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RAPID COMMUNICATION

Non-quantal Acetylcholine Release after Cholinesterase Inhibition in vivo

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Summary

After anticholinesterase treatment *in vivo*, depolarization of the postsynaptic muscle fibre membrane by about 4 mV develops due to non-quantally released acetylcholine from the motor nerve terminal. This conclusion was supported by experiments with the curarization of diaphragm slices from anticholinesterase treated mice during intracellular microelectrode recordings.

Key words

Acetylcholine release – Mouse diaphragm – Anticholinesterases

The mechanism of action of anti-cholinesterases (anti-ChEs) is still of general interest as these compounds are hazardous pesticides and potential chemical weapons. Anti-ChEs are also used for the treatment of myasthenia gravis, Alzheimer type dementias, in ophthalmology, surgery and other indications (see Aquilonius and Gillberg 1990).

With the help of anti-ChEs we described the electrophysiological symptom of non-quantal release (NQR) of acetylcholine (ACh) in rodents (Vyskočil and Illes 1977) as a depolarization of muscle fibres in the endplate zone after *in vitro* ChE inhibition (Vyskočil and Illes 1978). Subsequently, the question arose of whether signs of non-quantal release (NQR) also develop after systemic administration of anti-ChE, and further, to what extent this can account for the terminal failure of cholinergic transmission due to anti-ChE poisoning.

To check this, we injected i.p. LD50 (0.45 $mg.kg^{-1}$) armin (diethylparanitrophenylphosphate, USSR) or soman to white mice (both sexes, 20–25 g body mass). Diaphragms were then quickly dissected from nembutal-anaesthetized animals either sacrificed by cervical dislocation 15–20 min after the injection or from those that died under anaesthesia following the injection. The diaphragm slices were bathed in a standard oxygenated Krebs-Ringer solution at 20 °C. The NQR which causes depolarization of muscle fibres in the end-plate zone was statistically quantified by impaling, with glass microelectrodes (2.5 KCl, 10 MΩ), 20 or more fibres during a 5 min period before, and another 20 or more fibres during a 5 min period following the 5 min after the addition of 1×10^{-5} M d-tubocurarine (dTC) to the medium. The difference between the mean resting potentials (RP) under these two conditions is generally considered to be due to synaptic depolarization which reflects the NQR of ACh H-effect (Vyskočil and Illes 1977, 1978, Vyskočil *et al.* 1983, Zemková *et al.* 1987). The results with both anti-ChEs were very similar and therefore pooled.

In the diaphragms from sacrificed animals, the endplate resting potential (RP) was -76.2 ± 0.6 mV (mean ± S.E.M., n = 120 fibres). After dTC, the endplate zone became hyperpolarized to -80.4 ± 0.5 mV (n = 100). The synaptic depolarization was therefore equal to 4.2 ± 0.4 mV in anti-ChE pretreated mice. In those animals which had died spontaneously, a synaptic depolarization of 3.8 ± 0.2 mV developed. In both groups, the frequency of quantal miniature endplate potentials (MEPPs) was slightly increased to 3.7 ± 0.8 (n = 100) per second as compared with 2.6 ± 0.3 in control *in vitro* treated muscles. MEPPs of such frequency and with an exponential decay time of about 5 ms did not distort the RP estimation and apparently their contribution to the synaptic depolarization is very small.

When muscles from *in vivo* treated animals were further bathed in 10^{-5} M armin or soman for 15 min and then washed with a normal solution, the synaptic depolarization increased to 5.5 ± 0.3 mV indicating that *in vivo* treatment did not block the endplate ChE completely. Biochemical determinations (Ellman *et al.* 1961) revealed that only 53 ± 2 % of ChE activity was inhibited in diaphragm homogenates from *in vivo* treated mice (8 muscles).

Nevertheless, the present experiments demonstrated that the depolarization of postsynaptic muscle membrane by non-quantally released ACh does develop after anti-ChE injection *in vivo*. The physiological importance of this depolarization is evident in the light of earlier findings that NQR might desensitize the postsynaptic ACh receptors (Vyskočil *et al.* 1983) under certain circumstances and effectively arrest impulse transmission. Together with ACh release into the blood from other sources (viscera, blood vessels, etc., cf. Douglas and Paton 1954), the NQR from nerve endings might be one of the important factors determining synaptic failure and morphological changes on both pre- and postsynaptic parts of the endplate seen during anti-ChE poisoning.

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