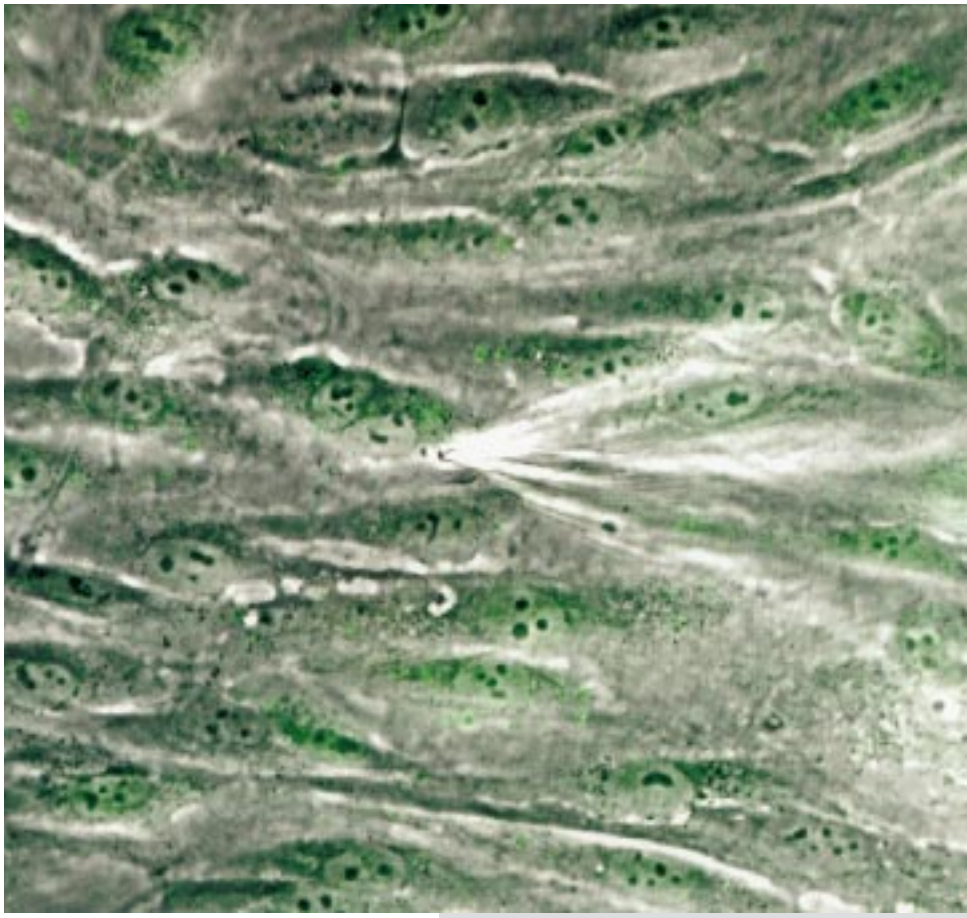
Simultaneous Measurement
Membrane Potential and Ca^{2+} Concentration

The membrane potential plays a central role in the regulation of the intracellular Ca^{2+} concentration. Therefore, many physiological and pharmacological investigations need the simultaneous measurement of these two parameters.
The laser scanning microscope LSM 510 from Carl Zeiss offers the possibility to combine confocal fluorescence detection with electrophysiological investigations.

Background

Voltage dependent L-type Ca^{2+} channels are responsible for the Ca^{2+} release from the sarcoplasmatic reticulum (Ca^{2+} sparks) in heart muscle cells. The electromechanical coupling of heart muscle cells was discovered investigating these processes /1,2/.
In blood vessel endothelial cells the membrane potential regulates the influx of Ca^{2+}. Vasoactive agonists like ATP lead to an increase of the intracellular Ca^{2+}concentration that in turn stimulates the NO synthetase. The NO release results in a vasodilatation.



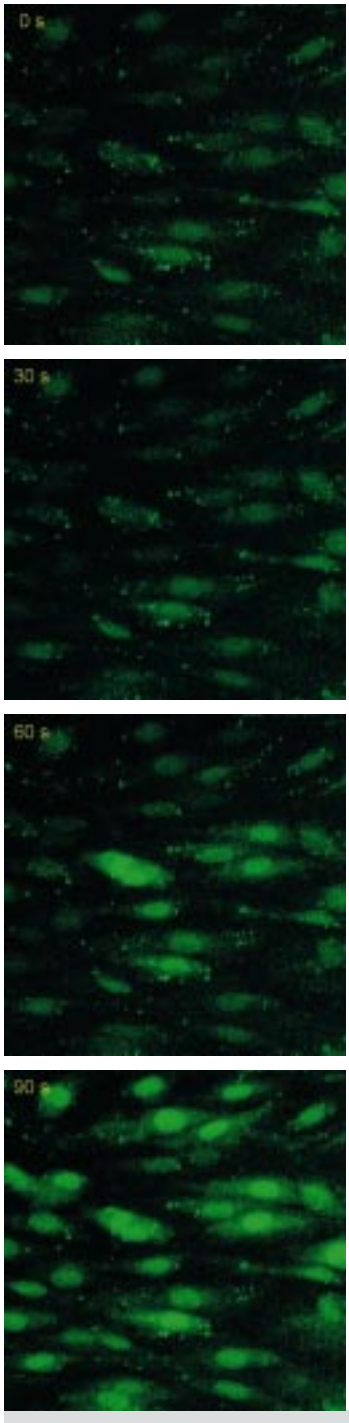
Human heart endothelial cells do also show the correlation between Ca^{2+}concentration and membrane potential which can be detected by simultaneous measurement of these parameters.

Fig. 1:
Patched confluent human heart endothelial cell under investigation with the LSM 510; (Objective: LD Achroplan 40x/0.60 corr. Ph2)

Transmitted light phase contrast image together with fluo-3 fluorescence of non stimulated cells; shadow = tip of patch pipette

Confocal Laser Scanning Microscopy
Electrophysiology using the LSM 510

ZEISS



Method

The experimental setup combines the technique of confocal laser scanning microscopy for Ca^{2+} flux imaging with patch clamp technology for electrophysiological measurements. On an active vibration damped table the inverted microscope stand Axiovert 100 M and a patch-clamp tower (List Electronic, Darmstadt, Germany) were installed. The scanhead was connected to the microscope in a base port position e.g. under the table.

Procedure

Living human fluo-3 stained heart endothelial cells were patched with a micropipette. The positioning of the patch pipette was done in the transmitted light mode of the LSM 510 at low laser light intensity controlled by AOTF (Acousto Optical Tunable Filter) to prevent any bleaching of the fluorescent dye or damage of the cultured cells (fig. 1). The membrane potential of the confluent monolayer of heart endothelial cells was measured by microelectrode and at the same time the fluo-3 fluorescence was imaged indicating the Ca^{2+} concentration (488 nm excitation, LP 505 nm emission filter). The application of the vasoactive agonist ATP during a time series experiment resulted in an increase of the intracellular Ca^{2+} concentration (fig. 2) and a simultaneous depolarisation of the whole monolayer. The mean fluorescence intensity was quantified for the whole monolayer (fig. 3). Mean fluorescence values for up to 99 regions of interest (ROI) can be detected and then evaluated automatically by the optional Physiology/Time Series software. The electrophysiological measurements where not affected by the fully motorised equipment of the LSM 510 detection unit.
Long distance objective lenses (such as LD Achroplan 40x/0.6 corr., 1.8 mm working distance) are very suitable for high resolution images and to insulate the electrophysiological setup completely from the optical system.

Fig. 2:
Time series of fluo-3 fluorescence in heart endothelial cells;
control: t = 0 s, t = 30 s;
change of Ca^{2+} concentration in the presence of ATP: t = 60 s, t = 90 s

S u m m a r y

For physiological investigations a LSM 510 base port configuration is useful. The installation of the scan head below the Axiovert 100 M microscope leads to high optical resolution and signal intensity due to a straight and short optical beam path. This setup also allows the use of the complete work bench for electrophysiological installations (micromanipulation unit) and easy operator access to the complete system. Measurement of the membrane potential by microelectrode and confocal imaging of the intracellular Ca^{2+} concentration by LSM 510 was possible without interference between both methods.

A c k n o w l e d g e m e n t s

The experiments were performed by Dr. B. J. Zünkler and S. Kühne, Federal Institute for Drugs and Medical Devices, Berlin, Germany.
Human heart endothelial cells by courtesy of Dr. Gräfe, German Heart Institute, Berlin, Germany.

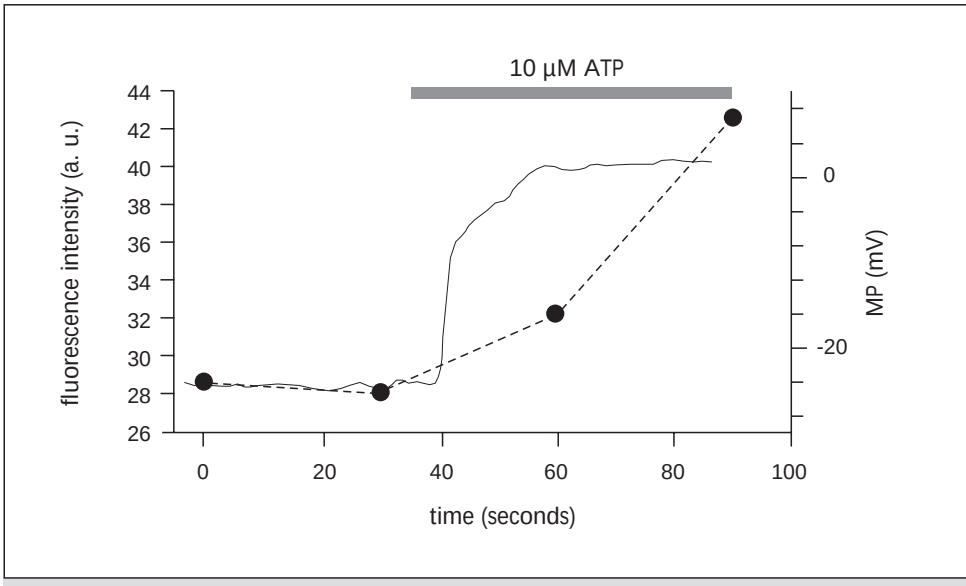


Fig. 3:
Fluo-3 fluorescence (dashed line) and simultaneously measured membrane potential (solid line) of the confluent heart endothelial cells of Fig. 2.

The fluorescence intensity of the whole monolayer is given as a mean fluorescence intensity (left axis).

The membrane potential (right axis) was measured simultaneously in the current-clamp mode of the whole cell configuration.

The application of 10 µM ATP (bar) results in an increase of the fluo-3 fluorescence intensity and in a simultaneous depolarisation from -25mV to +2mV.

Literature

/1/ Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ (1997). Defective excitation-concentration coupling in experimental cardiac hypertrophy and heart failure. Science 276: 800-806

/2/ Shorofsky SR, Izu L, Wier WG, Balke CW (1998). Ca^{2+} sparks triggered by patch depolarization in rat heart cells. Circ. Res. 82: 424-429.

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