Relationship between Membrane Depolarization and Intracellular Free Calcium in Individual Nerve Terminals from the Neurohypophysis

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The release of transmitter/hormone from nerve terminals is dependent on depolarization-induced changes in intraterminal free $[Ca^{2+}]$, yet anatomical limitations of vertebrate nerve endings have largely precluded direct monitoring of such changes at single axon endings. As a result the quantitative relationship of the change in $[Ca^{2+}]_i$ to exocytosis in vertebrate endings remains virtually uncharacterized. Using axon terminals isolated from the neurohypophysis of the rat, changes in $[Ca^{2+}]_i$ in individual endings in response to depolarizing stimuli were directly quantitated by dual wavelength microspectrofluorometry of cytoplasmic fura-2. The mean basal $[Ca^{2+}]_i$ was 66 ± 4 nM (n = 212, ±SEM), a value similar to most excitable and nonexcitable cells. Membrane depolarization evoked by elevation of extracellular $[K^+]$ resulted in a rapid, dose-dependent increase in $[Ca^{2+}]_i$ that was not present in medium lacking extracellular Ca^{2+} (no added Ca^{2+} plus 1 mM EGTA) and was greatly reduced by the inorganic Ca^{2+} channel blockers Cd^{2+} and La^{3+}. Application of the dihydropyridine nicardipine dose dependently reduced the rise in $[Ca^{2+}]_i$ evoked by 50 mM K^+ (93% block at 10 μM) as did desmethoxyverapamil (D888). These results suggest that the evoked rise in $[Ca^{2+}]$ is mediated by an L-type Ca^{2+} channel under these depolarizing conditions. Recovery of basal $[Ca^{2+}]_i$ on removal of elevated K^+ showed an initial rapid decline followed by a slower phase.

Initial studies have found the change in $[Ca^{2+}]_i$ in single nerve endings, in response to given K^+-induced depolarizations, to closely correlate to vasopressin release monitored from populations of isolated endings.[1] The close correlation suggests that Ca^{2+} influx by way of the L-type Ca^{2+} channel is associated with release under these depolarizing conditions. A strengthening of this conclusion was provided by the close relationship between dihydropyridine block of secretion (IC_{50} = 4 μM)[1] with the observed block of Ca^{2+} influx and rise in $[Ca^{2+}]_i$ (approx. IC_{50} = 2 μM). Furthermore, there is a close relationship between the kinetics of a change in $[Ca^{2+}]_i$ and that of vasopressin release in the present study when challenged with a 30-s pulse of 50 mM $^+$ [arginine vasopressin release graciously performed by Dr. J. J. Nordmann]. For depolarizations longer than 30 sec, however, $[Ca^{2+}]_i$ remained elevated, although release rapidly declined. Thus, the phasic nature of the vasopressin secretory response is not limited by inactivation of Ca^{2+} entry.

Vasopressin release from the neurohypophysis has been reported to be directly influenced at the level of the nerve endings by a variety of bioactive peptides. These include dynorphin[2] and cholecystokinin,[3] which are believed to be autoregulatory, and by the peptide hormone relaxin.[4] None of these peptides were found to affect either the basal $[Ca^{2+}]_i$ value or to alter the change in $[Ca^{2+}]_i$ evoked by either 25 mM

or 50 mM extracellular K^+. By contrast, the opioid receptor agonist, U50488 (a selective kappa agonist), did significantly reduce the evoked rise in $[Ca^{2+}]_i$ to K^+ depolarization without affecting the basal value.

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