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Mass transport at rotating disk electrodes: Effects of synthetic particles and nerve endings

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ABSTRACT

An unstirred layer (USL) exists at the interface of solids with solutions. Thus, the particles in brain tissue preparations possess a USL as well as at the surface of a rotating disk electrode (RDE) used to measure chemical fluxes. Time constraints for observing biological kinetics based on estimated thicknesses of USLs at the membrane surface in real samples of nerve endings were estimated. Liposomes, silica, and Sephadex were used separately to model the tissue preparation particles. Within a solution stirred by the RDE, both diffusion and hydrodynamic boundary layers are formed. It was observed that the number and size of particles decreased the following: the apparent diffusion coefficient excluding Sephadex, boundary layer thicknesses excluding silica, sensitivity excluding diluted liposomes (in agreement with results from other laboratories), limiting current potentially due to an increase in the path distance, and mixing time. They have no effect on the detection limit (6 ± 2 nM). The RDE kinetically resolves transmembrane transport with a timing of approximately 30 ms.

Rotating disk electrode (RDE)[1] voltammetry has been used in a variety of biological applications such as enzymology [1–4], neurochemistry, and neuropharmacology of neurotransmitters [5,6]. In our laboratory, the RDE has been used for the study of the kinetic mechanism(s) of the dopamine transporter (DAT) [7,8], the kinetics of dopamine (DA) depolarization stimulated release by K^+ and coupled reuptake by the DAT [9], the study of the binding chemistry of DA [10], structure activity studies of the transport of DA [10], kinetic mechanisms of DA transport in human DAT expressed in human embryonic kidney cells (HEK hDAT) [11], and the function of the DAT in the presence of cocaine and after withdrawal from the repeated administration of cocaine or methamphetamine [7,12–14].

RDE voltammetric experiments in these studies involve measurements of membrane fluxes in homogenized brain tissue or synaptosomes in physiological buffer [6,7]. Tissue homogenates contain synaptosomes, other particles, and inclusions with diameters ranging from 0.2 μm for nerve endings to 100 μm for some observed inclusions [15]. The interface between a particle and buffer solution has an unstirred layer (USL), a region of stationary fluid where convection is not significant. Thus, solutes encounter the USL and move through this region by diffusion [16], a process slower than convection. This "barrier" produces errors in estimation of transport rates, thereby affecting apparent values of V_{max} and K_m [16]. A number of techniques have been used to evaluate USLs at biological membranes [17,18], and the results have been reviewed [16,19,20]. Planar membranes have USL thicknesses from approximately 300 μm without convection [16] to 100 μm with stirring. Other USL thicknesses range from 0.6 to 800 μm depending on the nature of the material and stirring rate [16]. The diffusion time through the USLs represents a limit for resolution of kinetic measurements.

Electrolysis at the surface of still solid electrodes in quiescent solutions produces a diffusion-controlled concentration gradient [21]. In contrast, at an RDE, the signal is controlled by both convection and diffusion [21]. Here analyte in bulk solution is brought by laminar flow to the electrode from a field perpendicular to the electrode surface and spun away horizontally in a radial pattern across the electrode surface (Fig. 1A). Under these conditions and with the application of a sufficient potential, a maximal oxidative or reductive current (limiting current, i_L) is observed and both diffusion boundary (δ) and hydrodynamic boundary (δ_0) layers are formed (Fig. 1B). Thus, there are USLs at the electrode itself, perhaps affecting temporal resolution. The δ_0 is the thickness of the velocity gradient from the electrode surface where the closest layers are unstirred to the bulk solution [21], and the δ represents the concentration gradient of the electroactive analyte adjacent to the

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[1] *Abbreviations used:* RDE, rotating disk electrode; DAT, dopamine transporter; DA, dopamine; HEK hDAT, human DAT expressed in human embryonic kidney cells; USL, unstirred layer; i_L, limiting current; δ, diffusion boundary; δ_0, hydrodynamic boundary; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine.

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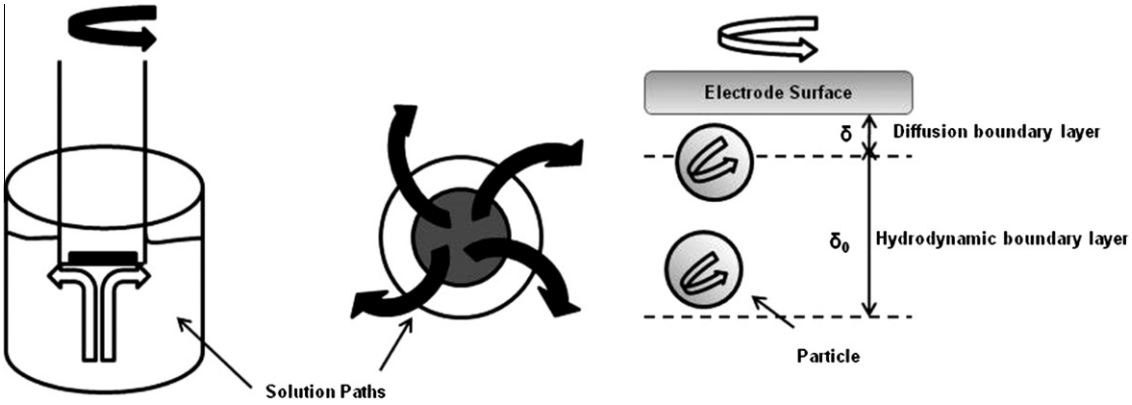

Fig.1. Schematic diagram of the solution paths and different boundary layers at the surface of an RDE. Panel A illustrates conditions of laminar flow. It shows fluid flow from a field perpendicular to the electrode surface and the radial paths of fluid flow across the disk surface. Panel B shows the boundary layers at the electrode surface. Particles may spin or rotate within the layers. δ, thickness of the diffusion boundary layer; δ_0, thickness of the hydrodynamic boundary layer.

electrode [21]. The velocity of the solution and the concentration of the electroactive substance in the region closest to the electrode surface is zero. The thickness of δ is several times smaller than the thickness of δ_0, and the thickness of δ_0 can be reduced by increasing the rotation rate that enhances the i_L [21].

Particles in the solution can affect the i_L at RDEs depending on their size and number [22–25]. Particles will rotate while the solution is stirring (Fig. 1B), increasing the rate of mass transport. Previous work studied the effect of different types of inert particles, ranging from 0.3 to 40 μm in diameter, on the i_L in large volumes (100s–800s of ml) of solution, including Soda Lime glass beads [24], SiC [23,25], Al_2O_3 [23], B_4C [23], and red blood cells [22]. De Ficquelmont-Loizos and coworkers [23] found that the i_L is reduced when the particle size is below the δ. Sonneveld and coworkers [25] found that the i_L is increased when the number and the rotation rate are increased in the presence of SiC particles at the RDE.

Our laboratory uses 200- to 1600-fold smaller volumes in containers where the walls are within 1.0 mm of the shaft and disk surface of the RDE. Convective flow as described above for an RDE requires "infinite" distance between the RDE and the walls of the solution container. It has been shown that at a fixed value of analyte concentration, the i_L is independent of the volume of the solution and the size of containers greater than 3.5 cm^3 [26,27] and depends on the rotation rate. Volumes less than 3.5 cm^3 have not been fully tested.

Because it is unknown whether the preparation of the brain tissue and the nerve endings in the physiological buffer will affect the mass transport, it is important to model the effect of inert particles to mimic the nerve endings. In addition, it is important to determine whether the USL in real samples of nerve endings affects the kinetic measurements.

The goals of this work were to (i) determine how i_L is affected by numbers and sizes of particles in the low-volume chamber, (ii) determine whether the sensitivity and detection limit are affected by the numbers and sizes of particles, (iii) determine how temporal resolution is affected by particles, (iv) estimate the thickness of the USL at membrane surfaces in real samples of nerve endings, and (v) estimate a temporal limit for measurements of kinetics in preparations of nerve tissue.

Materials and methods

Instrumental setup

The setup for the RDE experiments has been described previously [5]. The 3-mm-diameter glassy carbon electrode and MSRX precision rotator were obtained from Pine Instrument (Grove City, PA, USA). The reference electrode was AgCl-coated Ag, and the auxiliary electrode was a Pt wire. LC-4A or LC-4C potentiostats (Bioanalytical Systems, West Lafayette, IN, USA) were used to apply a potential of +450 mV and to measure the current. Data were recorded on a Nicolet 310 digital oscilloscope (Nicolet Instrument, Madison, WI, USA) set at 20 ms resolution. The custom-made (details provided on request) Pyrex glass incubation chamber has an inner diameter of 1 cm and a depth of 1.5 cm, containing a volume of 500 μl of physiological buffer.

Chemicals and materials

The reagents and composition of the pH 7.4 bicarbonate-based physiological buffer have been described previously [5]. Solutions were made in purified water from a Barnstead Nanopure water purification system (Dubuque, IA, USA). The particles and their diameters were 3 and 10 μm silica (Rainin, Woburn, MA, USA) and 40–300 μm Sephadex (Sigma, St. Louis, MO, USA). Dopamine hydrochloride and (−)-cocaine hydrochloride were also obtained from Sigma.

Liposomes

Liposomes (0.03–0.1 μm diameter) were made according to the methods of Davis and coworkers [28] by first making a 15-mM solution of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipids (Avanti Polar Lipids, Alabaster, AL, USA) in $CHCl_3$ (Fisher Scientific, Pittsburgh, PA, USA). Then 4 μmol of DMPC from the solution above was transferred to 2.1 ml of $CHCl_3$ in a round-bottom tube and dried under an N_2 stream for 2 h. This produced a lipid cake on the walls of the round-bottom tube. After the lipid cake was dried, it was fully dissolved in 1.0 ml of physiological buffer and sonicated (VWR Scientific model 50 Aquasonic) at 60 °C until the solution was translucent. The liposomes were transferred to an Eppendorf tube and stored at 4 °C for up to 1 week.

Animals

Male Sprague–Dawley rats (350–450 g) were obtained from the Washington State University internal breeding colony, housed 2–3 per cage in a university vivarium with a 12-h light/dark cycle, and maintained at 22–24 °C, and they had ad libitum access to food and water. The rat procedures were in strict accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the university laboratory animal care and use committee.