New and Notable

PresyNaptic Calcium Channels: Why Is P Selected before N?

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Genetic and evolutionary analysis formalizes the existence of three families of voltage-gated calcium channels CaV1, CaV2, and CaV3, with members that correspond well to previous classifications of L-type (CaV1), P-type (CaV2.1), N-type (CaV2.2), R-type (CaV2.3), and T-type (CaV3) made on the basis of biophysical and pharmacological properties. Members of all three families can gate small synaptic-vesicle (SV) fusion at transmitter release sites; however, at fast-transmitting synapses between axon terminals and their target cells, this function is performed almost exclusively by members of the CaV2 family. Interestingly, while some synapses rely virtually exclusively on CaV2.1 or CaV2.2, others use a combination that can also include CaV2.3.

Because transmitter release at frog neuromuscular junctions and chick autonomic synapses is gated by CaV2.2 channels while mammalian synapses rely predominantly on CaV2.1 channels, it was tacitly assumed that this shift reflected an evolutionary progression. However, other studies have suggested that the type of channel selected might reflect the need for channel-specific functional traits. This question was recently explored directly in a presynaptic terminal at the hippocampal mossy fiber synapse by comparing the biophysical properties of the three CaV2.2 channel types. Although CaV2.1 and 2.2 were quite similar, it was predicted by mathematical modeling that the former exhibits a higher opening efficiency with the brief action potential (AP) waveforms characteristic of this synapse (1). This study was something of a tour-de-force but was complicated by the challenge of reliably distinguishing inward calcium current through each of the three CaV2 channel types.

Wen et al. (2) were drawn to this question by the discovery that at the zebrafish (but not the frog (3)) neuromuscular junction, CaV2.1 (or CaV2.1-like) channels play a key role in neurotransmission. This observation contradicted the idea of an evolutionary CaV2.2-to-CaV2.1 switch, and prompted them to test whether differences in kinetic behavior might favor CaV2.1 channels. To avoid the complications of dissecting a mixed-channel calcium current and the extra challenges of recording from presynaptic nerve terminals, they took a reductionist approach and expressed the channels heterologously in a cell line. The obvious limitations of this approach (notably the need to guess at accessory channel subunit types) were mitigated by the advantage of near-ideal patch-clamp recording conditions and expression of one channel type at a time.

The main findings of the study were that, while many parameters of the CaV2.1 and CaV2.2 channels were rather similar, significant differences in channel kinetics were exposed when these were explored across a broad voltage range. Two key findings were that at depolarized potentials, CaV2.1 exhibited a faster opening rate than CaV2.2 while at hyperpolarized potentials, the two channel types closed at similar rates. Inward currents carried by the two channel types during a fast AP were then simulated using these kinetic parameters and mathematical modeling.

This analysis revealed two important findings. First, the duration of the inward currents triggered by the two channel types was very similar—thus, both would supply a short pulse of Ca^{2+} influx at the critical repolarization phase of the AP (4). Second, the study also predicted that during an AP, CaV2.1 channels should exhibit a significantly higher open probability (P_O) than CaV2.2 channels.

Two questions arise from this work. Do native presynaptic CaV2.1 and 2.2, with the naturally occurring accessory proteins and any other modifying associations, exhibit the same P_O difference?If so, is this difference the primary reason for the predominance of CaV2.1 at most neuromuscular junctions or at many synapses in the central nervous system?

The first question can only be further explored at the few presynaptic terminals that express both channels and are accessible for direct voltage-clamp analysis. If P_O is indeed the biophysical trait that has led to the favoring of CaV2.1 as the fast-transmitting, presynaptic calcium channel, then the next question is: what particular advantage does this have for transmission? In their study, Naranjo et al. (5) concluded, in line with the earlier mossy fiber synapse study (1), that the larger influx permits more efficient transmitter release with typical short-duration APs.

It is interesting to speculate on how the difference in CaV2.1 and CaV2.2 kinetics might affect transmitter release gating. There is a growing consensus (6–10) that, at fast transmitting synapses, SV discharge can be triggered by one or a very few local calcium channels (11). Virtually instantaneously after the calcium channel opens, a high concentration domain of Ca^{2+} forms, which is centered on the pore. According to the single-domain hypothesis, the SV is located sufficiently close to a single channel that its Ca^{2+} domain can saturate the SV calcium-sensor binding sites (≥ 4) and trigger fusion. Although considerable attention has been paid to the relationship between the single-calcium-channel current amplitude and

Submitted October 28, 2014, and accepted for publication December 8, 2014.

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Editor: Chris Lingle.

0006-3495/15/02/0451/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.12.021

gating of SV fusion (12), very little is known about the relationship between the duration of calcium channel opening and single domain secretion. The kinetics of ion binding to the calcium sensor, the sensor on-rate, can reasonably be presumed to be the key limiting factor. Models of release gating posit a sensor on-rate in the 10–200 μs range (13,14), values that are supported by direct recording of single domain-based secretion (11). However, calcium channel kinetics, as evaluated by single channel recording, are highly complex at these short time intervals. Two properties are of particular significance with respect to single domain secretion gating: reluctant openings, which are self-explanatory; and current flickers, which are very rapid, voltage-insensitive, open/closed cycles (15).

Reluctant openings have been reported to be more evident in CaV2.2 than CaV2.1 channels (16) and could provide the basis for the difference in whole-cell current activation rates observed by Naranjo et al. (5). Because reluctant openings make the CaV2.2 channel less reliable as a trigger for single domain-based secretion, this factor alone could favor the selection of CaV2.1 for AP-gated secretion. It is, however, far more difficult to predict which of the two channels types might be more effective with respect to single-channel open duration. Channel closing rates can be measured from the tail current decay at different voltages, and are similar for CaV2.1 and CaV2.2 (5), in particular across the hyperpolarized membrane voltage range at which transmitter release is gated after an AP (see above). This suggests that, at least for voltage-dependent kinetics, open durations do not favor one channel over another. However, single domain activation of SV fusion has been reported to require influx of up to 180 Ca^{2+} ions, a quantity that can be predicted to require a submillisecond single channel open duration (11)—in the realm of channel flickers. Kinetics of open channel flicker have not been compared in detail for single CaV2.1 and CaV2.2 channels. It is, however, reasonable to predict that the channel with the shorter closed-flickers should exhibit a higher apparent P_O—as observed for CaV2.1 (5)—and, hence, be more effective for the gating of secretion by single domains.

If CaV2.1 is selected as the presynaptic calcium channel due to its higher P_O, then it begs the question why other central or peripheral synapses favor CaV2.2 channels. The obvious explanation is that properties of these channels, other than P_O, are also important for the needs of specific synapses. Possibilities include channel type-specific scaffolding mechanisms, activation or inactivation during sustained changes in the resting membrane potential, or sensitivity to specific molecular modulation pathways.

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