spun in order to obtain pellicles less than 500 μm thick. After postfixation in situ with 1% buffered OsO_4, pellicles were embedded in a Epon-Araldite mixture. Sections were cut at right angles to the surface of pellicles through the whole pellet. Then they were stained and observed on a Siemens Elmiskop I A electron microscope. Proteins were measured according to Lowry et al.[6].

Results. At 16th day of incubation, the fraction obtained with osmotic shock from synaptosomal fraction appeared relatively poor in membranes, which were to some extent disrupted and almost devoid of synaptic thickenings (see figure 1). Microsomes, free mitochondria and a few membrane profiles containing residual synaptic vesicles adhering inside could be also observed.

At 18th day of incubation, the fraction appeared enriched in membrane profiles about the size of synaptosomes, many of which have attached synaptic thickenings. Free mitochondria and microsomes content was lower than in younger embryos (see figure 2). In chicks, the fraction appeared full of well-maintained membranes with very recognisable symmetrical and asymmetrical synaptic thickenings, whereas contaminating structures appeared sharply diminished (see figure 3).

Relative protein content of membrane fraction of 16 days old chicks was about 30% of 18 days old embryos value and about 25% of chicks value. In contrast, the difference in relative as well as in absolute content of proteins between synaptosomal fraction before and after osmotic shock appeared to decrease during maturation.

Discussion. Our morphological observations on these fractions enriched in membranes seem to be perfectly in agreement with data we obtained on synaptosomes isolated from chick embryonic optic tectum (see preceding paper)[4]. We observed a progressive increase in maturation of synaptosomes with augmentation of synaptic thickenings and a decrease of contamination of the synaptosomal fraction parallel to the progressive development of tectal synaptogenesis. Synaptosomal membranes isolated in older stages appeared also quite similar to that isolated from adult rat brain[5], and from 6–8 days old chicks[7]. Consequently, it is highly probable that membranes obtained after osmotic shock of synaptosomal fractions are largely synaptosomal and synaptic membranes which show a progressive maturation. However, no data are so for available about morphology of embryonic synaptosomal membranes. In addition, from a morphological point of view, it is not possible quantitatively to measure contamination by microsomal membranes and other subcellular elements which may appear during preparation of membrane fraction by centrifugation, even if a relative low speed has been used. Consequently, further biochemical and enzymatic studies are necessary for characterization of these membrane fractions. With this reservation, nevertheless, we think that this method can be used as a first step to obtain synaptosomal and synaptic membranes at relatively precocious stages of development, and it might provide an improved technique to prepare embryonic synaptosomal membranes from shocked mitochondrial fraction.

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Electro-mechanical noise in atrial muscle fibres of the carp[1]

S. Akselrod, J. Richter, E. M. Landau and Y. Lass

Department of Physiology and Pharmacology, Tel Aviv University Sackler Medical School, Tel Aviv (Israel),
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Summary. Steady membrane voltage fluctuations have been observed in atrial muscle fibres of the carp. These voltage fluctuations produce minute mechanical oscillations, as revealed by an interference contrast microscope. The steady voltage fluctuations may be related to abnormal automaticity in the heart.

Fluctuation phenomena have revealed useful information on molecular mechanisms operating in excitable membranes[2–6]. We now report the finding of steady membrane voltage fluctuations which produce minute mechanical oscillations in atrial muscle fibres of the carp. The voltage fluctuations can grow and lead to repeated spike activity, and may thus be linked to abnormal automaticity in the heart.

Materials and methods. Experiments were performed on isolated carp (Cyprinus carpio) atria, from which the pacemaker regions have been removed. Muscle fibres in the carp atrium are relatively large (up to 25 μm in diameter) and stable intracellular recording can be achieved for at least a few minutes. The atria were continuously perfused with carp Ringer of the following composition[7]: (mM) NaCl 120, KCl 2.7, $CaCl_2$ 2.9, $MgCl_2$ 1.0, $NaHCO_3$ 10, pH (7.8–8.0). The intracellular potential was recorded with glass micropipettes having a resistance of 15–25 MΩ when filled with 3 M K-citrate or KCl. Signals were recorded on paper chart (Grass Model 79) or on magnetic tape (Hewlett Packard 3955 System) and analysed by a digital computer (Varian 620L-100).

Results and discussion. Figure 1 shows a typical recording of voltage fluctuations in atrial muscle cells. Immediately after penetration, a DC potential of –79 mV was recorded, and there was a large increase in the voltage fluctuations. The average RMS value ± 1 SEM of the voltage fluctuations was 66 ± 11 μV (n = 14). Spectral analysis of the noise showed most of the power to be concentrated at frequencies below 3 Hz with the addition of a hump at

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1 Hz which may indicate some intrinsic time constant of the atrial fibre[8,9]. At frequencies above 3 Hz, the power was proportional to $1/f^2$ where f is the frequency. The table summarizes the effects of several pharmacological agents and ions on the voltage fluctuations. These experiments rule out a possible quantal release of transmitter from autonomic nerves[10] and suggest that Na^+ and Ca^{++} ions are directly involved in generating the noise. The ionic channel may also be permeable to K^+ and Cl^-, but as these ions are known to affect the passive membrane resistance[11,12], they may influence the amplitude of the noise indirectly. When atrial muscle fibres were observed with interference contrast microscope (Zeiss, $\times 500$) and the noise was maximized (in low K^+ or SO_4^{--} Ringer), extremely small mechanical oscillations could be seen. The mechanical oscillations were abolished by choline Ringer ($[Na^+]_0 = 0$, +atropine 5×10^{-8} M), or acetylcholine (5×10^{-6} M), or EGTA (2 mM). To show that the mechanical fluctuations are produced by the membrane voltage fluctuations, a second microelectrode was used to change the membrane potential (electrode separation: 5–20 μ). Small depolarization of less than 1 mV was sufficient to cause local contraction and small hyperpolarization caused local relaxation[13] (see figure 2). No mechanical artefacts were observed in the voltage trace during contraction or relaxation (see figure 3). We thus concluded that the mechanical fluctuations are induced by the membrane voltage fluctuations, possibly by a fine regulation of the Ca^{++} influx and the release of calcium from intracellular stores[13]. We often observed a transition from small steady noise (similar to figure 1) to large oscillations and finally to spike activity. A typical trace is shown in figure 4. Here the transition occurred spontaneously, but it could also be obtained by reducing the K^+ concentration or by ouabain (5×10^{-6} M). This phenomenon resembles the transient large amplitude oscillations leading to action potential which have been observed in Purkinje fibres[14].

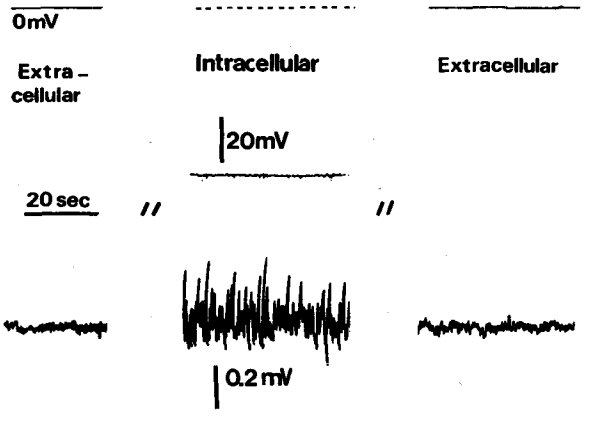

Fig. 1. Membrane voltage fluctuations in an atrial fibre ('intracellular') as compared to the background noise of the microelectrode in the bathing solution ('extracellular'), before and after recording from inside the cell. The membrane potential is recorded at a low gain in the upper trace (DC) and at a higher gain in the bottom trace (AC, −3 db at 0.07 Hz). When the microelectrode impales the cell, a membrane potential of −79 mV is recorded, together with prominent voltage fluctuations (Polygraph Record).

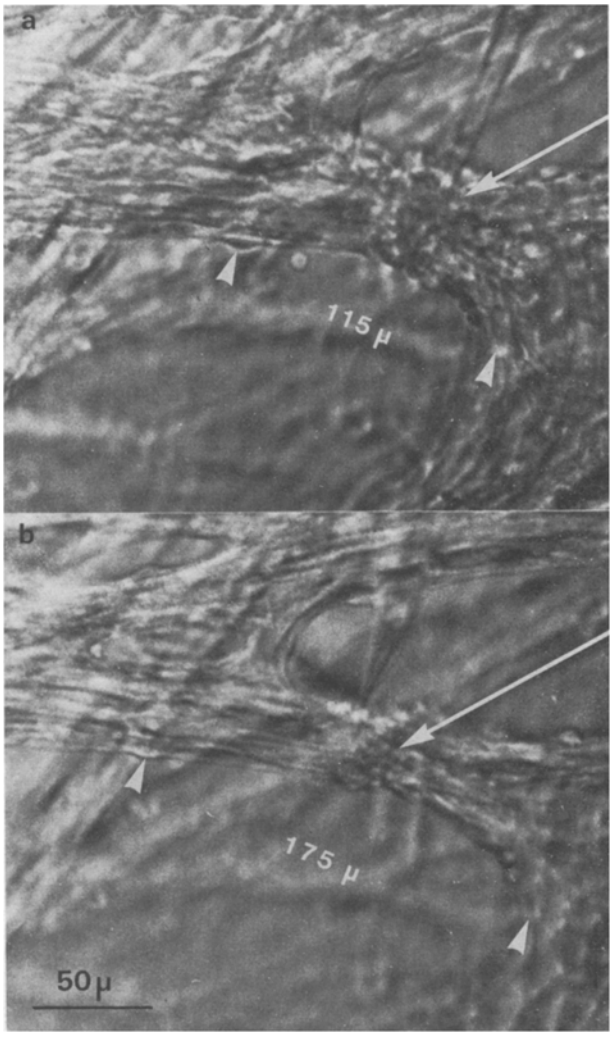

Fig. 2. Photograph of a bundle of atrial fibres in normal Ringer solution. The arrow at the right indicates a fibre impaled by a microelectrode for current injection. The arrowheads measure the distance between 2 landmarks on the fibres. a) Control: no current was injected through the current microelectrode. b) The same bundle of fibres while passing a hyperpolarizing pulse. The relaxation of the injected fibre and its neighbours is clearly seen. The separation between the 2 landmarks increased from 115 μm to 175 μm. In order to document this phenomenon, a particularly strong (~0.5 μA) and long (~2 sec) pulse was applied but relaxation or contraction could be observed with much shorter and smaller currents. The fact that polarizations smaller than 1 mV have a mechanical effect in cells with resting potential from −70 to −90 mV, confirms the results of Vassort et al.[13] (no mechanical threshold) rather than those of Einwächter et al.[19] (existence of a mechanical threshold). Strong relaxation of the fibres could be obtained after pressure injection of 3 M acetylcholine through a micropipette; a similar injection of 3 M NaCl did not affect the preparation.

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Effects of pharmacological agents and ionic changes

	Noise intensity	Membrane potential
Acetylcholine 5×10^{-6} M – 5×10^{-8} M	Strongly decreased	Hyperpolarization of up to ~20 mV – no desensitization
Acetylcholine 5×10^{-7} M + atropine 5×10^{-8} M	No change	No change
Atropine 5×10^{-8} M	No change	No change
Propranolol 10^{-7} M	No change	No change
EGTA 2 mM	Decreased	Depolarization ~20 mV
Verapamil 2×10^{-6} M	Slightly decreased	No change
Ouabain 5×10^{-6} M	Increased*	Small depolarization < 5 mV
Choline-Ringer $[Na^+]_o = 0$ mM, + atropine 5×10^{-8} M	Decreased	Small hyperpolarization ⩽ 5 mV
Sulphate-Ringer $[Cl^-]_o = 0$ mM	Increased*	Small hyperpolarization < 10 mV
High K^+ Ringer $[K^+]_o = 16.2$ mM	Decreased (to 25 μV RMS)	Depolarization ~25 mV
Low K^+ Ringer $[K^+]_o = 0$ mM	Increased*	Hyperpolarization ⩽ 10 mV

Changes in noise intensity and membrane potential as compared to normal Ringer (average RMS ± 1 SEM = 66 ± 11 μV).
* Up to 1.5 mV RMS; was then accompanied by mechanical oscillations and, especially with ouabain, could lead to spike activity.

The noise in the atrial fibres was probably distinct from the normal pacemaker potential, because both the low and the high amplitude noise (cf. figure 1 and figure 3) could be observed in cells with a resting potential of –80 mV or less, whereas the pacemaker mechanism is activated at resting potentials more positive than –60 mV[15]. The noise may be similar to that observed in Purkinje fibres after treatment with strophantidine[16]. In addition, the mechanical oscillations observed together with the large amplitude noise may be part of the feedback mechanism[17] responsible for the noise. We thus report a new type of membrane noise which may underly the appearance of ectopic centers and thus participate in the generation of arrhythmias[18]. It will be of great interest to study the mechanism of the noise growth, and its relationship to the generation of action potentials in the heart.

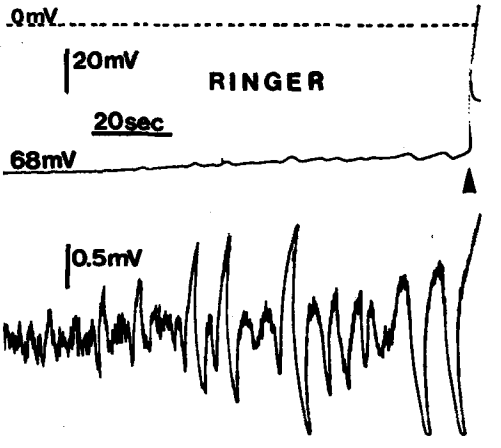

Fig. 4. An intracellular recording showing a change in the pattern of the noise leading to an action potential. The membrane potential is measured at a low gain (DC) in the upper trace and the fluctuations are shown at a higher amplification in the lower trace (AC, –3 db at 0.07 Hz). A cell was penetrated with a microelectrode and had a resting potential of –68 mV (upper trace). The broken horizontal line above the upper trace denotes zero potential. Initially, voltage fluctuations were of the usual type (lower trace, cf. figure 1). After some time, slower and larger oscillations appeared which grew until a spike was triggered (see overshoot of transient at the arrow) and a twitch was observed under the microscope. The same phenomenon has been observed in many cells, with resting potential as negative as –90 mV and is different from the normal pacemaker activity which develops in depolarized atrial cell[15] (Polygraph Record).

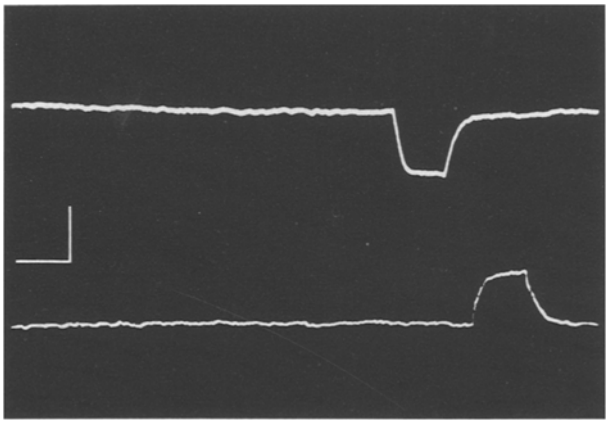

Fig. 3. Voltage recording from a cell with –70 mV resting potential. The lower trace shows a depolarization of about 2 mV, causing a local contraction. The upper trace shows a hyperpolarization which had no mechanical effect. It should be noted that, although the depolarizing pulse had a mechanical effect, whereas the hyperpolarizing pulse had not, the shape of both transients was similar and there was no evidence of a mechanical artefact. Time base: 5 msec/div. Voltage: 2 mV/div.

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