ULTRASTRUCTURAL AND FUNCTIONAL CHANGES IN RAT BRAIN SYNAPTOSOMES DURING ELECTRICAL STIMULATION IN VITRO

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Electrical stimulation of a suspension of rat brain synaptosomes leads to significant Ca\textsuperscript{++}-dependent liberation of endogenous noradrenalin and to a Ca\textsuperscript{++}-dependent increase in its concentration in the synaptosomes themselves. Cyclic nucleotide phosphodiesterase activity is lowered significantly under these same conditions. No disturbance of synaptosomal ultrastructure is found during stimulation. An increase in the number of electron-dense synaptosomes is observed.

KEY WORDS: ultrastructure of synaptosomes; electrical stimulation; secretion of noradrenalin; phosphodiesterase.

Synaptosomes (isolated nerve endings), isolated from different parts of the CNS of animals, are convenient objects for the neurochemical study of synaptic processes. During depolarizing procedures in vitro - electrical stimulation (ES), an increase in the K\textsuperscript{+} concentration, addition of veratrine and ouabain - biochemical changes associated with the Ca\textsuperscript{++}-dependent liberation of neurotransmitters contained in the synaptosomes take place [1, 6].

The object of this investigation was to study the effect of ES of a suspension of synaptosomes on their ultrastructure and also on functional indices such as the rate of Ca\textsuperscript{++}-dependent liberation of noradrenalin (NA) and cyclic nucleotide phosphodiesterase (PDE) activity.

EXPERIMENTAL METHOD

Synaptosomes were isolated from rat brain without the cerebellum by the method described previously [3]. After sedimentation in modified Krebs-Ringer solution (104 mM NaCl, 5 mM KCl, 1.2 mM CaCl\textsubscript{2}, 1.3 mM MgCl\textsubscript{2}, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 10 mM glucose, 20 mM Tris-HCl buffer, pH 7.6, at 37°C) the synaptosomes were suspended in a fresh portion of the same solution (1-3 mg protein/ml and preincubated (15 min, 37°C) with agitation by means of a magnetic stirrer. Part of the suspension was left under the same conditions during ES (control), the rest was subjected to ES by means of platinum ring electrodes at 37°C and with constant
agitation. The diameter of the platinum wire was 1 mm, the diameter of the electrodes and cuvettes 20 mm, and the distance between the electrodes 15 mm. Stimulation was by square pulses of alternating polarity with a frequency of 100 Hz, the duration of a single pulse was 0.4 msec, the interval between paired pulses of different polarity 0.2 msec, and the voltage of the electrodes 7-10 V; the conventional strength of the current, measured by means of a milliammeter graduated for sinusoidal voltage, was 12 mA. An ESU-1 electrostimulator was used and the amplitude of pulses of different polarity was measured and strictly equalized as shown by the readings of the oscilloscope. After ES the synaptosomes were separated by centrifugation (20,000 g, 10 min, 4°C). The acid-soluble fraction of the supernatant and the residue of synaptosomes were used for determination of the Na concentration [3]. In experiments in which PDE activity was measured, the residue of synaptosomes was frozen at −10°C and stored for not more than one week. PDE activity in synaptosomes disintegrated in this way was determined by a radioisotope method [4].

Synaptosomes were fixed for electron-microscopy in experimental and control samples 5-7 sec after the end of ES. Prefixation was carried out in the suspension by adding formaldehyde or OsO₄ up to a concentration of 1% (pH 7.4) [7]. The synaptosomes were then sedimented and, in the case of prefixation with formaldehyde, they were postfixed with 1% OsO₄. Similar procedures also were used to fix the previously sedimented synaptosomes. In all cases the residue was dehydrated and embedded in Durcupan.

Ultrathin sections stained with uranyl acetate and lead citrate by Reynolds' method were examined and photographed in the JEM-7A electron microscope. The state of the vesicular apparatus of the synaptosomes

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