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Chapter 2

Preparation, Calibration and Application of Potassium-Selective Microelectrodes

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Abstract

In most animal cells, potassium ions are kept at concentrations of around 130 mM, while extracellular potassium concentrations are usually in the low mM range. In the brain, the resulting outwardly directed electrochemical gradient for potassium together with a selective potassium conductance of the plasma membrane is the basis for the highly negative membrane potential of neurons and glial cells. Consequently, maintenance of this gradient, and of low extracellular potassium concentrations, is a fundamental requirement for proper brain function.

At the same time, excitatory electrical activity of neurons is accompanied by a loss of intracellular potassium and a resulting transient elevation in extracellular potassium, which is counteracted by potassium

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uptake into glial cells. Under many pathological conditions, this intricate interplay is disturbed and several neurological diseases can be causally linked to impairment of potassium homeostasis. To understand the properties and function of neural networks under physiological and pathophysiological conditions, determination of the extracellular potassium concentration and its activity-induced fluctuations is thus required. Double-barreled, potassium-selective microelectrodes are reliable tools for such measurement in the intact tissue. In the present chapter, we will provide a detailed description of the preparation of liquid carrier-based ion-selective microelectrodes with a tip diameter of about 1 µm.

Special emphasis will be given to the calibration of these electrodes including the testing of their ion specificity and the handling and correction of non-specific reactions to other ions or molecules. Finally, we will illustrate the application of potassium-selective microelectrodes for measurement of extracellular potassium transients in acute tissue slices of the mouse hippocampus evoked by inhibition of Na^+/K^+-ATPase, application of receptor agonists or in response to ammonium (NH_4^+).

Introduction

Normal brain function requires the maintenance of low extracellular potassium concentrations ($[K^+]_o$), as even small elevations in $[K^+]_o$ in the mM range alter neuronal excitability [1]. $[K^+]_o$ homeostasis is mainly mediated by transporter- and channel-mediated uptake of K^+ into astrocytes, and as such it is dependent on the astrocyte membrane potential and the K^+ distribution across the plasma membrane itself [2, 3, 4].

Furthermore, astrocyte membrane potential and K^+ distribution influence the capacity for electrogenic, Na^+-dependent glutamate uptake, and reducing its driving force disturbs extracellular glutamate homeostasis [5, 6, 7]. Because of the critical importance of $[K^+]_o$ homeostasis, quantitative determination of $[K^+]_o$ is often necessary and required to understand the function and dysfunction of neural networks under physiological and pathophysiological conditions.

For decades, double-barreled K^+-selective microelectrodes have proven to represent reliable tools for such measurements in the intact tissue. They allow the accurate determination of $[K^+]_o$ in a small volume and with high specificity. The high selectivity of K^+-selective microelectrodes is ensured by a specific sensor cocktail ("liquid membrane ionophore") that is filled into one

channel of the electrode and consists of the carrier molecule valinomycin. Valinomycin is a dodecadepsi-peptide that binds potassium in a fully enclosed internal polar cavity [8].

The lipophilic sensor is overlaid with an aqueous phase ("backfill" solution) in which the ion species to be measured (potassium) is dissolved in a defined concentration. The concentration gradient between the liquid outside of the electrode and the backfill solution inside the electrode drives the diffusion of potassium through the sensor layer and generates an electric potential. Because this channel additionally records fluctuations in the extracellular field potential, a second channel (”reference”), which has to be arranged in close proximity to the ion-selective channel, serves to subtract this “artifact” and to separate these two signals properly.

In the following, we will describe the preparation of the so-called twisted, double-barreled K^+-sensitive microelectrodes. These are constructed out of two separate channels, which guarantees minor interference between them. Preparation of another type of electrode, the so-called concentric ion-selective microelectrode, which is characterized by fast response times, has been described in detail elsewhere [9]. Besides the preparation of twisted, double-barreled K^+-sensitive microelectrodes, we also provide a detailed description of the calibration procedure and the testing and handling of cross-selectivities of the sensor.

Special Technical Requirements

Twisted double-barreled microelectrodes are constructed from two separate glass capillaries, which are pulled out together to result in a tip of an overall diameter of around 1 µm for their use in brain slices. To this end, special horizontal pullers, such as the Type PE-2 (Narishige, Tokyo, Japan) can be employed. The PE-2 has a revolvable chuck, so that the center area of both capillaries can be preheated, softened and twisted together to increase the overall stability of the double-barreled electrode.

Liquid membrane ionophores such as valinomycin inherit a very high resistance (10-20 GΩ), so a special electrometer amplifier with high input impedance (R_{in}) and a small bias current (I_{bias}) is needed. We use a custom-made amplifier with R_{in}=10 TΩ and I_{bias}=50fA-1pA. Alternatively, commercially available amplifiers can be employed (e. g. EPMS-07, NPI, Tamm, Germany). Because the ion-selective channel additionally records

fluctuations in the extracellular field potential, a second channel (”reference”) serves to subtract this “artifact” and to separate these two signals properly.

Preparation of Twisted Double-Barreled Potassium-Selective Microelectrodes

To prepare twisted, double-barreled microelectrodes, borosilicate glass capillaries with filament are used (Clark Electromedical Instruments, Harvard Apparatus, United Kingdom). Usually, we prepare our electrodes from capillaries of 1.5 mm outer diameter for the ion-selective channel and of 1.0 mm diameter for the reference channel (“GC150F-15” and “GC100-F-15”, respectively). It is important to carefully clean the glass before use. To this end, the capillaries can be soaked in aceton overnight, they must then be rinsed several times with distilled water and ethanol. The capillaries are cut to a length of 7.5 cm (ion-selective channel) and of 6.5 cm (reference channel). Both capillaries are then glued together at their respective centers using two-component glue (e. g. Araldite, Huntsman advanced materials GmbH, Switzerland). Alternatively, the capillaries can be fixed together by small stripes of aluminum foil. The fixed double-capillaries are then centered and pulled out together in two steps using the above-mentioned vertical puller. During the first step, the middle part of the capillaries is heated up (~60 sec) until the glass is soft enough to enable a horizontal rotation of the revolvable chuck by 180°, which results in a mechanical stabilization of both channels. After a short cool down phase (~30 sec), the capillaries are completely pulled out in a second step, resulting in two sharp, double-barreled microelectrodes.

While this procedure is time-consuming and specials pullers are needed, so-called theta-glass capillaries (Clark Electromedical Instruments) with two chambers can be used alternatively. Their septum, however, may get extremely thin and even porous when electrodes are pulled out, so that the ion-selective and reference channel may interfere with each other.

Because sensor cocktails are very hydrophobic, the hydrophilic inner surface of the glass capillary must be silanized to mask its surface charges, a procedure for which most laboratories use hexamethyldisilazane (HMDS; Fluka, Buchs, Switzerland). In our lab, the vapor pressure method is used for silanization [10]. To this end, the future ion-selective channel is exposed to vaporized HMDS for 90 minutes. At the same time, the reference channel is floated with pure air, e. g. through an inserted hollow needle, to prevent its

silanization. Another possibility to protect it from silanization is to fill the reference channel with distilled water [10]. To complete the silanization reaction, the double-barreled capillaries are then heated in a furnace at 200 °C for two hours. After this, they must be kept dry until use to protect the silanization. To fill the sensor into the silanized channel, a very fine glass pipette which reaches far into the electrode's tip or a fine plastic tube can be used. If filament-containing capillaries are used as recommenced above, capillary forces will drive the sensor into the very tip. If that does not happen sufficiently (the sensors are usually quite viscous), one can use a hair to push it down and force it into the tip. For potassium-selective microelectrodes, approximately 0.5-1µl of a liquid neutral K^+ carrier based on valinomycin (Ionophore I, Cocktail B, Fluka, Buchs, Switzerland) is used. Afterwards, the capillary is carefully backfilled with a saline that contains the ion for which the sensor is selective without touching the sensor with the glass pipette or tubing used. Because chlorinated silver wires are used for connection to the amplifier, the backfill solution must also contain Cl^-. For potassium-selective microelectrodes, a backfill saline of 100 mM KCl may thus be used. If silanization was successful and the sensor is filled properly, it will form a concave surface against the backfill (Figure 1 A). Touching the sensor while backfilling might destroy its surface (Figure 1 B). In a poorly silanized microelectrode, the sensor may retract from the capillary wall and form several spheres (Figure 1 C). Often, residual air bubbles are a problem. One can remove them by gentle heating with a soldering iron. However, it is of note that the sensor is temperature-sensitive and may be destroyed by too much heat.

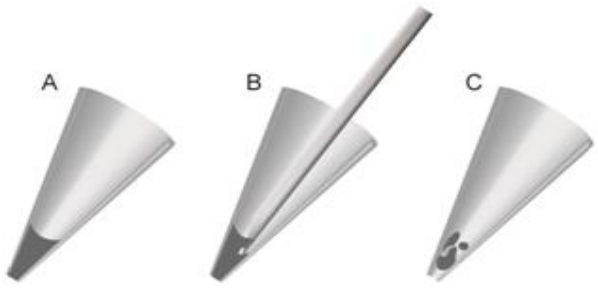

Figure 1. Schematic drawing of the tip of the ion-selective channel after its filling. (A) If the sensor is filled in properly, it forms a concave surface against the backfill. (B) Touching the sensor while backfilling it, might destroy its surface. (C) In a poorly silanized microelectrode, the sensor retracts from the inner capillary wall and may form several spheres.

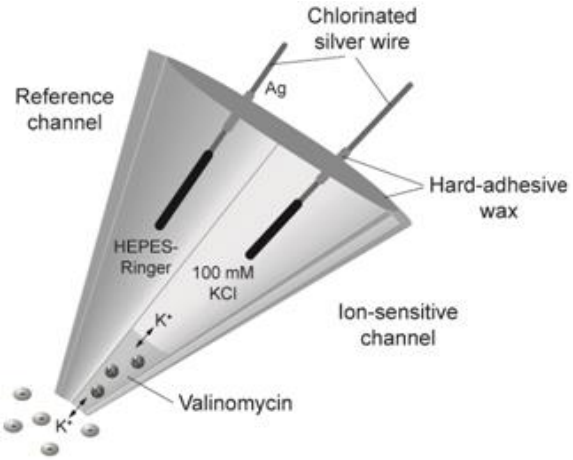

Figure 2. Schematic drawing of a double-barreled, potassium-sensitive microelectrode after its filling. For illustration purposes, the electrode is shown untwisted. The sensor barrel is filled with a liquid neutral K^+ carrier based on valinomycin and backfilled with 100 mM KCl.The reference barrel is filled with HEPES-buffered saline ("HEPES-Ringer"). Chlorinated silver wires are inserted into both channels and each barrel is sealed with dental wax.

The reference barrel can be filled with a saline containing 150 mM NaCl and 1 mM HEPES. Alternatively, HEPES-buffered physiological saline ("HEPES-Ringer") containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 25 HEPES, can be used. After filling both channels, chlorinated silver wires are inserted into the backfill and the reference barrel (Figure 2). They can be prepared using standard procedures (the easiest of which is plunging their tips into bleach). To prevent direct interference between the two channels, the outer parts of the silver wires can additionally be insulated by coating with hard dental wax (e. g. Deiberit 502 hard sticky wax, Siladent Dr. Boehme & Schoeps GmbH, Germany; Figure 2). In addition, each channel is sealed with dental wax (Figure 2). Filled electrodes can be stored for up to one day with their tips dipped in physiological saline.

Calibration of Potassium-Selective Microelectrodes

The chlorinated silver wires of the double-barreled microelectrode are connected to the inputs of a differential electrometer amplifier (see above) and

the electrode is then inserted into an experimental chamber which is filled with saline.

The potential of the sensor barrel (E_1) is composed of the ion potential (E_K) as well as the electrical potential of the saline (E_{ref}). While the latter is set to a value of zero per definition, it may change in an intact biological preparation such as an acute brain slice. Here, the extracellular field potential changes with neuronal activity and will thus overlay the pure ion signal of the ion-selective channel. Thus, it is necessary to separate these two. To this end, the potential of the reference channel (E_2) is subtracted (Figure 3).

Before and after their use, ion-selective microelectrodes must be calibrated. This is usually performed in an experimental chamber that is perfused with saline containing known potassium concentrations. The first step in this procedure is the determination of the electrode resistance. With an overall tip diameter of approximately 1 µm, the resistance of the reference channel should be 30-100 MΩ, the resistance of the ion-sensitive channel should amount to 10-20 GΩ (for potassium-selective microelectrodes). Because small tip diameters decrease the electrode's sensitivity and increase its time constants, higher resistances are usually not desirable. If necessary, the resistance of the channels can be reduced beveling its tip. To this end, the tip is exposed to a jet of abrasive (MicroPolish, Buehler GmbH, Duesseldorf, Germany) suspended in water for a couple of seconds until the desired resistance is obtained [11].

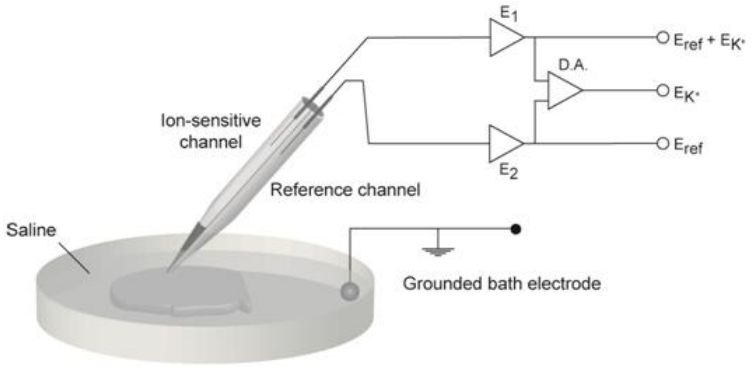

Figure 3. Schematic view of the recording arrangement. Shown is a dish filled with saline in which a brain slice is positioned. On the right, the bath electrode is indicated. The potential detected by the ion-selective channel (E_1) of the double-barreled electrode is composed of both the electrical field potential (E_{ref}) and the ion potential (E_{K+}), whereas the reference channel (E_2) only detects E_{ref}. To isolate E_{K+}, E_{ref} is subtracted from E_1 by means of a differential amplifier (D.A.).

For calibration of potassium-selective microelectrodes, a "potassium-free" saline containing 0 mM KCl, 125 mM NaCl and 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); and a "10 mM K^+" saline containing 10 mM KCl, 115 mM NaCl and 25 mM HEPES; both buffered with NMDG-OH (N-Methyl-D-glucamine) to a pH of 7.4 are prepared. These salines are mixed to result in calibration solutions with 1, 2, 4 and 10 mM potassium, which are used for calibrating the voltage response of the sensor to different potassium concentrations (Figure 4).

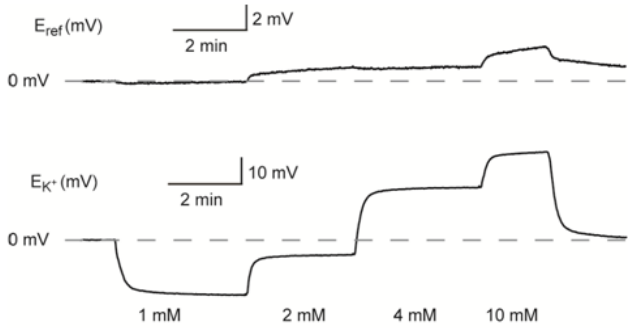

Figure 4. Typical calibration of a potassium-selective microelectrode in the experimental chamber. Shown are traces of the voltage-responses of the reference channel (E_{ref}, upper trace) as well as of the ion-selective channel (E_{K+}, lower trace) in response to stepwise changes in the potassium concentration in the perfusion saline from a basic level of 2.5 mM K^+ (defined as "zero"/ 0 mV) to 1, 2, 4, and 10 mM K^+ as indicated above.

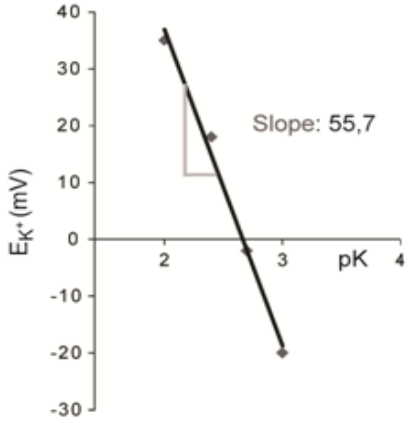

Figure 5. Plot of E_{K+} against the logarithm to the base ten of the potassium concentration (pK) and a linear fit of the data. The slope of this fit is 55.7 mV.

Because the voltage response of the sensor to potassium follows the Nernst equation (equation 1), plotting it against the logarithm to the base ten of the potassium concentration (pK) and fitting the data linearly (Figure 5) should result in a slope near -58 mV at room temperature.

If the sensor behaved ideally, it acted like a membrane that is permeable to one ion species only. Such behavior is described by Nernst equation:

$$E = E_0 + \frac{R \times T}{z \times F} \times \ln \frac{c\prime}{c\prime\prime} \quad (1)$$

E: measured electrode potential;
E_0: standard electrode potential, e. g. for a AgCl-wire at a temperature of 298.15 K and a partial pressure of 101.325 kPa (absolute); (see [12]))
R: gas constant (8.314 joules/degree Kelvin/mole)
T: absolute temperature (in Kelvin)
z: charge on the ion (+ 1 for potassium)
F: Faraday constant (96,500 coulombs)
c': extracellular ion concentration
c'': intracellular ion concentration

For practical purposes, $\frac{R \times T}{z \times F}$ and the natural logarithm are summarized to the Nernst slope *s*:

$$E = E_0 + \mathrm{s} \times \log \frac{c\prime}{c\prime\prime} \quad (2)$$

Some deviation of this ideal can be accepted (Figure 5), it is, however, vital that the voltage response of an individual electrode is essentially the same before and after its use in an experiment. Moreover, electrode drift (a slow change in the electrode potential without changing the saline nor the potassium concentration) must be minimal. For example, microelectrodes which show a drift of more than 2 mV during a 15 minute calibration, are discarded in our laboratory.

Another critical factor is the response time of the electrodes, which depends on their tip diameter, the form of the tip as well as on the thickness of the sensor phase in the electrode tip. Typically, electrodes based on valinomycin should have reached a stable potential within a maximum of 90 seconds after rapid changes in potassium. Finally, it is important to note that the time constants of the reference and the ion-selective channel are not equal due to their different resistance and capacitance. Thus, in extreme cases, the subtraction of E_{ref} (as measured by the reference channel) from E_1 (as

measured by the ion-selective channel) to reveal E_{K+} may not be valid during *changes* in the potassium concentration, but only when the electrode potentials are at steady-state.

Determination and Correction of Cross-Reactivity with Other Molecules

Ion-sensors should ideally act like a membrane permeable to only one ion, and thus strictly obey the Nernst-equation (see equation *(1)*), but all sensors have additionally a low sensitivity to other ions. Especially if the ion to be detected is present at relatively low concentrations as compared to interfering ions, this may exert a significant influence. The relationship between the electrode potential, the main ion species and the interfering ion activity is described by the Nikolsky-Eisenman-equation [13, 14, 15]):

$$E = E_0 + s \times log\left[a_i + \sum_i k_{i,j}^{pot} (a_j)^{\frac{z_i}{z_j}}\right] \quad (3)$$

E: measured electrode potential
E_0: standard electrode potential
s: Nernst slope
a: activity of ion. Only free ions are capable of reacting
a_i: activity of the measured ion
a_j: activity of the interfering ion
z_i: charge number of the measured ion
z_j: charge number of the interfering ion
$k_{i,j}^{pot}$: potentiometric selectivity coefficient of the ionophore (preference of the measured ion over an interfering ion)

Because of possible interference, it is essential to perform control calibrations and to test which ions affect the electrode potential. For valinomycin-based, potassium-selective microelectrodes, the following cations and molecules should be tested: H^+, Li^+, Na^+, Mg^{2+}, Ca^{2+} and NH_4^+ [11, 16, 8]. In our hands, only NH_4^+ significantly affects the electrode potential, a cross-reactivity which has been reported before [17, 18]. For determination of $[K^+]_o$ during measurements without interfering ions a formula based on the Nernst equation is used (see equation *(5)).* If, as in our case, the concentration of the interfering ion (NH_4^+: 5 mM) is higher than the baseline $[K^+]_o$ (2.5 mM), it

cannot be regarded as classical "interfering" ion. Thus, instead of the Nikolskii-Eisenman equation (3), equation 10 (see below) is used.

Application of Ion-Selective Microelectrodes for Measurement of Extracellular Potassium Concentration in Acute Brain Tissue Slices

We routinely use double-barreled potassium-selective microelectrodes in acute tissue slices of the mouse brain, which are prepared using standard procedures [19, 20]. Briefly, animals are anesthetized with CO_2, decapitated and brains are rapidly removed. Afterwards, transverse, 250 µm thick slices are obtained from hippocampi (Figure 6). After sectioning, slices are kept in physiological saline at 34 °C for 30 min and at room temperature thereafter until they are used for experiments. The composition of a typical physiological saline is as follows (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 20 glucose, bubbled with 95 % O_2 and 5 % CO_2 to result in a pH of 7.30. Osmolarity is 310 ± 5 mOsm/l.

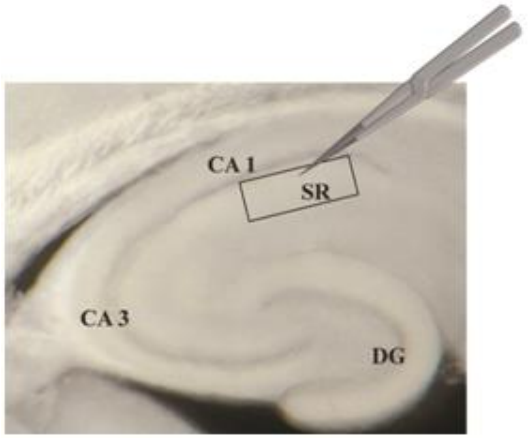

Figure 6. Transmitted light image of an acute hippocampal tissue slice. The two characteristic neuronal cell body layers of the hippocampus proper, including the CA1 and CA3 area, and the dentate gyrus (DG) are clearly visible as dark bands. The black box delineates the area of the *stratum radiatum* (SR) in the CA1 region, where measurements were performed. The position of the electrode is indicated schematically.

For measurement of $[K^+]_o$, twisted double-barreled, K^+-sensitive microelectrodes placed ~50 µm below the slice surface in the *stratum radiatum* of the CA1 region (Figure 6) are used.

Calculation of the Extracellular Potassium Concentration without Interfering Ions

The potassium gradient across cell membranes is mainly mediated by the action of the plasma membrane Na^+/K^+-ATPase, which exports three sodium ions in exchange for two potassium ions. It is ubiquitously expressed and can be blocked by either removing extracellular potassium or by applying ouabain, a plant alkaloid. Figure 7 shows the effect of blocking Na^+/K^+-ATPase, and cellular uptake of potassium, on $[K^+]_o$ in brain slices by bath perfusion of 100 µM ouabain. As reported and published before (e. g. [21, 3, 4, 22]), this manipulation causes an immediate, complex and variable increase in $[K^+]_o$, emphasizing the central role of the sodium pump in setting $[K^+]_o$.

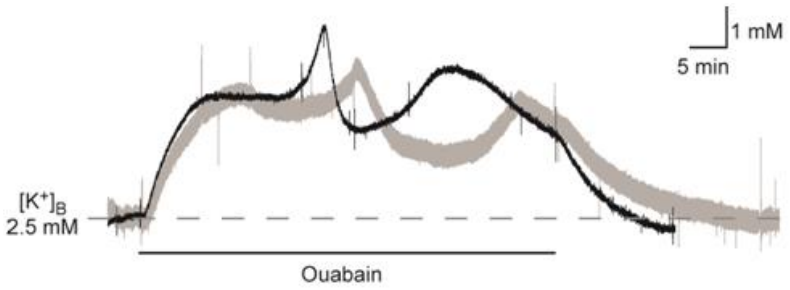

Figure 7. Changes in $[K^+]_o$ in the *stratum radiatum* of an acute hippocampal slice preparation induced by pharmacological inhibition of the Na^+/K^+-ATPase by 100 µM ouabain. Shown is an overlay of two different experiments, which illustrates the immediate, complex and variable increase in $[K^+]_o$ upon inhibition of Na^+/K^+-ATPase. Experiments were performed in the presence of the sodium-channel blocker tetrodotoxin (500 nM) to block neuronal action potential generation.

Glutamate is the most important excitatory transmitter in the vertebrate brain. It binds to ionotropic receptor channels, which are mainly permeable to both sodium and potassium. Their opening thus mediates the efflux of potassium from neurons. The resulting glutamate-induced increase in $[K^+]_o$ can be determined and its mechanism analyzed using double-barreled potassium-selective microelectrodes. Figure 8 shows that bath application of 1

mM glutamate for 120 sec causes a transient increase in the $[K^+]_o$, which is followed by an undershoot in the $[K^+]_o$ below its initial baseline upon removal of glutamate. The application was performed twice, demonstrating that the glutamate-induced $[K^+]_o$ transients can be induced repetitively.

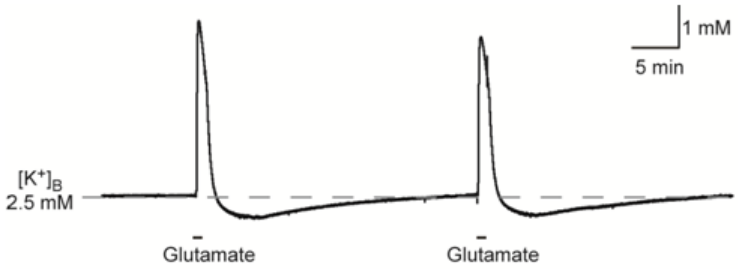

Figure 8. Transient changes in $[K^+]_o$ in the *stratum radiatum* of an acute hippocampal slice preparation induced by bath perfusion with 1mM glutamate for 120 sec. Shown are two consecutive applications.

Neither ouabain nor glutamate-induced changes in H^+, Na^+, Mg^{2+} or $Ca^{2+}$are large enough to significantly affect the electrode potential. For determination of $[K^+]_o$ during such measurements (without interfering ions) a formula based on the Nernst equation (equation 4) can be used. According to the Nernst equation, the change in the potential ΔE_{K+} (mV) is calculated as follows:

$$\Delta E_{K^+} = s \times lg \frac{[K^+]_B + \Delta[K^+]_o}{[K^+]_B} \quad (4)$$

ΔE_{K+}: changes in the potential of the valinomycin channel (mV)
s: Nernst slope
$[K^+]_B$: baseline concentration of potassium (in our case 2.5 mM K^+, see composition of the saline above)
$\Delta[K^+]_o$: changes in $[K^+]_o$ in the tissue during manipulations

Ouabain and glutamate induce an increase in $[K^+]_o$ ($\Delta[K^+]_o$). These changes and the underlying baseline concentration of 2.5 mM K^+, which corresponds to the K^+ concentration of the saline used (see above), determine the potential of the microelectrode (ΔE_{K+}, in mV). To calculate changes the $[K^+]_o$ ($\Delta[K^+]_o$, in mM), equation 4 is rearranged to:

$$\Delta[K^+]_o = [K^+]_B \times \left(10^{\frac{\Delta E_{K^+}}{s}} - 1\right) \quad (5)$$

For the Nernst slope s, an arithmetic average of the values determined by the calibration before and after the experiment is used.

Correction of Cross-Reactivity with Other Molecules

There is evidence for a K^+ dysregulation in the brain under hyperammonemic conditions [17, 23, 24, 25, 22]. We have analysed the effect of an NH_4^+ perfusion on the $[K^+]_o$ in acute brain slices [22]. Because the sensor shows significant response to NH_4^+ itself (cf. Figure 9: "bath control"), this artifact has to be corrected to uncover the NH_4^+-induced changes in $[K^+]_o$ only (Figure 9).

Figure 9 shows that addition of 5 mM NH_4^+ to the bath shifted the potential of the electrode by nearly 6 mV ($\Delta E_{K+\text{-bath}}$). Since the calibration curve is logarithmic, the artifact cannot simply be subtracted, but must be used to calculate the selectivity coefficient (*k*), of valinomycin for NH_4^+.

The factor *k* quantifies the effectiveness of NH_4^+ relative to K^+ to change E_{K^+}. To determine $k[NH_4^+]$, the change in the potential induced by addition of NH_4^+ to the bath, $\Delta E_{K+\text{-bath}}$, which is composed of $k[NH_4^+]$ and $[K^+]_B$, must be determined.

$$\Delta E_{K^+-bath} = s \times lg \frac{[K^+]_B + k\,[NH_4^+]}{[K^+]_B} \quad (6)$$

$\Delta E_{K+\text{-bath}}$: NH_4^+-induced changes in the perfusion saline
$[K^+]_B$: baseline concentration of potassium (in our case 2.5 mM K^+, see composition of the saline above)
k: selectivity coefficient of valinomycin for NH_4^+

Rearrangement of this formula for calculation of *k* results in:

$$k\,[NH_4^+] = [K^+]_B \times \left(10^{\frac{\Delta E_{K^+-bath}}{s}} - 1\right) \quad (7)$$

Our experiments revealed a *k* of 0.2 ± 0.1, and thus a K^+/NH_4^+ selectivity which is smaller than reported before [26, 16]. This illustrates the necessity to test and evaluate ion selectivities and interferences for a given preparation and type of electrode separately.

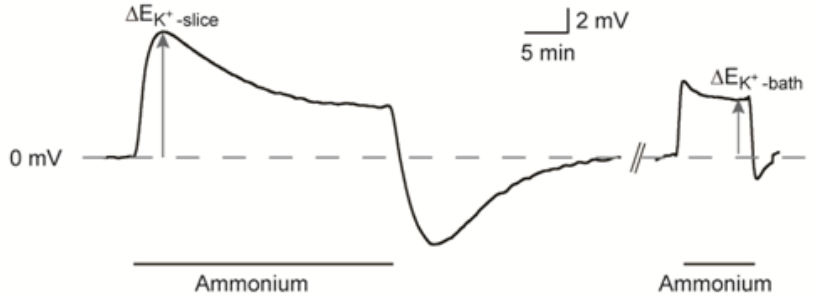

Figure 9. Changes in $[K^+]_o$ in the *stratum radiatum* of an acute hippocampal slice preparation induced by 5 mM NH_4^+. Shown is a typical experiment with an NH_4^+-induced increase in $[K^+]_o$ ($\Delta E_{K+\text{-slice}}$). After the experiment, the microelectrode was retracted from the slice and the NH_4^+-induced voltage change of the electrode ($\Delta E_{K+\text{-bath}}$) was determined in the perfusion saline. This potential change in response to NH_4^+ was used to calculate the selectivity coefficient (*k*), of valinomycin for NH_4^+.

Combination of the selectivity coefficient *k* with the Nernst equation,allows to calculate NH_4^+-induced changes of $[K^+]_o$ in the slice ($\Delta E_{K+\text{-slice}}$) as follows:

$$\Delta E_{K^+-slice} = s \times lg \frac{[K^+]_B + \Delta[K^+]_{app} + k\,[NH_4^+]}{[K^+]_B} \quad (8)$$

$\Delta E_{K+\text{-slice}}$: NH_4^+-induced changes in the potential of the valinomycin channel
$\Delta[K^+]_{app}$: consists of the NH_4^+-induced elevation in $[K^+]_o$ and the underlying NH_4^+-induced artifact.

During the experiment, three different components are involved in the NH_4^+-induced $[K^+]_o$-increase in the tissue. These are the baseline concentration ($[K^+]_B$) of 2.5 mM K^+, the underlying, NH_4^+-induced artifact ($\Delta E_{K+\text{-bath}}$) and the NH_4^+-induced changes in $[K^+]_o$, summarized here using the term $\Delta[K^+]_{app}$.

For calculation of $\Delta[K^+]_o$ (that is the NH_4^+-induced changes in the slice devoid of the NH_4^+-induced artifact), equation (8) has to be rearranged to:

$$\Delta[K^+]_o = [K^+]_B \times \left(10^{\frac{\Delta E_{K^+-slice}}{s}} - 1\right) - k\,[NH_4^+] \quad (9)$$

$\Delta[K^+]_o$: NH_4^+-induced $[K^+]_o$-changes

Replacing $k[NH_4^+]$ by combining equation *(7)* and equation *(8)* finally yields in:

$$\Delta[K^+]_o = [K^+]_B \times \left(10^{\frac{\Delta E_{K^+-slice}}{s}} - 10^{\frac{\Delta E_{K^+-bath}}{s}}\right) \quad (10)$$

$\Delta[K^+]_o$: NH_4^+-induced changes in the slice devoid of the NH_4^+-induced artifact (mM)
$[K^+]_B$: the baseline concentration 2.5 mM K^+ which is determined by the NR
$\Delta E_{K+\text{-slice}}$: NH_4^+-induced changes in the potential of the valinomycin channel determined in the slice (mV)
$\Delta E_{K+\text{-bath}}$: NH_4^+-induced changes in the potential of the valinomycin channel determined in the bath (mV) (see Figure 9)

With this formula the NH_4^+-induced changes can be calculated. For determination of the total $[K^+]_o$, the underlying baseline concentration is subtracted:

$$[K^+]_o = [K^+]_B \times \left(10^{\frac{\Delta E_{K^+-slice}}{s}} - 10^{\frac{\Delta E_{K^+-bath}}{s}} + 1\right) \quad (11)$$

This now allows to convert the NH_4^+-induced potential changes of the valinomycin channel determined in the slice (in mV) into changes in $[K^+]_o$ only (Figure 10).

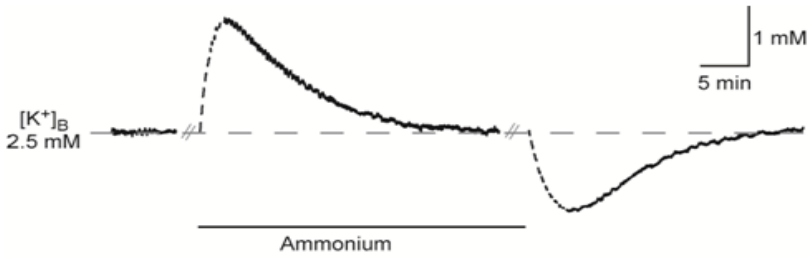

Figure 10. Shown is a calculated trace resulting from the correction of the NH_4^+-mediated voltage signal of the ion sensor, revealing the estimated change in $[K^+]_o$. Wash-in and wash-out phase of NH_4^+ are dotted to emphasize that NH_4^+ concentrations change and calculations may not be accurate during these periods.

Conclusion

Double-barreled K^+-selective microelectrodes have been introduced years ago. Employed with some care, including testing of interference with other ions and obeying strict standards for their calibration before and after the experiment, they represent reliable tools and are still the method of choice for dynamic measurement of even small extracellular $[K^+]_o$ changes in the mM range in the intact tissue. Because of their relatively large diameter (~1 µM), they can, however, be employed only in large cells, and are basically unsuited for detection of intracellular $[K^+]$ in small vertebrate neurons and glial cells. Although some potassium-selective fluorescence dyes are on the market for measurement of intracellular $[K^+]$, they are quite noisy and difficult to use. Here development of new and improved tools is highly desirable.

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