Slow, rhythmic *fluctuations of frog sartorius muscle* fibre membrane potential under the influence of "middle-frequency" (5 kc.p.s.) steady-state currents

By EDWARD SENN

It is well-known that in contrast to the method of stimulating nerve and muscle fibres with alternating currents (a.c.) at low frequency (up to 1 kc.p.s), the application of symmetrical sine wave a.c. at sufficiently high frequency (above 1 to 3 kc.p.s) has no physical effect on the membrane potential, the reason being that in the latter case outward and inward current flow across the membrane are identical. Their use therefore presents many advantages when examining changes of membrane potential during an electrical stimulation of long duration, to which should be added the possibility of exact compensation of the stimulating artifact. Such currents have been termed middle-frequency (m.f.) currents [3]. Any depolarization or consequent repolarization detectable under their influence is therefore alone the result of physiological membrane processes. The term "reactive depolarization" has been proposed for such an effect of m.f. current on the excitable membrane [5].

It has been shown [8, 9] that m.f. currents of appropriate form (drop or triangular shaped, [8]), i.e., m.f. currents of delayed intensity rise, provoke repetitive spike activity. The latter comes to an end as soon as the reactive membrane depolarization has reached a characteristic plateau, namely the level corresponding to about half the resting potential.

The present experiments are concerned with the further course of this plateau depolarization under the influence of m.f. current of steady-state intensity. A special form of m.f. current was used, namely m.f. current the intensity of which first increased slowly and was then kept constant as soon as the plateau level of depolarization was attained. We suggest the term "steady-state m.f. current" for this kind of current form.

Method

The experimental conditions were the same as those used in earlier studies [4, 7]: in vitro preparations of frog sartorius muscle; transverse flow of (steady-state) m.f. currents through the proximal (nerve-free) end of the muscle; insertion of the intracellular microelectrode into one fibre between the two activating electrodes; compensation of the m.f. current artifact by an RC bridge. The application of steady-state m.f. currents allowed us to study the influence of the latter on the membrane potential for any desired length of time.

Results

The plateau level of depolarization following the rhythmic spike activity could be maintained only when the rise in intensity of the m.f. current applied was sufficiently steep. Under such conditions the plateau state could be prolonged up to several seconds. If during the plateau state the m.f. current rise decreased in steepness or passed over into steady-state intensity, however, a repolarization process sets in (Figs. 1 and 2). This process was at first slow, i.e., the polarization gain was 10–15 mV over a period of several seconds. As soon as this descending plateau state reached a repolarization level in the range between −50 and −60 mV, a sudden repolarization drop terminated the plateau depolarization. The repolarization drop ended at a level comparable with the normal resting membrane potential. This newly reached polarization state was characterized by an unusual instability of the membrane potential which lasted as long as the steady-state m.f. current was applied.

The instability was accompanied by the following rhythmic fluctuations of the membrane potential (Figs. 1 and 2): Sudden jumps of the membrane potential up to the plateau level and immediate drops to the unstable resting level occurring at first frequently (max. 4 p.s.) and then with decreasing rate (down to only 8 per min.). The duration of the intermediate plateau states reached by the sudden jumps was never longer then 1 to $1\frac{1}{2}$ s.

Occasionally the depolarization jumps and repolarization drops were of complex shape: a spike could arise from the depolarization jump (Fig. 2), or one or more small depolarization humps could precede the repolarization drop.

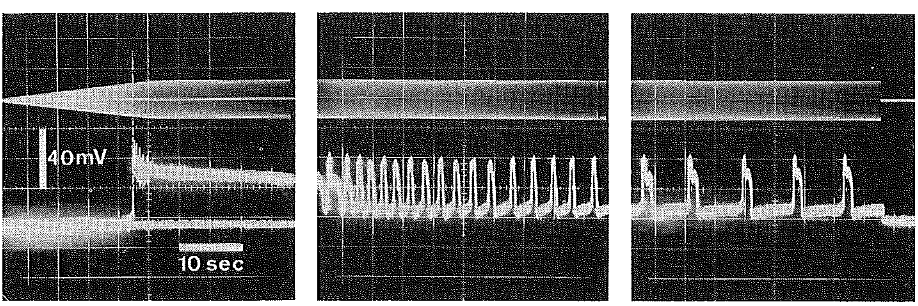

Fig. 1 The further course of the muscle fibre membrane potential after having reached the plateau level under the influence of a "steady-state m.f. current": a descending plateau followed by slow, rhythmic fluctuations. The repolarization drop from the plateau level down to the resting level occurs first in one and then in two steps. Note the existence of a repolarisation threshold.

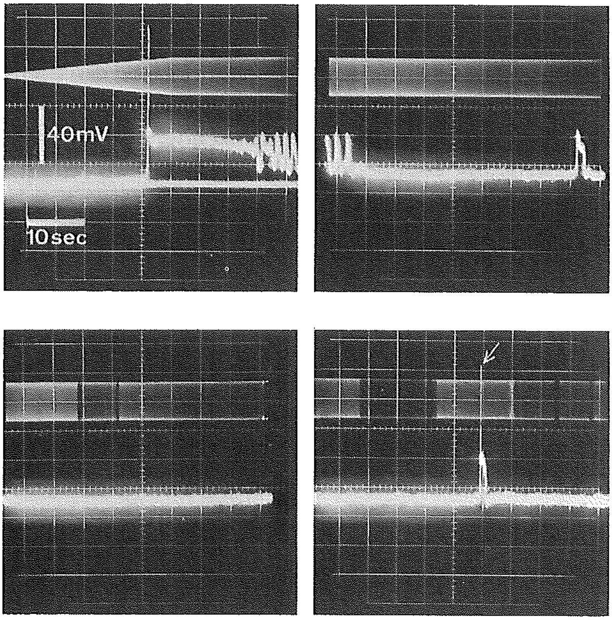

Fig. 2 The behaviour of a muscle fibre membrane potential under the continuous transverse flow of a "steady-state m.f. current". Duration of the m.f. current flow about 4 min. The slow repolarization during the plateau depolarization and the subsequent phase of slow, rhythmic fluctuations of the membrane potential is followed by an enduring state of almost full repolarization. This unstable resting level of membrane potential is interrupted twice by a depolarization jump up to the plateau level, first whithout, then with the occurrence of a spike of full amplitude (arrow).

Discussion

Earlier experiments have demonstrated that m.f. currents of continuously rising intensity represent an "adequate stimulus" for provoking "reactive depolarization" [8], i.e., a localized, active membrane reaction. The latter continues to develop even beyond the spike threshold. This further rise in reactive depolarization beyond the membrane threshold generates a rapid succession of spikes. This repetitive spike activity is terminated only when reactive depolarization has almost reached its peak, i.e., a plateau level of about half the resting membrane potential. During the whole of the plateau period the spike generation mechanism is inhibited by this special physiological membrane state. There is only one "adequate stimulus" capable of prolonging plateau depolarization, namely an m.f. current of continuously rising intensity. The sole factor limiting the plateau is attainment of a current strength of harmful intensity.

An m.f. current the intensity of which is maintained at steady-state strength instead of rising continuously cannot therefore prolong plateau depolarization but simply leads to characteristic instability of the membrane potential. The unstable membrane potential oscillates rhythmically between two different potential levels, i.e., the plateau level and a level comparable in value with the resting membrane potential. The oscillating membrane potential is marked by characteristic membrane thresholds for the depolarization jumps as well as for the repolarization drops. Because the jumps and drops are never the consequences of a particular m.f. current period or of a particular change in the current intensity, the membrane potential fluctuations must be considered to be spontaneous and can be designated as reactive.

Only few other methods have been shown to provoke comparable rhythmic membrane oscillations. In contrast to the physiological action of m.f. current, all these other methods involve profound changes in the extracellular milieu such as replacement of chloride ion with a non-permeating anion (muscle fibres, [2]) or the addition of scorpion venom (nerve fibres, [1]).

The persistence of such a plateau membrane state in nerve fibres may perhaps furnish an explanation of the "local electric anaesthesia" [6] provoked in nerves by the application of a.c. of several kc.p.s. This type of reversible interruption of nerve function is limited precisely to the time of current flow, i.e., to the duration of the plateau state.

The slow, rhythmic oscillations of the membrane potential of skeletal muscle fibres are reminiscent of the spontaneous membrane potential changes of cardiac pace-maker cells. Despite the almost complete absence of spikes in the membrane potential of skeletal muscle fibres kept under steady-state m.f. current, and despite the different levels of plateau state, the one (muscular) at half membrane potential, the other (cardiac) at nearly zero membrane potential, the two sorts of muscle cells show comparably unstable behaviour of the membrane characterized by a tendency to depolarization jumps and consequent repolarization drops occurring as soon as a certain threshold of depolarization or repolarization has been reached.

Since symmetrical m.f. currents do not cause physical changes in the membrane potential, the slow rhythmic fluctuations of the latter must be a consequence of a direct action of the m.f. current on the molecular membrane structures determining the permeability to sodium, potassium and chloride ions.

Zusammenfassung

Mittelfrequente Wechselströme (5 kHz) bewirken an der Muskelfasermembran (Musculus sartorius, Frosch) eine langdauernde aktive Membranpotentialsenkung (reaktive Depolarisation). Wenn die Intensität des mittelfrequenten Wechselstromes kontinuierlich ansteigt, dann nimmt auch die reaktive Depolarisation zu und geht dabei so lange mit dem Auftreten von repetierenden "spontanen" Spikes einher, bis das Maximum der reaktiven Depolarisation, eine plateauförmige Dauerdepolarisation, erreicht ist. Der Übergang des ansteigenden Mittelfrequenzstromes während dieser Plateaudepolarisation in einen Mittelfrequenzstrom konstanter Intensität leitet einen langsamen Repolarisierungsprozess ein. Das Plateau senkt sich

demzufolge langsam bis zum Erreichen einer charakteristischen Repolarisationsschwelle (zwischen −50 und −60 mV), an welche sich eine sturzartige Repolarisierung auf ungefähr die Ruhepotentialhöhe anschliesst. Das Membranpotential bleibt aber trotz der erreichten, fast vollständigen Repolarisation während der gesamten Stromflusszeit in einem unstabilen Zustand. Es "springt" dabei in einem zunehmend langsameren Rhythmus von 4 pro sec bis 8 pro min mehr oder weniger regelmässig auf die Plateauhöhe, um jedesmal unmittelbar anschliessend wieder sturzartig in den Repolarisationszustand zu "fallen". Später treten die Plateausprünge nur noch selten und unregelmässig auf, und das Membranpotential verharrt im "reaktiven" Repolarisationszustand, solange der Mittelfrequenzstrom weiterfliesst. Die Depolarisationssprünge können mit dem Auftreten eines Spike einhergehen.

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