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Application of the Bio-electric Law to Several Different Types of Mammalian Cell

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Abstract

According to the bio-electric law, all living cells have a negative trans-membrane potential produced by mitochondrial generation of electrons, the mitochondria having an even more negative intra-organellar potential. A change in trans-membrane potential initiates functional activation. Neural dendrites, the sino-atrial and ventricular cells of the heart, the vascular smooth muscle cell, the vascular endothelial cell, red blood cells, leukocytes, platelets, liver cells, adipocytes, brown fat cells and retinal cells have been studied. There are at least 3 types of electron movement. Complete depolarization and repolarization occurs in nerve, skeletal muscle and heart, whereas variable changes in trans-membrane potential occur in vascular smooth muscle cells and non-contracting cells. The retinal cell is unique in that activation is associated with hyperpolarization. It is concluded that study of electron movements within living cells requires much further attention in physiological research.

Keywords: Mammalian cell; Mitochondria; Heart; Vascular smooth muscle cell; Vascular endothelial cell; Brown fat cells

Introduction

It is recognized that organic chemistry depends on electron flow from high electron density sites to lower electron density sites [1], but this principle has not been followed very much by physiologists. It is well recognized that cells of excitable tissues such as nerve, heart and muscle have a negative trans-membrane potential. Yet when one estimates the sums of the charges of the ions (concentration times charge) in mammalian cells (Figure 1) one finds that they are equal. (This ignores the fact that intracellular pH is caused by hydrogen ion activity (H+), but even at the lowest pH value of 6.8, the extra positive charges are an order of magnitude lower, namely 159nEq/L, just a few more positive charges), but the cell has a negative electric charge.

The Bio-electric Law [1] postulates that the negative trans-membrane potential is the result of free electrons produced from mitochondria. This principle has been explored in cardiac ventricular cells [2] and sinus node cells [3,4]. The purpose of the present study was to explore whether this principle can be applied to other cell types, including those from non-excitable tissues.

Bio-electric law

The principle of the bio-electric law follows from the Nernst equation

- $E_{cell} = Eo_{cell} - (RT/nF)\ lnQ$
- E_{cell} = Cell potential under non-standard conditions, i.e., during activation.
- Eo_{cell} = Cell potential under standard conditions, in which there is a negative trans-membrane potential, i.e., a net excess of intracellular negative charges.
- R = Gas constant, which is 8.31 (volt-coulomb)/(mol)
- T = Temperature
- n = Number of moles of electrons exchanged in the electrochemical reaction (mol)
- F = Faraday's constant, 96500 coulombs/mol
- Q = Reaction quotient, which is the equilibrium expression with initial concentrations rather than equilibrium concentrations.

The only variable in the equation is n, so that the changes in trans-membrane potential, associated with cellular activation and function, are due to changes in the moles of electrons in the electrochemical reaction.

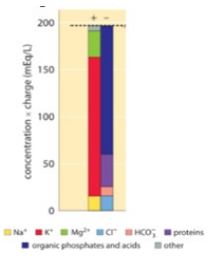

Figure 1: The distribution of ions within the intracellular cytoplasm; equal positive (left column) and negative (right column) electric charges [5]. Intracellular potassium ions (red) are electrostatically bound to the protein (blue) matrix of the cytoplasmic gel.

Literature Review

Is sodium ion (Na+) entry into cells solely responsible for cell depolarization?

The answer to this has to be, "No", because electric currents are carried by electrons, so that in the case of an inward current (using

conventional polarity) the outflow of electrons always exceeds any entry of positively charged particles. In addition, since an electron has one 10,000 of the mass of an Na+, it has 10,000 times the acceleration of an Na+, according to Newton's second Law of motion. The classical study of Hodgkin and Huxley [6] established that depolarization from the resting negative trans-membrane potential of squid giant nerve axon was sodium ion (Na+) dependent and that repolarization was potassium ion (K+) dependent. From these observations grew the hypothesis that depolarization of nerve and muscle was achieved by an ingoing current carried by Na+ and repolarization by an outward current carried by K+. To my knowledge, there has been no measurement of Na+ moving from the extracellular compartment into the cell during the rapid depolarization of action potentials, so Na+ dependence does not necessarily imply the carrying of current. There have been many studies demonstrating the opening of "Na+ channels"; these will increase the conductance of the membrane and thus increase the outflow of electrons and depolarization.

Is potassium ion (K+) exit from cells solely responsible for cell repolarization?

The only measurement of outward flow of K+ during repolarization is in sino-atrial node [7], but a previous analysis indicated that it was quantitatively only able to provide 25% of the increase in trans-membrane potential required to complete repolarization [4]. According to the Bio-electric Law the negative charge of polarized cells is achieved by the production of electrons during oxidative phosphating in mitochondria. Mitochondria have a voltage of -180 to -220 mV according to Perry [8], whereas the electrical potential of the general cell cytoplasm rarely exceeds -90 mV. As the mitochondrial membrane has a finite conductance, electrons flow at a variable rate (dependent on variable mitochondrial membrane resistance) from mitochondria to cell cytoplasm. Therefore, it is reasonable to conclude that the major contribution to cell repolarization is mitochondrial generation of electrons to replace the electrons lost during depolarization.

An example of this assumption is found in isolated liver cells that have a mitochondrial transmembrane potential of over -150 mV, falling to approximately -144 during stimulated respiration [9]. Ward et al. [10] recorded a -150 mV mitochondrial membrane potential at a cell (plasmalemmal) membrane potential of -60 mV in cultured cerebellar granule cells. Mitochondrial membrane potential is thus considerably higher than the plasma membrane potential, so that a current inward to the mitochondria, according to conventional polarity, is a mitochondrial outflow of electrons to the general cytoplasm, that would be generated because the mitochondrial membrane has a finite electrical conductance.

Energetic considerations

These have previously been addressed [11]. Where does the electrical energy for these processes come from? The conventional theory that inward Na+ movement causes depolarization and outward K+ movement causes repolarization must lead, in the absence of some form of compensation, to cellular Na+ overload and K+ depletion of the intracellular compartment. The compensation usually assumed is that of the Na+/K+ exchanger (the sodium pump), but this explanation must now be rejected because, in the first place, the exchange is not equal (3 Na+ out for 2K+ in); in the second place, the exchanger is controlled by intracellular Na+ only, whereas there is no stimulation of the pump by excess extracellular K+, as can be seen from analysis of the decay of extracellular K+ excess [3].Finally, it has been shown that there is insufficient chemical energy available to maintain trans-membrane potential with the Na+/K+ at Phase [12,13].

The field force of cell membranes

The interior of the resting cells, e.g., neuron or muscle cell is at an electrical potential about 50 – 100 mV more negative than the external medium. Although this potential difference seems small, it exists across a plasma membrane only about 5 nm thick, so that the resulting voltage gradient is about 100,000 V/cm [14]. This field force is sufficient to drive charged particles including protons out of the interlamellar space, greatly increasing its electrical resistance, until this is reduced by synaptic neurotransmitters that cause depolarization, or by a travelling wave of action potentials along a nerve axon or skeletal and cardiac conducting tissue. The consequence of this is that high trans-membrane potential causes high plasma membrane electrical resistance, while lower trans-membrane potentials have lower plasma membrane electrical resistance, leading to electron leakage.

Neurons

At present, one has to regard measurements made in the 1950's on isolated axons as rather crude compared with that which can be achieved today in an intact animal [15]. The earliest work on this subject [6] used axons separated from the cell body, which therefore contained no energy source. Electrical energy had to be applied from the experimental equipment, as is the case with patch clamp research, e.g. [16]. By contrast, Moore et al. [15] recorded spontaneous electrical activity from neural dendrites and from the neuron soma in freely behaving rats. In this case the electrical energy must be generated by the cell. A nerve cell depolarizes when activated by a neurotransmitter and a wave of depolarization travels down the axon. This occurs via a well-known cable theory that only involves electrical phenomena, i.e., electron movements, which are described in detail mathematically [17].

Myocardial cells

The first work on the Bio-electric Law was undertaken by considering cardiac ventricular cells [2,3] which are engulfed in a travelling action potential wave that spreads through tissue behaving like a syncytium. The ventricular cell has a diastolic potential of -80 to -90 mV and it depolarizes rapidly in an Na+ dependent manner to about +40 mV. No rise in intracellular Na+ has been observed during this very rapid phase of depolarization, but this could be due to inadequate methodology. That the spike of inward current reaching its maximum 1.5 msec or less (the "spike") was attributed to electron outflow follows from the fact that an electron has one 10,000th of the mass of an Na+, and therefore 10,000 times the acceleration in accordance with Newton's second law of motion. Na+ does enter the cell in the prolonged phase of the action potential ("plateau") that sub-serves the delivery of calcium ions (Ca^{2+}) to produce cardiac contraction. Sodium sensitive electrodes can record this Na+ entry via the sodium/calcium exchanger (NCX) in Purkinje fibers [18]. The slow loss of electrical positivity during the plateau of the ventricular action potential and the final repolarization to the diastolic potential of -80 mV are conventionally attributed to K+ outflow because they are K+ dependent, but this would lead to intracellular depletion of K+ in the absence of a K+ inward pump. These features of the action potential were therefore attributed to electron production by mitochondria to restore the negative diastolic charge of the cytoplasm [3] whereas some

cardiac ventricular cells may not depolarize spontaneously, spontaneous depolarization is a necessary attribute of pacemaker cells (sino-atrial node cells, SAN) that initiate the heart-beat. Under normal circumstances the peacemaking is performed by a healthy sino-atrial node. These cells depolarize slowly during diastole from about -60 mV to about -40 mV when an action potential is triggered that then propagates. The inability to find a specific cation dependency has led to this diastolic pacemaker current being named the "funny" current, If. It has been argued that the greater membrane conductance at -60 mV allows a contribution of slow inward current in diastole carried by electron outflow. There is evidence of K+ entry in diastole with an equal outflow of K+ during repolarization [7], but the maximum contribution of K+ has been calculated to be only about 25%, so the main contribution to repolarization (about 75%) was postulated to be due to electron production [4].

Vascular smooth muscle cells

As with the SAN cell, the vascular smooth muscle (VSM) cell has a resting membrane of -60 mV, with the same consequence that cation entry can be controlled by trans-membrane electric field force. The neurotransmitters, nor-adrenaline and serotonin, lower cell membrane resistance leading to depolarization. Data from VSM cells from Neild & Kotecha [18,19] yielded a single phase exponential decay of contractile force (dependent on calcium ion delivery to the actomyosin filaments) with increasing membrane field force (dependent on net electron excess). This is in contrast to the SAN cell, in which a fairly clear-cut threshold exists for the start of Ca^{2+} inflow to produce the cardiac cycling. In the VSM, there is a continuous control of the Ca^{2+} delivery to the actomyosin contractile mechanism (to maintain "tone"), due to compartmentalization of Ca^{2+} signaling [20]. In the actomyosin ATPase cycle, ATP is hydrolyzed by myosin and the subsequent interaction of myosin with actin produces force and/or displacement [20]. Thus, at this point in the discourse, we contrast those cells which depolarize completely (nerve and striated muscle) to those in which there is a variable change the level of polarization.

Variation of trans-membrane potential in the VSM cell and thus of tone is mainly achieved through nitric oxide production by the endothelium (endothelium-dependent relaxing factor EDRF). A very important aspect of vascular smooth muscle is the maintenance of vessel tone, and, in the case of arteries, vasodilation to accommodate increased flow in response to increased downstream demand [21]. This vasodilation is mediated by nitric oxide (NO) release from endothelial cells. NO also maintains some control of membrane potential during control conditions (so-called hyperpolarization), which is reversed in some species by N-nitro- L-arginine, which blocks NO synthase [22]. The dependence of hyperpolarization on cellular potassium ions and the opening of calcium activated potassium channels has led to the hypothesis that the increased negativity of membrane potential occurs through an outward flow of potassium ions. However, this would create an unsteady state of potassium ions and affect the ionic equilibrium. Alternatively, no inhibits the calcium ion inward current [23], which is accompanied by reduced electron outflow. The VSM cell is an example of control of function by varying a continuous generation and outflow of electrons with varying cellular negative charge, including some spikes [24]. The data of burbling [24] is compatible with this theory. Disproof of the theory can be achieved by measuring the extracellular potassium ion transient in response to NO and to determine whether it is large enough to account for all of the accompanying hyperpolarization.

Vascular endothelial cells, blood cells and the glycocalyx interaction

Vascular endothelial cells at normal extracellular K+ concentration sustain a trans-membrane potential close to -80 mV [25]. According to Zeng, the glycocalyx layer that separates the vascular endothelial cell from the blood is negatively charged, forming an electrostatic barrier for blood cells and proteins, like albumin [26]. The negative charge of albumen is part of the electrostatic barrier of the glycocalyx along with negatively charged heparin sulphate. The actual electrical potential of the glycocalyx does not seem to have been measured. In addition, the glycocalyx participates in the functions of the endothelial cell in sensing increases in shear stress at the vessel wall induced by increases in blood flow, thus triggering nitric oxide production and vasodilation (VSM relaxation). That endothelial cell depolarization (outward flow of electrons), associated with reduced cell stiffness [25] is involved in shear stress induced vasodilation remains a possibility. A test of this would be to monitor endothelial cell trans-membrane potential changes during increased blood flow, in addition to experiments in which layers of endothelial cells are electrically stimulated to induce depolarization [27]. The barrier provided by the endothelial cell glycocalyx may prevent adhesion of blood cells by means of electrostatic repulsion. Red blood cells also have negative trans-membrane potentials in the region of -8 to -10 mV [28,29]. Leukocytes and platelets are more damaging if in contact with glycocalyx-bare endothelial cells. Leukocytes have a trans-membrane potential of about -67 mV and depolarize upon activation [30]; human blood platelets have a membrane potential of -52 to -60 mV, depolarization of which increases the sensitivity to aggregating agents such as ADP [31].

Non-cardiovascular cells

The examples of cardiovascular cell negative transmembrane potentials above reflect my main area of interest in the cardiovascular systems. However, I postulate that this phenomenon is common to all living cells. An example of a non-cardiovascular cell is the adipocyte. Human fat cells have a trans-membrane potential of about -34 mV [32], whereas the more metabolically active brown adipose cell has a membrane potential of about -54 mV [33]. Moreover, adrenaline and noradrenaline in concentrations as low as 10-8 g/ml produced a prompt depolarization (electron loss) and cooling of the brown fatty tissue; lowering of the oxygen tension caused a marked and reversible decrease in the trans-membrane potential. In tissue obtained from cold adapted rats, the membrane potential was considerably diminished. These results are compatible with an outflow of electrons (depolarization) with generation of heat, i.e., the function of brown fat. In addition, nitric oxide was found to trigger mitochondrial biogenesis in brown adipocytes [34], thus increasing in the electron producing mitochondrial mass.

Retinal cells

Retinal rod and cone cells differ from most of those previously discussed, in that they show activation by an increase in negativity of transmembrane potential (photon to electron conversion is also found in clouds, plants and solar panels), in-stead of by depolarization. There is also a complex of cGMP-gated channel and Na/Ca^{2+}, K exchanger [35]. In the dark (at rest), the photoreceptors are quite active, constantly releasing neurotransmitter. After absorption of a photon, the resulting hyperpolarization decreases the amount of neurotransmitter released. This means that light actually turns off neurotransmitter receptors. Hyperpolarization and neurotransmitter

release are graded inversely according to light intensity. Retinal neurotransmitters have been reviewed by Wu & Maple [36] The rods and cones are connected to horizontal and bipolar cells that connect to the retinal ganglion cells [37].

Results and Discussion

The conventional explanation of the low electron charge at rest is that it is a K+ leak and that hyperpolarization in response to photons is caused by a closing of Na+ channels. This seems unlikely as it is not compatible with an ionic steady state and balance. According to the Bio-electric interpretation, the -40 mV resting potential is associated with a leak of electrons and the acquisition of electrons via the photoreceptor leads to a transmembrane potential between -40 mV and -80 mV that increases the electrical resistance of the membrane. The highest electric field force across the cell membrane at -80 mV would exclude all charged particles from the interlamellar space, described by Bauer μ [35] as "Closing of all channels" (Table 1).

Cell	Standard conditions	Mode of activation
Mitochondria	-180 to -220 mV	Depolarisation (Electron outflow)
Nerve	-50 to -100 mV	Depolarisation (Electron outflow)
Heart Ventricle	-80 to -90 mV	Depolarisation (Electron outflow)
Heart Sinus Node	Approx -60 mV	Depolarisation (Electron outflow)
Skeletal Muscle	-70 to -90 mV	Depolarisation (Electron outflow)
Vascular Smooth Muscle	Approx -60 mV	Depolarization (Electron outflow)
Vascular Endothelium	Approx -80 mV	--
Liver	Approx -37 mV	--
Red Blood Cell	-8 to -10 mV	--
Leukocyte	-67 mV	--
Platelet	-52 to -60 mV	--
Fat Cell	-34 mV	--
Brown Fat Cell	-54 mV	--
Retinal Cell	-40 mV	Hyperpolarisation

Table 1: Trans-membrane potentials.

Conclusions

There is sufficient evidence to justify the postulation of a Bio-electric Law that applies to all living cells, the features of which are:

(a) Negative trans-membrane potential indicating excess intracellular negative charge of electrons

(b) A source of electrons from mitochondria which have more negative charge than the general cytoplasm

(c) Electron production from mitochondria during oxidative phosphorylation

(d) Depolarization occurs by electron outflow

(e) Repolarization occurs by electron production.

References

1. Scudder PH (2013) Electron flow in organic chemistry, (2nd edn) John Wiley & Sons, Inc, NY, USA.
2. Noble MIM (2016) The bioelectric law conference paper. NHLI Imperial College seminar. University of Aberdeen, Scotland, UK.
3. Noble MIM (2018) A re-appraisement of key aspects of ventricular excitation and contraction. Int J Clin Cardiol Res 2: 1-7.
4. Andersen O (2013) Cellular electrolyte metabolism in Encyclopedia of metalloproteins, Springer, UK. pp: 580-587.
5. Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117: 500-544.
6. Kronhaus KD, Spear JF, Moore EN, Kline RP (1978) Sinus node extracellular potassium transients following vagal stimulation. Nature: 275: 322-324.
7. Noble MIM (2018) On the mechanism of depolarisation and repolarisation in the sino-atrial node. Open J Cardiol Heart Dis: 1: OJCHD.000507.
8. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA (2011) Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. Biotechniquies 50: 98-115.
9. Brown GC, Lakin-Thomas PL, Brand MD (1990) Control of respiration and oxidative phosphorylation in isolated rat liver cells. Eur J Biochem 192: 355-362.
10. Ward MW, Rego AC. Frenguelli BG, Nicholls DG (2000) Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. J Neurosci 20: 7208-7219.
11. Noble MIM (2017) Commentary: The number of Electrons in the Nernst Equation: Energetic considerations. Open J Cardiatic Res 1: OJCR. 000502.
12. Ling GN (1997) Debunking the alleged resurrection of the sodium pump hypothesis. Physiol Chem Phys Med NMR 29: 123-198.
13. Ling GN (2007) History of the membrane (Pump) theory of the living cell from its beginning in mid-19th century to its disproof 45 years ago though still taught worldwide today as established truth. Physiol Chem Phys Med NMR 39: 01-67.
14. Alberts B Johnson A, Lewis J (2002) Ion channels and the electrical properties of membranes. Molecular Biology of the Cell (4th edn) Garland Science, New York, USA.
15. Moore JJ, Ravassard PM, Ho D (2017) Dynamics of cortical dendritic membrane potential and spikes in freely behaving rats. Science p: 355.
16. Hamil OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution recording from cells and cell-free membrane clamps. Pflugers Arch 391: 85-100.
17. Tasaki I (2002) On the cable theory of nerve conduction. Bulletin of Mathematical Biology 64: 1069-1082.
18. Boyett MR, Hart G, Levi AJ, Roberts A (1987) Effects of repetitive activity on developed force and intracellular sodium in isolated sheep Purkinje fibres. J Physiol 388: 295-322.
19. Neild TO, Kotecha N(1987) Relation between membrane potential and contractile force in smooth muscle of the rat tail artery during stimulation by norepinepheine, 5-hydroxytryptamine, and potassium. Circ Res 60: 791-795.
20. Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, et al. (2016) Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. Pharmacol Rev 68: 476-532.

21. Markos F, Ruane O'Hora, T, Noble MIM (2013) What is the mechanism of flow mediated arterial dilatation? Clin Exp Pharmacol Physiol: 40: 489-494.
22. Kippeit-Drews P, Morel N, Godfraind T (1992) Effect of nitric oxide on membrane potential and contraction of rat aorta. J Cardiovasc Pharmacol: 20: S72-S75.
23. Zima AV, Belevich AE, Povstan AV, Kharkhun MI, Tsityura Ya D, et al. (1996) Mechanism of action of nitric oxide donors on voltage-activated calcium channels in vascular smooth mucle cells. Neurophysiol 28: 296-304.
24. Bulbring E (1985) Correlation between membrane potential, spike discharge and tension in smooth muscle. J Physiol 128: 200-221.
25. Callies C, Fels J, Liashkovich I, Kliche K, Jeggle P, et al. (2011) Membrane potential depolarization decreases the stiffness of vascular endothelial cells. J Cell Sci 124: 1936-1942.
26. Zeng G (2017) Endothelial glycocalyx as a critical signalling platform integrating the extracellular haemodynamic forces and chemical signalling. J Cell Mol Med 21: 1457-1462.
27. Trivedi DP, Hallock KJ, Bergethon PR (2013) Electric fields caused by blood flow modulate vascular endothelial electrophysiology and nitric oxide production. Bioelectromagnetics 34: 22-30.
28. Jay AWL, Burton AC (1969) Direct measurement of potential difference across the human red blood cell membrane. Biophys J 9: 115-1221
29. Zavodnik IB, Piasecka A, Szosland K, Bryszewsks M (1997) Human red blood cell potential and fluidity in glucose solutions. Scand J Clin Lab Invest 57: 59-63.
30. Mottola C, Romeo D (1982) Calcium movement and membrane potential changes in the early phase of neutrophil activation by phorbol mysristate acetate: A study with ion-selective electrodes. J Cell Biol 93: 129-134.
31. Friedhoff LT, Sonenberg M (1983) The membrane potential of human platelets. Blood 61: 180-185.
32. Al-Hilli S, Willander M (2009) Membrane potential measurements across a human fat cell using ZnO nanorods. Nanotechnology: 20: 175103.
33. Girardier L, Seydoux J, Clausen T (1968) Membrane potential of brown adipose tissue. J Gen Physiol 52: 925-940.
34. Nisoli E, Clementi E, Paulucci C, Cozzi V, Tonello C, et al. (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 299: 896-899.
35. Bauer PJ (2000) The Complex of cGMP-gated channel and Na/Ca^{2+}, K exchanger in rod photoreceptors. Madame Curie Database, Landes Bioscience, Austin, Texas, USA.
36. Wu SM, Maple BR (1998) Amino acid neurotranmitters in the retina: A functional overview. Vision Res 38: 1371-1384.
37. Visual Pathways. Retrieved from: https://en.wikipedia.org/wiki/Visual_system.