

## Analysis of Voltage Fluctuations in the Ventricular Myocardium

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**Abstract.** Spontaneous voltage fluctuations at a constant mean resting potential in small segments of papillary muscles obtained from the right rabbit ventricular myocardium were recorded. The voltage noise was quantified by its variance and its power density spectrum (PDS). Application of verapamil ( $17.6 \times 10^{-6}$  mol/l) decreased the voltage fluctuation of the ventricular preparation. The verapamil-sensitive variance (the difference between control and after application of verapamil) was found to be  $72.15 \times 10^{-10} \pm 37.81 \times 10^{-10} \text{ V}^2$ . The verapamil-sensitive PDS (taken as difference between control PDS and the PDS after application of verapamil) was described by a function  $S_v = S_0 \times (1 + (f \times f_c^{-1})^n)^{-1}$ . A corner frequency  $f_c$  of  $2.78 \pm 0.85 \text{ Hz}$  was obtained.  $n$  was found to be  $3.25 \pm 0.43$ . The results correspond with the assumption of a verapamil-blocked channel with an average open time of 63.6 ms at the resting potential.

**Key words:** Voltage fluctuation — Myocardium — Calcium channel

### Introduction

In excitable membranes fluctuation phenomena have revealed useful information about the mechanisms of opening and closing of membrane ionic channels (Anderson and Stevens 1973; Verveen and De Felice 1974; Neher and Stevens 1977). We have tried to expand the methods of analysing the fluctuations of electrical events to a cardiac multifibre preparation. The investigation of cardiac ionic channels, hitherto, was successful in case of the ACh-induced potassium channel in the mammalian atrial myocardium only (Noma et al. 1979a, b; Osterrieder et al. 1980). The study of the kind presented in this paper is a trial to obtain an approximate description of kinetic property of a single verapamil-blockable channel by means of the analysis of the voltage fluctuations only.

## Material and Methods

### *Preparations. Solutions. Drugs.*

Small preparations from the right rabbit ventricular papillary muscles were obtained by a procedure of tying up and fixing a small segment of the muscle on a perforated piacryl panel. The dimensions of the preparation were 0.2 to 0.5 mm in diameter and 0.7 to 1.3 mm in length (weight  $3.9 \times 10^{-5}$  to  $4.1 \times 10^{-4}$  g). Using a surface-volume ratio of  $0.52 \mu\text{m}^{-1}$  (Bassingthwaighe and Reuter 1972) the total area of the preparations was estimated between 0.27 and 0.81  $\text{cm}^2$ . The preparation fixed on the panel was mounted in a piacryl chamber (volume 0.5  $\text{cm}^3$ ) for electrical measurements and superfused by a solution of the following composition (mmol/l): NaCl 137, KCl 2.7,  $\text{CaCl}_2$  2.5,  $\text{NaHCO}_3$  12,  $\text{NaH}_2\text{PO}_4$  0.4, glucose 11, pH 7.3 (equilibration with 95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ) at a constant temperature of about 30°C (superfusion rate 7 ml/min). To block a Ca conductance verapamil (Knoll AG, Ludwigshafen, BRD) was used in a concentration of  $17.6 \times 10^{-6}$  mol/l.

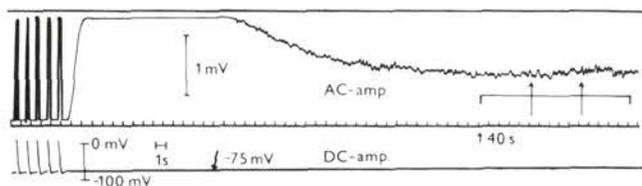
### *Recording system.*

Two micropipettes (tip diameter below 1  $\mu\text{m}$ ) were inserted into the preparation, one for potential measuring, the other for current injecting to stimulate the preparation. Pulling the microelectrodes with suitable geometric properties have to be done with greatest care. We used micropipettes with a very small and short tip and resistances of about 1.5 to 3  $\text{M}\Omega$  only. The potential reading pipette was connected via a follower (Hugo Sachs elektronik, BRD, microelectrode- and clamp amplifier). The voltage was recorded on a pen-recorder both at a DC channel with low amplification and at an AC channel with high amplification. The pen-recorder served as a continuous monitor for the constant mean resting potential. The AC channel output was recorded on a magnetic tape (Philips, Analog 7) for the later calculations of the power spectra.

### *Variance and Power Spectrum.*

The output of the tape recorder was fed into a computer system NTA 1024-EMG 666 (EMG production, Budapest). To diminish the noise generated by the tape, a steep Bessel low-pass filter with a cut-off frequency of 25 Hz (8 dB/octave) was used. Both the variance and the one-sided power spectral density function (using a discrete Fourier transformation) were calculated. For that reason each sample (lasting 5 s) was digitized into 512 points. We have found that the power spectrum generated by the cellular voltage fluctuations (the voltage electrode was inserted into the cell) disappeared in the instrumental noise (voltage reading microelectrode inserted into the bath only) beyond the frequencies higher than 10 Hz. Therefore the upper frequency of the Fourier transform was set to 10 Hz. The sampling rate was chosen to be 9.8 ms. Because of the 5 s lasting sampling the lowest frequency was 0.2 Hz. All the records were inspected by eye before processing to reject all the traces containing artefacts like non-constant DC potentials.

The "cellular" variance and power density spectra (PDS) were obtained by the difference between the values during control or after the application of verapamil ( $17.6 \times 10^{-6}$  mol/l) and the data obtained from the preceding analysis of the instrumental noise (microelectrode inserted into the bath). The "verapamil-sensitive" variances and PDS were taken as the difference between the "cellular" variance and PDS before and after application of verapamil. To present the spectra in the log-log form the obtained values were averaged: Between 0.2 and 0.5 Hz the points were not altered; from 0.5 to 1.0 Hz two points were averaged; from 1 to 2 Hz four points, from 2 to 10 Hz 8 points were averaged to a single value. The rough data were printed out in linear coordinates. For approximation by means of equation (1) three spectra of the same traces were averaged.

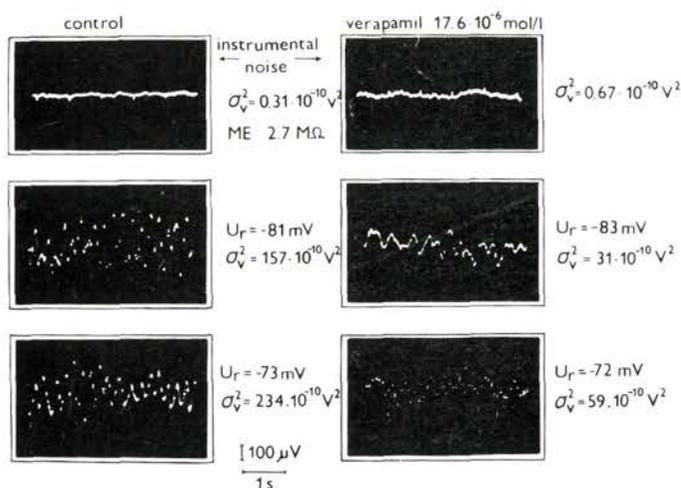


**Fig. 1.** Protocol of an experiment to record the voltage fluctuation on the pen-recorder. The preparation was driven with a rate of 1 Hz. After cessation of the drive the fluctuation of the "resting membrane" potential was detected by a high AC amplification. The arrows mark the sample time for processing.

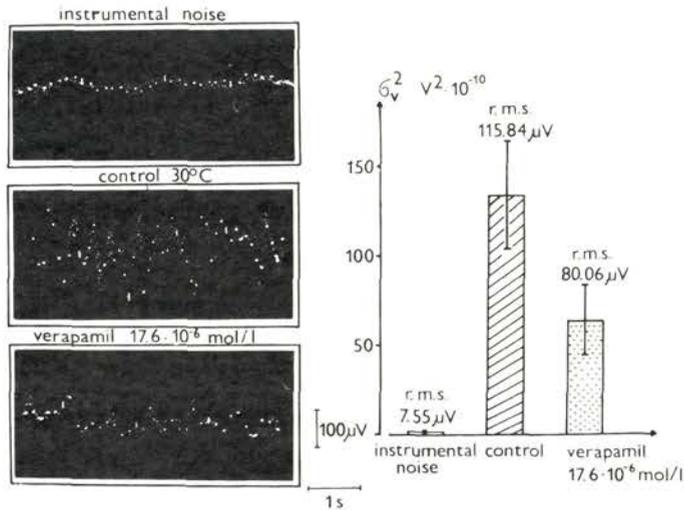
## Results

A typical experiment to study the voltage fluctuations is shown in Fig. 1. After a train of action potentials (pacing rate 1 Hz) the pacing was switched off and the DC potential was constant at a mean level of  $-75$  mV. The high AC amplification detects a large voltage fluctuation. The fluctuation signal was recorded for the calculation of the variance and the power density spectrum (PDS) 40 to 55 s after cessation of the pacing. From this trace a 5 s sample was chosen for processing.

If the micropipette was localized in the bath a very small voltage fluctuation was recorded representing the noise of the microelectrode and the set-up for the registration of the voltage signal. Inserting the voltage reading microelectrode into



**Fig. 2.** Original registrations of the digitalized voltage fluctuation obtained from the NTA 1024. Note the small instrumental noise and the decreased voltage fluctuation after application of  $17.6 \times 10^{-6}$  mol/l verapamil.  $U_r$ : resting potential,  $\sigma_v^2$ : variance of the voltage noise in the demonstrated example, ME: resistance of the used microelectrode for voltage reading.



**Fig. 3.** Analysis of the variances obtained from the voltage fluctuation of 6 experiments (32 single records). r. m. s.: root mean value of the voltage fluctuation in the considered range of the frequencies between 0.2 and 10 Hz.

the ventricular preparation the voltage fluctuation was found to be increased in spite of a nearly unchanged resistance of the microelectrode after the impalement. After application of verapamil ( $17.6 \times 10^{-6}$  mol/l) the voltage fluctuations were decreased. Fig. 2 demonstrates such an experiment: after application of verapamil the variance of the voltage fluctuations at a constant DC potential was found to be decreased. Note that the variance of the instrumental noise was about 0.2 % of the voltage noise measured after the impalement of the preparation. From 6 experiments (32 single records) the variance of the voltage fluctuation at a constant mean DC potential (Resting potential) between  $-73$  and  $-91$  mV are shown in Fig. 3. Verapamil decreased the variance of the voltage fluctuation significantly. The mean value of the "verapamil sensitive" variance (see Table 1) was found to be about  $72 \times 10^{-10}$  V<sup>2</sup> ( $17.6 \times 10^{-6}$  mol/l verapamil).

The spectral analysis of the voltage fluctuations was carried out to obtain some kinetic data for the characterization of a verapamil blockable channel knowing the uncertainty of such a measurement in a multifibre preparation with a lot of noise sources. Fig. 4 shows typical non-averaged rough PDS of the voltage fluctuation demonstrated above in the log-lin presentation. The "cellular" PDS was always obtained after subtracting the spectrum of the instrumental noise (microelectrode outside the cell before the penetration into the cell).

Most of the power was below 5 Hz. In some preparations a "hump" was observed at about 3 to 5 Hz (see Fig. 5). The spectra were characterized by a flat

**Table 1.** Synopsis of all the data obtained from the analysis of the verapamil-sensitive voltage noise from 6 experiments (mean values  $\pm$  standard deviation).  $U$ : resting membrane potential [mV];  $\sigma_v^2$ : variance of the voltage noise blocked by  $17.6 \times 10^{-6}$  mol/l verapamil [ $V^2$ ]. The power density spectra were described by

$$S_v = \frac{S_0}{1 + \left(\frac{f}{f_c}\right)^n}$$

$f_c$ : corner frequency [Hz];  $S_0$ : saturation term of the spectra (see formula (2')) [ $V^2/\text{Hz}$ ];  $\tau$  was obtained by  $\tau = (2\pi f_c)^{-1}$  [ms]

$U$ [mV]	$\sigma_v^2$ [ $V^2$ ]	$f_c$ [Hz]	$\tau$ [ms]	$n$	$S_0$ [ $V^2/\text{Hz}$ ]
-73	$80.8 \times 10^{-10}$	3.60	44.2	3.9	$2.01 \times 10^{-9}$
-81	$60.1 \times 10^{-10}$	1.42	112.1	3.2	$3.58 \times 10^{-9}$
-91	$126.4 \times 10^{-10}$	2.55	62.4	3.6	$4.35 \times 10^{-9}$
-82	$98.1 \times 10^{-10}$	3.33	47.8	2.7	$2.87 \times 10^{-9}$
-80	$47.2 \times 10^{-10}$	3.51	45.4	3.0	$1.11 \times 10^{-9}$
-75	$20.3 \times 10^{-9}$	2.29	69.5	3.1	$7.42 \times 10^{-10}$
mean values	$72.15 \times 10^{-10}$	2.78	63.57	3.25	$2.44 \times 10^{-9}$
$\pm$ standard deviation	$\pm$ $37.81 \times 10^{-10}$	$\pm$ 0.85	$\pm$ 25.87	$\pm$ 0.43	$\pm$ $1.41 \times 10^{-9}$

plateau from low frequencies to the corner frequency and a steep fall at higher frequencies. Therefore we tried to describe the spectra by means of a "Lorentz" like distribution of the power densities

$$S_v = \frac{S_0}{1 + \left(\frac{f}{f_c}\right)^n} \quad (1)$$

( $S_v$ : power density, [ $V^2/\text{Hz}$ ];  $f_c$ : corner frequency, [Hz]). Because of

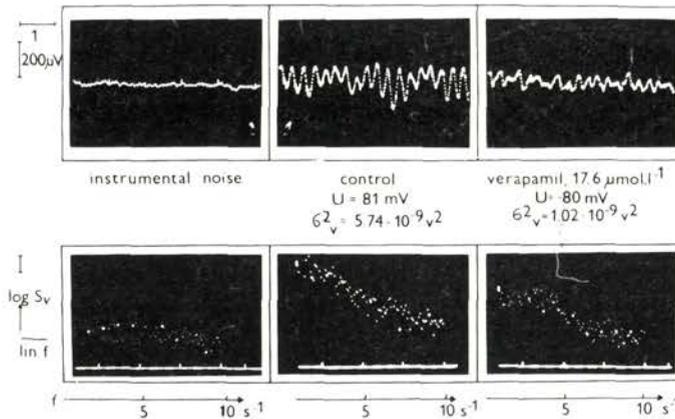
$$\sigma_v^2 = \int_0^\infty S_v(f) df \quad (2)$$

one obtains

$$\sigma_v^2 = \frac{f_c \cdot S_0}{n \cdot \sin \frac{\pi}{n}} \quad (2')$$

(Gröbner and Hofreiter 1961)  $\sigma_v^2$ : variance of the voltage fluctuation during a sample period). (2') was used to calibrate the PDS. By means of this procedure we obtained  $S_0$  for each PDS from the measured variance of the same frame.

In the most cases we found no "Lorentzian" spectra because of higher values

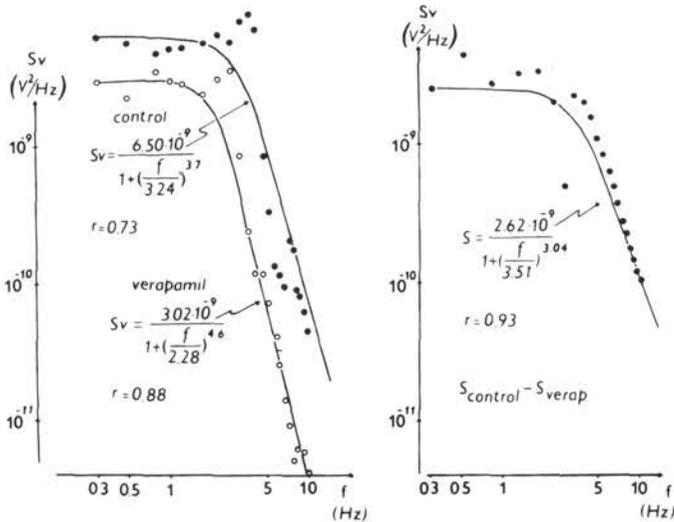


**Fig. 4.** Example of an original registration of the rough power spectra for the control case (the microelectrode penetrates the cell membrane), after application of verapamil and the instrumental noise (the same electrode, but outside the cell in the bath). The spectra are presented in the log-lin form.  $I$  means one decade of the power density  $S_v$ .

of  $n$  than 2. The displayed experiment (used for Table 1) was best fitted by equation (1) using parameters  $f_c$  between 1.3 and 3.5 Hz and  $n$  between 2.6 and 4.6. For each couple of control-PDS and PDS after the application of verapamil the "verapamil-sensitive" PDS were calculated. The corner frequency of the "verapamil-sensitive" PDS was found to be  $2.78 \pm 0.85$  Hz. We evaluated a slope parameter  $n$  for the "verapamil-sensitive" PDS of  $3.25 \pm 0.43$  which was larger than expected from a "Lorentzian" distribution of the power densities. The measure of adjustment  $r$  for all the examples converted into Table 1 was sufficient (between 0.7 and 0.93). Table 1 summarizes all the results describing the "verapamil-sensitive" voltage fluctuation in the ventricular rabbit myocardium.  $\tau$  in Table 1 was obtained from  $\tau = (2\pi f_c)^{-1}$ .

## Discussion

Because of a lot of experimental difficulties (undefined intra- and extracellular noise sources, low-frequent mechanical oscillations (Akselrod et al. 1979), spatial inhomogenities, low-resistant couplings, the uncertainty of the estimation of the noise generating membrane surface area...) the studies of fluctuation phenomena in the heart muscle are rare. We only discuss a voltage noise signal recorded from mechanical complete inactive preparations. This voltage noise was separated from the cellular voltage noise by application of the Ca channel blocker verapamil in a concentration of  $17.6 \times 10^{-6}$  mol/l which blocks the channel completely (Kohlhardt 1972; Kohlhardt and Mnich 1978). The outstanding reproducibility of the



**Fig. 5.** Power density spectra (two spectra are averaged as described in the method) of the verapamil-sensitive voltage noise. left side: control power density spectra (PDS) (●) and the PDS after application of verapamil (○) ( $17.6 \times 10^{-6}$  mol/l). The smooth curves are fitted by the function shown below. right side: difference spectrum from the left characterizing the verapamil-sensitive PDS. The smooth curve is calculated according to the demonstrated formula.  $r$  means the correlation coefficient between the measured and calculated values.

effects of verapamil on the variances and the power density spectra (PDS) of the voltage fluctuations and also the high ratio of the cellular to the instrumental (or background) noise encouraged us to the present analysis. If the noise generated by random opening and closing of the Ca channel is independent from the other noise sources and if verapamil blocks the Ca channel sufficiently selectively, the data characterizing the Ca channel can be obtained by subtracting the variance or PDS before and after application of verapamil. How strong the presumption are valid we cannot decide experimentally.

Table 1. summarizes our data recorded for the verapamil sensitive voltage noise. All the PDS were well fitted by equation (1). The approximation was sufficient ( $r$  between 0.7 and 0.93). However, we never found a real Lorentzian type of power distribution. The slope parameter  $n$  in the log-log presentation was always greater than 2 (mean value  $3.25 \pm 0.43$ ). Therefore the channel kinetics cannot be described by a simple two-state model of opening and closing the membrane channel. In some cases the PDS showed a resonant-like frequency (Fig. 4, 5) at about 3 to 5 Hz. An interaction between sarcolemmal channel and intracellular events as described by Akselrod et al. (1979) may be responsible for

the deviation from the Lorentzian type. Another source of error is the frequency-dependent membrane impedance. Because the membrane acts as a low-pass filter, it is necessary to correct the data obtained from the voltage fluctuation. Taking into account the measurements of Colatsky ((1980), membrane resistance  $R_m = 1.32 \text{ k}\Omega \cdot \text{cm}^2$ , membrane capacity  $c_m = 6.6 \text{ }\mu\text{F} \cdot \text{cm}^{-2}$ ) and

$$S_I = |Z|^{-2} S_V \quad (3)$$

( $S_I$  is the PDS for the current noise,  $Z$  means the membrane impedance  $|Z|^2 = R_m^2 \times (1 + (2\pi R_m c_m f)^2)^{-1}$  (Stevens 1972)) the corrected values for a current fluctuation from Table 1 are  $\tau = 58.52 \text{ ms}$ ,  $f_c = 2.72 \text{ Hz}$  and  $n = 2.80$ . The shape for these corrected PDS is improved and more similar to a Lorentzian distribution of the power spectral densities.

The most reliable measure obtained from the voltage fluctuation was the corner frequency  $f_c$ . If this frequency is related to the relaxation (or mean open) time of the verapamil blocked channel by  $\tau = (2\pi f_c)^{-1}$  the channel kinetic is quite slow ( $\tau$  about 63.6 ms). The value found for the channel's relaxation agrees well with the inactivation time constant of the Ca channel obtained from voltage clamp experiments extrapolated to the resting potential (Beeler and Reuter 1977; Reuter and Scholz 1977). Because of the good reproducibility of the quantification of the voltage fluctuations it seems to be suitable to extend methods for measurements of the current fluctuations to a cardiac multifibre preparation, too.

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