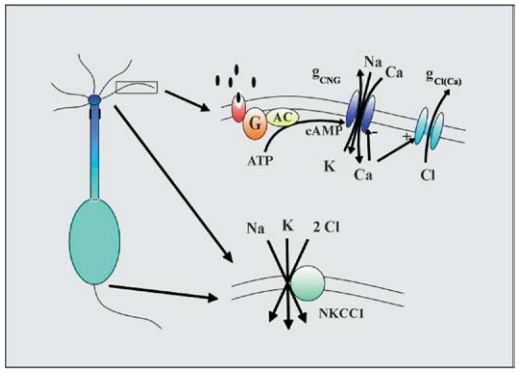

Figure 1. Diagram Showing the Proposed Mechanism for Regulation of Intracellular Chloride by NKCC1 in Olfactory Receptor Neurons

Olfactory transduction takes place in the cilia of the olfactory receptor neuron (top panel). Odors (black ovals) bind to olfactory receptors that stimulate a G protein (G_{olf}) that in turn activates adenylyl cyclase type III (AC). The subsequent increase in intralumenal cAMP elicits opening of a cyclic nucleotide-gated channel (g_{CNG}) allowing Ca^{2+} to enter the cilium. The elevation in $[Ca^{2+}]_i$ activates a Ca^{2+}-activated Cl^- channel allowing efflux of Cl^-, thereby causing cell depolarization. Intracellular Cl^- is kept elevated above electrochemical equilibrium by the Na^+-K^+-$2Cl^-$ cotransporter NKCC1.

nide produces a similar decrease in odor-activated Cl^- current. The combined pharmacological and genetic approaches provide solid evidence that the transporter involved in Cl^- uptake in ORNs is NKCC1. The authors also characterized the distribution of the NKCC1 using immunofluorescence and make the surprising observation that NKCC1 is found distributed uniformly throughout the dendritic and soma plasma membrane of the ORNs, *but not in the cilia*. They suggest the NKCC1 increases intracellular chloride above electrochemical equilibrium throughout the ORN, allowing the soma and dendrite to serve as a large Cl^- reservoir that the cell can utilize to maintain intralumenal Cl^- elevated within the cilia during the response to odors.

While the observation of a uniform distribution of NKCC1 in the plasma membrane of the ORNs appears contradictory to the finding of a standing $[Cl^-]_i$ gradient by Kaneko and coworkers, potentially interesting explanations may exist. For example, while the distribution of the cotransporter may indeed be uniform, the $[Cl^-]_i$ may vary from knob to soma due to spatial variation in the driving force caused by a standing gradient of intracellular Na^+. In addition, the functional status of NKCC1 may differ depending on location along the dendrite.

Regardless, the work of Reisert and coworkers shows that NKCC1 is the cotransporter responsible for Cl^- accumulation in ORNs. Thus, like CNS neurons in neonates, ORNs in adult rodents express the Na^+-K^+-$2Cl^-$ cotransporter NKCC1, resulting in intracellular chloride concentrations above electrochemical equilibrium. Unlike most neurons in the adult nervous system, ORNs avoid the "chloride switch": they do not express KCC2, the main Cl^- extrusion cotransporter operating in the majority of neurons of the adult CNS. As a result, ORNs depolarize in response to opening of Ca^{2+}-activated Cl^- channels.

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Selected Reading

Buck, L.B. (2000). Cell *100*, 611–618.

Frings, S., Seifert, R., Godde, M., and Kaupp, U.B. (1995). Neuron *15*, 169–179.

Gold, G.H. (1999). Annu. Rev. Physiol. *61*, 857–871.

Kaneko, H., Putzier, I., Frings, S., Kaupp, U.B., and Gensch, T. (2004). J. Neurosci. *24*, 7931–7938.

Kleene, S.J., and Gesteland, R.C. (1991). J. Neurosci. *11*, 3624–3629.

Kurahashi, T., and Yau, K.-Y. (1993). Nature *363*, 71–74.

Leinders-Zufall, T., Greer, C.A., Shepherd, G.M., and Zufall, F. (1998). J. Neurosci. *18*, 5630–5639.

Lindemann, B. (2001). Biophys. J. *80*, 1712–1721.

Lowe, G., and Gold, G.H. (1993). Nature *366*, 283–286.

Payne, J.A., Rivera, C., Voipio, J., and Kaila, K. (2003). Trends Neurosci. *26*, 199–206.

Reisert, J., Lai, J., Yau, K.-W., and Bradley, J. (2005). Neuron *45*, this issue, 553–561.

Reuter, D., Zierold, K., Schroeder, W.H., and Frings, S. (1998). J. Neurosci. *18*, 6623–6630.

Schild, D., and Restrepo, D. (1998). Physiol. Rev. *78*, 429–466.

DOI 10.1016/j.neuron.2005.02.002

Minis: Whence and Wherefore?

In this issue of *Neuron*, Sara et al. find that spontaneously released miniature synaptic potentials arise from a pool of vesicles distinct from those released by neural activity. This modification of a basic tenet of the quantal hypothesis has important implications for the analysis of changes in synaptic transmission.

Let's face it—minis (miniature synaptic potentials) are boring. The spontaneous release of the transmitter contents of a vesicle from nerve terminals seems to exist but for one purpose: to provide abstruse amusement to neuroscientists wanting to define the size of a quantum and to statisticians wanting to characterize the rules for random release or to know how many quanta were released by an action potential to generate the real signal of interest— the full-size postsynaptic potential that transmitted information across the synapse.

However, attitudes toward minis are changing. A turning point occurred in 1995, when Stevens and Wang demonstrated that cortical neurons sometimes

communicate via a single synapse, which releases only one quantum of glutamate in response to an action potential (Stevens and Wang, 1995). At such synapses, communication occurs by the fusion of a single vesicle, having exactly the same postsynaptic consequence as a mini. Clearly, then, minis could no longer be ignored as too small to be of any consequence.

Interest in minis increased with the demonstration that an increase in mini frequency can produce a significant inhibitory tone in postsynaptic neurons (Lu and Trussell, 2000). Moreover, single spontaneously released excitatory quanta can generate an action potential in small neurons, while single inhibitory quanta can suppress or delay postsynaptic firing (Carter and Regehr, 2002). Finally, nicotinic activation of presynaptic receptors can release enough minis to strongly excite even large principal cortical neurons (Sharma and Vijayaraghavan, 2003).

Minis not only may play a signaling role at synapses, but also may be important in development. Murphy and colleagues (1994) found that NMDA antagonists reduced calcium/calmodulin-dependent kinase II activity (presumably in dendrites) more than tetrodotoxin block of activity, suggesting that spontaneous miniature glutamate release modulates postsynaptic enzymatic activity. In 1999, McKinney and colleagues showed a drop in size and number of dendritic spines when miniature glutamate release was blocked by botulinum toxins, or their effects were blocked by AMPA antagonists; blocking activity-evoked release with tetrodotoxin was ineffective (McKinney et al., 1999). At *Drosophila* neuromuscular junctions, the normal postsynaptic clustering of glutamate receptors requires the spontaneous release of minis: in *syntaxin-1A* and *shibire* mutants lacking minis and evoked release, the clusters were absent, while in *synaptobrevin* or *cysteine string protein* mutants or flies treated with tetrodotoxin, in which only evoked release was blocked, receptor clustering was normal (Saitoe et al., 2001). Blocking minis with botulinum toxin A can also lead to an *increase* in dendritic protein synthesis in hippocampal neurons that had previously been silenced with tetrodotoxin (Sutton et al., 2004), suggesting that minis keep resting protein synthesis in check but responsive to stimuli that strengthen synapses.

As minis assume functional significance, attention turns toward understanding their origin. Differences in temperature and calcium sensitivity (Angleson and Betz, 2001; Delaney and Tank, 1994) have led to the suggestion that minis are released by a different mechanism than the quanta evoked by an action potential. Different sensitivities to calcium and strontium were interpreted similarly, although these may reflect instead how these ions are buffered and extruded (Xu-Friedman and Regehr, 2000). And the different sensitivities of evoked release and minis to mutations of synaptotagmin (Geppert et al., 1994) and the other synaptic proteins mentioned above (Saitoe et al., 2001) further supported the view that minis and evoked quanta were different.

Nevertheless, the findings published in this issue of *Neuron* by Sara et al. (2005) will surprise anyone familiar with the classical evidence that a mini looks exactly like a quantum evoked by an action potential (Katz, 1969). The present study demonstrates that minis are released from a pool of vesicles distinct from those released by activity. Using styryl membrane dyes, the authors first confirmed that both spontaneously released and activity-released vesicles are labeled by endocytosis and are destained by exocytosis. Successive loading of spontaneously released vesicles with an antibody to the luminal domain of synaptotagmin and a labeled secondary antibody showed recycling of the vesicles involved in minis. The key experiment involved staining vesicles by spontaneous recycling or by stimulated exo/endocytosis, and comparing rates of destaining by spontaneous recycling or stimulation. The surprising result is that vesicles are destained more rapidly by the same procedure used to load them: vesicles loaded by spontaneous recycling are more rapidly destained by spontaneous release than by activity, and vesicles loaded by activity (action potentials or potassium-induced depolarization) are more rapidly destained by activity than by spontaneous release. An extensive series of controls eliminated confounding variables and interpretations. When refilling spontaneously released and recovered vesicles was blocked by inhibition of vacuolar ATPase with folimycin, minis almost vanished, while evoked release was hardly affected except by repetitive stimulation, which then rapidly depleted the readily releasable pool (RRP). In contrast, selective reduction of evoked release in *synaptobrevin* mutants eliminated the fast destaining of vesicles loaded and released by stimulation.

These results reveal two pools of vesicles that intermix relatively slowly. Minis are released from a pool that is preferentially refilled by recovered mini vesicles, and quanta that are released by activity (or hyperosmotic shock) are preferentially released from an RRP, which they reenter along with a reserve pool on endocytosis. Activity also eventually empties the pool loaded by minis, so the minis enter a reserve pool that is reluctantly released by stimulation. Activity fills both the RRP and a reserve pool, so that subsequent destaining by activity is biphasic, with fast unloading of the RRP followed by unloading of the reserve pool, as observed previously (Klingauf et al., 1998). Minis seem unable to deplete this pool. The pool loaded by minis does not appear to be identical to the reserve pool loaded by activity, because it is smaller and does not intermix with the RRP. Thus, there is no fast phase of destaining by activity after dye loading by spontaneous activity. Kinetic modeling shows that vesicles reentering the mini pool mix with it randomly, so that its destaining rate is slower than that of the mini frequency, which includes recovered unstained vesicles.

Ultrastructural labeling of spontaneously recycling and stimulation-dependent recycling of vesicles using horseradish peroxidase showed that both pools of vesicles are distributed randomly throughout the active zone. Thus, the two pools of vesicles are not physically segregated, unlike the reserve and RRP at some neuromuscular junctions (Kuromi and Kidokoro, 1998; Rizzoli and Betz, 2004). However, in *synaptobrevin* mutants, spontaneous destaining was similar after either spontaneous or activity-driven loading; and depolarization-

induced destaining was similar whether the mini pool or the activity-dependent pool was loaded. Thus, synaptobrevin appears to be necessary to prevent mixing of the two pools.

One might imagine that the pool of recycling minis is not responsive to elevations in $[Ca^{2+}]_i$, being perhaps a Ca^{2+}-independent form of secretion. This appears not to be the case, however, since modest elevation of external $[Ca^{2+}]$ speeds both the loading of the mini pool and its destaining, while destaining by activity still reveals no fast component of release from the RRP. Thus, Ca^{2+} increases recycling of minis within its own special pool.

Where does this leave us in understanding the origin of minis? The present results mesh nicely with genetic studies showing that minis are selectively spared by knockout of *synaptotagmin I*, *complexins*, *rab-3-interacting molecule 1*α, and *synaptobrevin* and have different temperature and ionic sensitivities. It now appears that minis are released from a molecularly distinct pool of vesicles whose mixing with the RRP is prevented by synaptobrevin. Is synaptobrevin absent from or altered in spontaneously released vesicles? Are there other detectable molecular differences? Are mini vesicles recovered by a clathrin/dynein-dependent process? Is it the same as the slow component of endocytosis of activity-dependent vesicle release (Klingauf et al., 1998)? Combination of GFP-tagged proteins or antibodies with labeling of spontaneously recycling vesicles with horseradish peroxidase or photoconverted styryl dyes should soon provide answers to such questions.

Finally, the present results question a basic tenet of the quantal hypothesis of synaptic transmission—that the unitary quantal event that is the building block of evoked release is identical to the spontaneously released quantum. This assertion must now be tested as a part of any quantal analysis, rather than simply assumed. Given the emerging roles of minis in synaptic signaling and development, molecular mechanisms regulating their release and recycling are likely to attract more attention for some time.

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Selected Reading

Angleson, J.K., and Betz, W.J. (2001). J. Neurophysiol. *85*, 287–294.

Carter, A.G., and Regehr, W.G. (2002). Nat. Neurosci. *5*, 1309–1318.

Delaney, K.R., and Tank, D.W. (1994). J. Neurosci. *14*, 5885–5902.

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., and Sudhof, T.C. (1994). Cell *79*, 717–727.

Katz, B. (1969). The Release of Neural Transmitter Substances (Springfield, IL: CC Thomas).

Klingauf, J., Kavalali, E.T., and Tsien, R.W. (1998). Nature *394*, 581–585.

Kuromi, H., and Kidokoro, Y. (1998). Neuron *20*, 917–925.

Lu, T., and Trussell, L.O. (2000). Neuron *26*, 683–694.

McKinney, R.A., Capogna, M., Durr, R., Gahwiler, B.H., and Thompson, S.M. (1999). Nat. Neurosci. *2*, 44–49.

Murphy, T.H., Blatter, L.A., Bhat, R.V., Fiore, R.S., Wier, W.G., and Baraban, J.M. (1994). J. Neurosci. *14*, 1320–1331.

Rizzoli, S.O., and Betz, W.J. (2004). Science *303*, 2037–2039.

Saitoe, M., Schwarz, T.L., Umbach, J.A., Gundersen, C.B., and Kidokoro, Y. (2001). Science *293*, 514–517.

Sara, Y., Virmani, T., Deák, F., Liu, X., and Kavalali, E.T. (2005). Neuron *45*, this issue, 563–573.

Sharma, G., and Vijayaraghavan, S. (2003). Neuron *38*, 929–939.

Stevens, C.F., and Wang, Y. (1995). Neuron *14*, 795–802.

Sutton, M.A., Wall, N.R., Aakalu, G.N., and Schuman, E.M. (2004). Science *304*, 1979–1983.

Xu-Friedman, M.A., and Regehr, W.G. (2000). J. Neurosci. *20*, 4414–4422.

DOI 10.1016/j.neuron.2005.02.003