DIFFUSION CHARACTERISTICS OF SKATE (RAJA ERINACEA) CEREBELLUM MEASURED WITH TETRAMETHYLAMMONIUM AND ION-SELECTIVE MICROELECTRODES

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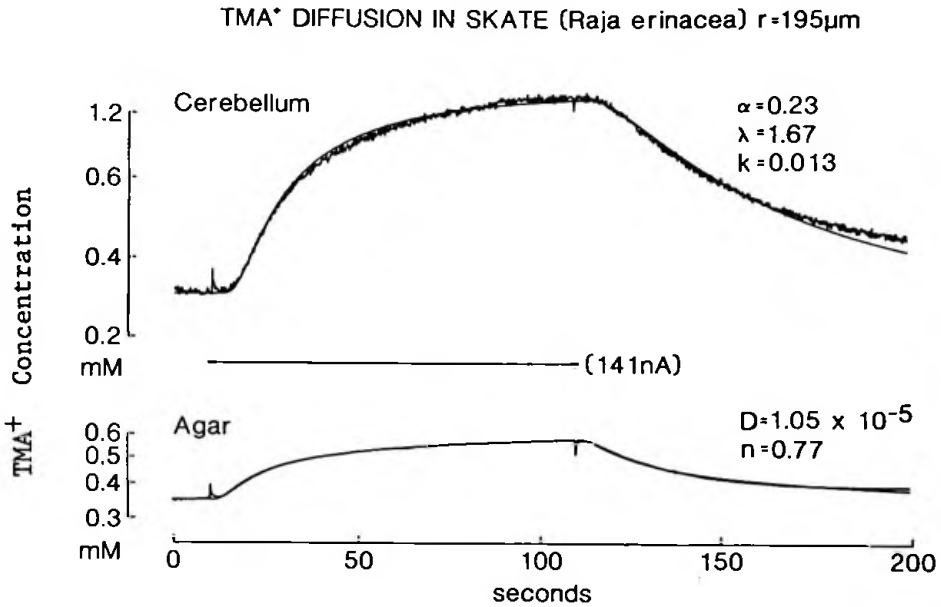
Diffusion of substances in the neuronal microenvironment is crucial for the movement of metabolic substrates and the transmission of informational substances, as well as for the delivery of therapeutic agents. Studies on a variety of neuronal tissues and general theoretical arguments indicate that diffusion in the brain is modified by the volume fraction of the extracellular space and the tortuosity, or increase in path length, imposed by local obstructions. These parameters are typically about 20% for the volume fraction and 1.6 for tortuosity in a variety of neuronal tissues (see Nicholson & Rice, Annals NY Acad Sci, in press).

The brain of the elasmobranch differs significantly from that of most other species. The osmotic strength of the interstitial fluid is approximately 1000 mosmoles and the blood-brain barrier is not formed from the endothelial elements typical of other species (Cserr & Bundgaard Am. J. Physiol. 246: R277-R288, 1984). Furthermore an early study of the brain of Scyliorhinus canicula using morphometry based on electron micrographs suggested a volume fraction of only 5%. Later experiments at MDIBL using radio-labelled sucrose in the brain of Squalus acanthias (Fenstermacher & Patlak 1975. In: Fluid Environment of the Brain. eds. Cserr et al, pp 206-214) indicated volume fractions in the range 16%-22% and a tortuosity of 4.0. In the present study we have re-examined diffusion in the elasmobranch brain using a relatively recent method involving the iontophoresis of tetramethylammonium (TMA^+) and its detection with ion-selective microelectrodes a few hundred micrometers away.

Skates were anesthetized with sodium pentobarbital (30mg/kg), respirated by forcing running sea water through the spiracles and the cerebellum exposed. Artificial extra dural fluid (NaCl: 288 mM, KCl: 6 mM, CaCl: 5 mM, MgCl: 3 mM, $NaHCO_3$: 8 mM, urea: 350 mM) to which was added 0.2 mM TMA chloride as a reference concentration for the ISM, was continuously flowed over the brain. The diffusion measurements followed the general scheme described elsewhere (Nicholson & Phillips, J. Physiol. 321: 225-257, 1981). ISMs were fabricated from single barrel capillaries using Corning exchanger 477317. Separate reference and iontophoresis electrodes were glued to the ISM. The iontophoretic electrode contained 150 mM TMA chloride and the shank was bent so that it could be mounted parallel to the ISM at 100-300 micrometers away. No potentials were generated in the tissue by the iontophoresis so the purpose of the reference electrode was to subtract out noise. A continuous forward bias current was applied to the iontophoretic electrode and, for measurements, the current was stepped by about 150 nA. The resulting signals were recorded on a Nicolet 3091 digital oscilloscope and transferred via a serial interface to an Apple II computer equipped with an accelerator. This computer performed data storage, curve fitting and plotting on an HP7475A digital plotter. Control measurements were performed in 0.3% agar gel with similar ionic composition to that of the brain.



Measurements were made at depths 500 - 1000 micrometers below the surface of the cerebellum and gave results similar to those depicted in the Figure. The volume fraction and tortuosity were extracted by curve-fitting over both the rising and falling portions of the curve. A theoretical curve was generated from these parameters and superimposed on the experimental data. Curves were only accepted where the fit was good on both rising and falling phases. Similar measurements in the agar gel gave curves like that shown in the Figure and enabled the transport number of the iontophoretic electrode to be calculated (see Nicholson & Phillips 1981). As seen in previous studies there was considerable 'amplification' of the diffusion signal in the brain due to the combination of the volume fraction and tortuosity factors. Analysis of 104 curves revealed a value of 0.24 ± 0.02 (mean $\pm$ SEM) for the volume fraction and 1.62 ± 0.02 for tortuosity. These values implied that the mean volume of the extracellular space in the skate cerebellum was 24% and the apparent diffusion coefficient, relative to a free medium, was reduced by a factor of 2.62 (the square of the tortuosity). A somewhat unusual finding, compared to studies in other cerebella, was that most curves fitted better when some concentration-dependent uptake was present ($k=0.0067 \pm 0.0006\ s^{-1}$).

In six experiments, skates were injected with hyperosmotic NaCl (see report by Cserr et al, this volume) and volume fraction and tortuosity determined every 10 minutes for the following hour according to the above paradigm. With the accuracy available we were not able to detect any consistent changes in the extracellular parameters following the injection. This is in accord with the concept that injection of sodium does not perturb extracellular space volume significantly, however these data are preliminary.

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