CALCIUM DIFFUSION IN THE CEREBELLUM OF THE SKATE (RAJA ERINACEA)

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Controversy exists concerning the ability of Ca^{2+} to diffuse freely in the brain extracellular space. Since extracellular concentrations of this ion determine the amount that enters a cell and subsequently performs a wide variety of functions, the issue of Ca^{2+} mobility is important.

We used a modification of our previous methods based on the use of ion--selective microelectrodes (ISM) and the controlled release into the brain of the substance under study (Nicholson and Rice, MDI Bulletin. 25: 54-55, 1986.) Since the iontophoresis of Ca^{2+} from micropipettes is unreliable, we used pressure ejection by compressed gas under the control of an electronic valve (Figure 1). In order to determine the volume of material ejected we added tetramethylammonium (TMA) to the calcium solution in the ejection pipette. The two ions then co-diffused to the measuring ISMs and the resulting concentration-versus-time curves were recorded on a digital oscilloscope and then analyzed with an IBM PC (Nicholson and Phillips, J. Physiol., 321: 225-257, 1981; Nicholson, Brain Res., 333: 325-329, 1985)

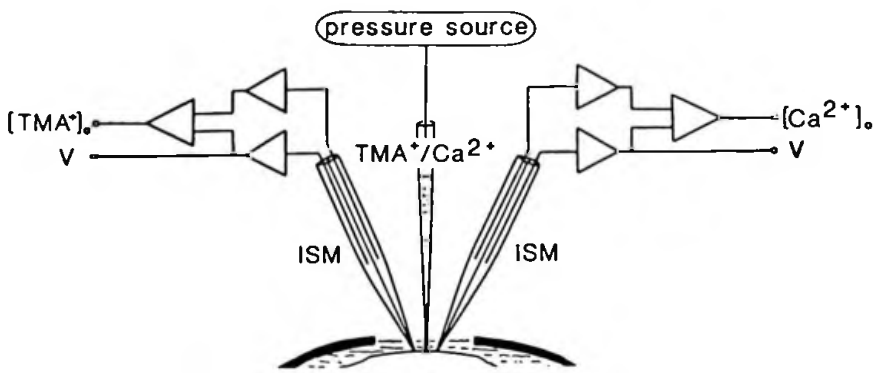

Figure 1. Experimental setup. Two ISMs, one selective for TMA^{+} and the other for Ca^{2+}, were glued on either side of a third micropipette that contained both ions. When a pressure pulse was applied to the central electrode, the ions were released into the brain tissue and diffused to the measuring electrodes. Each ISM consisted of two barrels, one containing the ion-exchanger and the other a reference barrel to monitor local brain potentials. The latter was electronically subtracted from the signal on the ISM barrel.

Measurements were made on skate of approximately 1 kg in weight anesthetized with both pentobarbital and urethane. Our previous results for TMA^+ diffusion in the skate cerebellum indicated a tortuosity of 1.62 and a volume fraction of 0.24 (Nicholson and Rice, MDI Bulletin, 25: 54-55, 1986). Using these parameters as a basis we measured 77 pairs of curves (see Figure 2) and found that calcium exhibited similar values of tortuosity (based on free diffusion coefficient for Ca^{2+} of 5.32 x 10^{-6} x cm^2 x sec^{-1} at 13 °C) and volume fraction to those for TMA^+.

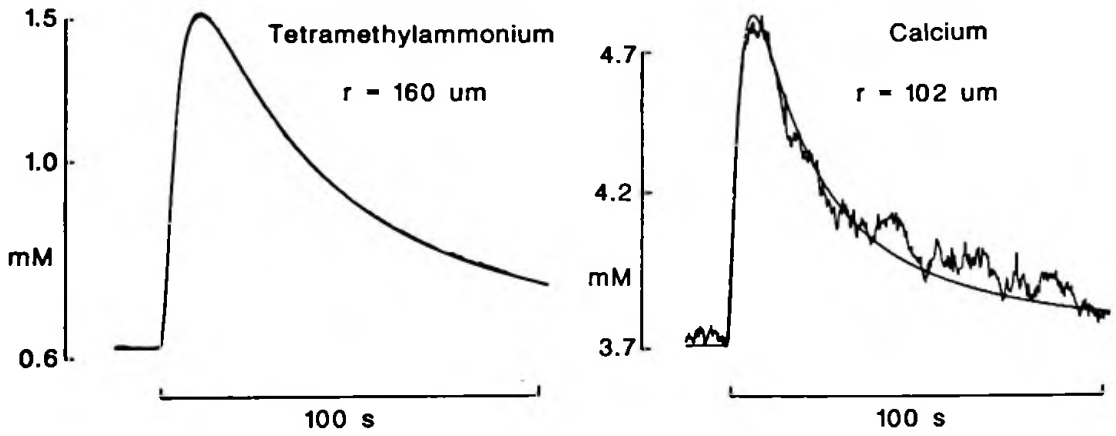

Figure 2. Simultaneously recorded ion concentration curves on two separate ISMs following release of a solution of TMA^+ and Ca^{2+} from a third micro- pipette. TMA-ISM was separated from the source by a distance of 160 um, while the Ca-ISM was 102 um from the source. The pulse of ions was delivered at t= 10s with a duration of 400 ms and an estimated volume of 0.12 nl. The concentration of released TMA was 100 mM and that of the Ca was 30 mM. In this experiment both curves were consistent with values of 0.22 for the volume fraction and 1.6 for the tortuosity.

We conclude that, at least for elevations of Ca^{2+} concentrations above baseline levels, the diffusion of calcium is only determined by its free diffusion coefficient, the volume fraction and tortuosity of the extracellular space. This means that it moves in a similar manner to small monovalent ions.

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