A Test of K⁺-Sensitive Electrode Responses to Changes in K⁺ Concentration

Tsuneo Tomita, Osamu Tamura,* Machiko Abe,* and Kiyoshi Shimbo

Department of Physiology, School of Medicine, St. Marianna University, Kawasaki, 213 Japan
*Department of Ophthalmology, School of Medicine, Ehime University, Ehime, 791-02 Japan

Summary Tests of K⁺-sensitive microelectrodes were made using a plastic chamber, which consisted of upper and lower compartments separated by a partition with a small opening in the center. After the upper and lower compartments were filled with 25 and 100 mm KCl respectively, the tip of a K⁺-sensitive electrode was moved up and down through the partition opening, while recording potential changes. Some hydrodynamic events associated with electrode movement and effects of gravity upon spherical diffusion are discussed.

Key words: density gradient, diffusion, K⁺-sensitive electrode.

In 1987, one of the authors (T.T.) visited the laboratory of Hanitzsch in Leipzig and observed her experiment in the isolated rabbit retina. The experiment appeared to show that the slow PIII preceded by several seconds the light-induced decrease in extracellular K⁺ concentration around photoreceptors (Hanitzsch, 1988). This result would contradict the hypothesis that the slow PIII represents a Müller cell response to changes in light-induced extracellular K⁺ concentration (Ripps and Witkovsky, 1985). At the same time, it was thought possible that the apparent slowness of the light-induced K⁺ concentration changes might result from sluggishness in the response of K⁺ electrodes. As a test of this possibility, the response of K⁺ electrodes was measured while quickly moving their tips between solutions of different K⁺ concentrations in a chamber shown schematically in Fig. 1A. The test showed that the response of K⁺ electrodes to changes in K⁺ concentration was fast enough to exclude the above possibility. In this paper we deal with two unexpected observations during recording of the response of K⁺ electrodes to changes in K⁺ concentration.

The recording chamber and electrode arrangement used are shown

Received for publication July 23, 1990
schematically in Fig. 1A. A cylindrical plastic chamber, approximately 2 cm in height and 2 cm in diameter, had a partition with an opening 2 mm in diameter in the center. The lower compartment was filled with 100 mM KCl, and the upper compartment with 25 mM KCl.

A single-barreled K⁺ electrode was used. It contained K⁺-selective liquid-ion exchanger (Corning, #477317) at the tip, and 100 mM KCl filling the remaining entire length. A chlorided silver wire, set at the bottom of the lower compartment, served as the reference electrode. The pair of the electrodes were fed into a probe of high input impedance (Nihon Kohden, MEZ-7101), and the output, once converted into digital data with a signal analyzer (Nihon Kohden, ATAC-350), was introduced into a computer (Nihon Denki, PC-9801VM21). Conversion from the measured K⁺ potential, \( E \), to relative K⁺ concentration, \( C \), was made using the expression,

\[
C = e^{E/a},
\]

where \( a \) is constant. This expression was derived from the Nernst equation.

The up-and-down movement of the K⁺ electrode through the partition opening was controlled by a stepping motor connected to an oil pressure microdriver.
A TEST OF K⁺-SENSITIVE ELECTRODES

(Narishige, MO-81). This microdriver allowed the electrode tip to be moved at various speeds over a total distance of 10 mm, i.e., ±5 mm from the level of the partition opening.

The theoretical analysis of spherical diffusion of K⁺ ions through the partition opening was primarily made by numerical, step-by-step computation. We have confirmed that the results obtained are in good agreement with analytical solutions of differential equations for spherical diffusion by K. Yonemitsu (personal communication).

Figure 1B shows computer-generated lines of spherical K⁺ diffusion through the partition opening from the lower compartment (100 mM KCl) to the upper (25 mM KCl), obtained under the conditions that (1) gravity is free, (2) the compartments are large enough compared to diffusion range, and (3) diffusion constant of KCl as $D = 200 \times 10^{-7}$ cm²/s. The lines plot the K⁺ concentration as a function of distance from the partition opening at 5, 10, 15, and 20 min after addition of K⁺ solutions. To simplify the calculation of spherical diffusion, the 2 mm partition-opening was assumed to be a sphere of 1 mm radius and this sphere to be the source of K⁺ at a constant concentration of 62.5 mM (the mean of the K⁺ concentrations initially added to the two chamber compartments). The zero point

![Graph showing relative K⁺ concentration vs. electrode position](image)

Fig. 2. Two complete recordings of relative K⁺ concentration with a K⁺ electrode traveling at a rate of 0.3 mm/s across the partition opening in Fig. 1A. The two tracings obtained at 10 and 120 min after the start were superimposed photographically. A downward-pointing arrow is positioned next to tracings obtained during electrode withdrawal from 100 to 25 mM KCl, while the phase of electrode advancement is shown by an arrow pointing up.
on the abscissa corresponds to a point 1 mm away from the sphere's center. For comparison, a line of one-dimensional $K^+$ diffusion at 20 min is also shown in Fig. 1B, where the line plots theoretical KCl diffusion at the state wherein the partition between the two compartments has been removed.

Figure 2 shows relative $K^+$ concentrations ($C$ in Eq. (1)) measured during two passes of a $K^+$ electrode through the recording chamber. The electrode passes were made 10 and 120 min after the chamber compartments were filled with their respective solutions. For each measurement, the electrode tip was withdrawn at a rate of 0.3 mm/s from a depth of 5 mm in the 100 mM KCl-containing compartment (+5 to 0 mm in Fig. 2), to a level of 5 mm in the 25 mM KCl-containing compartment (0 to −5 mm), and then advanced back into the 100 mM KCl compartment. A complete electrode passage took slightly more than 1 min.

Figure 2 reveals an unexpected result, that is, a hysteresis-like loop in each tracing. We carefully checked the microdriver and found no mechanical backlash. We next suspected that when a $K^+$ electrode is advancing with its tip ahead, the tip would be in contact with solution whose $K^+$ concentration is less disturbed by the advancing $K^+$ electrode, but as the electrode is being withdrawn, higher-$K^+$ solution in the lower compartment would eddy and form a stream of higher-$K^+$ solution to fill the space left behind the withdrawing electrode. This would tend to produce an apparent lag in the response of the $K^+$ electrode to the surrounding $K^+$ concentration.

![Fig. 3. Effects of temporarily halting electrode movement for 1 min during electrode withdrawal and advancement. Explanation in the text.](image-url)
Figure 3 supports the above suspicion. During one complete up-and-down passage of the electrode, electrode movement was halted for 1 min during the up (withdrawing) and for another minute during the down (advancing) phases. The pause during electrode withdrawal (left side) resulted in a change in $K^+$ concentration reading which was far larger than that produced by the pause during electrode advancement (right side). The above results strongly suggest that the unexpected hysteresis-like loop in Fig. 2 was not due to sluggish responses of the $K^+$ electrode to $K^+$ changes, but instead was a manifestation of hydrodynamic events associated with electrode movement.

Figure 2 manifests another unexpected result. The tracings at 10 and 120 min are more or less identical and show practically no sign of diffusion during nearly 2 h. However, this result may be mainly attributable to the effect of gravity upon the system shown by the diagram in Fig. 1A. Diffusion tends to spread spherically around the opening in accordance with Fick's formula, or with the $K^+$ distribution shown in Fig. 1B. However, in the presence of gravity, this undergoes a change. Let us consider a hemisphere around the opening in the upper compartment initially filled with 25 mM KCl solution. As diffusion proceeds with time, the solution of KCl around the opening becomes thicker, i.e., heavier than the solution elsewhere in the upper compartment. This causes a movement of heavier solution downward to settle as a thin layer of heavier solution just above the partition. In the lower compartment initially filled with 100 mM KCl, an event similar to the above but in the opposite direction would occur. With the progress of diffusion, KCl around the opening becomes thinner and hence lighter than the solution in the rest of the lower compartment, and the lighter solution floats to form a thin layer just under the partition. This explains our failure to detect a sign of diffusion in the tracings obtained with a considerable time interval between them. Experimental reproduction of a spherical diffusion pattern would be possible only at the gravity-free state. In fact, we have observed in a one-dimensional diffusion experiment, made by use of a plastic tube containing 25 mM KCl solution supernatant over 100 mM KCl, that diffusion does proceed faster, at a theoretically predictable speed.

Before our conclusion, we want to discuss briefly the problem of why the observation of Hanitzsch (1988) contradicted the hypothesis of Ripps and Witkovsky (1985) that the slow PIII represents a Müller cell response to changes in light-induced extracellular $K^+$ concentration. There seem to be at least two other possible interpretations of Hanitzsch's observation: (1) The long delay of $K^+$ decrease to the slow PIII could be the result of recording of $K^+$ changes and slow PIII from a retinal depth different from the depth of the origin of $K^+$ changes. As is evident, the latency of slow PIII is independent of the depth of recording, but the latency of $K^+$ decrease should depend highly on the depth of recording. If the depth of recording is different from the depth of origin of $K^+$ decrease, the $K^+$ decrease should appear with a delay of time required for $K^+$ diffusion. (2) It is difficult to isolate the slow PIII from the receptor potential, and this is particularly so under the condition that their currents flow across the retina. It should be
carefully checked whether the peak latency of slow PIII has been affected by contaminations such as receptor potential.

As far as the technical aspects dealt with in this paper, we may conclude that the chamber used in the present experiment provides a useful tool for testing K⁺ electrodes, and perhaps other ion-sensitive electrodes also, and that knowledge of hydrodynamic events associated with electrode movement would serve for appropriate interpretation of the test results. Gravity deforms diffusion pattern from spherical to flattened, allowing the chamber to serve for repetitive electrode tests for many hours without renewing the solutions.

We thank Kiyoshi Yonemitsu, Professor Emeritus of Physics, Tokyo Metropolitan University, for his guidance in analytical solution of a differential equation of spherical diffusion. Our thanks are also due to Andrew T. Ishida, Ph.D., Department of Animal Physiology, University of California, Davis, for his valuable comments and careful check of English usage in the manuscript.

REFERENCES
