DIFFERENT MODES OF FLUORESCENTLY LABELLED PEPTIDERIC VESICLES IN RAT ASTROCYTES

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Introduction. Astrocytes, the most abundant type of glial cells in the central nervous system, play an important role in synaptic transmission and metabolic coupling between neurons and microvessels. Astrocytes store and release many neuroactive substances (1). It appears that intracellular calcium signalling may have an important role in their release, such as in the release of glutamate (2), neuropeptides, such as atrial natriuretic peptide (ANP) (3) and prostaglandins (4). It has been shown that the mechanism of glutamate and ANP release may involve exocytosis (3, 5-7), since these chemical messengers are found in membrane bound vesicles. In astrocytes these vesicles possess several components of SNARE molecules, key elements for vesicular exocytosis (3, 7-9). However, much less is known about the delivery and transport of vesicles to the sites of exocytosis in astrocytes. We studied the characteristics of vesicle mobility in primary cultured rat astrocytes by labelling single vesicles and used dynamic microscopy to analyze vesicle movements.

Methods. Astrocytes were isolated from the cortex of neonatal rats (3 days) (10). Cells were grown in a culture medium composed of Dulbecco's Modified Eagle's Medium (DMEM), 10% Fetal Bovine Serum (FBS), 1 mM Sodium Pyruvate, 2 mM L-glutamine, 25 mg/ml Penicilnine-Streptomycine at 37°C, 8% CO₂. We used green fluorescent protein tagged ANP to label single vesicles (ANP-Emd; 11). DNA encoding ANP-Emd was introduced into cells by lipofection. Cells were observed by an inverted microscope (Zeiss Axiovert 135) equipped with a CCD camera and a water-immersion objective C-Apochromat 63 x / NA 1.2. Excitation wavelength of 470 nm was provided by a monochromator (Polychrome IV, Till Photonics, Gräfelfing, Germany), emission light was collected with the 510-533 nm filter and a dichroic mirror of 500 nm (Omega Optical, Inc., Vermont, USA). Images were recorded every 300 ms, exported into 8-bit TIFF files and analyzed with the single vesicle tracking software (ParticleTR, Celica, Slovenia). Vesicles selected for tracking were from 2-16 pixels large and were tracked over a period of 15 s. From the coordinates of vesicle position we estimated parameters in each frame for all vesicles analyzed: current time, step length, track length, square displacement and velocity.

Results. Trajectories of vesicle movements were performed by fitting a 2D Gaussian curve on the selected pixels. The peak of the 2D Gaussian curve provided x and y coordinates of the vesicle. These coordinates were converted into tracks of vesicle movement by connecting the coordinates in a series of images separated by 300 ms over the period of 15 s, as indicated by the curves associated with labelled vesicles (Figure). The tracks of individual vesicles revealed two types of vesicle mobility (Figure). In one group vesicle tracks remained close to the origin of tracking, as if vesicles moved in a non-directional manner. In the other group the elongated vesicle tracks indicated an apparent directional mobility, as if vesicles travelled in a preferential direction. We estimated the total track length and velocity of vesicle movements. The average length of the vesicle pathways in 15 s was in the range of a few μm with an average velocity of less than 1 μm/s and with maximal velocities up to 3 μm/s, similar to the velocity of transporting
vesicles in neurons. The maximal displacement (a measure for a net displacement of a vesicle) of 35% of vesicles studied (n=300) was more than 1 μm in 15 s. We termed vesicles, which translocated for more than 1 μm to a preferential direction as directional vesicles. In contrast to the non-directional vesicles that attained a maximal displacement of less than μm. By observing time-dependent vesicle displacements from the origin of tracking it was noticed that directional vesicles frequently changed their pattern of mobility in time. In contrast to this, such switching between distinct modes of mobility was at large undetected in vesicles termed non-directional. To understand the mechanism(s) of vesicle mobility a mean-square displacement (MSD) was calculated as a function of time for both groups of vesicles. These results show that free diffusion describes well the mobility of non-directional vesicles. On the other hand, in the group of directional vesicles, which have a much higher mobility than non-directional vesicles, the mechanism appears to be coupled to a mode of rapid directional motion.

Figure: (a), primary cultured rat astrocyte labelled with the fusion protein ANP-Emd, expressing inside vesicles. Vesicles are seen as bright fluorescent dots. (b), trajectories of the movements of the vesicles (white) superimposed to the fluorescent image of the cell. Scale bars: 10 μm. (c), trajectories of the movements of the vesicles (z-axis). White trajectories denote saltatory movement of directional vesicles, grey trajectories denote non-directional vesicles.

References