SPATIOTEMPORAL INHOMOGENEITY OF [Ca]_i IN NEURONS

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INTRODUCTION

The concept that Ca might serve as an intracellular messenger to couple stimulus to response in excitable tissues is well established. An understanding of the precise mechanisms by which second messengers act, requires information on the temporal nature of changes in second messenger concentration and their spatial distribution within the cell. By optical imaging with fluorescent Ca-indicators, it was found that the change in intracellular free Ca-concentration is often transient and spatially inhomogeneous (for a recent review see Tsien and Tsien, 1990). Appreciation of this particular paradox leads to the recognition that Ca-cycling in the target cell has an important, spatially and temporally distinct messenger function. The purpose of this communication is to consider the intracellular messenger role of Ca in generation of specific transients and gradients in the cytoplasm of nerve cells.

Ca-RECOVERY AND Ca-RELEASE MEDIATED BY INTERNAL Ca-STORES

The mechanisms of Ca-regulation have been studied using snail neurons and rat sensory neurons. The results obtained were qualitatively similar. Intracellular Ca was calculated from fura-2 signal measured with a photomultiplier in epi-fluorescence mode, giving either the average, \(<Ca>_i\), or local, \([Ca]_p\), concentration of free Ca. Fig. 1A shows that pulse trains of various frequencies established a new steady level of \(<Ca>_i\), and after the end of stimulation it recovered with a time course, corresponding to a single exponent. The Ca-recovery is mainly determined by Ca-pump (CaP) of internal stores (CaS).

This can be argued (Kostyuk et al. 1990; Miller 1991) first by excluding all other mechanisms known from biochemical studies, namely mitochondria, Ca-pump or Na/Ca-exchange in the surface membrane. Second, the temperature coefficient was close to that of CaP and injection of vanadate, an inhibitor of ATPases, counteracted with Ca-recovery (Fig. 1).
Ca pumped into CaS could be subsequently released via a pathway, corresponding to the Ca-induced Ca-release. When cell stimulation raised $<\text{Ca}_i>$, to a certain threshold, which in neurons is low enough only in the presence of caffeine, it triggered a further spontaneous $<\text{Ca}_i>$ increase (Mironov and Usachev, 1990). Then $<\text{Ca}_i>$ recovered with the time course, similar to that observed after Ca-load by electric stimulation. Periodic Ca-transients were also observed. They occurred either spontaneously or could be more consistently triggered by Ca-influx as discussed below.

**Ca-INDUCED Ca-RELEASE: MECHANISM, MODEL AND IMPLICATIONS**

The model of Ca-release (CaR, Mironov and Tupikin, 1988) was based on the concept proposed earlier by Fabiato (1983) which is shown schematically in Fig. 2A. The functioning of Ca-dependent activation and inactivation gates of CaR were represented by variables $m$ and $h$, respectively, in line with the model of nerve excitability (Hodgkin and Huxley, 1952). The basic model consisted of two differential equations

\[
d\text{C}/dT = P \, m^2(1 - h)^2 - g\text{C}/(K' + \text{C}) + j \tag{1a}
\]

\[
dh/dT = k_+ \text{C}(1 - h) - k_- h \tag{1b}
\]

For CaP the Michaelis-Menten equation was used with the maximal rate $g = 10 \, \mu\text{M}/\text{s}$ and the dissociation constant $K' = 0.5 \, \mu\text{M}$. CaR activation was assumed to proceed instantaneously with the maximal rate $P = 50 \, \mu\text{M}/\text{s}$ and it was described by a steady variable $m_{\infty} = C/(K + C)$ with $K = 0.2 \, \mu\text{M}$. On- and off-rate constants $k_+ = 4 \, \mu\text{M}^{-1}\text{s}^{-1}$ and $k_- = 1 \, \text{s}^{-1}$ were estimated from the experimental data (Fabiato 1983; Bezprozvanny et al.; Finch et al. 1991).

When Ca-influx $j$ into the cytoplasm was large enough, the model started to generate periodic Ca-transients (Fig. 2B). Further increase in influx decreased the peak $[\text{Ca}]$ and the period of oscillations due to accumulating inactivation after each spike, until only damped oscillations could be observed. The frequency of spikes was dependent on different model parameters, but most dramatically it can be changed, varying the rate constants for inactivation (Fig. 2C). It produced a 100-fold change in