On the Liquid Junction Potential in the Sucrose-gap Method

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The sucrose-gap method[1] was used for the recording of the electrical activity of smooth muscle[2,3,4]. When observing effects of various ions on the electrical activity of cell membrane by the sucrose-gap method, it must be considered on the liquid junction potential due to replacing with various solutions. When the main ionic composition of the normal solution was changed by other ionic composition, for example chloride ion in NaCl was replaced by other monovalent or divalent anions, the liquid junction potential between the normal solution and the sucrose solution is changed into the junction potential between the test solution and the sucrose solution. The recorded membrane potential change is the summation of the true membrane potential change and the liquid junction potential change. Therefore, for estimating the changes of the cell membrane potential, the recorded membrane potentials must be corrected by the liquid junction potential changes. In this paper, the liquid junction potential in the sucrose-gap method was studied theoretically and experimentally.

METHODS

The changes of the liquid junction potentials were measured by the apparatus shown in Fig. 1. Each bath (A, B and C) contained 100 ml of solution. Bath A and B contained Ringer-Locke solution (or 50 ml of Ringer-Lock solution + 50 ml of sucrose solution). The original level of potential was set from A and B solution. The liquid junction potential change was measured by connecting the bath B and C which contained the test solution. From this value, the change of the liquid junction potential in the sucrose-gap method was estimated.

In the sucrose-gap method, the outlet of the normal solution and the sucrose solution or the test solution and the sucrose solution was same. In this part, it assumed that these solutions were mixed at a certain rate. Therefore the solution A, C and B were prepared by mixing 50 ml of the normal solution or the test solution and 50 ml of the sucrose solution. The composition of the Ringer-Locke solution was as follows ; NaCl, 154 ; KCl, 5.6 ; CaCl₂, 2.2 ; NaHCO₃, 2.0 and

glucose, 5.0 (mM).   NaCl was replaced by isomolar NaNO$_3$, NaI, NaBr, NaSCN, Na aspartate, Na glutamate, Na methylsulfonate and Na p-toluensulfonate.   When NaCl was replaced with Na$_2$SO$_4$, the concentration of Na$_2$SO$_4$ was 75 mM.
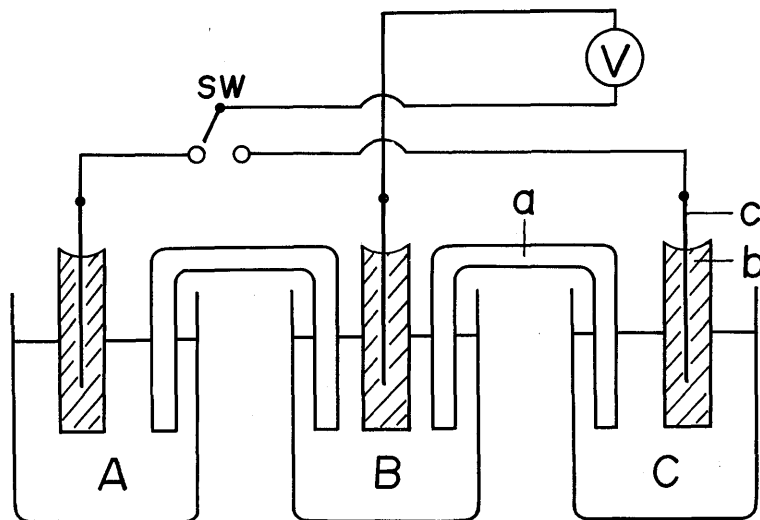

Fig. 1. The circuit for the measurement of changes of the liquid junction potentials.
a ; 10% sucrose-agar bridge, b ; 3M-KCl-agar electrode and c ; Ag-AgCl wire.

RESULTS

I. Liquid junction potentials

NaCl and sucrose

The liquid junction potential between the normal solution and the sucrose solution was estimated by two different methods.   For calculating the potential differences, the 0.15M of NaCl solution was used as the normal solution.

Method A ; The concentration of the sucrose solution in the sucrose-gap method was 10 % (v/w).   Acetate and other substances were considered as the trace materials in the sucrose solution.   The commercial sucrose (special grade) contains about 0.006 % of acetate.   When making the 10 % sucrose solution which was prepared by above sucrose, the concentration of acetate is 10^{-4}M.   The liquid junction potential between the sucrose solution and NaCl solution (0.15 M) was calculated from Henderson's equation

$$E = \frac{RT}{zF} \frac{(C_1 u_1 - C_1 v_1) - (C_2 u_2 - C_2 v_2)}{(C_1 u_1 + C_1 v_1) - (C_2 u_2 + C_2 v_2)} \ln \frac{C_1(u_1 + v_1)}{C_2(u_2 + v_2)} \quad \cdots\cdots\cdots\cdots (1)$$

where solution 1 = sucrose solution, $u_1 = u_H = 315$, $v_1 = v_{CH_3COO} = 35$, $C_1 = 10^{-4}$ (M) and solution 2 = NaCl solution, $u_2 = u_{Na} = 43.5$, $v_2 = v_{Cl} = 65.5$, $C_2 = 0.15$ and $t = 18°C$.

Then $E = 31.35$ mV and the potential of the NaCl solution was positive. When $C_1 = 10^{-5}$M, $E = 42.91$ mV.

Method B ; The sucrose solution and the normal solution make a boundary phase along the stream. At this part, if cation and anion in NaCl solution are diffused into the sucrose solution at a certain rate, the difussion potential is produced at the boundary phase. The diffusion potential is given by the equation,

$$E = \frac{RT}{zF} \frac{u-v}{u+v} \ln \frac{C_1}{C_2} \cdots\cdots\cdots (2)$$

where solution 1 = sucrose solution, solution 2 = NaCl solution, C_1 is the concentration (x M) of the diffused NaCl. The diffusion potentials were calculated for various x values. The result was shown in Table IA, the results calculated on the case of $C_2 = 0.075$ were also shown. As shown in Table IA, when $x = 1.5 \times 10^{-4}$, $E = 35.00$ (mV) and the potential of NaCl solution was positive.

Table I. A Calculated liquid junction potentials (mV) at various x (C_1).

$C_1 = x$(M)	1.5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}
NaCl	35.00	25.50	17.32	13.78
	(31.45)	(21.86)	(13.70)	(10.20)
KCl	1.20	0.86	0.59	0.47
LiCl	56.22	40.38	27.76	22.04
NaNO$_3$	29.96	21.83	14.82	11.80
	(26.96)	(18.74)	(11.75)	(8.74)
NaI	36.22	26.37	17.91	14.25
	(32.58)	(22.65)	(14.20)	(10.57)
NaBr	36.84	26.87	18.25	14.52
	(33.14)	(23.04)	(14.44)	(10.75)
Na$_2$SO$_4$	17.39	12.08	7.58	5.64
	(15.49)	(10.24)	(5.67)	(3.80)

$C_2 = 0.15$; ( ) indicates the case of $C_2 = 0.075$.
$C_2 = 0.075$; ( ) indicates the case of $C_2 = 0.038$ for SO$_4$ solution.

Sucrose and isotonic KCl

In the sucrose-gap method, the isotonic potassium solution was flowed into one side of the U-shaped tube. The cell membrane was depolarized by this external isotonic potassium solution. The liquid junction potential between the sucrose

solution and the isotonic potassium solution was calculated by above two methods. For calculation, the concentration of 0.15 M potassium chloride was used.

Method A ; When the concentration of acetate is 10^{-4}M, E = 1.33 mV and the potential of potassium solution was positive.

Method B ; The calculated values for various x (from 1.5 x 10^{-4} to 10^{-2}M) were shown in Table IA. The potential of potassium solution was positive.

The calculated values by two methods were very small. The result indicates that it is not neccessary to consider the liquid junction potential due to the sucrose solution and the potassium solution.

Cation replacement

When observing the effect of sodium ion on the electrical activity of the cell membrane, sodium ion in normal solution is replaced by other mono-valent cation. The liquid junction potential of isomolar LiCl solution to the sucrose solution was calculated by above two methods.

Method A ; When $u_{Li} = 33.4$, $v_{Cl} = 65.5$ and $C_2 = 0.15$ M, E = 49.64 mV and the potential of LiCl solution was positive.

Method B ; The concentration (x) of C_1 was changed from 1.5 x 10^{-4} to 10^{-2}M. The calculated values were shown in Table IA. The values of the liquid junction potential of LiCl were larger than that of NaCl. This due to the difference of u_{Na} and u_{Li}.

Anion replacement——large values of v

For the study on effects of anions on the electrical activity of the cell membrane, chloride ion in normal solution was replaced with other mono-valent or divalent anions (for example chloride ion was replaced by nitrate ion). The liquid junction potential of the normal solution to the sucrose solution was transfered to that of the test solution to the sucrose solution. Obviously these values of liquid junction potentials were different.

Method A ; The liquid junction potentials between the sucrose solution and various anion solutions were as follows; NO_3, 26.85 mV ; I, 32.60 mV ; and Br, 33.20 mV. The potential of these solutions were positive and the order of values are lyotropic order.

Method B ; The calculated values of $C_1 = x$ (M) in various anion solutions were shown in Table IA. In Table IA, the values in the case of $C_2 = 0.075$ M were also shown. The values of the mobility for various anions are $v_{NO_3} = 61.7$, $v_I = 66.5$, $v_{Br} = 67.0$ and $v_{SO_4} = 68.5$. When $u < v$, $(u-v)/(u+v)$ is negative, $C_1 < C_2$, so ln (C_1/C_2) is negative. Therefore E > 0 under the conditions. The potentials of these anion solutions are positive.

Anion replacement——small values of v

When using an anion replacement solution, it is considered that the anion which has a smaller hydrated radius than that of chloride ion is permeable to the cell

membrane, while an anion which has large molecular structure is less or non-permeable to the cell membrane. In this experiment, aspartate, glutamate, methyl-sulfonate and p-toluensulfonate were used as a less or non-permeable anion. If the value of anion mobility (v) is smaller than the value of u_{Na}, then $(u-v)/(u+v) > 0$. As the diffused concentration ($C_1 = x$ M) is smaller than C_2, then ln $(C_1/C_2) < 0$. Therefore the potential of these solutions is negative. The diffusion potential of the solutions was calculated by Method B. The result was show in Table IIA. Only the cases which values of v were smaller than that of u_{Na} were calculated. As shown in Table IIA, the values of the liquid junction potential were taken large value with decreasing the values of v and x. The following values of the mobility of the mono-valent anion were known ; acetate, 35 ; cyanacetate, 41.8* ; propionate, 35.8* ; butyrate, 32.6* ; benzoate, 32.3* and picrate, 30.4* (* indicates the limiting equivalent conductivity).

Table I.B Calculated $\triangle E$ (mV) at various $X(C_1)$

$C_1 = X(M)$	1.5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}
LiCl	21.22	14.88	10.44	8.26
NaNO$_3$	–5.04	–3.67	–2.50	–1.98
	(–4.49)	(–3.12)	(–1.98)	(–1.46)
NaI	1.22	0.87	0.59	0.47
	(1.13)	(0.79)	(0.50)	(0.37)
NaBr	3.06	1.37	0.93	0.74
	(1.69)	(1.18)	(0.74)	(0.55)
Na$_2$SO$_4$	–17.61	–13.42	–9.74	–8.14
	(–15.96)	(–11.62)	(–11.65)	(–6.40)

$C_2 = 0.15$; ( ) indicates the case of $C_2 = 0.075$.
$C_2 = 0.075$; ( ) indicates the case of $C_2 = 0.038$ for SO$_4$ solution.

Changes in the liquid junction potential ($\triangle E$)

The liquid junction potentials in the sucrose-gap method were produced at the phase of the normal solution or the test solution and the sucrose solution, the phase of the potassium solution and the sucrose solution. On the measurement of the membrane potential, the isotonic potassium solution was flow continuously through one side tube of the sucrose-gap. Therefore only the change of the liquid junction potential due to the normal solution and the test solution to the sucrose solution is affectable. The potentials at both ends in the sucrose solution are assumed to be equal and continuous in the sucrose solution. The degree of the diffusion rate is assumed to be equal at both ends. Under the conditions, the change of the liquid junction potentials can be estimate from the values in

Table IA and IIA.

Cation replacement solution ; When sodium ion in NaCl solution was replaced by Li ion, the change of the liquid junction potential was calculated from the values in Table IA, e.g., $\triangle E = E_{NaCl} - E_{LiCl}$. The result was shown in Table IB. Anion replacement solution ; The values of the case of NO_3, I, Br and SO_4 solution were also calculated from Table IA. The result was shown in Table IB. The changes in the liquid junction potentials were generally small except in SO_4 solution. The direction of change in NO_3 and SO_4 solution was negative and that in I and Br solution was positive.

When the chloride ion was replaced by the anion of which mobility (v_2) was smaller than that of sodium ion (u_{Na}), the liquid junction potential had large value with decreasing the concentration (x) and the potential was negative to the sucrose solution. Therefore the changes in the liquid junction potentials were considerably large values. The result was shown in Table IIB.

Table II.A   Calculated liquid junction potentials (mV) at various v (20–40) and x (C_1)

x (M) \ v	1.5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}
20	−64.10	−46.70	−31.72	−25.24
	(−57.66)	(−40.08)	(−25.12)	(−18.71)
30	−31.81	−23.22	−15.77	−12.55
	(−28.67)	(−19.93)	(−12.49)	(−9.31)
40	−7.26	−5.39	−3.66	−2.91
	(−6.55)	(−4.55)	(−2.85)	(−2.12)

$C_2 = 0.15$; ( ) indicates the case of $C_2 = 0.075$.

Table II.B   Calculated $\triangle E$ (mV) at various v and x (C_1)

x (M) \ v	1.5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}
20	−99.10	−72.20	−49.04	−39.02
	(−89.11)	(−61.94)	(−38.82)	(−28.91)
30	−66.81	−48.72	−33.09	−26.33
	(−60.12)	(−41.79)	(−26.19)	(−19.51)
40	−42.24	−30.89	−20.98	−16.69
	(−38.00)	(−26.41)	(−16.55)	(−12.32)

$C_2 = 0.15$; ( ) indicates the case of $C_2 = 0.075$.

II.   Experimental results

Anion replacement——large values of v

The mean values of the measured potential changes in NO_3, I, Br, SCN and

SO_4 solution were shown in Table III. In Table III, $+$ and $-$ indicate positive and negative potential change to NaCl solution respectively. The obtained values in Table III except SCN solution were compared with Table IB. The direction of the change in potentials consisted with the estimated one. The values of change in I and Br solution ($\triangle E$) were correspond to the values at $x = 1.5 \times 10^{-4}M$. The value of mobility of SCN was not clear. If the order of the mobility is same as the lyotropic order ($Br > I > Cl > NO_3 > SCN$), the value of mobility of SCN is smaller than v_{NO_3}. For $v_{SCN} < v_{NO_3}$, the liquid junction potential of SCN solution is larger than that of NO_3 solution and negative. When NaCl solution was replaced by NaSCN solution, the change of the liquid junction potential was larger than that of NO_3 solution. The obtained result consisted with above consideration.

Table III. Mean values of measured $\triangle E$ (mV) and calculated values of C_2' in various anion solutions

solutions	$\triangle E$ (mV)	C_2'	solutions	$\triangle E$ (mV)	C_2'
SO_4	-9.0	0.6984	methylsulfonate	-19.7	0.4558
SCN	-6.0	0.7872	p-toluensulfonate	-29.3	0.3108
NO_3	-0.7	0.9728	aspartate	-33.6	0.2618
I	1.8	1.0745	glutamate	-44.2	0.1712
Br	2.2	1.0917			

Anion replacement——small values of v

The potential changes by aspartate, glutamate, methylensulfonate and p-toluensulfonate replacement were measured and the results were summarized in Table III. As shown in Table III, these values were $-20 - -40$ mV and negative to NaCl solution. The large values in changes may due to that the mobility of above anions is smaller than u_{v_a} value. In Table II B, the values which were correspond to above $\triangle Es$ were at $x = 1.5 \times 10^{-4}M$ and $v = 40$ in aspartate and glutamate solution. The changes of the liquid junction potential in the p-toluensulfonate and methylensulfonate solutions were $-20 - -30$ mV. These values were lower than the liquid junction potential of NaCl solution (31.45 mV), therefore, it seems that the liquid junction potential of these solutions is positive to the sucrose solution and the values of the mobility are to be $u_{Na} < v < v_{Cl}$. As the changes of the liquid junction potentials at $v_{NO_3} = 61.7$ was -5 mV ($C_2 = 0.075, x = 1.5 \times 10^{-4}M$), the mobility of these anions may be less than $v = 61.7$.

Estimation of the diffused concentration

In this experiment, the solution B and C were connected by the sucrose bridge,

these two solutions were not mixed. If a certain amount of cation and anion in these solutions are diffused into the sucrose agar, the phase boundary potential is produced. The potential difference in this system is given by

$$E = E_1 + E_2 = \frac{RT}{F} \ln \frac{C_1}{C_1'} + \frac{RT}{F} \ln \frac{C_2'}{C_2} = \frac{RT}{F} \ln \frac{C_2'}{C_1'} \cdots\cdots\cdots (3)$$

where C_1 and C_2 are the concentration of NaCl solution and the test solution, C_1' and C_2' are the diffused concentration of NaCl solution and the test solution into the sucrose-agar. The diffusion potential between C_1' and C_2' is negligible. From above equation, the concentration of C_1' and C_2' can be estimatd by using the measured values in Table III. When $C_1' = 1$, the relative values of C_2' were shown in Table III. The result in Table III suggests that the diffused concentrations at both ends of the sucrose-agar bridge are almost same in NO_3, I and Br solutions. In glutamate and other solutions C_2' values were small. This suggests that the diffusion rates at both ends of the sucrose bridge are different.

SUMMARY

1. For estimating the liquid junction potentials in the sucrose-gap method, the liquid junction potentials between various ionic solutions were measured.

2. The changes of the liquid junction potentials between anion replaced solutions (NO_3, I, Br, SCN and SO_4) and NaCl solution were $-9.0 - 1.8 \, mV$. These values were correspond to the values calculated from the diffusion potentials at a certain concentration in various anion solutions.

3. The anion replaced solution (aspartate, glutamate, p-toluensulfonate and methylensulfonate) showed the large values of the changes in the liquid junction potentials ($-20 - -40 \, mV$). The values of the mobility in above anions were estimated theoretically.

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