DYNAMICS OF NUCLEIC ACIDS AND NUCLEOPROTEINS IN THE MOTOR NEURONS OF THE FROG'S SPINAL CORD DURING THEIR EXCITATION

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The object of this investigation was the cytochemical study of the motor neurons of the frog's spinal cord during their reflex and antidromic stimulation for periods of between 15 sec and 6 min.

EXPERIMENTAL METHOD

Experiments were carried out under Nembutal anesthesia. The motor neurons of the lumbar enlargement were investigated at the level between the points of exit of the 8th and 9th pairs of spinal nerves, where the nucleus of the motor neurons innervating the triceps femoris muscle has been located experimentally. Excitation of the motor neurons was produced by electrical stimulation of the central end of the sciatic nerve on one side by means of a continuous series of rectangular pulses with an amplitude of 4-6 mA and a frequency of 100 per sec, the duration of each pulse being 0.2 msec (slightly higher than the threshold level of stimulation). Against the background of excitation of the motor neurons, recorded by the contraction of the triceps femoris muscle, they were fixed rapidly by perfusion with a cold fixing solution (Carnoy's, Brodskii's, or Shabadash's mixture), followed by further fixation in the corresponding solution. Paraffin sections, 6 μ in thickness, were stained by Feulgen's method, with pyronine and methyl green by Brachet's method, with gallocyanin by Einarson's method, with thionine by Nissl's method, with eosin-azure by Maximow's method, and with methylene blue by Shabadash's method. The excited motor neurons, situated ipsilaterally, were compared with the contralateral motor neurons at the same level, which served as controls. In addition, intact frogs were used as controls. Altogether 80 animals (57 experimental and 23 control) were investigated.

EXPERIMENTAL RESULTS

No difference between the excited motor neurons and the controls could be found in the localization and amount of DNA. In both the intact and the excited neurons DNA was localized in the nucleus, where it was distributed as dispersed granules and a number of larger granules.

Investigation of RNA showed changes in the intensity of staining of the nucleolus and tigroid of the excited motor neurons (see figure), and a relationship was detected between sorption of the dye and the time of stimulation. On the assumption that the intensity of pyroninophilia corresponds to the RNA content, it may be concluded that after excitation for 1-6 min changes took place in the concentration of the RNA localized in the nucleolus and tigroid. No changes in the intensity of staining were found during the investigation of motor neurons stimulated for 15-30 sec.

After stimulation for 1 min the intensity of staining was slightly reduced, evidently indicating utilization of the RNA. Two minutes after the beginning of stimulation the changes in the RNA concentration could not be taken as definite, because along with intensively stained cells there were others showing reduced sorption of the dye. After stimulation for 3 min, the reaction of the motor neurons became more definite and was characterized by an increase in the intensity of straining, probably associated with the beginning of compensatory reactions leading to an increase in the concentration of RNA essential for maintaining the normal course of protein synthesis. When the motor neurons were investigated after reflex and antidromic stimulation for 4-6 min, the RNA concentration in the nucleolus and tigroid was increased, and the RNA localized in the tigroid and between its granules stained so intensively that often the granules seemed to merge into a single mass. Double nucleoli appeared in the stimulated motor neurons;

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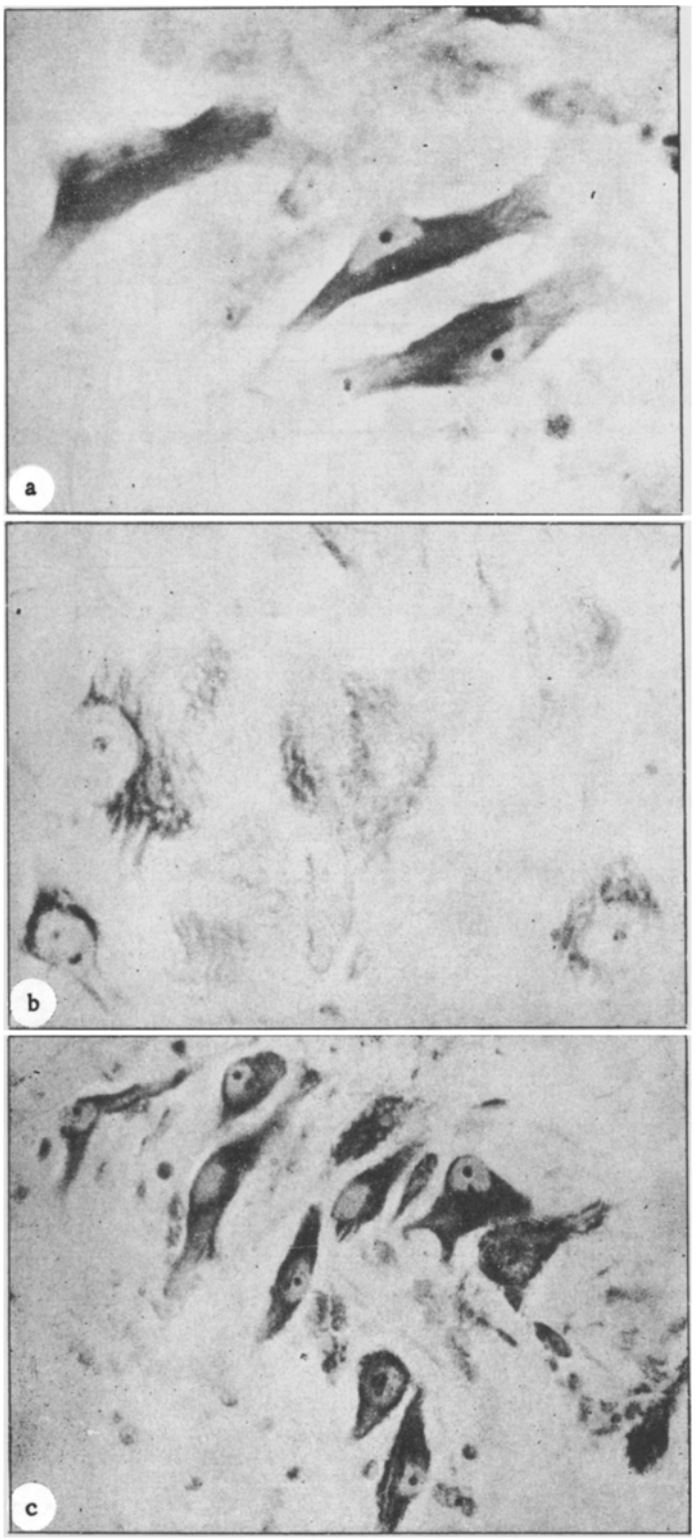

Motor neurons of the spinal cord of a frog. a) Intact; b) after stimulation for 1 min. A sharp decrease in the sorption of pyronine by the stimulated motor neurons can be seen; c) after stimulation for 6 min. An increase in pyroninophilia of the excited neurons by comparison with the intact is visible. Photomicrograph. Fixation by Brodskii's method. Brachet's reaction. a) Objective 40 ×, ocular 6.6 ×; b) objective 20 ×, ocular 6.6 ×.

they are also found in intact motor neurons, although much more rarely. Finally, on the side of stimulation, motor neurons with a nucleolus expelled from the nucleus into the cytoplasm were sometimes seen. This phenomenon was evidently connected with an increase in the activity of the nucleolus as the site of protein synthesis. However, not all the motor neurons of a particular nucleus reacted in the same way. Among the neurons of the excited zone with an increased RNA content there were others with a normal, or in some cases a reduced, intensity of staining.

Using the reaction of most of the motor neurons of the stimulated nucleus as criterion, the experimental results may be subdivided into four groups depending on the duration of stimulation: 1) absence of visible changes (15-30 sec); 2) a decrease in the intensity of staining by Brachet's method, evidently corresponding to a decrease in the RNA concentration (1 min); 3) indefinite results in which the RNA concentration was sometimes increased, sometimes decreased (2 min); 4) an increase in the intensity of staining with pyronine, probably connected with an increase in the RNA concentration (3-6 min).

By staining for nucleic acids by Einarson's method results similar to those described above were obtained, and in each individual case the relationship between the excited and the intact zone corresponded to that found after staining by Brachet's method.

As a result of staining of the tigroid by histological methods (thionine, eosin-azure) changes were found which were in general agreement with those described above. However, the changes were less marked, presumably because of the lower sensitivity of the method. Histochemical detection of the ribonucleoproteins of the tigroid was carried out by Shabadash's method. For the intact motor neurons the optimal pH was 4.4. During stimulation changes in the intensity of staining with methylene blue at pH 4.4 were observed, and these were analogous to, and coincided in time with, the changes in staining for RNA. The intensity of staining at pH 4.4 was reduced 1-2 min after the beginning of stimulation (increase in pH to 4.6) and the sorption of methylene blue was increased after stimulation for 4-6 min, at pH 4.4 (decrease in pH to 4.2). The changes in the RNA in the stimulated motor neurons thus occurred in phases corresponding to the changes in pH.

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SUMMARY

Stimulation of the spinal motoneurons in a frog provokes changes in the state of ribonucleic acid. These changes are phasic in character, RNA concentration decreasing in one minute after stimulation and increasing in 3-6 minutes' time.