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Sequential Release of ATP and Acetylcholine from Cholinergic Synaptic Vesicles Modulated by $Ca^{2\oplus}$ and Synaptic Membranes in vitro

Cholinergic synaptic vesicles isolated from the electric organ of *Torpedo marmorata*[1] contain 0.2 molecules of ATP per molecule of acetylcholine (ACh)[2]. When the electric organ is stimulated in situ, both constituents are released concomitantly from the vesicles[3]. It is generally accepted that $Ca^{2\oplus}$ ions are the trigger for transmitter release. Therefore we investigated whether this metal cation can induce changes in vesicle composition during in vitro incubation, either on its own or on addition of isolated presynaptic membranes.

Synaptic vesicles were purified by a combination of several differential centrifugation steps and separation on a discontinuous isoosmolar sucrose/glycine gradient[4], then incubated in 0.8M glycine for 1 min at 4 °C. The intrinsic Ca concentration in the incubation medium is 50μM. The addition of further $Ca^{2\oplus}$ ions to the incubation medium induced a marked release and hydrolysis of ATP (– 50% at 700μM Ca), presumably by intrinsic ATP-ase activity of the vesicles[5] or of contaminating membranes.

When presynaptic membranes are isolated from a nerve terminal sac fraction (T-sacs[6]), and added to the incubation medium, the release of ATP is drastically enhanced. However, membranes are ineffective after boiling. The presence of high concentrations of metal ions is not essential for membrane induced release of vesicular ATP in vitro.

Under these conditions only slight changes in the ACh content of the vesicles are observed (– 10%).