Synaptic Transmission at Single Boutons in Sympathetic Ganglia

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Synaptic transmission has traditionally been studied at the level of the entire nerve terminal rather than at one of its constituent boutons. Autonomic ganglia provide preparations for recording from individual boutons, as well as for determining the calcium transients necessary for transmitter release at these boutons. The results suggest a new paradigm for synaptic transmission.

The analysis of synaptic transmission at the level of the single release site or active zone in a whole preparation has been a goal of synaptic physiologists for some time. There are a number of questions that can only be answered directly at this spatial level of resolution. For example, does transmission occur in units determined by the relative size of a quantum of transmitter released or by the size of the postsynaptic receptor patch that is saturated by the release of the packet of transmitter? Other problems can also be met, such as whether all active zones undergo a transient increase in calcium concentration on arrival of the nerve impulse and whether this calcium then recovers to normal levels at the same rate independent of which active zone is being considered. Until recently, there have been two main approaches to the technical problem of recording from single boutons. One involves the use of preparations that possess relatively large boutons, such as the crayfish neuromuscular junction, although here it seems likely that each of these boutons has more than a single active zone (8). The other approach is to use cultured preparations, often of hippocampal neurons, in which typically small boutons occur (10). However, there is uncertainty as to whether the distribution of receptors at these boutons and indeed the functioning of the active zones in the boutons is normal compared with that in a whole preparation. The introduction of new techniques as applied to autonomic ganglia has now allowed the analysis of synaptic transmission at single boutons, which possess single active zones, to be carried out in whole preparations. This review describes the approach and some of the results obtained.

Transmitter release at single boutons with single active zones

The amplitude-frequency histogram of spontaneous synaptic potentials at the amphibian neuromuscular junction is described by a Gaussian distribution that has been used to define a unit or quantum of acetylcholine (ACh) release (9). An alternative interpretation of the origins of this Gaussian distribution is that the size of the receptor patch at an active zone is such that it is saturated by the number of ACh molecules released in a packet, so that the size of the quantum is determined by the size of the receptor patch. However, a number of experiments now point to the likelihood that the original hypothesis is correct for the neuromuscular junction, namely that the quantum arises from the size of the unit of ACh release (11). The question may then be asked as to whether this is likely to be the case at the next most-frequently studied synapse, namely that between sympathetic preganglionic boutons and postganglionic neurons. We approached this problem by developing a technique for placing loose-patch electrodes over single boutons, previously visualized by loading with dextran-rhodamine, and recording the excitatory postsynaptic current (EPSC) due to transmission from a singlebouton in the size range of 1–2 μm (Fig. 1A). For this purpose, the preparation chosen was the rodent pelvic ganglion, which possesses monopolar neurons that receive in general just a single preganglionic nerve terminal consisting of 10–20 boutons, each with a single active zone (4). The EPSCs recorded from individual boutons were each preceded by the electrical signs of the nerve terminal action potential, with both the EPSC and the action potential remaining at an almost invariant amplitude from impulse to impulse (Fig. 1B). The amplitude-frequency histogram of the EPSCs for individual boutons was therefore well described by a single Gaussian distribution that possessed a variance similar to the electrical noise. Non-stationary fluctuation analysis of the EPSCs at a bouton indicated that ~120 ACh receptor channels were available beneath boutons for interaction with a packet of released ACh. Given that a synaptic vesicle contains ~10,000 ACh molecules, Monte Carlo simulation of the process of ACh release from a vesicle and its subsequent interaction with these receptors generated an EPSC with the same temporal characteristics as the observed EPSC and with the same stochastic fluctuations in amplitude. Clearly, in this case, the average amplitude of the EPSC does not give a measure of the size of the unit of transmitter release but is rather determined by the relatively small number of ACh receptors beneath a bouton. The concept of a quantum in this case is that of a unit of action rather than one of transmitter release.

Multiquantal spontaneous release of transmitter

Many synapses in the peripheral and central nervous system possess amplitude-frequency histograms of spontaneous synaptic potentials, when recorded intracellularly, that are...
generally not described by a Gaussian distribution but rather by multimodal distributions (1, 14). In the case of autonomic ganglia, these multimodal histograms of spontaneous EPSPs have been described as a mixture of Gaussian distributions in which the mean of the successive Gaussians are integer multiples of the mean of the first Gaussian in the mixture; such multimodal distributions are a Poisson mixture of Gaussians (5). Some of the neurons on which these multimodal distributions have been observed are monopolar, so that the complexity of their histograms of spontaneous EPSCs cannot arise as a consequence of spatial attenuation of the spontaneous EPSCs along dendrites. At such synapses, the concept of a quantum of transmission, whether arising as a consequence of a unit of transmitter release or from a transmitter-saturated receptor patch of relatively constant size, might not be appropriate. If evoked release uses as a unit of transmission the potential fluctuations given by the spontaneous release, then these are distributed according to a Poisson distribution rather than a Gaussian, indicating a multiquantal unit of transmission. It is clearly important to determine the origins of such a multiquantal unit for transmitter release.

**Spontaneous calcium transients in adjacent boutons**

One possibility, originally described in rodent pelvic ganglia (5), is that multimodal histograms of spontaneous EPSPs arise as a consequence of spontaneous multiquantal transmitter release. However, this does not now seem possible, because the unit of transmitter release saturates the receptor patch beneath a single bouton to within stochastic limits set by the interaction between 10,000 released ACh molecules and ~120 ACh receptors. The only possible way in which multimodal histograms of spontaneous EPSPs can then arise involves the near-simultaneous release of a unit of transmitter from each of two or more active zones, each with its own receptor patches (3). How could this arise? The most likely possibility is provided by recent observations on spontaneous calcium transients in adjacent boutons (13). Calcium indicators can be introduced into preganglionic boutons using a technique in which the indicator is conjugated with dextran, allowing it to be carried by orthograde transport from the cut preganglionic nerves into their terminals. With this method, spontaneous fluctuations in the calcium concentration can be observed in boutons, even in the presence of tetrodotoxin to block any possibility of action potentials (Fig. 2A). These changes in calcium concentration are frequently observed in adjacent boutons and are sometimes of long duration (Fig. 2B). If these transients reflect changes in calcium concentration at active zones that are high enough to trigger vesicle exocytosis, then near-simultaneous exocytosis from the zones similar to that which is proposed for the active zones of adjacent small boutons. This would then give rise to multimodal amplitude-frequency histograms of spontaneous EPSPs. Larger sympathetic boutons, several micrometers in extent, frequently possess more than one active zone, and each of these zones is associated with its own receptor patch beneath the bouton (11). Spontaneous changes in calcium have also been observed in these larger boutons that could trigger synchronous exocytosis from the zones similar to that which is proposed for the active zones of adjacent small boutons. This would then offer an alternative source of spontaneous synaptic potentials that contribute to the multimodality of amplitude-frequency histograms (7). It is interesting in this regard that postganglionic sympathetic nerve terminal varicosities also show spontaneous calcium transients that often occur synchronized in adjacent varicosities (6). These could also contribute to the multimodality of the amplitude-frequency histograms of spontaneous potentials often recorded at these varicosities.

**Autoreceptors on single boutons**

The spatial resolution of the optical systems available at present do not allow determination of the calcium transient...
that occurs at the active zone due to a nerve impulse; therefore, it is not possible to relate the probability of exocytosis of a vesicle with that of the calcium influx. However, it may be noted that all boutons of about the same size in both the paravertebral and prevertebral sympathetic ganglia respond with a calcium transient of about the same amplitude when measured ~5 ms after the arrival of an impulse (13). If the size of this transient is proportional to the calcium influx at the active zone, then there does not seem to be any basis for assuming that there will be differences in the probability of secretion at different boutons related to differences in calcium influx. However, autoreceptors on these boutons probably play a major role in determining the probability of secretion by an impulse, particularly those for purines and endorphins (2). The question arises as to how endogenous cotransmitters such as ATP and enkephalin exert their effects on autoreceptors of sin-

FIGURE 2. Spontaneous calcium transients in single boutons. A: image of oregon-1,2-bis(2-aminophenoxy)ethane-N,N',N'-tetraacetic acid (BAPTA) intensity in 3 boutons of a single preganglionic nerve terminal in a rat superior cervical ganglion 110 ms after beginning of a calcium transient. B: simultaneous, spontaneous changes in calcium within 3 adjacent boutons in rat superior cervical ganglion in a, b, and c. Amplitude of spontaneous events (within 0–100 s) is largest in bouton shown in b. For comparison, calcium transient following an action potential is shown at 112 s into recording. Note that amplitude of response to an action potential is similar in all 3 boutons, whereas that due to spontaneous events is significantly different [from Lin et al. (13)].

FIGURE 3. Evoked calcium transients in single boutons and their modulation by autoreceptors. A: line scan through a single bouton in response to a single action potential measured with a line scan confocal recording through center of bouton. Action potential arrived at bouton at \( t = 0 \). With a temporal resolution of 5 ms, fluorescence intensity from oregon-BAPTA attains a peak within <10 ms. Subsequent decline in calcium concentration is best described by a double exponential, with rate constants of \(-2\ s^{-1}\) and \(0.25\ s^{-1}\). B: effect of adenosine (100 \( \mu M\); open squares) on amplitude of calcium transient and calcium recovery in a bouton after an action potential compared with a transient in same bouton in absence of adenosine (closed squares). Curves show double exponential curve fits to data. In presence of adenosine, amplitude of calcium transient to a single action potential falls, with no significant change in rate of recovery (rate constants of \(-2\ s^{-1}\) and \(0.23\ s^{-1}\) ) [from Lin et al. (13)].
ingle boutons. Calcium transients in a sympathetic bouton that are evoked by a single impulse decline with two different rate constants, reflecting rates of calcium recovery to baseline conditions, one at ~2 s⁻¹ and the other at ~0.25 s⁻¹. Even if the confocal microscope is used in line scan mode, with a temporal resolution of a few milliseconds, no other rates are detected after the first 5 ms after arrival of the nerve impulse (Fig. 3A). Exogenous adenosine, the metabolic breakdown product of ATP, and enkephalin both exert a profound depression on the amplitude of the calcium transient in single boutons without affecting the rates of recovery of calcium (Fig. 3B). It seems then that the autoreceptors for at least these cotransmitters are present on each bouton and that their principal mode of controlling the probability of secretion is through regulation of the influx of calcium ions.

Conclusion
This short review has indicated the extent to which methods for recording the electrical signs of transmission at single sympathetic boutons, as well as the calcium transients in these boutons, has illuminated our understanding of synaptic transmission. The observations arising from these techniques indicate a new paradigm for transmission. This is one in which the amplitude of each quantum is determined by the size of the receptor patch beneath a bouton that is saturated by the released transmitter. Calcium transients in the boutons that arise either spontaneously or in response to a nerve impulse can couple release from adjacent active zones, so that the unit of transmission must be regarded as composed of multiquanta. Cotransmitters, acting on autoreceptors, control the extent of the calcium transients in each of the boutons and hence the probability of secretion from the boutons.

References