

Bio Currents Research Center Tutorial

Applications

The material below has been adapted from several primary papers and reviews originating from the BRC. These should be referred to in publications and for the original source materials.

References:

1. *Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007) Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes. In: Methods and New Frontiers in Neuroscience. Ed. Adrian C. Michael. CRC Press. Ch. 18: 373-405.*
2. *Messerli, M.A., Robinson, K.R. and Smith, P.J.S. (2006) Electrochemical sensor applications to the study of molecular physiology and analyte flux in plants. In: Plant Electrophysiology - Theory and Methods. Ed. Alexander G. Volkov. Springer-Verlag. 73-107.*

As the purpose of this tutorial is to introduce advances in a method the many applications of self-referencing to biological systems will not be reviewed. A collection of references to publications from the BRC, and others, can be found at the BRC web site (www.biocurrents.org). However, selected studies and publications, relevant to the neurosciences, are briefly introduced below.

Neural repair and disease

Microglia

These reactive cells of both vertebrate and invertebrate nervous systems have been the subject of three studies using self-referencing microelectrodes.

Chromogranin A (CGA) is a recently identified endogenous component of neurodegenerative plaques in Alzheimer's and Parkinson's diseases. CGA stimulates microglial secretion of NO and tumor necrosis factor- α , resulting in neuronal and microglial apoptosis. Using an electrochemical H₂O₂ microelectrode it was demonstrated that CGA produces a rapid oxidative burst in primary microglia in culture.³

Using both self-referencing K⁺ and H⁺ ISMs the presence of an K⁺/H⁺ - ATPase in the microglia, with a KD in the physiological range of the [K⁺]_o, has been reported.⁴ The pump was ouabain insensitive but modulated by Omeprazole and Sch28080. In this study comparisons were also made between current values measured locally with an ISM and those calculated from whole cell patch clamp as related to the inward rectifying potassium channel. The ISMs followed ~0.5 of the whole cell current. Better signal processing methods developed since this study, and taking into account the problem of the electronics speed-of-response (**discussed above**), should now result in better correspondence to the total cell current.

In an invertebrate system, the leech, damage to the interganglionic connectives is hypothesized to stimulate the activity of eNOS, with the resultant production of nitric oxide causing the accumulation of migratory microglia at the lesion site. The production of the NO was measured directly from the lesion site using the electrode design discussed **above**.⁵

Neural Tube Defects

One of the numerous complications of diabetes is the occurrence of embryonic congenital malformations, notably neural tube defects. Li et al. correlated this to the expression of Pax3 and tested the hypothesis that expression and embryopathy is related to an hypoxic state.⁶ Hypoxia arises through diffusion limited oxygen delivery caused by excessive glucose metabolism. In this study the self-referencing oxygen microelectrode was used to demonstrate that embryonic oxygen consumption was suppressed after exposure to high maternal glucose levels. Along with other experiments it was concluded that a depletion of oxygen in the embryo gives rise to impaired embryonic gene expression and defective development through induced oxidative stress.⁶

Sensory Neurobiology

Hair Cells

The increase in K^+ flux through hair cells upon stimulation - whether by sound in the cochlea or acceleration in the vestibular labyrinth - is known to increase the perilymph $[K^+]$ on the basolateral side. Self-referencing K^+ ISMs, in conjunction with several other techniques, including micro-Ussing chambers, have followed these fluxes from both the strial marginal cells and the vestibular dark cells.⁷⁻¹⁰ In both tissues, the potassium channel responsible for the flux is the slowly activated K^+ channel (KsK). The laboratories involved in this research have also made extensive use of the older vibrating voltage probe. This device has not been covered in this review. Interested parties are directed to Lee and Marcus for applications to the current studies in the ear and to Nuccitelli for an applications overview.^{11, 12}

Hair cells of both the auditory and vestibular systems use calcium to control aspects of performance and tuning.¹³ Self-referencing Ca^{2+} ISMs have been used to follow the role of the plasma membrane Ca^{2+}/H^+ ATPase (PMCA) in the regulation of this flux. A substantial calcium efflux could be measured, suggesting, in conjunction with pump density, that the PMCA activity should generate a substantial membrane current as the bundles expel cytosolic Ca^{2+} .¹³

Vision

Horizontal cells isolated from the retina of the skate have provided a model system in vision research. Self-referencing hydrogen-selective ISMs have been applied to examine the relationship between glutamate and regional changes in extracellular pH.¹⁴ The results suggested that glutamate modulation of H^+ flux arises from calcium entry into the cells and the subsequent activity of the plasma membrane Ca^{2+}/H^+ ATPase. The data from this reduced preparation did not support the action of hydrogen ions as part of an inhibitory feedback loop but further experiments are underway (R. Paul Malchow, unpublished).

Olfaction

Olfaction in estuarine animals, moving through different salinities, poses unique problems. To sample for chemical cues the sensillae must be exposed to changing osmotic and ionic conditions. Gleeson et al. used ISMs selective for K^+ and Ca^{2+} to question whether at low salinities the integrity of the olfactory dendrites of the Blue Crab are sustained by a diffusion generated ionic microenvironment within the aesthetascs.^{15, 16} Both ISMs confirmed this hypothesis with a clear loss of these ions from the hymolymph at low salinities. Further, the flux rates fell well within the range estimated from $^{22}Na^+$ flux data from previous studies.¹⁶

Additional Electrically Active Cells

Neurons

Isolated neurons have been the subject of several self-referencing studies although limited by technical aspects of the technique. With the availability of positional control, as discussed above, and faster temporal resolution, one can anticipate more work on isolated neurons or networks, in addition to those mentioned above. Knox et al. in an early study of an isolated neuron observed calcium influx and activation of a cation current, coupled to intracellular Ca^{2+} release, in peptidergic neurons of *Aplysia californica*.¹⁷ Ca^{2+} released from a thapsigargin-sensitive store activated a non-selective cation current that may sustain depolarization of the somata. Using the same cell, Duthie et al. measured calcium fluxes in oxidatively challenged neurons.¹⁸ Preliminary data on oxygen consumption, at the level of a single neuron, has been shown and correlated to membrane potential under voltage clamp conditions.¹⁹

Muscle

Using self-referencing approaches on muscle are complicated by contraction. Contraction, particularly rapid contraction, will disturb the chemical gradient the microelectrodes are coupling to. However, under some conditions the technique can be successfully used, for example in muscle from the Sea Cucumber.²⁰ In this case Ca^{2+} flux changes were correlated with

neurotransmitter application. Devlin also recorded Ca^{2+} flux modulation, to FMRFamide and 5-HT application, from the muscular trabeculae of the gastropod ventricle.^{21,22}

A mammalian muscular system studied with self-referencing is within the cerebral arteries. Membrane depolarization from -65mV to -35mV is associated with myogenic contraction. Depolarization is graded to transmural pressure and sufficient to act on the open probability of the dihydropyridine-sensitive Ca^{2+} channel, augmenting the calcium contraction mechanism.²³ Using a self-referencing Cl^- ISM arterial Cl^- efflux can be measured with a clear temperature dependent pressure component that correlated closely to myogenic contractility.²³ This study concludes that Cl^- efflux, through Cl^- channels, contributes to the depolarization of the muscle cell. An additional attribute of this study is the realization that the ISM is affected by Cl^- channel blockers, such as DIDS, NPPB and IAA-94. Pharmacological experiments are conducted using tamoxifen.

Amperometric detection has also been applied to L6 myotubes. Individuals suffering from type 2 diabetes mellitus have reduced expression of genes for key proteins in oxidative metabolism and mitochondrial function in muscle.²⁴ Measurements of glucose oxidation from myotubes are unaltered by moderate overexpression of mitochondrial uncoupling protein 3 (UCP3) but markedly increased with the chemical uncoupler dinitrophenol (DNP). Glucose oxidation is measured using $\text{U-}^{14}\text{C}$ Glucose and scintillation counting. This result is closely matched to the pattern of oxygen uptake monitored using self-referencing technologies. No difference in UCP3 expressing cells and control myotubes is found but a significant increase in oxygen uptake was seen with DNP treatment.²⁴

Pancreatic β -Cells

Pancreatic β -cells are the electrically active component of the Islets of Langerhans and secrete insulin in response to depolarization through voltage dependent Ca^{2+} channels. Part of the control pathway is the closure of the KATP channel regulated by changes in the ATP/ADP ratio as this relates to increased metabolism and circulating glucose levels. This predicts an oscillation in single β -cell oxygen consumption at similar frequencies to insulin vesicle fusion. Porterfield et al. studying INS cells in culture, show this at the level of a single cell.²⁵ Damon Osbourn and Emma Heart have confirmed this observation on primary b-cell cultures and correlated activity with Ca^{2+} signaling (unpublished). Using INS cells, a method has been developed to simultaneously monitor oxygen flux with sub-micron spatial resolution of the microelectrode.²⁶

Additional Pumps and Channels

Apoptosis and Volume Changes

Apoptosis occurs in most cell types and can be triggered by a variety of physiological and pathological stimuli. It is a key mechanism in structuring tissues during development. This phenomenon has been studied using a mouse zygote (the condition prior to the first cleavage) and a self-referencing K^+ ISM.^{27,28} Cell shrinkage is an incipient hallmark of apoptosis and relates to changes in potassium flux that decrease the intracellular K^+ concentration, regulating apoptotic progression. This flux is measured at the single zygote level with apoptosis induced by H_2O_2 treatment.²⁸ Using a collection of pharmacological blockers for various K^+ channels, the mechanism underlying this change in K^+ flux is identified as the two-pore domain K^+ channel (K2P).²⁷

Ion fluxes, moving as a result of hyposmotic challenge and cell swelling, have been studied using single human epithelial tsA201a cells.²⁹ A role for an anion flux is critical in the induction of a recovery mechanism that ultimately involves the activation of voltage dependent potassium channels, a reduction in cytoplasmic osmolarity, and the initiation of flow of osmotically obligated water out of the cell. A clear chloride flux was followed using a self-referencing Cl^- ISM and was correlated to cell volume changes and membrane currents. This paper is of particular interest for the assessment of interferents to what would be better described as an anion selective ISM.

Acidification and Alkalinization

Self-referencing microelectrodes, although designed for applications to single or small groups of cells, can equally be applied to semi-intact epithelial structures. A series of papers studying the acidification of the vas deferens and epididymus of the rat is a good example.³⁰⁻³⁵ The lumen of the mammalian vas deferens and epididymus is held at a pH of around 6.6, an important factor in the immobilization and maturation of sperm. Using a self-referencing H^+ ISM Breton et al. scanned the surface of the vas deferens locating hydrogen transport hotspots inhibited by Bafilomycin A1 - a highly selective blocker for the vacuolar type proton ATPase (V-Type pump), a non-phosphorylating H^+ pump.³⁰ Subsequent studies have shown the involvement of chloride independent bicarbonate transport and an inhibition by

cadmium.^{31,34} Monitoring the localized H⁺ flux has also allowed the study of the vesicle fusion event and a role for the cytoskeleton in trafficking and recycling of the V-Type pumps.^{32,35} These studies have been conducted in conjunction with molecular and imaging techniques.

Using another epithelial structure, larval mosquito midgut, processes driving the extreme alkalization of the anterior segment (pH ≈ 11) have been studied with both H⁺ and Cl⁻ self-referencing ISMs.^{36,37} Proton and chloride regional fluxes were mapped down the length of the midgut and pharmacological blockers used to determine their inter-relationship. Application of DIDS produced consistent results when measuring either H⁺ or Cl⁻ flux. However, at 10⁻⁴ M DIDS caused a small reversal of the Cl⁻ flux, a phenomenon not seen with methazolamide treatment.³⁷ This difference may be attributed to the interference of DIDS with the electrode performance as discussed above and by Messerli et al.³⁸ The authors conclude that an H⁺ V-Type ATPase energizes an anion exchange across the apical membrane. The electrophoretic Cl⁻/HCO₃⁻ exchanger and carbonic anhydrase are seen as critical components in the alkalization of the anterior midgut.³⁷ This study also presents an alternative approach to buffer correction when the diffusion coefficient of the buffer is unknown.³⁷

Literature Cited:

3. Twig, G., Graf, S.A., Messerli, M.A., Smith, P.J.S., Yoo, S.H., and Shirihai, O.S. (2005) Synergistic amplification of beta-amyloid and interferon-gamma-induced microglial neurotoxic response by the senile plaque component chromogranin A, *Am. J. Physiol.*, 288, C169-C175.
4. Shirihai, O., Smith, P.J.S., Hammar, K. and Dagan, D. (1998), H⁺ and K⁺ gradient generated by microglia H/K ATPase, *Glia*, 23, 339-348.
5. Lindner, E., Gyurcsányi, R.E., and Buck, R.P. (1999), Tailored transport through ion-selective membranes for improved detection limits and selectivity coefficients, *Electroanalysis*, 11, 695-702.
6. Li, R., Chase, M., Jung, S.-K., Smith, P.J.S. and Loeken, M.R.. (2005) Hypoxic stress in diabetic pregnancy contributes to impaired embryo gene expression and defective development by inducing oxidative stress, *Am. J. Physiol.*, 289, E591 - E599.
7. Wangemann, P., Liu, J. and Marcus, D.C. (1995) Ion transport mechanisms responsible for K⁺ secretion and the transepithelial voltage across marginal cells of stria vascularis in vitro, *Hearing Res.*, 84, 19-29.
8. Marcus, D.C., Sunose, H., Liu, J., Bennett, T., Shen, Z., Scofield, M.A. and Ryan, A.F (1998)., Protein kinase C mediates P2U purinergic receptor inhibition of K⁺ channel in apical membrane of strial marginal cells, *Hearing Res.*, 115, 82-92.
9. Marcus, D.C. and Shipley, A.M. (1994). Potassium secretion by vestibular dark cell epithelium demonstrated by vibrating probe, *Biophysical J.*, 66, 1939-1942.
10. Shen, Z., Liu, J., Marcus, D.C., Shiga, N. and Wangemann, P. (1995), DIDS increases K⁺ secretion through an Isk channel in apical membrane of vestibular dark cell epithelium of gerbil. *J. Membrane Biol.*, 146, 283-291.
11. Lee, J.H. and Marcus, D.C. (2003) Endolymphatic sodium homeostasis by Reissner's membrane. *Neurosci.*, 119, 3-8.
12. Nuccitelli, R. (1990) Vibrating probe technique for studies of ion transport. In: *Noninvasive Techniques in Cell Biology*, Wiley-Liss, pp. 273-310.
13. Yamoah, E.N., Lumpkin, E. A., Dumont, R. A., Smith, P.J.S., Hudspeth, A. J. and Gillespie, P.G. (1998), Plasma-membrane Ca²⁺-ATPase ensures low Ca²⁺ concentration in hair-cell stereocilia, *Neurosci. J.*, 18, 610-624.
14. Molina, A.J, Verzi M.P., Birnbaum, A.D., Yamoah, E.N., Hammar, K., Smith, P.J.S. and Malchow, R.P.(2004), Neurotransmitter modulation of extracellular H⁺ fluxes from isolated retinal horizontal cells, *J. Physiol.* 560, 639-657.
15. Gleeson, R.A., Hammar, K. and Smith, P.J.S. (2002) Sustaining olfaction at low salinities: mapping ion flux associated with the olfactory sensilla of the blue crab *Callinectes sapidus*, *J. Exp. Biol.*, 203, 3145-3152.
16. Gleeson, R.A., McDowell, L.M., Aldrich, H.C., Hammar, K. and Smith, P.J.S. (2000) Sustaining olfaction at low salinities: evidence for a paracellular route of ion movement from the hemolymph to the sensillar lymph in the olfactory sensillar of the blue crab, *Callinectes sapidus*, *Cell Tissue Res.*, 301, 423-431.
17. Knox, R.J., Kao, L.S., Jonas, E., Connor, J., Smith, P.J.S. and Kaczmarek, L.K. (1996) Calcium influx and activation of a cation current are coupled to an intracellular Ca²⁺ mobilization in peptidergic neurons, *J. Physiol.*, 494, 627-63.

18. Duthie, G.G., Shipley, A. and Smith, P.J.S. (1994) Use of a vibrating electrode to measure changes in calcium fluxes across the cell membranes of oxidatively challenged *Aplysia* nerve cells, *Free Rad. Res.* 20, 307-313.
19. Hammar, K., Malchow, R.P. and Smith, P.J.S. (2003) Electrochemical measurement of O₂ consumption in single excitable cells, *Biophysical J.*, 84, 457A.
20. Devlin, C.L. (1997) A vibrating Ca²⁺-selective electrode measures Ca²⁺ flux induced by the neuropeptide FMRFamide in a gastropod ventricle, *Comp. Biochem. Physiol.*, 116A, 93-100.
21. Devlin, C.L. (2001) 5-Hydroxytryptamine stimulates Ca²⁺ flux in the ventricular muscle of mollusk (*Busycon canaliculatum*) during cardioexcitation, *Biol. Bull.*, 200, 344-350.
22. Devlin, C.L. and Smith, P.J.S. (1996) A non-invasive vibrating calcium-selective electrode measures acetylcholine-induced calcium flux across the sarcolemma of a smooth muscle, *J. Comp. Physiol.*, 166, 270-277.
23. Doughty, J.M. and Langton, P.D. (2001) Measurement of chloride flux associated with the myogenic response in rat cerebral arteries, *J. Physiol.*, 534, 753-761.
24. MacLellan, J.D., Gowing, A., Gerrits, M., Smith, P.J.S., Sivitz, W., Wheeler, M.B. and Harper, M.-E. (2005) Increased uncoupling protein 3 stimulates fatty acid, but not glucose oxidation, and decreases reactive oxygen species in muscle cells. *Diabetes*, 54(8), 2343-2350.
25. Porterfield, D.M., Corkey, R.F., Sanger, R.H., Tornheim, K., Smith, P.J.S. and Corkey, B.E. (2000) Oxygen consumption oscillates in single clonal pancreatic b-cells (HIT), *Diabetes*, 49, 1511-1516.
26. Osbourn, D., Sanger, R.H. and Smith P.J.S. (2005) Determination of single cell oxygen consumption with impedance feedback for control of sample-probe separation, *Anal Chem.*, in press.
27. Trimarchi, J.R., Liu, L., Smith, P.J.S. and Keefe, D.L. (2002) Apoptosis recruits two-pore domain potassium channels used for homeostatic volume regulation, *Am. J. Physiol.*, 282, C588-C594.
28. Trimarchi, J. R., Liu, L., Smith, P.J.S. and Keefe, D. L. (2000) Non-invasive measurement of potassium efflux as an early indicator of cell death in mouse embryos, *Biol. Reprod.*, 63, 851-857.
29. Garber, S.S., Messerli, M.A., Hubert, M. Lewis, R. Hammar, K., Indyk, E., and Smith P.J.S. (2005) Monitoring Cl⁻ movement in single cells exposed to hypotonic solution, *J. Memb. Biol.* 203, 101-110.
30. Breton, S., Smith, P.J.S., Lui, B. and Brown, D. (1996), Acidification of the male reproductive tract by bafilomycin-sensitive H⁺ ATPase. *Nature Medicine*, 2, 470-472.
31. Breton, S., Hammar, K., Smith, P.J.S. and Brown, D. (1998) Proton secretion in the male reproductive tract: Involvement of chloride-independent bicarbonate transport, *Am. J. Physiol.*, 275, C1134-C1142.
32. Breton, S., Nsuma, N.N., Galli, T., Smith, P.J.S. and Brown, D (2000) Tetanus toxin-mediated cleavage of cellubrevin impairs proton secretion in the male reproductive system. *Am. J. Physiol.*, 278, F717-F725.
33. Brown, D., Smith, P.J.S. and Breton, S. (1997), Role of V-ATPase rich cells in acidification of the male reproductive tract, *J. exp. Biol.*, 200, 257-262.
34. Herak-Kramberger, C.M., Sabolic, I., Blanusa, M., Smith, P.J.S., Brown, D. and Breton, S., Cadmium inhibits vacuolar H⁺ ATPase-mediated acidification in the rat epididymus, *Biol. Reprod.*, 63, 599-606, 2000.
35. Beaulieu, V., Da Silva, N., Pastor-Soler, N., Brown, C.R., Smith, P.J.S., Brown, D. and Breton, S., Modulation of the actin cytoskeleton via gelsolin regulates vacuolar H⁺ATPase (V-ATPase) recycling, *J. Biol. Chem.*, 280, 8452-8463, 2005.
36. Boudko, D., Moroz, L., Linser, P., Trimarchi, J., Smith, P. and Harvey. W., In situ analysis of pH gradients in mosquito larvae using non-invasive, self-referencing, pH-sensitive microelectrodes, *J. exp. Biol.*, 204, 691-699, 2001.
37. Boudko, D.Y., Moroz, L.L., Harvey, W.R. and Linser, P.J., Alkalinization by chloride/bicarbonate pathway in larval mosquito midgut, *Proc. Nat'l. Acad. Sci.*, 98, 15354-15359, 2001.
38. Messerli, M.A., Smith, P.J.S., Lewis, R.C., and Robinson, K. R., Chloride fluxes in lily pollen tubes: a critical reevaluation, *Plant J.*, 40, 799-812, 2004.