Local Oscillations of Frog Skeletal Muscle Sarcomeres Induced by Subthreshold Concentration of Caffeine

J. POLEDNA and A. ŠIMURDOVÁ

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Czechoslovakia

Abstract. Different intracellular processes are selectively controlled by a signalling system based on transient rises or oscillations of cytoplasmic calcium concentration, which transmit extracellular signals at subcellular level. When treated with a subthreshold concentration of caffeine, skeletal muscle cells provide a suitable preparation to study mechanisms which generate repetitive calcium transients. Based on optical diffraction measurements of local contractions of individual sarcomeres, we have shown substantial enhancement of spontaneous repetitive calcium release in the presence of subthreshold caffeine concentration. Calcium release propagates to neighbor calcium sources and forms slow contraction waves. A power spectra density analysis has revealed parameters of the time course of these events. However, substantial amounts of calcium released in sarcomeres are not synchronized.

Key words: Skeletal muscle — Caffeine — Oscillation of sarcomere tension — Power spectra density analysis

Introduction

Caffeine is known to potentiate skeletal muscle contraction. In higher concentrations it induces contractures (Axelsson and Thesleff 1958). Recent findings have shown direct effects of caffeine on the calcium release channel in sarcoplasmic reticulum, the ryanodine receptor (Xu et al. 1989). Caffeine facilitates calcium induced calcium release (Endo 1977).

Kumbaraci and Nastuk (1982) described a subthreshold oscillatory activation of skeletal muscle. Frog sartorius muscle bathed in 1 mmol/l caffeine generated brief asynchronous contraction of individual sarcomeres, "sarcomeric oscillations", which evolved into more orderly waves with time. These waves travelled at rates of 50–200 μm/s.

Caffeine in a concentration of 2 mmol/l caused a subthreshold oscillatory activation of single sarcomeres. They occurred independently of membrane potential and were blocked by agents which directly interfere with Ca release from the sar-

coplasmic reticulum (Herrmann 1986). In her more recent work, Herrmann-Frank (1989) provided a more comprehensive characterization of caffeine-induced isometric force and sarcomeric oscillations studied in single skeletal muscle fibers of the frog. Both force and sarcomeric oscillations stopped when calcium release from the sarcoplasmic reticulum was prevented by drugs or when the sarcoplasmic reticulum membranes were solubilized by detergent. These experiments revealed that the oscillatory processes were caused by a cyclic release of calcium ions from the sarcoplasmic reticulum.

Oscillations of cytosolic calcium concentrations are a common process in a wide variety of cells (Rink and Jacob 1989). In physiological conditions, changes of calcium concentrations transmit information at subcellular level. Action potentials evoke spikes of cytosolic calcium (in cells with significant voltage gated calcium entry) or internal release controlled by membrane potential (as in skeletal muscle). A variety of agonists at submaximal concentrations are able to evoke a series of calcium spikes in nonexcitable cells lasting a few seconds, with amplitudes and width not much affected by the increasing concentration of the ligand, but with increasing frequencies. The mechanism of calcium oscillations reflects mutual interactions of processes controlling influx of calcium from extracellular space and intracellular stores, and its uptake. Relevant processes of intracellular concentration changes include voltage dependent Ca inward current, Ca induced Ca release, Ca dependent inactivation of Ca influx into the cytoplasm, Ca pumps and exchangers.

Oscillations of individual sarcomeres in a skeletal muscle cell provide a model for studying repeated release of calcium from intracellular stores. We used optical diffraction methods to characterize periodic release of calcium from the sarcoplasmic reticulum in skeletal muscle fibers induced by subthreshold concentrations of caffeine.

Materials and Methods

Isolated muscle fibers were dissected from m. semitendinosus of the frog *Rana temporaria*. A single muscle fibre was placed into experimental chamber with one tendon tied to a tension transducer (Marko et al. 1986) and stretched to approx. 1.2 of its slack length. The frog saline had the following composition (mmol/l): Na^+ 120, K^+2.5, Ca^{2+} 1.8, Cl^- 121, $Tris^+$ 4, pH 7.1. Experiments were performed at room temperature (22–25 °C).

The fibre was illuminated by a 632.8 nm He-Ne laser beam perpendicular to the fiber axis. Intensity and structure of the first diffraction maximum were analyzed using either a photodiode or a linear 256 photodiode array (CCD) placed 40 mm beneath the fibre, in parallel with the axis of the latter. The photodiode array was composed of 256 sensing elements arranged linearly in 13 μm intervals. Signals from the photodiode and photodiode array were displayed on a Tektronix 11201 Digitizing Oscilloscope. The oscilloscope was interfaced to personal computer via GPIB interface.

The photodiode signal was amplified and filtered by a antialiasing low pass filter with cut-off frequency at (40 Hz, −3 dB, 4-pole Bessel type), then digitized at 100 Hz

sampling rate by a 9-bits analog-digital convertor of the Tektronix 11201 oscilloscope. The data were stored on diskettes for further analysis. DC level was subtracted from each record of 1024 samples, the spectral densities were calculated and subsequently averaged to obtain the mean power spectrum.

Results

A sarcomere consists of A and I bands with different optical properties. They form a three-dimensional quasiperiodic array in muscle. It therefore behaves as a three-dimensional diffraction grating. Analysis of diffraction patterns can provide information about distribution of sarcomere lengths in a muscle fibre. The lengths of sarcomeres reflect the state of activation under different experimental conditions.

We studied the diffraction of a laser beam by single fibers of frog skeletal muscle and effects of subthreshold doses of caffeine in a range of 1 to 1.5 mmol/l. The diffraction pattern had the form of maxima lines perpendicular to the fibre axis and placed symmetrically with respect to the zero order maximum.

The distribution of light intensities in the first maximum usually had a fine structure, as has been reported by Cleworth and Edman (1972) and Tameyasu et al. (1982).

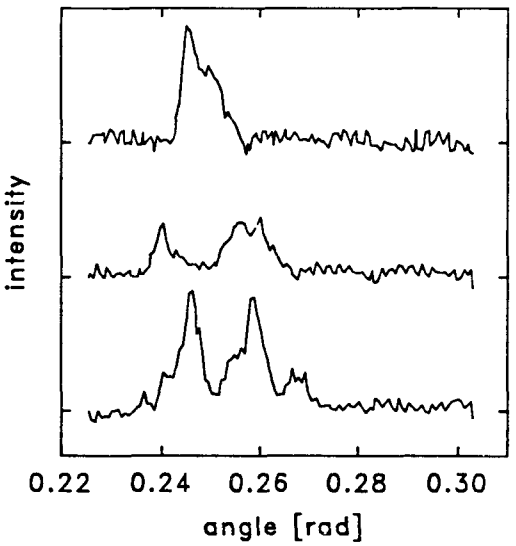

Figure 1. Angular distributions of intensity measured by linear diode array in three different points along the first diffraction maximum line. Increasing angles correspond to decreasing sarcomere lengths. The distribution of light intensities in the maximum usually contains several peaks. The pattern changes moving along the diffraction line.

To analyze processes of calcium release from the sarcoplasmic reticulum, light diffraction of muscle fibre under normal conditions was compared with that under caffeine effect. In the rest, the distribution of light intensities in the maximum usually contained several peaks. In addition, this pattern changed along the diffraction line (Fig. 1) and also when the fibre was moved along its axis. Rüdel and Zite-Ferenczy (1979) suggested that the pattern results from small differences of sarcomere length between the various clusters.

Working from fundamental diffraction theory, Judy et al. (1982) have derived equations that predict the effects of light diffraction caused by periodic and nonperiodic aberrations in the regular spacing of a three-dimensional sarcomere array. Sarcomere length was described by a distribution function. This function may be discrete or continuous and may contain one or more subpopulations. When sarcomeres of different lengths are arranged randomly in myofibrils, no subpeaks of diffraction maximum are present. Diffraction peak amplitude decreases, and peak width increases with the increasing standard deviation of the length population. The subpeaks are present only if (*a*) the distribution of the sarcomere length population is multimodal, i.e., has two or more discrete length populations, and (*b*) serially contiguous sarcomeres of the same length population are clustered together within myofibrils to form domains.

After addition of caffeine in subthreshold concentrations this intensity pattern started to change. Peaks decreased, their shape was altered and new peaks appeared at other angles than observed under resting conditions. Such pronounced changes were detectable only in a narrow concentration range, just below the contracture threshold. This process lasted about 30 min., and a new pattern of intensity distribution occurred. Changes in fibre tension were not detected.

Fig. 2 illustrates an example of the first-order diffraction pattern and its change at subthreshold caffeine concentrations. This result corresponds to the theoretical conclusions of Judy et al. (1982). This means that, in physiological solution, almost all sarcomeres of a fibre are in resting state, and the light intensity profile reflects the length distribution within the fibre. When caffeine is added in subthreshold concentration, it facilitates the local release of calcium from sarcoplasmic reticulum, which propagates to neighbor sarcomeres. These sarcomeres contract and their length decreases, while resting sarcomeres are stretched. Local contractions form clusters of shorter sarcomeres which influences the intensity distribution in diffraction maxima.

This qualitative result corresponds well with theoretical predictions. However, quantitative tests were not applicable due to nonhomogenities of intensity distribution along the line of diffraction maximum. Therefore only changes at the same point could be compared.

To obtain quantitative characteristics of calcium release processes, fluctuations of light intensity in the first maximum were measured by a photodiode. A

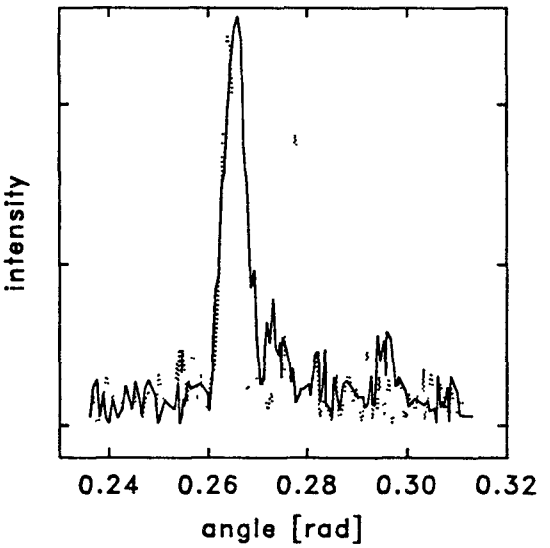

Figure 2. Intensity distribution of the first maximum diffraction line (solid line) and its change after 1.5 mmol/l caffeine (dotted line). Under caffeine effect, the main peak was splitted reflecting two subpopulations of sarcomere lengths. The sarcomere length, d, can be computed from the Bragg equation, $d = k \cdot \lambda / \sin \theta_k$, where k is the order of the diffraction line, θ_k is the angle between the kth and zero-order lines, and λ is the wavelength of the laser beam. In this case, $k = 1$ and $\lambda = 632.8$ nm. The main peak in the rest corresponds to 2.44 μm.

commonly used method to analyze irregular oscillations is to construct a power spectrum in which the number of occurrences of oscillations of a given frequency is plotted against the frequency. The power spectrum can be obtained from the Fourier transformation of time series. Contractions of individual sarcomeres, which are not synchronized, decrease the order of the diffraction lattice and the integral intensity of the first diffraction maximum also decreases. An analysis of intensity fluctuations revealed an increase of spectral density below 5 Hz after addition of subthreshold concentrations of caffeine. This effect is shown in Fig. 3.

Changes of the power spectrum density can provide more information about time characteristics of primary processes causing light intensity fluctuation in the first maximum. The simplest approximation of light intensity changes due to sarcomere contraction can be the difference of two exponentials. The first one corresponds to relaxation and the second to activation of contraction. This approximation can be used to derive the analytical form of the power spectrum density function (Kristian et al. 1991).

The power spectrum density function of Poisson wave with unit events equal

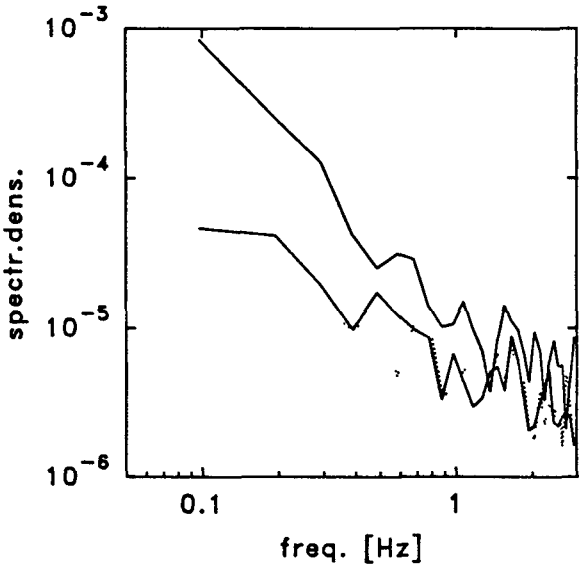

Figure 3. The power spectrum density of intensity fluctuations of the first diffraction maximum (lower solid line) and its changes in 1.5 mmol/l caffeine (upper solid line). This disturbation returns to the rest state (dotted line).

to the difference between two exponential functions

$$i(t) = i_0 \cdot (\exp(-t/\tau_2) - \exp(-t/\tau_1)) \tag{1}$$

has the form

$$S(f) = \frac{2 \cdot v \cdot i_0^2 \cdot (\tau_2 - \tau_1)^2}{1 + w^2(\tau_1^2 + \tau_2^2) + w^4 \tau_1^2 \tau_2^2} \tag{2}$$
$$w = 2\pi f$$

where v is the average frequency of current fluctuations. The power spectra of experimental records were fitted by equation (2) to obtain parameters of elementary events. An example of data processing is in Fig. 4. The inset shows the time course of an elementary intensity change corresponding to contraction of a sarcomere or a small cluster of synchronized sarcomeres. The relation of sarcomere contraction and light intensity change might be nonlinear, however both should cover the same time interval. Therefore time parameters of activation and relaxation can be sufficiently estimated and cannot differ substantially.

The mean value of activation time constant was 0.23 ± 0.12 s and that of relaxation time constant was 1.43 ± 0.65 s. These time constants are rather large compared to muscle contraction. They reflect either slower activation in the presence

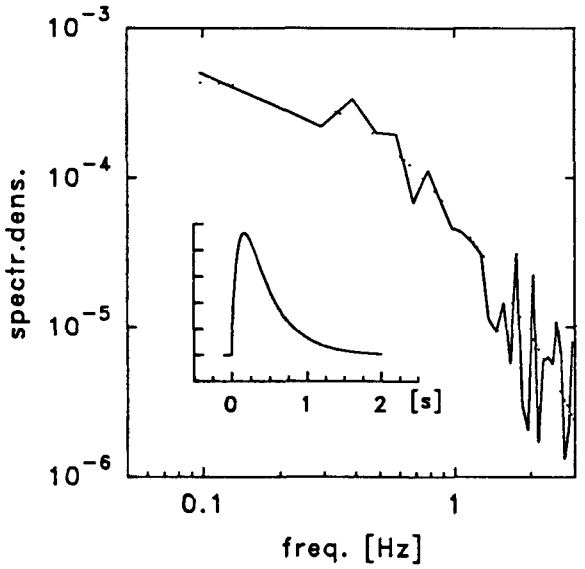

Figure 4. The approximation (dotted line) of the power spectrum density (solid line) of intensity fluctuations evoked by 1 mmol/l caffeine. Spectra in normal condition and under caffeine were subtracted and approximated by equation (2). The inset shows an elementary event. In this experiment, estimated time constants of activation and relaxation were 0.0929 s and 0.3645 s, respectively.

of caffeine or a summation of individual sarcomere contractions due to propagated activation.

This point can be elucidated by a comparison of the record variance and its contribution to overall decrease of maximum intensity. The variance of a record was calculated by the relation

$$\sigma^2 = \int S(f)\mathrm{d}f \tag{3}$$

The ratio of intensity decrease in the presence of caffeine over σ ranged between 14.4 and 98.1. This means that fluctuations are small compared to overall intensity decrease. These values favor the explanation that most sarcomeres are not synchronized and do contribute only to the intensity decrease while fluctuations reflect clusters of sarcomeres where activation propagates.

Discussion

Calcium ions mediate extracellular signals on intracellular targets. The signalling system based on transient rises or oscillations of the cytoplasmic calcium concentra

tion has potential advantages and can selectively control different processes in the cell. Skeletal muscle cells treated with subthreshold concentrations of caffeine provide a suitable preparation to study mechanisms which generate repetitive calcium transients. Contraction is a natural indicator of intracellular calcium concentration. Based on optical measurements of local contractions of individual sarcomeres we have shown substantial enhancements of spontaneous calcium release in the presence of subthreshold caffeine concentrations. Calcium release propagates to neighbor calcium sources and forms slow contraction waves. Many biological phenomena exhibit, as their most apparent feature, a coherent pattern or waveform that moves in space. An analysis of power spectra density can reveal parameters of the time course of these events. These spectra were measured also by Herrmann-Frank (1989). Our aim was to relate spectra changes with processes at the sarcomere level. We could show cluster formation of activated sarcomeres as detected by the structure of the first diffraction maximum, and its dynamics revealed by an analysis of intensity fluctuation spectra at the diffraction maximum.

Caffeine is known to potentiate twitch tension in skeletal muscle. Caffeine, at higher concentrations, causes an increase in baseline tension or induces large and maintained contractures (Luttgau and Oetliker 1968).

Simon et al. (1989) have found that under control conditions, tubular membrane repolarization appeared to rapidly terminate release even when cytosolic calcium was elevated from a preceding release, indicating that calcium induced calcium release driven by cytosolic calcium was insignificant. However, in fibers exposed to caffeine the release of calcium from the sarcoplasmic reticulum continued well after tubular membrane repolarization, consistent with the appearance of substantial component of Ca induced Ca release.

Fluctuating sarcomere contractions are activated by increasing intracellular calcium concentrations due to the self-regenerative release of Ca^{2+} from intracellular stores, potentiated by caffeine. This activation can propagate to neighbor sarcomeres and form clusters of synchronously contracting sarcomeres or even propagate over longer distances forming slow contracting waves. Then, the calcium concentration begins to rapidly decrease due to sequestration of Ca^{2+} ions into the intracellular stores.

Spontaneous local activations appear under different conditions which favor calcium induced calcium release. For instance, they are initiated either spontaneously or by external stimulation during disruption of tubular system by the glycerol procedure (Zachar et al. 1972, 1973). In this case the fiber is overloaded with calcium from the extracellular space. Slow contraction waves spread along the frog fiber with a velocity of a few mm/s. They start at one or several places and propagate in both directions. If propagated towards each other they cancel.

There are several mechanisms that can lead to oscillations of internal calcium independent of changes in membrane potential. For instance, a repetitive calcium

discharge and reuptake, based on calcium induced calcium release from sarcoplasmic reticulum, is such a possible mechanism (Poledna 1991).

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