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A BASIC PROGRAM FOR TEACHING MEMBRANE POTENTIAL

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Abstract : A qualitative discourse on membrane potential, albeit simple, may be equivocal at crucial points while a purely mathematical treatise on the subject, for all its exactitude, may be incomprehensible to a student of Physiology. A quasi-quantitative approach has therefore been attempted as a compromise between clarity and exactness. A BASIC program is utilised to compute the stereotype calculations and to give instant displays of the results through figures and graphic animations.

Key words : physiology teaching computer simulation membrane potential BASIC program

INTRODUCTION

The equilibrium potential of a membrane is the result of a dynamic balance between depolarizing and hyperpolarizing membrane currents. Many text-books try to simplify the matter by underpinning the discourse to the Nernst equation for the equilibrium potential of either Cl^- (1) or K^+ (2), adding a rider that the membrane potential at equilibrium is best represented by the Goldman Equation. Notwithstanding such caveats, many students attribute the resting membrane potential predominantly to either Cl^- or K^+ and dismiss the Na^+ current in the resting state as insignificant. An exclusively mathematical analysis on the other hand may be well beyond the comprehension of a medical undergraduate. Rather, a quasi-quantitative approach should give the right mix of facts and figures by simplifying the calculations markedly and at the same time, lending an insight into the broad, qualitative aspects of the subject.

A BASIC program has therefore been written using a logic that strikes a compromise between the complexity of the calculations and the accuracy of the end-result. The equilibrium potential is not directly calculated but "arrived at" by slicing the event into shorter ones, calculation of the instantaneous ionic currents, membrane potential, intracellular concentration changes and time elapsed at each stage using simple deductions, and the summation of the changes at the end of each stage.

CALCULATIONS

The ionic currents are calculated from the ionic concentration gradients and conductances. The resultant potential change is calculated from the ionic flux and the membrane capacitance. This calculation of potential is done after a fixed number of ions (10 billion) have crossed the membrane. Since the development of the electrical potential affects the subsequent ionic diffusion, the ionic currents are calculated afresh, and the whole cycle is repeated. After each cycle the change in potential diminishes, and converges towards the equilibrium potential.

(A) Calculation of cell dimensions: Calculations are done for a length of giant squid axon. Its diameter is assumed to be 0.5 mm and the length of the piece of axon is taken to be 6 cm. This length has been arbitrarily chosen to obtain convenient values of the volume (12 ml approx.) and surface area (1 sq. cm approx.) of the axon.

(B) Calculation of ionic currents: The current AMP (X) will be given by (E(X) + MP) × G(X), where E(X) is the equilibrium potential for the ion X, G(X) is the membrane conductance for the ion X, and MP is the instantaneous membrane potential.

The relevant BASIC statements are:

2010 AMPK = (26.5 * LOG (C(1)/C(2)) + MP) * GK

2020 AMPCL = (26.5 * LOG (C(6)/C(5)) + MP) * GCL

2030 AMPNA = (26.5 * LOG (C(4)/C(3)) + MP * GNA

(LOG here refers to the natural logarithm)

(C) Calculation of potential changes following diffusion : When a monovalent ion (Na^+, K^+ or Cl^-) adheres to one side of the membrane, it electrostatically attracts an ion of equal charge to the opposite side of the membrane, and the entire configuration resembles a parallel plate capacitor. Thus, when 10^9 (1 billion) monovalent ions adhere to the same side of a 1 cm^2 membrane, a potential of 0.16 millivolts will develop. (The charge of the monovalent ion is 4.8×10^{-10} esu and the membrane capacitance is 1 $\mu Farad/cm^2$).

(D) Calculation of the equilibrium potential : With the different ionic currents known, the resultant membrane potential is reviewed after a total of 10 billion ions have diffused across the membrane. The absolute number of the different ions diffusing will obviously be in direct proportion to the currents since all the ions under consideration are monovalent. The ratio of AMPK, AMPCL and AMPNA (the ratios adding up to 10) which are designated respectively as IK, ICL and INA is calculated by the following BASIC statements :

2040 DENOM = ABS (AMPK) + ABS (AMPCL) + ABS (AMPNA)

2050 IK = AMPK/DENOM * 10: ICL = AMPCL/ DENOM * 10: INA = AMPNA/DENOM * 10

Since IK, ICL and INA are only ratios, and since IK + ICL + INA=10, it is convenient to equate the total current to 10 billion ions/unit-time, the proportionality constant being absorbed in the value of the unit-time (T). Since diffusion of 1 billion ion changes the membrane potential by 0.16 mVolts, the membrane potential change following the diffusion of IK billion ions is equal to IK × 0.16. This is expressed by the following BASIC statements :

2400 MP = MP-IK * 0.16

2600 MP = MP-ICL * 0.16

2800 MP = MP+INA * 0.16

The control is then directed back to statement 2010 for a fresh review of the ionic currents. Equilibrium is attained when the hyperpolarizing currents eg. the outward K^+ or the Cl^- current (if it is directed inwards) balance the depolarizing currents eg. the inward Na^+ current or the Cl^- current (if it is directed outwards). Since looping through the above statements will generate a convergent but infinite series of values, equilibrium is declared when the values (of membrane potential) change minimally (arbitrarily fixed at <0.01 mVolt) after a solitary looping. It is expressed by the statement :

3000 IF ABS (MP-PREVMP) <0.01 THEN PRINT MP ELSE MP=PREVMP

(E) Calculation of time taken : The time taken (T) for 10 billion ions to diffuse is calculated by dividing the number of any particular ion diffusing by its current. The charge carried by IK billion K^+ is equal to IK × 10^9 × 1.6 × 10^{-19} coulombs. If the K^+ current = AMPK microAmperes then T = IK × 10^9 × 1.6 × 10^{-19}/ [AMPK × 10^{-6}] seconds. Since IK + AMPK = 10 + DENOM (cf. statement 2050 above), therefore T=1600/ DENOM microsecond. This is expressed by the following BASIC statement:

3100 INTERVAL=1600/DENOM: TOTALTIME = TOTALTIME+INTERVAL

As expected, the result obtained is independent of the particular ion considered. Also, as DENOM is the sum of the three ionic currents at any instant, and since these currents generally tend to decrease as equilibrium approaches, it follows that "T" increases progressively. Thus, the state of the membrane potential

and the ionic currents which are displayed on the screen depict the situation after successively greater intervals.

(F) Calculation of intracellular concentration changes: The change in concentration (C) when a certain number of ions (N) diffuse across the membrane can be calculated from the formula: C=N/[A × cell volume], where A = 6.023×10^{23}, the cell volume is given in Litres and C is given in moles/Litre. Thus, when 1 billion ions diffuse out of the cell, the intracellular concentration drop = 0.0138 picomoles/ 100 mL.

The relevant BASIC statements are:

2410 AMTK =AMTK+ IK

2610 AMTCL = AMTCL + ICL

2810 AMTNA = AMTNA + INA

2900 A = 0.0138 : AMTK = AMTK * A : AMTCL = AMTCL * A : AMTNA = AMTNA*A

DISPLAY ENHANCEMENTS

The screen shows the outline of a cell. The intracellular and extracellular concentrations and the conductances of the ions are displayed on it. The magnitude and direction of the ionic current is depicted by an arrow. The width of the arrow is proportional to the magnitude of the current. The magnitude of the current is also indicated in figures alongside the arrows. The ions are shown to be flitting across the membrane long the arrows. Each moving "+" or "-" sign depicts 1 billion ions. The membrane potential, the time taken for the potential changes, and the change in the intracellular concentrations of K^+, Na^+ and Cl^- are given in each screen. Two vertical bars depict the total depolarizing and hyperpolarizing currents. When their heights approximate, the screen heralds the equilibrium. The relevant BASIC statements are:

2100 BM = 0:BP = 0 'BM = HYPERPOLARIZING & BP = DEPOLARIZING CURRENT.

2110 IF AMPK>0 THEN BM = BM + ABS (AMPK) ELSE BP = BP + ABS (AMPK)

2120 IF AMPCL > 0 THEN BM=BM + ABS (AMPCL) ELSE BP=BP + ABS (AMPCL)

2130 IF AMPNA < 0 THEN BM = BM + ABS (AMPNA) ELSE BP+BP+ABS (AMPNA)

INTERACTIVE AREAS

The student can alter the values of ionic concentrations and conductances and also initialize the value of membrane potential.

DISCUSSIONS

Two samples of the run-time on-screen display have been illustrated here. Fig. 1 shows the screen

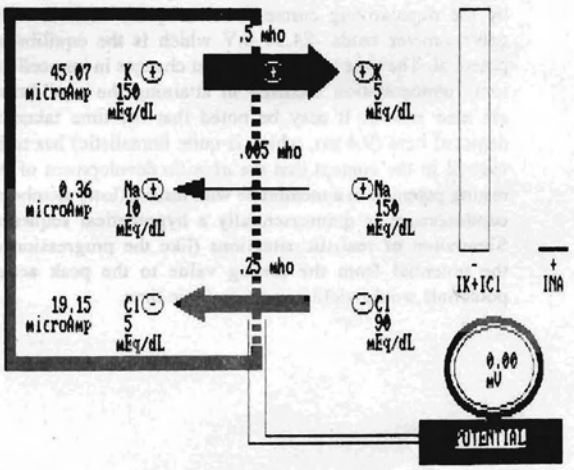

Fig. 1 : A reverse graphics dump of the first of a series of screens depicting the sequential changes in ionic currents, membrane potential, intracellular ionic concentrations, and time. The values of the ionic concentrations and conductances displayed are the default values. The vertical bar labelled "-" represents the strong hyperpolarizing current (in arbitrary units) carried by K^+ and Cl^-.

display as obtained with the default values of ionic concentrations and conductances. The vertical bars show that there is an overwhelming hyperpolarizing current attributable to both K^+ and Cl^-. Fig. 2 shows the equilibrium at -84.54 mV which is attained in about 9.4 ms and is attended with negligible changes in

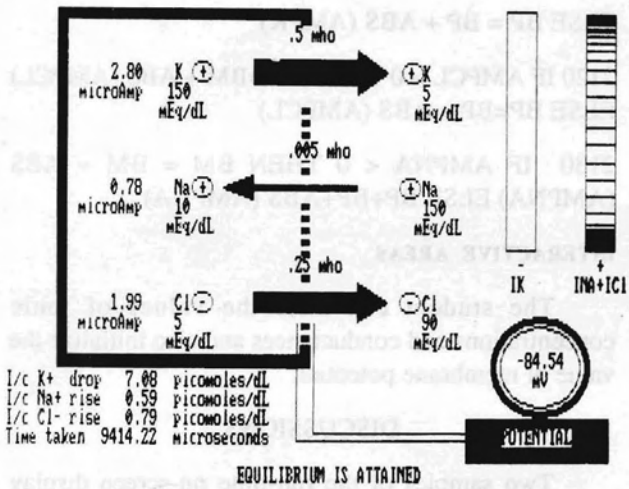

Fig. 2 : A reverse graphics dump of the final screen of the series, depicting equilibrium. The vertical bars show that the hyperpolarizing current, now carried by K^+ alone is matched by the depolarizing current carried by Na^+ and Cl^-. The galvanometer reads -84.54 mV which is the equilibrium potential. The time taken and the net changes in intracellular ionic concentration incurred in attaining the equilibrium are also shown. It may be noted that the time taken as depicted here (9.4 ms, which is quite unrealistic) has to be viewed in the context that the *abinitio* development of the resting potential in a membrane with resting (low) membrane conductances is quintessentially a hypothetical sequence. Simulation of realistic situations (like the progression of the potential from the resting value to the peak action potential) would yield a more realistic time.

intracellular ionic concentrations. The Cl^- current has reversed and the vertical bars show the hyperpolarizing current, now constituted by K^+ alone, balanced against the depolarizing Na^+ and Cl^- currents.

CONCLUSION

The merits of a problem solving approach to teaching elecrochemical driving force to undergraduates have been discussed at length by Nolan (3). Based broadly on the same precepts, and addressed to the same category of students, the present work uses a BASIC program to sequentially analyze the different ionic currents and the resultant potential and ionic concentration changes, realizing a more holistic treatment of the subject.

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