to the sensory transduction process is unclear (*2, 3*). Studies of transepithelial currents in lingual epithelium suggest that the transduction of salty, sweet, and sour tastes involves vectorial ion movements through the epithelium (*4, 5*). Bitter substances, however, induce no change in the transepithelial currents in canine lingual epithelium (*6*). To study taste transduction we developed a preparation of dissociated rat taste cells.

Taste cells from rat circumvallate papillae were identified with a monoclonal antibody (MAb) that recognizes the Lewis b blood group epitope. This epitope is expressed selectively on the taste cells (Fig. 1A) and on the overlying squamous cells (*7*). After dissociation of the epithelium by a combination of enzymatic digestion and mechanical agitation (legend to Fig. 1), the MAb labeled small aggregates of cells (Fig. 1B) and rare single cells. About 50% of the aggregates were labeled. Further evidence that the labeled aggregates were composed of taste cells was obtained with a Ca^{2+}–adenosine triphosphatase (Ca^{2+}-ATPase) histochemical staining procedure (*8*). Ca^{2+}-ATPase activity is associated with the taste cells in the lingual epithelium (*9*) (Fig. 1C) and, in the dissociated cell preparation, was found in the aggregates of cells (Fig. 1D, right). Thus, after dissociation, the taste buds remain as small aggregates, but the remainder

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of the epithelium dissociates into single cells.

We characterized the electrophysiological properties of the rat taste cells with the patch clamp technique (*10*). The resting membrane potential of the taste cells is about −69 mV (± 6) (mean ± SEM, $n = 18$) (*11*). At this resting potential, in the whole cell recording mode the membrane impedance is between 1 and 5 gigaohms. Whole cell recordings of the taste cells revealed two distinct populations of cells. All of the cells in antibody-labeled aggregates have a delayed rectifier K^+ current (Fig. 2A). A small subset of the taste cells (<5%) also have a voltage-dependent Na^+ current that is blocked by tetrodotoxin (TTX) (Fig. 2, B and C). Recordings from many unlabeled, nonsensory, lingual epithelial cells, whether singly or in clumps, did not display voltage-activated currents. Thus, the taste cells differed from the remainder of the lingual epithelial cells both antigenically and physiologically. In addition, the taste cells are not a homogeneous population of cells but rather consist of distinct subpopulations that express different ionic currents.

Because epithelial polarity is lost after dissociation, a stimulus added to the bath has access to both the apical and basolateral domains of the cell, thus complicating studies of ionic tastes. We therefore examined the process of bitter taste transduction. We did not use quinine, the archetypal bitter stimulus, for three reasons. First, it is fluorescent and therefore totally obscures the fluorescence of fura-2, the Ca^{2+}-sensitive dye used in our experiments. Second, it is membrane permeant and therefore its site of action is ambiguous. Third, it is a K^+ channel blocker and could have secondary effects unrelated to its bitterness. We used denatonium chloride, the most bitter chemical for humans, as the test substance (*12*). It is neither membrane permeant nor fluorescent, and it does not affect the K^+ channels at concentrations less than 0.5 m*M*. We have found that 1 μ*M* denatonium chloride is aversive to rats in a two-bottle preference test.

On the basis of morphological criteria, taste cells form chemical synapses with the gustatory neurons, where the taste cells are

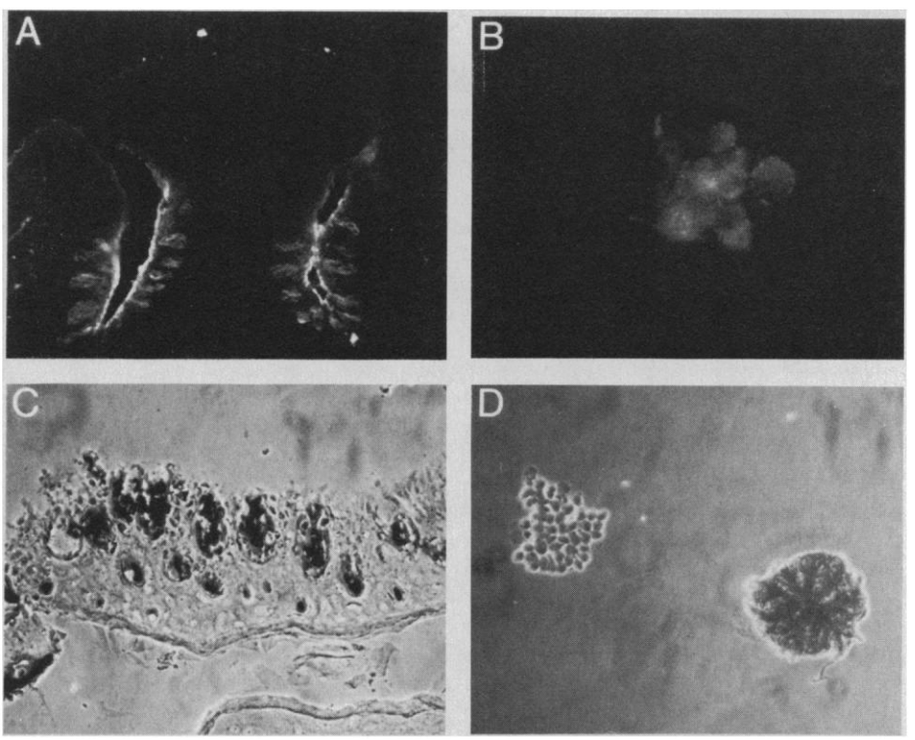

Fig. 1. Immunofluorescent and histochemical labeling of taste buds in situ and in the dissociated cell preparation. (**A**) A section of rat tongue through the circumvallate papillae. (**C**) A section of lingual epithelium after excision from the tongue. (**B** and **D**) Cell aggregates from dissociated cell preparations. (A) and (B) were labeled with antibody to the Lewis b antigen (143-2-A6, American Type Culture Collection, Rockville, Maryland); (C) and (D) were labeled with Ca^{2+}-ATPase histochemical stain. In (D) the aggregate to the right is stained, but the other aggregate, presumably a group of epithelial cells, is not. Magnification: (A) 100×, (B and D) 250×, (C) 160×. After the tongue was removed from a Sprague-Dawley rat (200 g), 0.4 ml of 0.25% collagenase (w/v) (Sigma) in Hank balanced saline solution (HBSS) was injected into it subcutaneously. The tongue was incubated at 37°C for 30 min. The epithelium was excised and incubated in a mixture of 0.25% collagenase (Sigma) and 0.25% trypsin (Cooper) in HBSS for 20 min at room temperature; EDTA was then added to a concentration of 3.5 m*M*. The epithelium was triturated 25 times, incubated for 20 min, triturated 25 times, washed 3 times in HBSS, and resuspended for use. Sections and dissociated cells were processed for immunocytochemistry by the procedures of Dodd and Jessell (*26*). The Ca^{2+}-ATPase staining was performed with the following modifications (*8*). The solutions contained 2.5 m*M* $CaCl_2$, and the sections were incubated for 10 min. After staining, they were dehydrated and a cover slip was applied with Permount. To label the dissociated cells we incubated them in 0.4*M* sodium glycine buffer, *p*H 9.0, for 15 min.

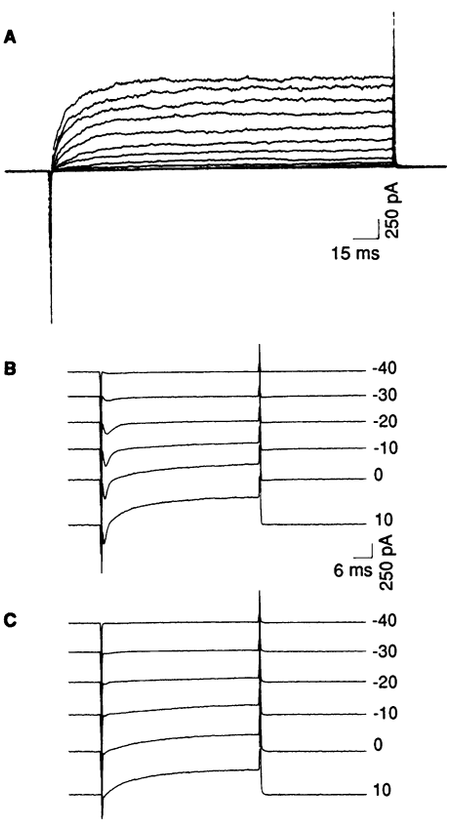

Fig. 2. Electrophysiology of taste cells. Whole cell recordings from two taste cells. (**A**) Superimposed current responses to a series of depolarizing steps from −20 mV to +80 mV at 10-mV intervals with a holding potential of −80 mV. (**B**) Recording from a taste cell containing voltage-dependent Na^+ current, holding potential −80 mV; the step potential (in millivolts) is listed at the right of each trace. (**C**) The same cell after treatment with 2 μ*M* TTX; the early inward current has been abolished. To cells prepared as described in Fig. 1, hyaluronidase (Cooper) and neuraminidase (Sigma) were added to final concentrations of 1 mg/ml and 0.1 U/ml, respectively; the cells were incubated for 20 min, then triturated 25 times, washed 3 times in buffer A (140 m*M* NaCl, 4 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10 m*M* Na Hepes, *p*H 7.4, and 5 m*M* glucose), and used. Bath contained buffer A. Patch pipettes (soda lime glass, Fisher) contained 140 m*M* KCl, 1 m*M* $MgCl_2$, 10 m*M* K EGTA, 10 m*M* Na Hepes, *p*H 7.2. Current records were recorded with a List EPC-7 amplifier. They were low pass–filtered and stored on hard disk for subsequent analysis by digital computer (Indec Systems, Sunnyvale, California). Capacitive and linear leak currents have been subtracted.

the presynaptic cells (*13, 14*). This suggests that a rise in the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ leading to neurotransmitter secretion could represent part of the sensory transduction process. Figure 3 shows the response of individual cells in a taste cell aggregate, loaded with the fluorescent Ca^{2+}-sensitive dye fura-2 (*15*), to the addition of 1 μM denatonium chloride to the bath. One of the cells in the aggregate exhibited a rise in $[Ca^{2+}]_i$ in response to denatonium, whereas there was no effect in the remainder of the cells. Similar responses were seen in a single cell in six other aggregates and in two cells in another aggregate. In these cells, $[Ca^{2+}]_i$ rose from a resting level of 50 nM ($\pm$ 7, n = 8) to a peak of 105 nM ($\pm$ 12, n = 8) ($P < 0.005$, Student's t test) after stimulation with denatonium. No rise in $[Ca^{2+}]_i$ was observed in any epithelial cell in response to denatonium. To demonstrate that the rise in $[Ca^{2+}]_i$ is specifically due to the bitter nature of denatonium, we examined the response of taste cells to a sweet stimulus. There was no change in $[Ca^{2+}]_i$ in any cell in 22 taste cell aggregates after stimulation with 1 mM saccharin, a chemical that rats perceive as sweet (*16*).

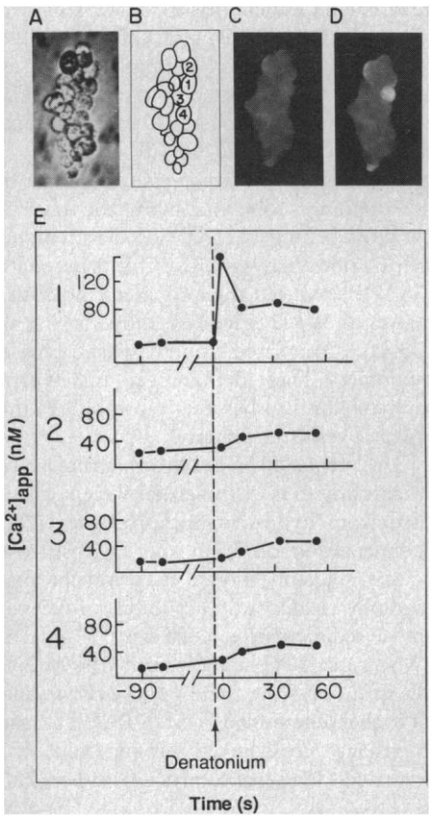

Fig. 3. Apparent response of $[Ca^{2+}]_i$ in individual taste cells loaded with fura-2 to bath application of 1 μM denatonium. (**A**) Phase micrograph of a taste cell aggregate. (**B**) Tracing of the cell outlines. The numbers on the cells correspond to the numbers to the left of each graph in (E). (**C**) Ratio image (350 nm/380 nm) of the fura-2 fluorescence before stimulation with denatonium. Black corresponds to low Ca^{2+} and white to high Ca^{2+}. (**D**) Ratio image immediately after the addition of denatonium to the bath. Note the change in cell 1 and in the cell at the bottom of the aggregate. (**E**) Quantitation of $[Ca^{2+}]_i$ in the responsive cell, 1, and in three adjacent cells. Cells, prepared as in Fig. 1, were incubated with 10 μM fura-2 acetoxymethylester (Molecular Probes) in buffer A for 10 min, washed three times in buffer A, and allowed to stick to a polystyrene petri dish. The cells were examined with a Zeiss microscope fitted with epifluorescence optics and a silicon intensifier target camera (Dage-MTI). Image pairs due to excitation at 350 and 380 nm were digitized with a computer-controlled image analyzer (Datacube) and stored on the hard disk for subsequent analysis. We calculated $[Ca^{2+}]_i$ by using maximum and minimum fluorescence signals obtained on the same aggregate (*15*).

Influx of Ca^{2+} from two sites, intracellular stores or the extracellular space, can lead to a rise in $[Ca^{2+}]_i$. In the absence of extracellular Ca^{2+}, denatonium induced a rise in $[Ca^{2+}]_i$ (Fig. 4) from a resting level of 67 nM ($\pm$ 9, n = 6) to a peak of 120 nM ($\pm$ 8, n = 6) ($P < 0.001$, Student's t test). The source of Ca^{2+} for this rise must be intracellular stores.

Depolarization-induced opening of voltage-dependent Ca^{2+} channels is one of the ways by which neurons generate a rise in $[Ca^{2+}]_i$ (*17*). To ascertain the involvement of this mechanism in taste cells, we examined the effect of K^+-induced depolarization on $[Ca^{2+}]_i$. In four cells that responded to denatonium, K^+ depolarization caused no further rise in $[Ca^{2+}]_i$. Immediately before K^+ was added $[Ca^{2+}]_i$ was 101 nM ($\pm$ 11), and after K^+ depolarization it was 83 nM ($\pm$ 19). These experiments suggest that voltage-dependent Ca^{2+} channels are not involved in the increased $[Ca^{2+}]_i$ induced by denatonium. They do not preclude the possibility that Ca^{2+} may also enter the cytoplasm through plasma membrane channels that are opened by second messengers, as has been described in lymphocytes (*18*).

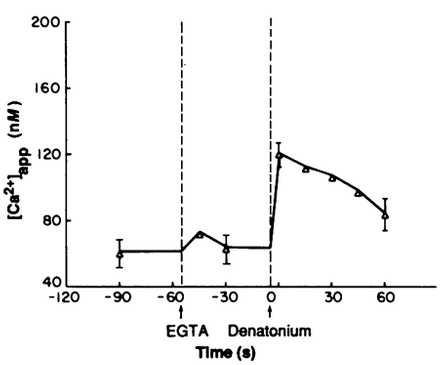

Fig. 4. Apparent response of $[Ca^{2+}]_i$ to stimulation with denatonium in the absence of extracellular Ca^{2+}. The mean response of six cells is plotted. The initial time point was obtained in the presence of 2 mM $CaCl_2$. The chamber was then perfused with eight volumes of Ca^{2+}-free buffer A with 2 mM EGTA. Finally, eight volumes of Ca^{2+}-free buffer A with 2 mM EGTA and 1 μM denatonium were perfused into the chamber. Standard error bars are shown on illustrative points and were comparable on the other points.

Our experiments indicate that the transduction of bitter taste occurs via a series of biochemical processes rather than by the more "classical" mechanism of depolarization-induced Ca^{2+} influx (*19, 20*). Because denatonium is membrane impermeant, the first step must involve binding to an apical cell surface receptor. In view of the structural heterogeneity of bitter compounds (*21*), it is uncertain whether there is a single receptor or multiple receptors for all bitter substances. Our experiments suggest that the interaction of a bitter ligand with its receptor results in the generation of an intracellular second messenger. The rise in $[Ca^{2+}]_i$ and the release of Ca^{2+} from internal stores suggests the involvement of the inositol trisphosphate system in bitter taste transduction (*22*). However, studies in epithelia indicate that adenosine 3',5'-monophosphate (cAMP) may also induce release of Ca^{2+} from internal stores (*23, 24*). Further experiments will be necessary to identify definitively the second messenger.

We have also demonstrated that on the basis of several physiological criteria, the taste buds consist of a heterogeneous group of cells. They can be divided into subpopulations of cells in view of their electrophysiological properties and their response to a bitter stimulus. This functional specialization is presumably important for the detection of specific taste modalities. The other cells within the taste bud may be involved in the transduction of other taste modalities or they may be supporting or basal cells. Previous work has shown that there are morphologically distinct subpopulations of cells within the taste buds (*13, 14, 25*). Further work will be necessary to determine whether the functional specialization that we have observed correlates with morphologically defined subpopulations of cells.

REFERENCES AND NOTES

1. C. Pfaffmann, in *Taste, Olfaction, and the Central Nervous System*, D. W. Pfaff, Ed. (Rockefeller Univ. Press, New York, 1985), pp. 19–44.
2. P. Avenet and B. Lindemann, *J. Membr. Biol.* **97**, 223 (1987).
3. S. C. Kinnamon and S. D. Roper, *J. Gen. Physiol.* **91**, 351 (1988).
4. S. A. Simon and J. L. Garvin, *Am. J. Physiol.* **249**, C398 (1985).
5. S. Mierson *et al.*, *Biochim. Biophys. Acta* **816**, 283 (1985).
6. S. A. Simon, R. Robb, J. L. Garvin, *Am. J. Physiol.* **251**, R598 (1986).
7. J. Dodd, in preparation.
8. T. Ando, K. Fujimoto, H. Mayahara, H. Miyajima, K. Ogawa, *Acta Histochem. Cytochem.* **14**, 705 (1981).
9. A. A. Zalewski, *J. Comp. Neurol.* **200**, 309 (1981).

10. O. P. Hamill *et al.*, *Pfluegers Arch.* **391**, 85 (1981).
11. We obtained this estimate from the reversal potential of the K^+ channel current-voltage plots observed in cell-attached patches, using electrodes filled with 140 m*M* KCl. It is unlikely that the intracellular K^+ concentration is as high as 140 m*M*, so the reversal potential will underestimate the actual membrane potential, which is likely to be even more negative.
12. A. Saroli, *Naturwissenschaften* **71**, 428 (1984).
13. R. G. Murray, A. Murray, S. Fujimoto, *J. Ultrastruct. Res.* **27**, 444 (1969).
14. J. C. Kinnamon, B. A. Taylor, R. J. Delay, S. D. Roper, *J. Comp. Neurol.* **235**, 48 (1985).
15. R. Y. Tsien *et al.*, *Cell Calcium* **6**, 145 (1985).
16. S. S. Schiffman, A. J. Hopfinger, R. H. Mazur, in *The Receptors*, P. M. Conn, Ed. (Academic Press, New York, 1986), vol. 4, pp. 315–377.
17. R. W. Tsien *et al.*, *Annu. Rev. Biophys. Biophys. Chem.* **16**, 265 (1987).
18. M. Kuno and P. Gardner, *Nature* **326**, 301 (1987).
19. In addition to releasing Ca^{2+} from internal stores, second messengers may increase ion conductances. This may account for the changes in transmembrane potential reported by several investigators (*20*). However, the low resting membrane potentials and input impedance reported in many of those studies, compared to those reported here, suggests that damage to the plasma membrane at the electrode penetration site may complicate the interpretation of their results. Further experiments will be necessary to resolve these issues.
20. T. Sato and L. M. Beidler, *Comp. Biochem. Physiol. A* **73**, 1 (1982); *ibid.* **75**, 131 (1983); M. Ozeki, *J. Gen. Physiol.* **58**, 688 (1971).
21. J. B. Harborne, *Introduction to Ecological Biochemistry* (Academic Press, New York, 1982).
22. M. J. Berridge, *Annu. Rev. Biochem.* **56**, 159 (1987).
23. C. E. Semrad and E. B. Chang, *Am. J. Physiol.* **252**, C315 (1987).
24. H. S. Chase and S. M. E. Wong, *ibid.* **254**, F374 (1988).
25. A. Farbman, *J. Ultrastruct. Res.* **12**, 328 (1965).
26. J. Dodd and T. M. Jessell, *J. Neurosci.* **5**, 3278 (1985).
27. We thank S. Siegelbaum, H. S. Chase, and D. Landry for helpful discussions and for allowing us to use their equipment during the early phases of this work; we thank A. Finkelstein for helpful comments on this manuscript. M.H.A. is the recipient of an American Heart Association clinician-scientist award. Supported in part by Public Health Service grants DK 34742 and DK 20999 (to Q.A.) and by grant NS22993 (to J.D.).

20 June 1988; accepted 9 September 1988

Identification of Synaptophysin as a Hexameric Channel Protein of the Synaptic Vesicle Membrane

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The quaternary structure and functional properties of synaptophysin, a major integral membrane protein of small presynaptic vesicles, were investigated. Cross-linking and sedimentation studies indicate that synaptophysin is a hexameric homo-oligomer, which in electron micrographs exhibits structural features common to channel-forming proteins. On reconstitution into planar lipid bilayers, purified synaptophysin displays voltage-sensitive channel activity with an average conductance of about 150 picosiemens. Because specific channels and fusion pores have been implicated in vesicular uptake and release of secretory compounds, synaptophysin may have a role in these processes.

NEUROTRANSMITTER RELEASE FROM presynaptic nerve terminals is thought to involve specialized vesicular compartments, synaptic vesicles, for both storage and exocytosis of transmitter molecules (*1*). Despite extensive investigation, the molecular mechanisms of accumulation and release of synaptic vesicle contents remain obscure. Morphological (*2*) and recent electrophysiological (*3, 4*) studies on other secretory systems, however, have provided evidence for the involvement of defined channels and "fusion pores" in these processes. In particular, a proteinaceous pore of diameter and conductance similar to that of hexameric gap-junction proteins has been inferred to initiate exocytosis of vesicle contents (*4*).

Synaptophysin (p38) is a major integral protein of the synaptic vesicle membrane (*5, 6*). It is expressed in central and peripheral neurons as well as in neuroendocrine cells (*7, 8*) and represents a major cytoplasmic Ca^{2+} binding site of synaptic vesicle preparations from rat brain (*9*). On the basis of analysis of its primary structure deduced from the cDNA sequence (*10–12*), it has been proposed that synaptophysin spans the vesicle membrane four times, with both its NH_2- and COOH-termini located on the cytoplasmic surface. This transmembrane topology is reminiscent of that of hexameric gap-junction proteins (*13*), raising speculations that homo-oligomeric forms of synaptophysin (*14*) also may form a transmembrane channel (*10, 11*).

Upon SDS–polyacrylamide gel electrophoresis (PAGE), brain synaptophysin exhibits a relative molecular mass of 38 kD (*5, 6*), which is reduced to 34 kD on deglycosylation (*8, 9*). Cross-linking of purified synaptophysin preparations isolated by a new chromatofocusing method (*15*) resulted in five adducts of the 38-kD polypeptide of apparent molecular masses of 72 kD, 110 kD, 145 kD, 180 kD, and 220 kD, all ±5 kD (Fig. 1A) (*16*). On immunoblots, all adducts were recognized by the monoclonal antibody SY38 (Fig. 1A) (*17*), which reacts with the cytoplasmic COOH-terminal region of synaptophysin (*5, 10*). Cleavage of the oligomers obtained with the reversible cross-linking reagent *N*-sulfosuccinimidyl(4-azidophenyl)-1,3′-dithiopropionate (SSADP) reduced their apparent molecular masses to 38 kD (Fig. 1B), showing that all adducts comprised only synaptophysin monomers. These data indicate that synaptophysin forms a hexameric complex in the synaptic vesicle membrane.

Consistent with a hexameric structure of synaptophysin is its molecular weight calculated from hydrodynamic properties (*18*). Sedimentation on H_2O and D_2O sucrose density gradients and gel exclusion chromatography yielded a Stokes radius of 6.5 nm and a sedimentation coefficient ($S_{20,w}$) of 8.9*S* (Table 1). The molecular mass of 230 kD estimated from these values (*19*) is similar to that determined by SDS-PAGE for the extensively cross-linked protein and that predicted for a synaptophysin hexamer (228 kD).

Ultrastructural analysis of negatively stained preparations of synaptophysin that had been solubilized in Triton X-100 and purified revealed a largely homogeneous population of particles of mean diameter of 7.8 nm (Fig. 2). In regions of shallow staining, a number of these randomly oriented particles exhibited a "rosette-like" structure with a central 1- to 2-nm density of heavy metal deposition, apparently resulting from filling of a central depression with uranyl salt (Fig. 2, inset). This appearance is reminiscent of the negative staining pictures of the 8- to 8.5-nm particles of hepatic gap junction protein preparations (*20*). The structure also resembles the 9- to 10-nm "doughnut-shaped" particles or rosettes reported for detergent-solubilized preparations of the Na^+ channel protein (*21*) and the nicotinic acetylcholine receptor (*22*) from fish electric organ. Similar 8-nm particles have also been described for a delipidized protein preparation proposed to me-

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