Intracellular Recording of Electrical Response of Muscle Fibre to Transversely Applied Middle-Frequency Pulse Stimulation

Middle-frequency alternating current was investigated with regard to the mechanism of its excitatory action by GILDEMEISTER[1], SCHWARZ[2,3], and WYSS[4–6]. It was suggested that depolarization occurs in the same way at both poles (KATZ[7], GILDEMEISTER[1]), and it was shown that impulses of middle-frequency current applied to nerve through bipolar electrodes consequently stimulate simultaneously at both poles ('ambipolar' stimulation, WYSS[8]). Middle-frequency stimulation therefore cannot be based on the polarity principle, the only principle of electrical stimulation so far known, but should be ascribed to a 'nonpolarity' mechanism ('stimulation apolaritaire', WYSS[6,9]). Thus, true transverse stimulation of nerve can be performed when the two electrodes are applied opposite each other, hence producing a uniform action in one and the same stimulation area (WYSS[4,6,10]).

In order to throw further light on the nature of the depolarization produced by middle-frequency sine-wave current, transverse stimulation was applied to striated muscle, and the changes of the transmembrane potential of a single muscle fibre were studied at the site of stimulation, using the intracellular microelectrode recording technique.

The sartorius muscle of the frog was prepared with a small piece of bone at its pelvic end, and kept overnight in Ringer's solution at nearly 0 °C. The solution contained in m*M*/l: NaCl 115; KCl 2.5; $CaCl_2$ 1.08; $NaHCO_3$ 2,38; and NaH_2PO_4 0.08.

In some experiments, choline chloride was substituted for NaCl. Before use, 5 mg/l of *d*-tubocurarine chloride and 11.10 m*M*/l of glucose were added to the solution, which was saturated with oxygen. The experiments were carried out at room temperature. Rectangular pulses of sine-wave current of 20 kc/sec, obtained from a middle-frequency oscillator operated by a 'square-wave' generator (General Radio Company, Type No. 1217-C) were used for stimulation (WYSS[11]). The stimulation current was fed to the preparation transversely through two square plate-electrodes, 1.5 · 1.5 mm surface area, which were placed on the muscle opposite each other. The inter-electrode distance was within 3–5 mm, depending on the size of the muscle. The KCl-filled glass microelectrode was inserted into a muscle fibre within the area between the two stimulating electrodes. The plate-electrodes as well as the axial wire of the microelectrode consisted either of platinized platinum or, in some experiments, of silver coated with silver chloride. Stimulation artifacts were compensated by a resistance-capacity bridge (WYSS[9,10]). Changes of the membrane potential fed through a negative capacitance electrometer (Argonaut LRA 043) were recorded on the lower beam of a dual beam oscilloscope (Tektronix 502) along with the stimulation voltage recorded on the upper beam.

The threshold responses of a single fibre to pulses of middle-frequency current of 30, 20, 10, 7, 5, 3, 2, 1 and 0.5 msec duration are shown in Figure 1. Application of the stimulus causes a progressively increasing depolarization, the gradient of which increased with the stimulation voltage. As soon as depolarization reached a given level, an action potential was evoked, as holds for conventional stimulation. A line joining the end-points of the lower limits of the stimulation voltage records (upper and left part of Figure 1) thus represents part of a strength-duration curve. It was also found that reversal of the poles of stimulation in no way affected the appearance of the action potential. Recordings were made from muscle fibres situated at various distances from the two stimulating electrodes. These tracings showed that intracellular action potentials of muscle fibres could be evoked from any point explored, and that no consistent difference could be established between the stimulation effects obtained from fibres situated in the immediate vicinity of either electrode. A certain degree of difference could, however, be encountered in regard to the threshold of response, depending on the spacing of the muscle fibres and the distances between the recording microelectrode and the stimulating plate-electrodes.

Figure 2 shows the characteristic behaviour of the membrane potential under the influence of a stimulating middle-frequency pulse prolonged beyond its utilization time, thus outlasting spike and negative after-potential. It can be seen that membrane depolarization produced by the steady state of middle-frequency current flow is

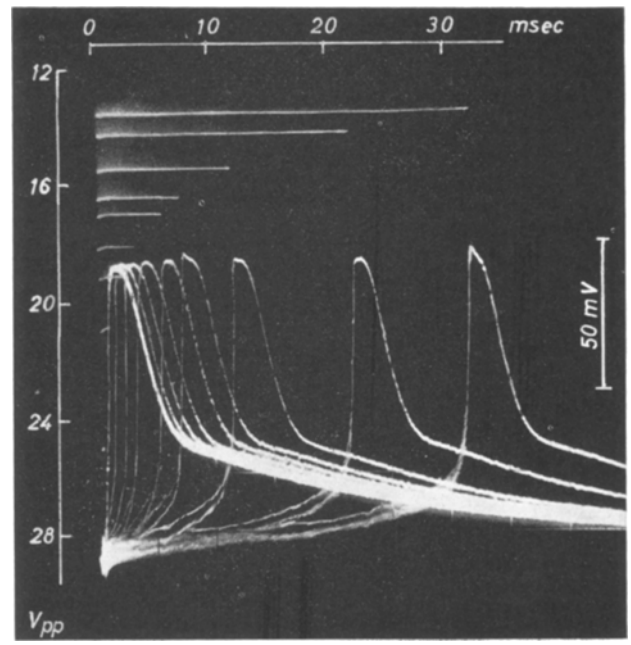

Fig. 1. Threshold responses of single muscle fibre to middle-frequency pulse stimulation. Peak-to-peak voltages as related to pulse duration ('voltage-duration relationship'). 9 successive exposures corresponding to 30, 20, 10, 7, 5, 3, 2, 1 and 0.5 msec duration. Upper beam: downward amplitude of the stimulating middle-frequency pulses as recorded with regard to scale of peak-to-peak values on the left. Lower beam: transmembrane potentials with regard to scale on the right. Note correlation between voltage of stimulation and gradient of local depolarization.

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maintained during the after-potential and outlasts the after-potential at the same depolarizing level if the pulse duration is prolonged beyond the end of the after-potential. The spike of the action potential is not modified by the middle-frequency current flow, but deformation of the late falling phase appears. The negative after-potential cannot appear because of the formation of a plateau, and final repolarization is postponed to the end of the middle-frequency pulse.

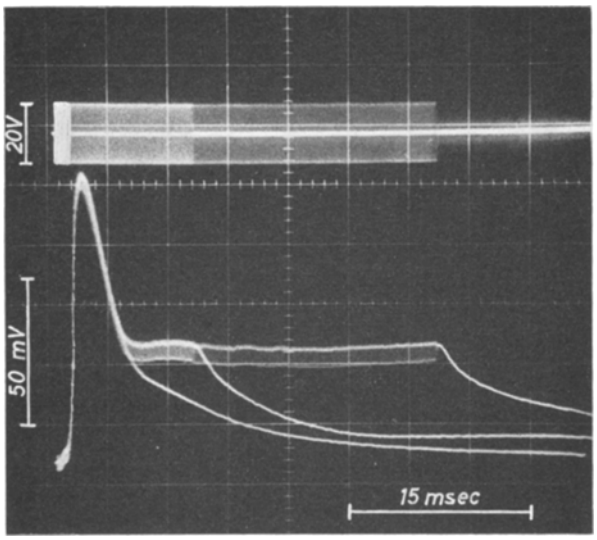

Fig. 2. Effect of prolongation of middle-frequency pulses beyond utilization time on the shape of the transmembrane potential change. Three responses of the same fibre to pulse durations of 1, 10 and 30 msec at same voltage (upper beam with scale on the left) recorded as transmembrane potential (lower beam with scale on the left). Note that compared with 1 msec response (utilization time) the pulses of 10 and 30 msec duration cause a maintained depolarization after the spike, without deforming the latter and postpone the final repolarization to the end of the middle-frequency pulse.

Further experiments demonstrated the local nature of the initial depolarization which occurs during the utilization time of middle-frequency pulses (see Figure 1). This was shown by comparing the shape of the action potential recorded at the site of stimulation with that obtained on the same fibre stimulated at a distance of 25 mm from the site of recording. In experiments to be reported in detail in a later paper, it was also shown that application of middle-frequency pulses to muscle immersed in sodium-free Ringer's solution, no longer produced local depolarization, and hence no action potential. This may suggest that middle-frequency stimulation causes primarily an increase of the sodium permeability of the membrane that consequently leads to an increase in sodium influx across the cell membrane.

Zusammenfassung. Der Froschsartorius wird mit Mittelfrequenz(20 kHz)-Stromstössen quer gereizt. Die Reizantwort wird mit intracellulärer Mikroelektrode am Reizort abgeleitet. Die lokale Depolarisierung und die daraus sich ergebende Auslösung des Aktionspotentials werden auf ihre Abhängigkeit von Stärke und Dauer der Reizimpulse untersucht.

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Effects of Angiotensin on the Superior Cervical Ganglion of the Cat

HAEFELY et al.[1] have recently reported that very low doses of bradykinin and angiotensin produce an inhibition of synaptic transmission in the superior cervical ganglion of the cat.

In this paper, we wish to report a dose-effect relationship obtained with minute quantities of angiotensin on the same preparation.

Material and methods. The effects of preganglionic cervical sympathetic nerve stimulation were estimated either by recording nictitating membrane contraction with an isotonic myograph transducer or by the oscilloscopic measurement of the amplified postganglionic nerve action potentials. Close retrograde intra-arterial injections were made towards the superior cervical ganglion through a polyethylene cannula inserted into the ipsilateral lingual artery.

Results and discussion. When angiotensin was injected towards the ganglion in amounts too small to contract directly the nictitating membrane (0.5–10 ng), contraction of the ipsilateral membrane was nevertheless elicited. Section of the postganglionic trunk or extirpation of the ganglion abolished this contraction, whereas it was not prevented by bilateral adrenalectomy. Hexamethonium (0.1–1 mg) as well as atropine (100–200 μg) effectively blocked membrane contraction induced by angiotensin (0.5–10 ng) or by preganglionic stimulation. However, hexamethonium, in similar amounts, failed to abolish the contractions obtained when higher doses of the hormone were applied to the ganglion (0.1–1 μg), as previously observed by LEWIS and REIT[2].

In addition to stimulating the postganglionic fibres, angiotensin may alter ganglionic transmission when injected towards the ganglion at a concentration insufficient to elicit membrane contraction. In the course of stimulating the preganglionic nerve with submaximal shocks (0.1 sec square waves at a frequency of 15–25 c/sec during 5 sec every 2 min), a retrograde injection of angiotensin (0.01–0.1 ng) resulted in a one- to fourfold enhancement of the response to subsequent stimulations. The response was evaluated by measuring nictitating membrane contractions or by recording postganglionic action potentials. In some preparations, this potentiation of synaptic transmission lasted for 3 h or more (lower tracing, Figure).

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