REACTIVE CHANGES IN AUTONOMIC SYNAPSES DURING HIGH-FREQUENCY ELECTRICAL STIMULATION

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Intravital investigations of neurons, axons, and synaptic and sensory endings have shown that it is possible, in principle, to explain their nonspecific reaction to a disturbance of homeostasis by colloid-chemical changes in the cytoplasm [7, 10]. However, it is not yet clear whether these structural changes can take place in response to specific stimulation, connected with action potential (AP) generation. Studies of the effect of electrical stimulation and of chemical activating agents on synapse morphology and ultrastructure are numerous but highly contradictory [12-15]. Often they were unaccompanied by any physiological control of stimulation or by comparison with the results of intravital studies.

The object of this investigation was to study reactive changes in synapses in the peripheral autonomic nervous system in more detail, and with electrophysiological control and parallel intravital observations, during high-frequency orthodromic electrical stimulation and to examine the colloid-chemical changes taking place under these circumstances in the synaptoplasm.

EXPERIMENTAL METHOD

Three series of experiments were carried out on 30 frogs and five cats. In the experiments of series I autonomic synapses of isolated neurons of the frog vagosympathetic trunk were studied intravitally by means of an inverted MBI-13 phase-contrast microscope, accompanied by electrophysiological control, before, during, and after electrical stimulation. The technique of isolating the neuron and the combined morphological and electrophysiological procedure were described previously [9]. High-frequency orthodromic stimulation of the proximal end of the vagosympathetic trunk was applied by means of double-barreled coaxial suction electrodes. Stimulation of supramaximal strength was used. The frequency of electrical stimulation was 20-60 Hz and its duration 5-15 min. The integral APs were recorded from one branch of the vagosympathetic trunk. In the experiments of series II the synapses were stained in a neutral 0.005% solution of methylene blue and fixed in a saturated solution of ammonium molybdate for 15 min before the experiment and also during electrical stimulation for 10 min. In series III axosomatic synapses of the vagosympathetic trunk of frogs and axodendritic synapses of the cranial cervical sympathetic ganglion of cats were fixed in 2.5% glutaraldehyde solution in phosphate buffer (by perfusion in the cats) and in 1% OsO_4 solution for electron microscopy after orthodromic electrical stimulation for 10 min (but without stopping the stimulation).

EXPERIMENTAL RESULTS

The intravital observations showed the presence of a pericellular synaptic apparatus on many isolated autonomic neurons of the vagosympathetic trunk (Fig. 1d-i), concentrated in the region of the axon hillock. Cross-striation corresponding to the spiral pericellular winding of the preganglionic fiber (Fig. 2d, e), and multiple long elliptical cross sections through individual synaptic enlargements along its course, and "en passant" axosomatic

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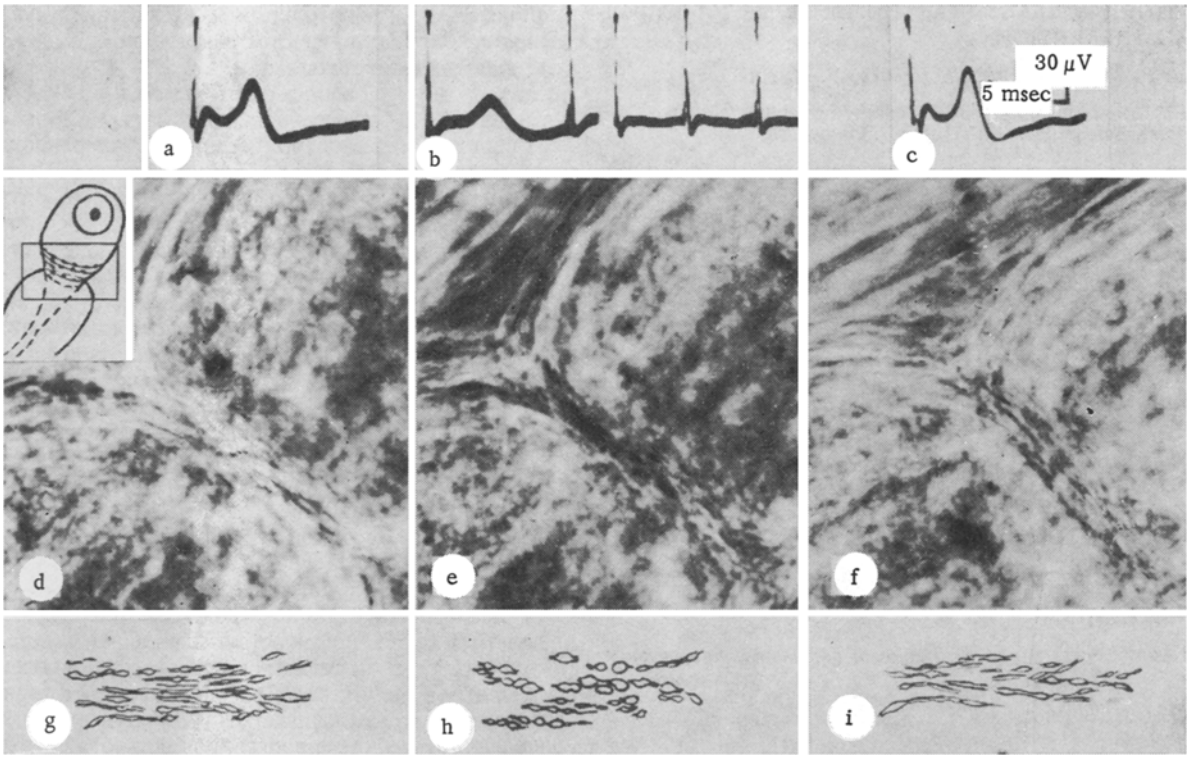

Fig. 1. Simultaneous intravital morphological and physiological changes in synapses during high-frequency electrical stimulation: a) integral three-component AP of vagus nerve during stimulation at 1 Hz; b) diminution and disappearance of late components of AP at 20 and 40 Hz; c) recovery of late components during stimulation at a frequency of 1 Hz; d) elongated cross sections through synaptic boutons of pericellular apparatus in initial state (inset shows diagram of arrangement of synapses in this particular preparation); e) rounding of synaptic boutons of the same preparation during stimulation at 40 Hz; f) recovery of shape of boutons after stopping high-frequency stimulation; d-f) intravital microscopy, phase contrast, objective 70, ocular 10; g-i) diagram of synaptic boutons illustrated in d-f respectively.

synapses could be seen. The ratio of the width to the length of the enlargements was about 1:3. During the first 1-3 min of stimulation, when the late components of AP decreased considerably or disappeared (Fig. 1b) the synaptic enlargements became round in shape and larger in size, and their optical density decreased (Fig. 1e, h). This morphological picture in vital preparations usually corresponds either to true swelling or to a redistribution of the liquid fraction of the neuroplasm. The ratio of width to length of the boutons under these circumstances was 1:2 or even 1:1. Sometimes some degree of rounding of the neuron body could be observed (postsynapse, Fig. 2a-c). The morphological changes observed, like the blocking of synaptic conduction, were reversible (Fig. 1c, f, i), although the exact time of recovery of the structures after stimulation for 10 min was difficult to determine. It was measured in minutes (1-5 min).

A more complex picture was recorded in stimulated and fixed preparations stained with methylene blue. Some synaptic boutons were sharply increased in volume, with spherical outlines, and were deeply stained. Other equally large boutons were already decolorized (Fig. 2e). There were also decolorized shrunken boutons. The cell body was not yet stained but granules of dye typical for control autonomic neurons either were absent or their numbers were sharply reduced. This variegated picture corresponds most probably to the beginning of the intermediate phase according to Maiorov's classification of strucural and staining changes in synapses [7].

Electron-microscopic investigation of frog axosomatic and cat axodendritic autonomic synapses after stimulation revealed similar changes. The first noteworthy feature was the variegated electron density of cross sections through the synaptic boutons, dendrites, and

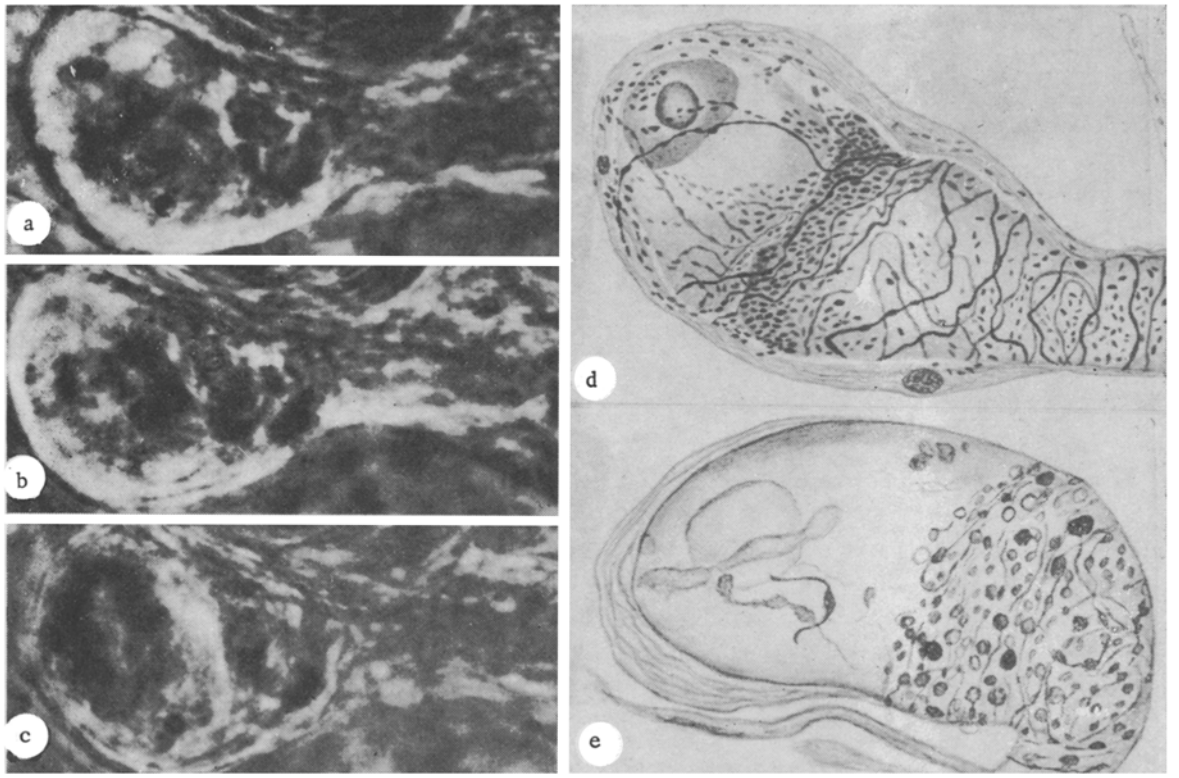

Fig. 2. Vital and fixed autonomic neurons in the frog vagosympathetic trunk before and after electrical stimulation: a) neurons in initial state; b, c) gradual rounding of body of postsynaptic neuron during high-frequency stimulation (intravital microscopy, phase contrast, objective 40, ocular 10); d, e) axosomatic "en passant" synapse of control (d) and stimulated (e) neurons (methylene blue, objective 40, ocular 10, diagramatic).

unmyelinated axons. They had a clear translucent matrix or areas of it were translucent (Fig. 3d, e), whereas other areas appeared as dark profiles. Typical pictures of "light and dark degeneration" of the synapses were not observed. In the zone of the translucent matrix, clumps of floccules of condensed, low-contrast material or aggregates of filamentous-tubular material could regularly be seen (Fig. 3b). Pale vesicles had low contrast; they were shrunken and had a tendency to agglutinate (Fig. 3d, e). Around their circumference floccular material was often adherent. The cristae of the mitochondria in the boutons were either reduced or destroyed (Fig. 3b). Modified mitochondria also were present in the postsynaptic dendrites. The number of pale vesicles was reduced, but instead of them there were many "dark vesicles" with a dense core and with fine osmiophilic bodies of varied structure (Fig. 3b, d, e). Osmiophilic vesicles and bodies were found inconstantly in the control preparations (Fig. 3a), only as single structures, and their structure was more uniform. Among the variants of structure of the granular "dark" bodies and vesicles there were intermediate forms, similar in their external appearance and size to the pale polymorphic vesicles, or variants resembling lysosomes, myelin bodies, and "lipid droplets." Dark granules similar in size to the core of the granular vesicles (Fig. 3c), and resembling glycogen, also were found. The distribution of these "dark" granules seemed to obey a certain topical rule: They were never adjacent to an active zone on the synapse but occupied the proximal zone of the bouton and they were clearly demarcated from the region occupied by pale vesicles. Glycogen-like granules appeared in the modified synapses of both cats and frogs. In frogs, however, they were much larger, they were more frequent, and they clearly occupied sites which in the control belonged to pale vesicles. A few of them were constantly found also in control preparations from frogs. Changes were found in the postsynaptic neuroplasm of the neuron soma after stimulation more frequently in the peripheral juxtamembranous zone. Here the rough endoplasmic reticulum had disappeared and ribosomes were arranged freely or in the form of rosettes near the cisterns of the smooth endoplasmic reticulum. Nearer the membrane the cisterns were swollen and modified mitochondria and true vacuoles appeared (Fig. 3c), evidence of hydration of this cytoplasm. In

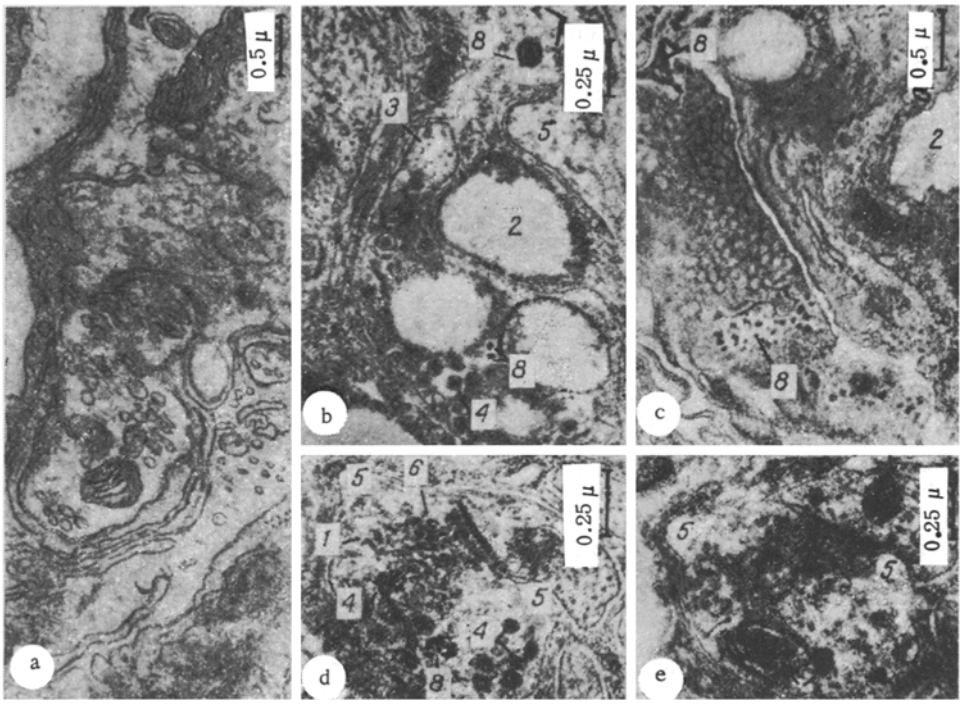

Fig. 3. Ultrastructural changes in synapses during electrical stimulation: a) synapse in control; b) changes in presynaptic axon; c, d) serial sections through modified axodendritic synapse in cat; e) changes in axosomatic synapse of frog. 1) Floccular condensation; 2) structureless mitochondria; 3) filamentous-tubular bundle; 4) dense bodies and vesicles; 5) translucency of matrix; 6) aggregation of synaptic vesicles; 7) myelin-like bodies; 8) glycogen-like granules. Initial magnification 18,000 ×.

the region of disorganization there were usually bundles of neurofilaments, and vesicles resembling synaptic and osmiophilic granular bodies.

The combination of changes described above cannot be regarded as strictly specific, for its elements have been described in synapses exposed to many types of influence and in the early stages of various diseases [2, 6]. At the same time, it is functional in character. Structural changes in the synapses developed comparatively quickly (10 min), and the structure and function of the living synapses were easily restored. There is as yet no satisfactory hypothesis to explain all the changes found in the autonomic synapses during high-frequency orthodromic stimulation. Nevertheless, there are good grounds for considering that the changes connected with activation are also based on colloidal-chemical conversions of the neuroplasm, like the nonspecific response of the neuron to changes in homeostasis. We know that any action exerted on the cytoplasm must be accompanied by conformational changes in its proteins [8] with an increase in their adhesive properties, which implies corresponding aggregation of protein structures. The changes described in the synapses can be taken as evidence of increased adhesion and aggregation of its components: fusion of the pale vesicles with one another and with the active zone, aggregation of filamentous-tubular material, and condensation of the substance of the matrix into clumps and floccules. These condensation phenomena during electrical stimulation were observed by other workers also [3-5]. Aggregation of proteins must lead simultaneously to displacement of the hydrated fraction of the neuroplasm, with an increase in the content of weakly bound water [10]. In the preparations described above this could correspond to local translucency of the matrix and the appearance of vacuoles in the peripheral juxtamembranous layers of the neuroplasm. Displacement of the hydrated fraction of the neuroplasm must be accompanied regularly by the appearance of a force of surface tension, moving the liquid from neighboring regions into the region of the bouton, enlarging it, and tending to make it spherical. Changes of this sort were indeed found in the intravital observations. Pale outlines of synapses on electron micrographs also corresponded to hydration. The presence of shrunken synaptic boutons in fixed preparations stained with methylene blue and

also of dark profiles of pre- and postsynapses on the electron micrograph can be partly explained by the removal of weakly bound water from the hydrated fraction during fixation and dehydration. Finally, conformational changes in proteins weaken their bonds with lipids. The splitting up of lipoprotein complexes and displacement of the lipids could even take place. One manifestation of this process may probably be the newly formed "dark" osmiophilic bodies, "lipid droplets," and also the myelin-like structures, lysosome-like bodies, and so on, observed during activation by other workers [1, 3, 11, 15] as well as ourselves. The various kinds of dark-core vesicles, which appeared at the site of ordinary pale synaptic vesicles, may perhaps also belong to the same group of bodies with increased osmiophilia and may reflect a phenomenon of segregation of lipids in modified or newly formed vesicles. The suggested explanations are naturally by no means final but they require expansion and experimental verification.

LITERATURE CITED

1. N. I. Artyukhina, Structural and Functional Organization of Neurons and Interneuronal Junctions [in Russian], Moscow (1979).
2. N. N. Bogolepov, Ultrastructure of the Brain in Hypoxia [in Russian], Moscow (1979).
3. Yu. A. Darinskii, "Morphological and physiological investigations of neurons of the central nervous system during various forms of activation," Author's Abstract of Doctoral Dissertation, Leningrad (1979).
4. L. N. D'yachkova, Dokl. Akad. Nauk SSSR, 155, No. 1, 227 (1964).
5. E. M. Zamoskovskii and Yu. A. Darinskii, Arkh. Anat., No. 2, 22 (1975).
6. A. S. Iontov, V. A. Kislyakov, T. F. Kuleshova, et al., Morphology of Connections of the Cortical Vestibular Area [in Russian], Leningrad (1980).
7. V. N. Maiorov, Morphology of Reactive States of the Autonomic Interneuronal Synapse [in Russian], Leningrad (1969).
8. D. N. Nasonov, Local Reaction of Protoplasm and Spreading Excitation [in Russian], Moscow–Leningrad (1962).
9. O. S. Sotnikov, in: Intravital Microscopy of the Neuron [in Russian], Leningrad (1978), pp. 45-57.
10. O. S. Sotnikov, A. L. Kurchikov, V. N. Kazachenko, et al., Fiziol. Zh. SSSR, No. 4, 497 (1980).
11. S. Fujimoto, K. Yamamoto, K. Kuba, et al., Brain Res., 202, 21 (1980).
12. K. Lynch, Brain Res., 194, 249 (1980).
13. R. A. D. O'Brien, A. I. Österg, and G. Vrbova, Neuroscience, 5, 1367 (1980).
14. I. I. Push and R. C. Wiley, Science, 172, 191 (1972).