Analysis of Gated Flux from or into Sealed Membrane Fragments

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Electrical excitability of biological cells involves the flow of ions through transmembrane channels. There is good evidence that the time course of electrical excitation events is controlled by specialized gating molecules. Investigation of the flux of ions into or from sealed membrane fragments (microsacs), cells, or vesicles, is a generally applicable method for studying the function of gating molecules. The overall flux signal is explicitly dependent on a number of physico-chemically well-defined variables. Flux amplitudes are dependent on the vesicle number and on the average volume of a vesicle, while flux rates depend on the average number of transmembrane channels per vesicle. Gating processes leading to channel opening and/or closing affect both amplitudes and rates. Averaging over inhomogeneities in vesicle size and channel density leads to an explicit expression for the time (t) dependent content of tracer ions X(t) in the vesicles. For efflux measurements, $X(t)=DVC_o \cdot \bar{v} \cdot \exp\{-\bar{n}\kappa(t)\left[1-\frac{\sigma^2}{2\bar{n}}\kappa(t)\right]\}$ where D=density of the suspension (microsacs/unit volume), V=volume of suspension, C= initial concentration of tracer ions, $\bar{V}$= average volume of a microsac, $\bar{n}$= mean total number of activatable channels per microsac, σ^2=variance in number of activatable channels per microsac and $\kappa(t)$= amplitude factor. Aside from $\kappa(t)$, all variables in Eq.(1) are functions of the method of preparation and of the materials used; as such they can be determined prior to the flux experiment - thus correcting for all sources of variability between preparations. All information about the gating process is contained in $\kappa(t)$, given by $\kappa(t)=k\cdot\int_0^t \alpha(\tau)d\tau$, where k is the rate constant for ion transport through a single channel, and $\alpha(t)$ is the time dependent fraction of open channels.

For the gating process regulated by acetylcholine receptors in sealed membrane fragments of T. marmorata electric organs it has been shown that ^{22}Na efflux can be correctly described if a cyclic reaction scheme is assumed for receptor inactivation. Furthermore, the activated-permeable conformation of the receptor is intrinsically metastable and short-lived. This parallelism to electro-physiological data demonstrates that flux measurements constitute a valuable method to study receptor-mediated membrane transport. (Natl. Acad. Si. USA 75, 3756-60, 1978; J. Theoret. Biol. 84, (1980), in press.