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Neutral Carrier-Based "Ca^{2+}-Selective" Microelectrodes for the Measurement of Tetraphenylphosphonium

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Ca^{2+}-selective microelectrodes with Simon's neutral carrier ETH 1001 are commercially available and have been widely used for the measurement of both extra- and intracellular calcium. The electrodes demonstrate high selectivity against other cations such as magnesium, sodium, and potassium. We report, however, that the ETH 1001-based microelectrode is a superior tetraphenylphosphonium (TPP^+)-sensitive electrode. The electrode exhibits a Nernstian response for $[Ca^{2+}] > 10^{-5}$ M but for $[TPP^+] > 10^{-7}$ M. Using two different methods, we found that log k_{TPPCa} (selectivity coefficient for TPP^+ with respect to Ca^{2+}) is in the range of −3.0 to −5.3. We argue that the ETH 1001 microelectrode can be used as a commercially available TPP^+ electrode. We illustrate this application by making membrane potential recordings in respiring mitochondria. The results are identical to those obtained using conventional ion-exchange TPP^+ electrodes.

Calcium microelectrodes utilizing Simon's "Ca^{2+}-selective" neutral carrier ETH 1001 (9) are commercially available and have been successfully used in the measurement of both extra- and intracellular calcium (1, 2, 7). The ETH 1001 ionophore and cocktail, available from Chemika, have been shown to be highly selective against physiological cations such as Mg^{2+}, K^+, and H^+. To date there have been no published reports of significant selectivity for other cations (12). One paper has indicated that this electrode receives interference from the pharmacological convulsant pentylenetetrazol (PTZ),[1] but the level of cross selectivity is negligible and becomes marginally apparent only at millimolar concentrations of PTZ (8).

In this paper, we report that the ETH 1001 microelectrode is actually more selective for the lipophilic cation tetraphenylphosphonium (TPP^+). TPP^+ is widely used as a membrane potential probe for interior negative cells and organelles. The cation distributes between internal and external spaces according to the Nernst equation, $\Delta\psi = RT/F \ln(C_e/C_i)$, where $\Delta\psi$, R, T, and F refer to the membrane potential with respect to the outside, the gas constant, absolute temperature, and Faraday's constant, respectively. C_e and C_i represent the concentration, or strictly the activity, of the probe externally and internally. Continuous measurement of $[TPP^+]$ is typically made by selective electrodes constructed with a polyvinyl chloride-based membrane containing tetraphenylboron as an ion exchanger (10). Steady-state measurements can be made from cell extracts that have been incubated with $[^3H]TPP^+$. With proper calibration and correction for probe binding (4), accurate measurements of membrane potential can be made with TPP^+.

We demonstrate herein that the ETH 1001 calcium-selective electrode is actually more selective for TPP^+. This is an important observation for groups attempting to make concomitant calcium and membrane potential recordings using electrode techniques. We argue that ETH 1001 microelectrodes can be used as commercially available TPP^+ electrodes, replacing time-consuming and toxic in-lab ion-exchange electrode fabrication (10). We illustrate this application by successfully tracking mitochondrial membrane potentials during respiratory-state transitions.

MATERIALS AND METHODS

Ca^{2+} and TPP^+ Response Curves

An ETH 1001-based calcium-selective microelectrode (MI 600) and Ag/AgCl reference electrode (MI 402) were purchased (Microelectrodes, Inc., Londonberry, NH). The electrodes were connected to a high-impedance pH

[1] Abbreviations used: PTZ, pentylenetetrazol; TPP, tetraphenylphosphonium.

0003-2697/96 $18.00

meter (Orion, Boston, MA), and the output was amplified, channeled into an A/D converter, and recorded using Analog Connection WorkBench (Strawberry Tree Inc., Sunnyvale, CA) software on an Apple Macintosh system.

The response of the electrode was tested in our "experimental buffer" (137 mM KCl, 10 mM Hepes, 2.5 mM MgCl, pH 7.2) with varying concentrations of $CaCl_2$ (J. T. Baker, Phillipsburg, NJ) or TPPCl (Aldrich, Milwaukee, WI). Our test chamber was water jacketed for temperature control (30°C) and equipped with a stirring bar for continuous mixing. Decade response plots and selectivity coefficients were determined using this chamber with an automated micropipetter (Hamilton, Reno, NE), capable of accurately delivering microliter volumes of TPPCl and $CaCl_2$ stock solutions prepared in the experimental buffer.

Ca^{2+} and TPP^+ Traces during Respiratory-State Transitions

Mitochondria were isolated from male Sprague–Dawley rat livers using a standard isolation protocol (6). Mitochondria were placed in the experimental buffer at a final mitochondrial protein concentration of 0.7 mg/ml. TPP^+ (10 μM) was added for membrane potential recordings. Confirmatory recordings of $[TPP^+]$ were made using an ion-exchange TPP^+-sensitive electrode constructed in our laboratory (10).

Transitions in mitochondrial respiratory states were elicited by the addition of inorganic phosphate (P_i), adenosine diphosphate (ADP), and respiratory substrates such as the glutamate/malate combination (11). All chemicals were purchased from Sigma (St. Louis, MO) and stock solutions were prepared in water and the pH adjusted to 7.2. Oxygen consumption was monitored with a Clark-type electrode.

RESULTS

The purpose of this study was to determine if ETH 1001 microelectrodes can be applied to the measurement of TPP^+. The response of the electrode to varying concentrations of $CaCl_2$ and TPPCl was studied, and using two different methods, the selectivity coefficient, k_{TPPCa}, was computed. The electrode was then used to monitor the mitochondrial membrane potential during respiratory-state transitions.

Sensitivity of the ETH 1001 Microelectrode

The microelectrode was attached to our incubation chamber containing 3 ml of the experimental buffer. Varying amounts of $CaCl_2$ and TPPCl were added to the chamber using an automated micropipetter. The response times to Ca^{2+} and to TPP^+ were comparable.

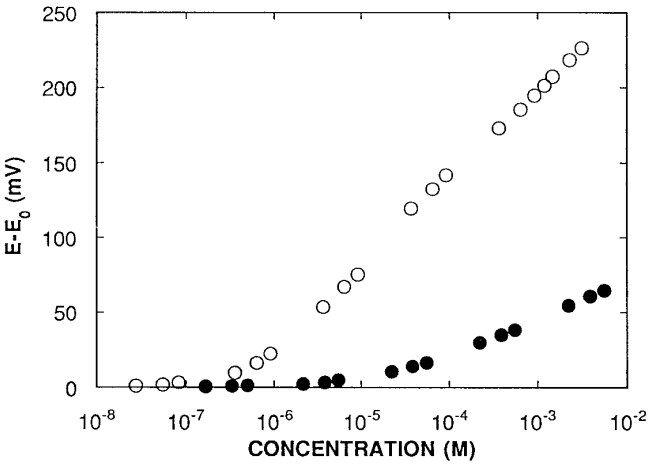

FIG. 1. ETH 1001 microelectrode response plots for Ca^{2+} (●) and for TPP^+ (○). 3 ml of experimental buffer (137 mM KCl, 10 mM Hepes, 2.5 mM MgCl, pH 7.2) was placed in a water-jacketed chamber to control the temperature at 30°C. The chamber was well mixed with a magnetic stirring bar. Microliter volumes of either $CaCl_2$ or TPPCl were delivered to the chamber with an automatic pipetter. The voltage response to Ca^{2+} (●) or TPP^+ (○) was monitored with a commercially available ETH 1001 "calcium-selective" microelectrode connected to a high-impedance pH meter. Plots are representative of three different decade response curves.

Figure 1 depicts the response curves for the two cations. The electrode demonstrates a linear response ($R = 0.999$) to $[TPP^+] > 10^{-7}$ M. The Nernstian slope, S, was found to be 56.9 mV/decade, which is close to the theoretical 59 mV/decade. In contrast, the ETH 1001 electrode is only weakly responsive at calcium concentrations below 10^{-5} M and linear ($R = 0.997$) above 10^{-5} M. The calcium response is consistent with the manufacturer's specification for the electrode.

Selectivity of the ETH 1001 Microelectrode

We were interested in quantitating the selectivity coefficient of the microelectrode for TPP^+ over Ca^{2+}. We have used two different "methods" for determining the selectivity coefficient. The first, known as the "separate solution" method, is based on the Nicolsky Eisenman (12) equation:

$$E = E_0 + RT/z_AF \ln(a_A + k_{AB}(a_B)^{z_A/z_B}),$$

where E is the potential measured with an electrode selective for ion A and E_0 is the constant potential. RT/z_AF is the Nernstian factor (slope), z_A and z_B are the valences of ions A and B, respectively, and a_A and a_B are the activities of ion A and the interfering ion B. k_{AB} is the selectivity coefficient of the electrode for ion A over ion B. The Nicolsky Eisenman equation is rearranged and modified to yield the separate solution equation (3), given by

$$k_{TPPCa} = a_{TPP}/\sqrt{a_{Ca}} \times 10^{(E_{Ca}-E_{TPP})/S},$$

where E_{TPP} and E_{Ca} are the potentials measured in experimental solutions with activities a_{TPP} or a_{Ca}, respectively, and S is the Nernstian factor for TPP^+.

The "matched potential" method defines the selectivity coefficient as the ratio of primary ion to the interfering ion concentration which gives the same potential change in a reference solution (12).

By using the activities (concentrations) and voltages in Fig. 1, we found, using the separate solution method, that log k_{TPPCa} varied between −3.0 and −5.3. The matched potential method yielded a log k_{TPPCa} of −3.0 at an $E - E_0$ of 50 mV.

Respiratory-State Transition Traces

The ETH 1001 microelectrode was used to measure extramitochondrial calcium and tetraphenylphosphonium during respiratory-state transitions.

Figure 2A depicts an experiment in which oxygen and calcium levels are monitored using the Clark-type and ETH 1001 electrodes, respectively, and shows that addition of either 500 nmol ADP or 30 μmol substrate (glutamate + malate) leads to calcium uptake by the mitochondria. To verify that this is indeed a calcium trace, 5-nmol $CaCl_2$ calibration pulses were injected at the end of the experiment. Note that this entire experiment was performed in the absence of TPP^+.

Figure 2B shows the same experiment performed in the presence of 10 μM TPP^+. The ETH 1001 electrode now selectively tracks TPP^+ distribution. Addition of 500 nmol ADP leads to rapid consumption of O_2 (state III respiration) and dissipation of membrane potential, while addition of 30 μmol substrate dramatically increases membrane potential (indicating an energized respiratory state IV). Figure 2C is the same experimental trace using a conventional ion-exchange TPP^+-sensitive electrode, confirming that the membrane potential can be accurately tracked with the ETH 1001 electrode.

The calcium signal seen in Fig. 2A could not be detected when the experiment was performed in the presence of 0.5 mM EDTA (data not shown). However, the membrane potential trace (Fig. 2B) remained unchanged in the presence of 0.5 mM EDTA (data not shown).

DISCUSSION

The ETH 1001 microelectrode responded so strongly to increasing concentrations of TPP^+ that we set out to determine if it is actually a better TPP^+ electrode. In practice, we found that the electrode was Nernstian from 10^{-7} to 10^{-2} M TPP^+. Based on selectivity coefficient determinations, we found that these electrodes

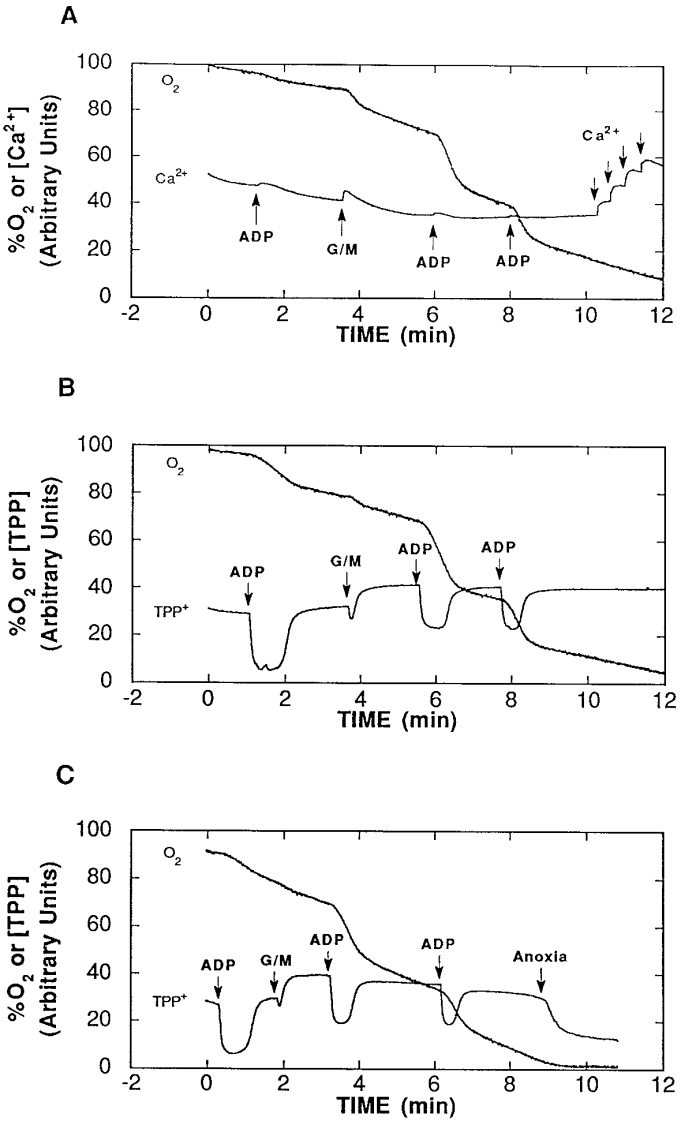

FIG. 2. Calcium and membrane potential traces during ADP-P_i-driven respiratory state transitions. 0.7 mg/ml mitochondria and 15 μmol K_2HPO_4 were added to 3 ml of the experimental buffer in the temperature-jacketed chamber described in the legend to Fig. 1. 10 μM TPPCl was added to the chamber for membrane potential traces (B, C). Oxygen saturation levels were monitored with a Clark-type electrode. 500 nmol ADP, 30 μmol glutamate/malate (G/M), and 5 nmol $CaCl_2$ additions are indicated by arrows. (A) An ETH 1001 microelectrode was used to monitor the uptake of calcium during respiratory-state transitions. At the end of the experiment, 5-nmol pulses of Ca^{2+} were injected to confirm that this is indeed a calcium trace. (B) The same experiment was performed, using the identical ETH 1001 microelectrode, in the presence of 10 μM TPPCl. The resulting trace represents the membrane potential. Addition of ADP dissipates the membrane potential and leads to rapid consumption of oxygen (state III respiration). When substrate (G/M) is added, the mitochondria are energized and membrane potential is maximal (state IV respiration). (C) This experiment is identical to B, except a laboratory-manufactured ion-exchange-sensitive TPP^+ electrode tracks [TPP^+]. The experiment was carried out to anoxia and shows the loss of membrane potential when oxygen is fully dissipated. Note the similarity in traces B and C.

are 1000 to 200,000 times more selective for TPP^+ than for Ca^{2+}. For submicromolar calcium concentrations, the electrode receives a negligible contribution from calcium. For $[Ca^{2+}] > 10^{-5}$ M, however, the electrode begins exhibiting a linear response and the observed voltage will receive a contribution from calcium. Therefore, groups intending to use the ETH 1001 microelectrode for TPP^+ measurements must determine how the background calcium fluctuations might contribute to the TPP^+ signal; this can be done simply by performing the experiment in the absence of TPP^+.

Studies of isolated mitochondria have demonstrated that the extramitochondrial calcium concentration must be maintained below micromolar levels. With increasing calcium, the mitochondria exhibit the pathologic mitochondrial permeability transition, the hallmarks of which include loss of membrane potential and calcium phosphate precipitation in the matrix (5). Experimentally, efforts are made to maintain the calcium concentration at submicromolar levels. Under these conditions, we were able to utilize the ETH 1001 microelectrode as a superb TPP^+ electrode, without any interference from calcium. Our traces of membrane potential using this technique matched perfectly with those accomplished using a conventional TPP^+ electrode. The resulting membrane potential tracing remained constant in the absence or presence of the calcium-chelating agent EDTA, indicating that Ca^{2+} did not interfere with our TPP^+ signal.

We have demonstrated that the neutral carrier Ca^{2+}-selective electrode (ETH 1001) responds strongly to TPP^+. This is extremely important for groups attempting to make simultaneous electrode measurements of calcium and TPP^+. Many studies have relied on the ETH 1001 electrode for calcium monitoring, and the use of TPP^+ is one of the most popular techniques for membrane potential recordings. Hence, groups undertaking efforts to make such simultaneous measurements should be aware of the potential artifacts that can arise from the use of ETH 1001 electrodes in combination with TPP^+.

Under proper experimental conditions, the ETH 1001-based Ca^{2+}-selective electrode can be used as a very sensitive and selective TPP^+ electrode. Because TPP^+ electrodes are not commercially available and must be constructed using toxic compounds, this novel use of the ETH 1001 microelectrode could be very useful when applied properly.

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