Asynchronous Ca\(^{2+}\) current conducted by voltage-gated Ca\(^{2+}\) (Ca\(_{V}\))-2.1 and Ca\(_{V}\)2.2 channels and its implications for asynchronous neurotransmitter release

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AUTHOR SUMMARY

The transmission of information from one nerve cell (neuron) to another nerve cell takes place primarily through chemical neurotransmitters that are released into the narrow space between neurons at synapses. The arrival of an electrical signal, termed an action potential, at a nerve terminal activates voltage-sensitive calcium (Ca\(^{2+}\)) channels, through which Ca\(^{2+}\) ions enter the nerve terminal. This Ca\(^{2+}\) entry triggers both rapid and sustained release of neurotransmitters (Fig. P1). We explored the role of Ca\(^{2+}\) channels as a potential source of Ca\(^{2+}\) entry that activates the sustained release mechanism, and we found a form of Ca\(^{2+}\)-dependent regulation of presynaptic Ca\(^{2+}\) channels that may trigger asynchronous neurotransmitter release.

Voltage-gated ion channels open in response to changes in the electrical potential across the nerve cell membrane, allowing ions to enter the cell. The voltage-gated Ca\(^{2+}\)2 (Ca\(_{V}\)2) subfamily of voltage-gated Ca\(^{2+}\) channels conducts Ca\(^{2+}\) currents into presynaptic nerve terminals, and Ca\(^{2+}\) entry through these channels initiates neurotransmitter release at synapses (1). This neurotransmitter release occurs in two phases: a fast, synchronous (phasic) component (Fig. P1A) and a slow, asynchronous (tonic) component (Fig. P1B) (2). It has been proposed that the slower, asynchronous component results from residual Ca\(^{2+}\) remaining after an action potential, which acts on a different Ca\(^{2+}\) sensor than the one that triggers synchronous release (3). Remarkably, when synchronous release is blocked, the asynchronous release process can release the entire readily releasable pool of neurotransmitter in synaptic vesicles, which are the organelles in which neurotransmitters are stored (3). This process suggests a functional competition between the synchronous and asynchronous release processes for the same pool of synaptic vesicles. In contrast to synchronous release, the source of Ca\(^{2+}\) entry for asynchronous neurotransmitter release remains unidentified.

Presynaptic Ca\(_{V}\)2.1 channels are regulated by Ca\(^{2+}\) sensor proteins, leading to short-term facilitation and depression of neurotransmitter release (4). Therefore, we searched for an additional mechanism of regulation of presynaptic Ca\(^{2+}\) channels that might contribute to asynchronous neurotransmitter release. We first studied Ca\(_{V}\)2.1 or Ca\(_{V}\)2.2 channels that were synthesized in the nonneuronal human embryonic kidney cell line tsA-201 after introduction of DNA encoding these Ca\(^{2+}\) channels. We detected small Ca\(^{2+}\) currents that remained active after repolarization and were slowly and progressively activated by prolonged Ca\(^{2+}\) entry during long single depolarizations (Fig. P1A) or trains of depolarizations (Fig. P1B). We have termed this Ca\(^{2+}\) current I\(_{\text{Async}}\), because it is activated asynchronously with respect to depolarization of the cell membrane. L-type Ca\(^{2+}\) currents of similar size through Ca\(_{V}\)1.2 channels do not activate I\(_{\text{Async}}\). This I\(_{\text{Async}}\) current is both Ca\(^{2+}\)-selective (i.e., it only conducts Ca\(^{2+}\)) and Ca\(^{2+}\)-activated (i.e., it is increased by Ca\(^{2+}\)). It is not permeable to other ions (including Na\(^{+}\), K\(^{+}\), H\(^{+}\), or Cl\(^{-}\)), and it is not affected by inhibitors or activators of other ion channels. However, application of Zn\(^{2+}\) ions blocks I\(_{\text{Async}}\) and the Ca\(_{V}\)2.1 channel (as measured by tail currents) with similar affinity. These results indicate that I\(_{\text{Async}}\) is conducted by Ca\(_{V}\)2.1 and Ca\(_{V}\)2.2 channels but not by other ion channels present in tsA-201 cells.

Global rises in the intracellular level of Ca\(^{2+}\), which spread beyond the vicinity of the Ca\(^{2+}\) channel molecule within the cell, are required to activate I\(_{\text{Async}}\) because it is blocked when extracellular Ca\(^{2+}\) is substituted with Ba\(^{2+}\) or the entering Ca\(^{2+}\) is bound by the slow intracellular Ca\(^{2+}\) binding molecule EGTA. The response to this rise in intracellular Ca\(^{2+}\) does not require the ubiquitous calcium sensor molecule calmodulin (CaM): mutations that prevent Ca\(^{2+}\) binding to CaM do not block I\(_{\text{Async}}\).

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See full research article on page E452 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1121103109.
and mutations in the CaM binding site on Cag2.1 channels also fail to block I\textsubscript{Async}. Therefore, although I\textsubscript{Async} is a Ca\textsuperscript{2+}-dependent and Ca\textsuperscript{2+}-selective current, it is not regulated by Ca\textsuperscript{2+} sensor proteins bound to the channel. Trains of repetitive depolarizations of tsA-201 cells increase I\textsubscript{Async} in a pulse-wise manner. The rise of I\textsubscript{Async} during trains of impulses and the kinetics of decay of I\textsubscript{Async} after repolarization resemble the rise and decay of asynchronous neurotransmitter release at conventional fast synapses, suggesting that I\textsubscript{Async} is a potential Ca\textsuperscript{2+} source for asynchronous neurotransmitter release.

To directly examine the possibility that I\textsubscript{Async} can contribute to asynchronous release of neurotransmitters, we measured I\textsubscript{Async} in microcultures of single neurons that form synapses on themselves (autapses), at which transmission is initiated by Cag2.1 and Cag2.2 channels that are naturally present within the nerve terminals. As in the tsA-201 cells that we constructed to express these channels artificially, prolonged Ca\textsuperscript{2+} entry through Cag2.1 and Cag2.2 channels in nerve cells induced I\textsubscript{Async} (Fig. P1A) that was blocked by Ba\textsuperscript{2+} substitution. In addition, trains of depolarizations increased both I\textsubscript{Async} and asynchronous neurotransmitter release in a pulse-wise manner (Fig. P1B). Thus, I\textsubscript{Async} conducted by Cag2.1 and Cag2.2 channels in nerve cells in microcultures closely resembles asynchronous transmitter release at the synapses formed by these nerve cells. Thus, this previously unrecognized Ca\textsuperscript{2+} signal may contribute to the residual intracellular Ca\textsuperscript{2+} that triggers asynchronous release of neurotransmitters at synapses.

Asynchronous neurotransmitter release is thought to play an important role in processing and transmission of information at synapses by converting the information contained in the frequency of a series of action potentials into a change in the extent of release of neurotransmitters at synapses. I\textsubscript{Async} may trigger this information-encoding and -transmitting process by providing a source of asynchronous Ca\textsuperscript{2+} entry; this source integrates the frequency of action potentials and initiates asynchronous neurotransmitter release that increases progressively with the frequency and duration of action potential generation.