AN INTRACELLULAR MICROELECTRODE STUDY OF THE MECHANISM OF ORIGIN OF RHYTHMIC MUSCLE FIBER ACTIVITY

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Investigations of the electrical activity of the nervous system show that many excitable elements (cells, axons, receptors) are able to generate rhythmic discharges in response to single stimuli. A suitable model for the study of rhythmic activity is the spontaneous electrical activity which develops in striated muscle fibers when the muscle is placed in Ringer solution with a reduced calcium concentrate.

METHODS

Experiments were carried out on isolated nerve-muscle preparations of the sartorius muscle of the frog Rana pipiens. The muscle was placed in a chamber containing modified Ringer solution in which the concentration of calcium chloride was reduced to 20 - 50 per cent of normal. Muscle potentials were recorded with glass intracellular microelectrodes by the standard method [2, 8]. The motor nerve was stimulated with rectangular impulses from an electronic generator.

RESULTS

Muscles placed in the solution with a reduced concentration of calcium ions showed spontaneous rhythmic activity. When recorded with intracellular microelectrodes, the activity of single fibers took two main forms: spike potentials, and slow waves of negativity with spike potentials superimposed (Fig. 1a and c). In some instances, slow waves appeared that were not accompanied by spikes. As a rule, each spike was preceded by an independent slow, local negative potential change—the prepotential [3]—regardless of whether the spike developed at the peak of a slow wave of negativity or without any such slow wave. In some cases prepotentials were absent, which may be explained by the fact that the spontaneous spikes being recorded had developed some distance away from the point where the microelectrode entered the cell, so that the local variations in potential accompanying the spikes were not detected. The propagated spike was able to generate new waves of activity, with clearly distinguishable prepotentials (Fig. 1c) near the site of insertion of the electrode into the cell. The rhythmic activity recorded from different fibers was marked by great variability, as regards both the frequency and the duration of the waves. Some fibers were capable of rhythmic activity over a period of many seconds, while in others rhythmic activity stopped after a few discharges had appeared. The cause of this dying out of rhythmic activity is not clear. It might be connected either with cessation of the activity of the stimulating focus that gives rise to the observed rhythm, or with elevation of the fiber's threshold of excitability to the stimulating action of the focus of excitation [4]. Data are also available to indicate that in the absence of sufficient ionized calcium, there is a gradual inactivation of the process of migration of sodium ions into the cell and expulsion of sodium ions out of the cell during the recovery period by the "sodium pump" [7]. For this reason, it is of great interest to determine the fiber's capacity for activity immediately after its rhythmic activity stops.

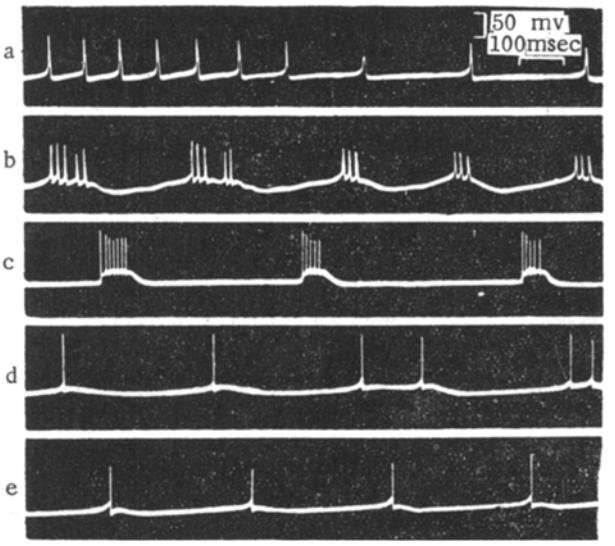

Fig. 1. Rhythmic activity of a muscle fiber. a, b, c) Spontaneous activity; d) rhythmic activity evoked by application of Ditilin (succinylcholine iodide); e) rhythmic activity evoked by nerve impulse.

For this purpose we investigated the effects of certain substances that are able to excite muscle fibers. It was found that such substances as decamethonium, Ditilin (succinylcholine), tetramethylammonium, veratrine, and caffeine, when added to calcium-deficient Ringer solution, almost always cause rhythmic activity to appear in cells that were inactive before the application of these preparations. The activity produced by application of depolarizing substances was indistinguishable in character from spontaneous activity (Fig. 1d). When calcium ions were added to the solution bathing the muscle, depolarizing substances produced rhythmic activity in only a small number of fibers. Curarization (tubocurarine chloride in a concentration of 5×10^{-6}) almost always prevented the appearance of activity in response to the application of tetramethylammonium, Ditilin, or decamethonium, and had little effect on the action of veratrine and caffeine. It follows that the development of rhythmic activity under the influence of substances in the first group is primarily related to their selective depolarizing action on the endplate region, but the effect of veratrine and caffeine is explained by depolarization of the remainder of the muscle fiber surface. Thus, rhythmic activity may arise as the result of prolonged depolarization of different regions of the cell: the endplate and the electrically excitable membrane.

An interesting feature of the action of depolarizing substances was their capacity to evoke rhythmic activity in fibers in which spontaneous activity had stopped before the preparation was applied. In this case, the potential variations which developed in response to the depolarizing substances sometimes corresponded exactly to the original potential variations, and sometimes differed markedly from them both in frequency and in the form and amplitude of the recorded potentials. This indicates that the cessation of rhythmic muscle fiber activity is not the result of inactivation of processes of active transport of sodium ions. It is probable that activity stops as a result of accommodation of the fiber to the stimulating action of the focus of excitation [1]. Moreover, we may suppose that the secondary rhythmic activity produced by the depolarizing substance, when it is identical to the initial spontaneous activity, is a consequence of depolarization of exactly the same region of the cell in which the rhythmic activity had arisen spontaneously. When changes occur in the form and frequency of the potentials, we may suppose that the preparation has a depolarizing effect on some other region of the cell, which now becomes the "pacemaker."

In order to test the assumption that spontaneous rhythmic activity arises as a result of activation of individual regions of the cell, from which the excitation process then passes to the rest of the fiber in the form of local and propagated potentials, experiments were performed with electrical stimulation of the motor nerve of the muscle being investigated.

It is well known that when ionized calcium is absent from the solution bathing the muscle, neuromuscular transmission is blocked [6]. Even so, under conditions where the calcium concentration in the Ringer solution was reduced to 20–50 per cent of normal, we were still able to record endplate potentials as well as spike discharges of the muscle fibers. The muscle retained its ability to display spontaneous rhythmic activity.

Stimulation of the motor nerve was often accompanied by development of prolonged rhythmic activity in "quiescent" fibers, which remained even after stimulation was discontinued. This activity completely corresponded in character to spontaneous rhythmic activity (Fig. 1e). Stimulation of the nerve when spontaneous activity was already present caused the spontaneous rhythm to be eliminated, and to be replaced by a rhythm synchronized with that of stimulation (Fig. 2a and a_1). In other instances, stimulation was followed by cessation of previous activity. Thus, a spontaneously active muscle fiber has the distinct capacity to react to excitation propagated from the endplate region. In some cases the muscle fiber does not respond immediately to nerve stimulation, but assumes the rhythm of stimulation gradually, at first giving potentials that are irregular and complex in form (e.g., the paired discharges in Fig. 2b and b_1), which then change, as stimulation continues, into spikes of the usual form, strictly synchronized with the stimulating rhythm.

Finally, when rhythmic activity has developed in the fiber, it may proceed quite independently of the rhythm of nerve stimulation (Fig. 2c and c_1). But usually even in these cases uninterrupted stimulation ultimately leads to synchronization of muscle potentials with the rhythm of stimulation.

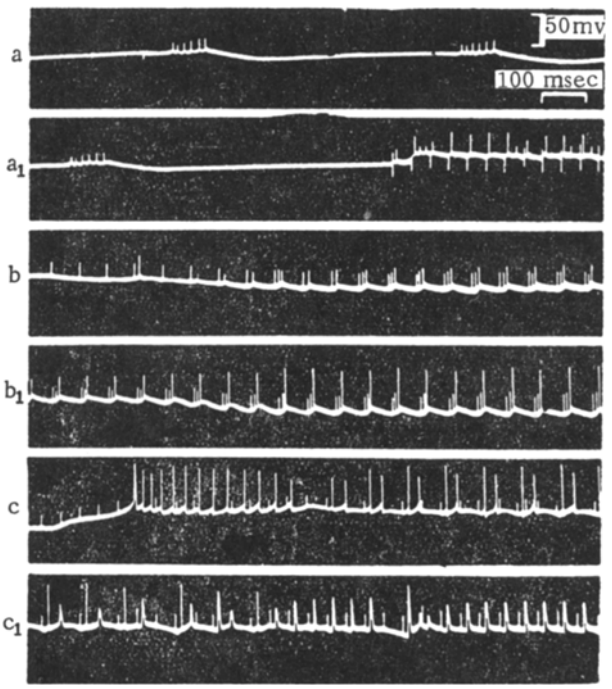

Fig. 2. The effect of stimulation of the motor nerve on rhythmic activity of the muscle fiber.
a, a_1) Effect observed with spontaneously active muscle fibers; b, b_1 and c, c_1) effect observed in absence of automatic activity.

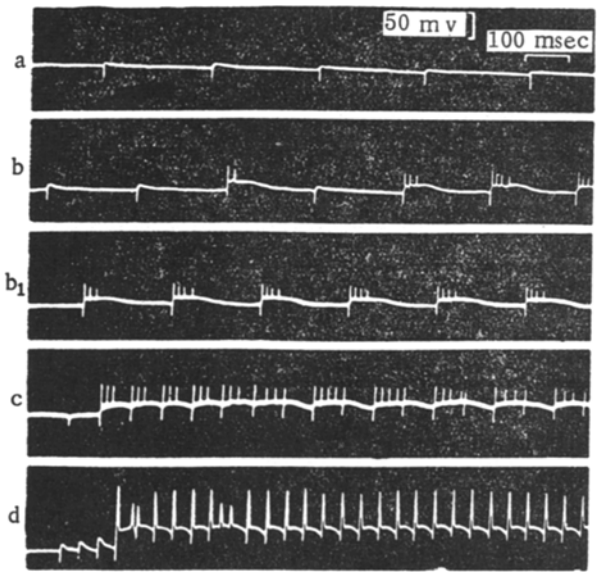

Fig. 3. Effect of frequency of motor nerve stimulation on character of rhythmic activity of muscle fiber. a, b, c, d) Rhythmic activity of fiber with increasing frequency of stimulation. Microelectrode in endplate region: b, b_1) continuous recording during repeated stimulation.

We were interested in the dependence of rhythmic muscle responses on the frequency of electrical stimulation of the motor nerve. It was found that there exists a definite optimum range of frequencies (10–50 c/s), at which rhythmic responses synchronous with stimulation developed in a muscle fiber.

The significance of the stimulation frequency is illustrated in Fig. 3, which shows recordings of muscle potentials with an intracellular microelectrode inserted in the region of the endplate. The magnitudes of the endplate potentials were greatly reduced in response to a reduced calcium ion concentration. At a slow stimulus frequency only local potentials develop, which do not give rise to spikes. With increase in frequency of stimulation, rhythmic discharges occur in response to some stimuli, and become more and more stable as tetanic stimulation continues. As the stimulus frequency continues to increase, this process is accelerated, and at the beginning of the stimulus the muscle begins to show rhythmic responses after each nerve impulse. As the rate of stimulation increases still further, assimilation of the rhythm develops rapidly but the rhythmic character of each response is lost, and the muscle develops a single spike in response to almost every nerve impulse.

This relationship between the rhythmic responses of the muscle and the frequency of nerve firing can be explained by the fact that at low rates of stimulation the endplate potentials are too small to excite the rest of the muscle fiber surface. With increase of stimulus frequency, the endplate potentials become larger (postactivation potentiation), and the level is reached which is necessary for excitation of the neighboring portion of the membrane, in which rhythmic responses are generated. As the size of the endplate potentials increase further, the muscle fiber generates only single responses, since a large depolarization depresses the capacity of the fiber to give rhythmic spike potentials, although in calcium deficiency a successive increase in the endplate potentials certainly occurs in the course of tetanic stimulation [6] and is seen in the illustration presented (see Fig. 3).

Nevertheless, the possibility is not excluded that assimilation of the stimulus rhythm by the muscle fiber, particularly in the presence of spontaneous muscle fiber activity, may also depend on other causes. By analogy with data obtained on smooth muscle [5], which behaves very much like striated muscle in a calcium-deficient solution, we can assume that assimilation of the stimulus rhythm by a fiber that is capable of spontaneous activity is related to its capacity to generate slow waves. Actually, group discharges developed, as a rule, both to spontaneous slow negative potential variations and to slow waves produced by a nerve impulse. We have not made direct determinations of fiber refractoriness, but the fact that a subsequent discharge may develop in the fiber, even when the effective stimulus coincides with the falling phase of the previous spike or comes immediately after the end of this phase, shows that the refractory period is very brief and does not exceed 1–3 msec. Therefore, the refractory period does not determine the muscle fiber's capacity to assimilate the stimulus rhythm and to respond to a single impulse with a rhythmic discharge.

Thus, the data we have presented show that rhythmic muscle fiber potential variations in a calcium-deficient medium may arise as a result of local depolarization of the fiber endplate under the influence of various pharmacological substances or of a nerve impulse, and also as a result of a depolarizing influence on the rest of the muscle fiber surface. When rhythmic discharges are produced by a nerve impulse, the frequency of nerve firing is of great importance.

SUMMARY

Experiments were performed on an isolated nerve-muscle preparation of frog sartorius muscle kept in a solution with reduced calcium ion concentration. The appearance of rhythmic activity of the muscle fibers may be caused by depolarization of either the endplate area or other portions of the membrane. Depolarization resulting in rhythmic activity may be caused by the application of some pharmacological substances (succinylcholine, decamethonium, tetramethylammonium, caffeine, veratrine) or by a nerve impulse. The involvement of the muscle fiber in rhythmic activity is especially effective when the motor nerve is stimulated at a frequency of 10 to 50 stimuli per second.

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