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Diffusion from an iontophoretic point source in the brain: role of tortuosity and volume fraction

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It is commonly assumed[3,7,9] that the solution to the diffusion equation with point source, derived from the equivalent heat problem[2], describes ion migration in the brain. We show both theoretically and experimentally that the above solution must be generalized for iontophoresis of ions which are confined to the extracellular space of the brain. We further show that K^+ behaves anomalously and therefore probably migrates by transcellular routes.

To interpret diffusion in a locally inhomogeneous medium, two effects have to be explicitly allowed for: tortuosity and volume fraction. Tortuosity is related to the increase in path length which a diffusing particle encounters in a complex medium. In principle this can occur without diminution in the volume accessible to the particle, for example by the imposition of infinitely thin baffles in the medium. Tortuosity may be formally represented by a factor λ such that the diffusion coefficient in the complex medium, D*, is related to the value in free solution, D, by $D^* = D/\lambda^2$ (ref. 15). Volume fraction relates to the fact that, given a source emitting particles at a fixed rate, the increase of particle density at any point in the accessible volume of a complex medium will be inversely proportional to the available volume fraction, α, at that point. This will effectively increase a source of strength Q, in free solution, to Q/α in the complex medium. This effect can be independent of tortuosity if, for example, the available volume takes the form of radial channels in the case of a point source.

The commonly used diffusion formula[3,7,9] accommodates the tortuosity as a reduced diffusion constant, D*, but does not incorporate the volume fraction α. The factor should be taken into account by dividing the source by α. Thus the diffusion equation solution for a point source becomes:

$$\triangle C\,(r, t) = (Q/4\pi D^* \, r \, \alpha)\ \mathrm{erfc}\ (r/2\sqrt{D^* t}) \qquad (1)$$

where r is the distance from the source, t time, and erfc the complementary error function. In the case of an iontophoretic point source $Q = In/F$, where I is the applied current, n the transport number for the ion and source being used and F the Faraday.

The source is switched on at time t = 0. Electrophoretic effects within the medium can be neglected[3,9,12].

The accuracy of Equation 1 was tested by iontophoresing tetraethylammonium ions (TEA^+) from a micropipette and detecting the ions at a fixed distance (35–200 μm) away with an ion-selective micropipette (ISM).

ISMs were prepared from theta-capillary tubing[11]. Corning 477317 resin was used as ion-exchanger for TEA^+ ISMs because this compound, although designated as a K^+-exchanger, is primarily a tetraalkyl ammonium sensor[5,10].

It was necessary[5] to back-fill the ion-selective barrel of the ISM with TEA chloride (150 mM) to avoid non-Nernstian behavior[10]. Interference from the 3 mM K^+ and 160 mM Na^+ in the physiological saline was equivalent to less than 0.03 mM TEA^+, as determined during the ISM calibration at the end of each experiment. The ISM was glued to a micropipette containing 150 mM TEA chloride and iontophoresis was accomplished with an isolated constant current source[8]. To obtain reliable iontophoresis and avoid the transport number changes reported by Dionne[5], it was necessary to apply a small bias current which ejected the ion at all times. This accounts for the baseline TEA^+ values. This does not affect the validity of Equation 1, as the response to a current step applied on top of the bias value.

Sprague–Dawley rats were anesthetized with urethane (160 mg/kg), the posterior cerebellum exposed and superfused with warmed Ringer solution[11]. The electrode array was lowered into the cerebellar cortex to depths of 200–600 μm, indicated by locally evoked field potentials. TEA^+ was ejected from the source electrode and the response of the ISM recorded on a chart recorder. At the conclusion of the experiment, a thick layer of agar (0.3 % in Ringer) was gelled over the brain, the array was withdrawn into it and the diffusion measurements repeated. This provided a check of the free diffusion value for the ion (taking $\alpha = 1$; $\lambda = 1$ for agar) and enabled the source electrode transport number, n, to be estimated. Finally the ISM was calibrated in an appropriate series of flowing solutions[11]. Values of D* in the brain were determined by fitting the complementary error function to the curves with linear regression analysis. Graphs of $\triangle C/\lambda^2$ against $\triangle C_a\,(r, \infty)$ erfc $(r\lambda / 2\sqrt{Dt})$ enabled values of α to be estimated. $\triangle C_a\,(r, \infty) = In/4\pi FDr$ is the asymptotic value of $\triangle C$ in agar.

The results of a TEA^+ experiment are shown in Fig. 1A. During passage of the iontophoretic pulse, in both brain and agar, the ISM recorded an S-shaped rise in TEA^+ which was followed, at the end of the pulse, by a falling phase with similar characteristics. The response in agar was faster and had a smaller amplitude than that in the brain. When the data was calibrated and plotted in accordance with Equation 1, excellent linear fits were obtained (Fig. 2). The slopes of the graphs in Fig. 2 are inversely proportional to the volume fraction α. It is seen from Fig. 2 that the percentage volume fraction for the cerebellum is less than 100 % and, in fact, falls in the range 8–29 % (α = 0.08–0.29) from the TEA^+ experiments shown. This is consistent with other studies of the volume of extracellular space in the nervous system[13,17]. The diffusion constants determined from the curve fitting averaged $9.8 \pm 0.5 \times 10^{-6}$ sq. cm/sec (Mean ± S.E.M. for data used in Fig. 2) for the free diffusion of TEA^+, a value

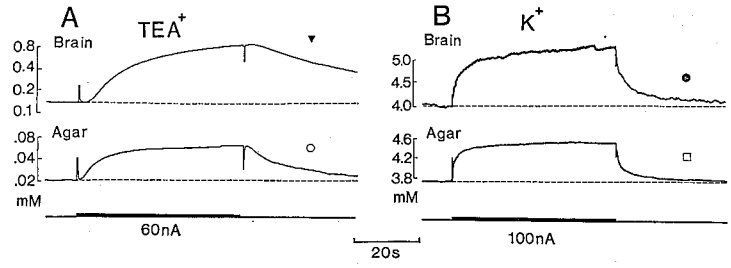

Fig. 1. Iontophoretically induced increases in $[TEA^+]_o$ and $[K^+]_o$. A: upper signal shows rise in $[TEA^+]_o$ at a depth of 250 μm in the cerebellum produced by a 50 sec step of 60 nA in the iontophoretic current. ISM and source micropipette tips separated by 122 μm. Lower signal shows $[TEA^+]_o$ rise in 0.3% agar in Ringer gel with same electrode array and current pulse. B: upper signal shows rise in $[K^+]_o$ at a depth of 300 μm in the cerebellum produced by 50 sec step of 100 nA in the iontophoretic current. ISM and source micropipette tips separated by 42 μm. Lower signal shows $[K^+]_o$ rise in agar gel with same electrode array and current pulse. Note that K^+ signal is faster than TEA^+ signal because of smaller source–ISM separation.

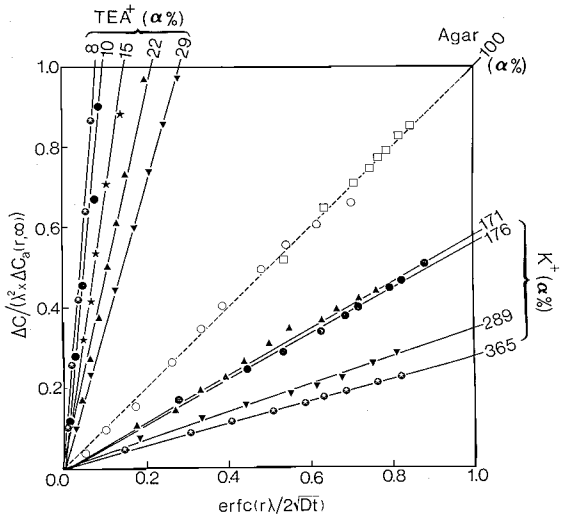

Fig. 2. Comparison of apparent volume fractions revealed by TEA^+ and K^+ iontophoresis. For 5 TEA^+ and 4 K^+ experiments (8 animals, several trials in each) the parameters D, λ, n, α and $C_a(r, \infty)$ were obtained. A plot of $\triangle C/\lambda^2 \triangle C_a(r, \infty)$ against $\text{erfc}(r\lambda/2\sqrt{Dt})$ then gives lines the slope of which is $1/\alpha$, the reciprocal volume fraction. The equivalent values of α as a percentage are indicated at the ends of linear regression lines. For the actual curve fitting between 10 and 20 points were selected along the rising diffusion curves, such as those depicted in Fig. 1 and a function of the form $A\,\text{erfc}(\beta/2\sqrt{t})$, where A and β were undetermined constants, fitted with zero intercept, using linear regression. For all the brain and agar data shown in this figure the correlation coefficient was greater than 0.99 for the fitting of this function. TEA^+ results cluster in the range 8–29%. K^+ results cluster in the range 171–365%. These latter values are physically impossible, since they exceed 100%. Different symbols indicate different experiments; data shown in Fig. 1 depicted by symbols indicated in that Figure. All agar points lie close to $\alpha = 100\%$; only points corresponding to experiments in Fig. 1 are shown for agar.

close to that determined from the limiting conductivity [(9×10^{-6} sq.cm/sec) (ref. 4)], and $3.7 \pm 0.4 \times 10^{-6}$ sq.cm/sec for the apparent diffusion constant in the brain. Thus the tortuosity, λ, was 1.6 ± 0.1, a value similar to that obtained for labeled extracellular markers[15]. Transport numbers, n, for the source-electrode fell in the range 0.26 ± 0.06. The theoretical transport number for TEA chloride is 0.3, as estimated from the limiting conductivity[16]. Note that if α is not incorporated into Equation 1, the apparent transport number in brain would average 1.6, an impossible value. Confirmation that TEA^+ remained extracellular was obtained in other experiments where we superfused the brain with 10 mM of TEA^+ in Ringer solution for several hours and saw no change in locally evoked field potentials; had the TEA entered neurons a blockage of some of the K^+ conductance in presynaptic axons would have been expected[1].

These results show that both the tortuosity, λ, and the volume fraction, α, must be taken into account when applying quantitative arguments to iontophoresis in the brain. When these factors are used, the migration of ions accurately obeys classical Fickian diffusion. The diffusing ions must remain extracellular, however; the consequences of apparently non-extracellular migration are illustrated in experiments with K^+.

Similar experiments to those outlined above were carried out using K^+ ions and K^+-ISMs[11]. Apparently similar responses were seen (Fig. 1B) which were fitted well by a complementary error function and a tortuosity of 1.9 ± 0.2. The volume fraction, however, exceeded 100% (Fig. 2) and application of the *F*-test (analysis of variance) to the populations of α values for TEA^+ and K^+ showed that the difference was significant with $P < 0.001$. The α values for K^+ are obviously physically impossible and showed that Fickian diffusion was not sufficient to explain K^+ migration in the brain. This anomaly may be due to transcellular K^+ movement[6] or uptake by cells[14]. Similar experiments by Lux and Neher (9) were interpreted to mean that K^+ moved primarily by extracellular diffusion. This disagreement with our interpretation may be resolved by noting that Lux and Neher did not incorporate the volume fraction, α, into their analysis.

We conclude that the combination of iontophoresis and ISM for extracellular ions can provide a rapid and localized method of measuring the tortuosity and extracellular volume of the brain.

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