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A bioenergetic approach to the nerve terminal

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Synaptosomes are isolated nerve terminals prepared by controlled homogenization of defined brain areas [1–3]. The axon is torn off the terminal, which reseals and remains bioenergetically competent for several hours, showing high respiratory control [4], maintaining a plasma membrane potential of −60 to −80 mV in low K^+ medium [5,6] and a cytoplasmic free Ca^{2+} concentration of 0.1–0.2 μM [7,8] and releasing neurotransmitter by mechanisms which respond to the same inhibitors and modulators as more complex preparations. The synaptosome can, thus, be used to study the integration of metabolism, mitochondrial and plasma membrane ion circuits and the machinery for the uptake, storage and exocytosis of neurotransmitters. This paper will concentrate on this final aspect.

The amino-acid transmitters glutamate, γ-aminobutyrate (GABA) and glycine are released at approx. 90% of the synapses in the mammalian brain. Glutamate is the dominant excitatory neurotransmitter and is of particular current interest, since its post-synaptic receptors are strongly implicated in pathways of memory and learning and mechanisms of ischaemic brain damage [9]. Glutamate is accumulated into synaptic vesicles within the terminals as an anionic species driven by the membrane potential generated by an inwardly-directed H^+-translocating ATPase [10].

In vivo transmitter exocytosis is initiated by the arrival of an action potential at the terminal, as a consequence of which presynaptic voltage-activated Ca^{2+} channels open, directing Ca^{2+} onto a so-far uncharacterized 'Ca^{2+}-trigger' which initiates, within a fraction of a millisecond, the exocytosis of the synaptic vesicle contents. Although the synaptosome preparation has lost its physiological means of stimulation, it is possible to depolarize the terminal either by elevated KCl, or more interestingly, by inhibiting a class of K^+-channels with 4-aminopyridine, causing the terminals to undergo spontaneous repetitive action potentials [8].

Using fluorescent techniques for measuring glutamate release, the average cytoplasmic free Ca^{2+} concentration, ($[Ca^{2+}]_c$), and membrane potential, exploiting specific presynaptic neurotoxins, and with a knowledge of the bioenergetic behaviour of the terminal, it is possible to perform a great variety of experiments to investigate the factors which regulate the release of the transmitter. Findings with this approach include:

(a) Characterization of a non-inactivating Ca^{2+} channel which appears to be closely coupled to glutamate release [11] and which can be inhibited by novel neurotoxins from the funnel web spider which are being characterized in our laboratory [12].

(b) Evidence that the Ca^{2+} entering through this channel triggers glutamate exocytosis by interacting with a low-affinity binding site before it has equilibrated with the bulk cytoplasmic $[Ca^{2+}]_c$, whereas neuropeptides from the same preparation are released by a low-affinity receptor which responds to bulk cytoplasmic $[Ca^{2+}]_c$ [13]. Thus, the two classes of transmitter are released by fundamentally different mechanisms.

(c) Evidence that the potent clostridial neurotoxins botulinum neurotoxin A and tetanus toxin inhibit transmitter release by acting at a probable cytoskeletal site common to both classes of neurotransmitter [14].

(d) A mechanism for the presynaptic activation of glutamate release by protein kinase C, by inhibition of K^+-channels regulating the duration of action potentials [15].

(e) A bioenergetic mechanism to account for the massive and extremely toxic release of glutamate from neurones which occurs in brain ischaemia, resulting from the collapse of the Na^+-electrochemical gradient across the plasma membrane, and the reversal of the Na^+-glutamate co-transporter [16].

(f) An analysis of the functional role of protein phosphorylation in the control of transmitter release.

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All of these investigations have employed techniques which represent a fusion between cellular biochemistry and classical mitochondrial bioenergetic approaches, illustrating the potential of the latter to answer questions far beyond the artificial boundaries of 'energy-conserving' membranes.

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