Resting Potential of the Muscle-Cell in Toad's Stomach.*

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It is, nowadays, a class-room experiment to record intracellular potential with the glass capillary electrode (11) and a vast number of experiments upon a membrane potential of physiologically excitable cell, such as nerve, skeletal-, cardiac- or smooth muscle, have been reported.

As for a resting potential of the smooth muscle of stomach, however, little success has been achieved since Greven (10) reported the experiment on this material from *Salamandra maculosa* in 1954. Although it is likely that the small size of muscle-cell in another species has resulted the difficulty to record intracellular potential, the recent advances in the techniques to prepare the electrode and amplifier or to record the potential changes are overcoming this difficulty. In this paper, the resting potential of Toad's stomach-muscle in Ringer's solution was statistically evaluated and its changes in abnormal ionic solutions were examined.

METHODS

Throughout the experiments, the stomach muscle of winter toads (*Bufo vulgaris*) were used at room temperature (17～23°C). Muscle-strips (length: 2～3 cm, width: 3～5mm) were dissected from the pyloric or corporal portion under 20% urethane anesthesia (2～3cc intraperitoneally injected); and the mucous layer (mucosa and submucosa) was removed. The preparation was mainly the circular muscle and retained excitability to electrical stimuli and/or to pharmacological drugs. Records from the muscle-cell were obtained with glass capillary electrodes (11) with tips less than 0.5μ in diameter. They were filled with 3M-KCl and had a d. c. resistance of 20～60MΩ.

The preparation was loosely fixed on paraffin block in a convenient vessel by pinning it with its mucous side upward. It was immersed in the vessel filled with Ringer's solution or test solution. Ringer's solution contains 111mM NaCl, 2.0mM KCl, 3.4mM $CaCl_2$ and 0.3mM $NaHCO_3$ (pH: 7～8).

The capillary serving as an exploring electrode was attached to a half-rigid holder on a micromanipulator and the tip was inserted into the preparation.

* An experiment, directed by Prof. G. KAWABATA in the Department of Physiology.

The Ag-AgCl electrode as an indifferent one was placed in the bathing solution. The high input d. c. preamplifier (Nihon-Koden, MZ-3B) was used, whose output was amplified and recorded by both cathoderay oscilloscope and ink-writing electromagnetic one.

RESULTS and DISCUSSION

Resting potential in Ringer's solution.

According to Burnstock, Dewhurst & Simon (7) the circular muscle cell in a stomach of toad (*Bufo marinus*) is spindle-shaped, about 260μ in length and 5$\sim$8μ in middle width. It is likely that the material in this experiment had the values of similar magnitude, although they were not measured precisely.

In measuring the resting potentials the same or another electrode was inserted 3$\sim$4 times into one preparation, and some arbitrary criteria were adopted in estimating the validity of the results obtained. For instance, if the electrode-tip had not been inserted into the cell the potential difference would not be recorded. If the impalement of electrode-tip in a cell could not be attained, the potential would not be stable, and if the insertion of electrode had damaged the cell-membrane the potential would not be recorded, or would gradually decrease owing to the short-circuit between the electrode-tip and the bathing fluid. In the case where the electrode-tip was broken after the insertion, the spot of the cathoderay oscilloscope would not come back to the original level after pulling back the electrode into the bathing fluid.

From these criteria, following procedures were considered to result in a satisfactory measurement. When the electrode was inserted into the preparation the negative (glass capillary- against indifferent electrode) potential was suddenly observed on the recording apparatus. This potential was steady for at least 3 minutes. After pulling the electrode out of the muscle cell the original potential, which is 0-level, could be deflectively recorded in the range of $\pm$2mV.

The standard potential from a potentiometer was similarly recorded and the measured potential was calibrated. These values, however, were not the true resting potentials because a glass capillary electrode has the so-called "tip-potential" (23). Therefore, the tip of each electrode was artificially broken after the experiment and the potential difference before and after the breaking was measured. With other words, the potential level when the electrode-tip was in the bathing fluid was recorded at first. Then, the electrode was inserted into the preparation. The negative potential observed in this case is the "not-corrected" resting potential. After its measurement, the electrode was pulled back into the fluid and the original potential was observed again, as noted above. Thereafter, the tip was broken in the fluid. The potential difference, if any, is the tip-potential; which is negative against the indifferent electrode and each

electrode has a different value. In this experiment, it ranged between 0 and 28 mV in Ringer's solution, and the results obtained with the electrode having a tip-potential greater than 20 mV were discarded. As both the "not-corrected" resting potential and the tip-potential are negative, the additive value of the two is the "corrected" resting potential (cf. 23).

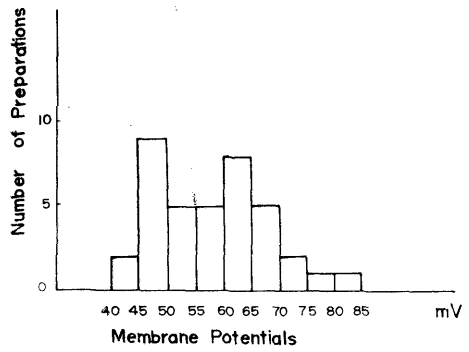

Fig. 1 Hitogram of the membrane resting potentials in the Ringer's solution. Tip-potential of the microelectrode was corrected. Preparation, from pyloric portion: Mean, 58.3: S. D., 10.2: N=35.

Figure 1 is a histogram of the corrected resting potential values of the muscle-cell in pyloric portion. In this figure, the distribution is flat, number of samples, 35, mean value, 58.3 mV and the standard deviation, 10.2. In the similar experiment with the preparation from gastric body, those values are 36, 52.9 and 8.7, respectively. The difference between two mean values is statistically significant ($p=0.01$).

It is well known that the resting potential of skeletal twitch muscle (14) or of heart muscle (26) takes the value from 90 to 95 mV. However, somewhat low values have been reported in skeletal slow muscle or in smooth muscle; e. g. the resting potential of slow muscle of frog's *M. iliofibularis* was average value of 60 mV (19), and that of taenia coli of guinea pig, 50～60 mV (2, 3). On the other hand, it is also generally accepted that the value of membrane potential is mainly determined by extracellular and intracellular concentrations of potassium ($[K]_0$, $[K]_i$ resp.) and that it is modified by those of sodium ($[Na]_0$, $[Na]_i$) (11, 12). In the toad stomach, Burnstock et al. (7) reported that the intracellular concentration of potassium was lower (68.5 mM) and that of sodium was higher (61～68 mM after the preparation was washed with Ringer's solution) than in skeletal muscle. Those ($[K]_0$ and $[Na]_0$) in Ringer's solution in this experiment were 2.0 mM and 111.0 mM, respectively. When their values of $[K]_i$ and $[Na]_i$ are applicable to the present preparation, the potassium-equilibrium potential is - 89 mV and that of sodium 15 mV. The measured resting potential (in Fig. 1) does not greatly differ from the value expected from these equilibrium potentials. On the other hand, the ratio of permeabilities of potassium and sodium can be calculated from the Goldman's constant field equation, assuming that

contribution of chloride-ion to the resting potential is negligible; that is about 1 : 0.04 at 18°C, using 58.3 mV as the membrane potential. The ratio in skeletal or heart-muscle is said to be 1 : 0.027 or 1 : 0.01. It may be concluded that the sodium-permeability of stomach muscle is slightly higher than that of skeletal- or heart-muscle. Kiessling (17) reported in 1960 that the permeability had been also higher in frog's tonic skeletal muscle (skeletal slow muscle in above notation).

Resting potential in sucrose solution.

A histogram of the "not-corrected" resting potential measured in isotonic sucrose solution (230 mM) is shown in Fig. 2. In the figure, the distribution is more flat than in Fig. 1, number of samples, 41, mean value, 82.4 mV and standard deviation, 9.0. It seems that the value of resting potential is greater (hyperpolarized) in sucrose solution than in Ringer's solution.

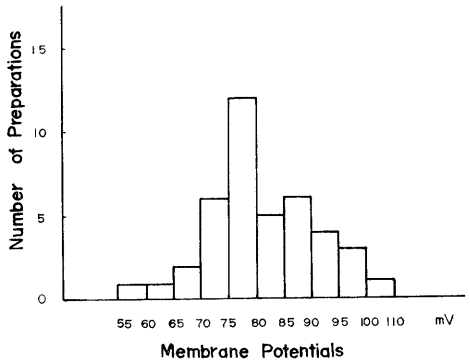

Fig. 2 Membrane resting potentials in isotonic sucrose solution, without the correction for the tip-potential.
Pyloric portion; Mean, 82.4; S. D., 9.0: N=41.

The preparation was pyloric portion and the criteria for the measurements were the same as above. The correction, however, was somewhat different because of the tip potential and of the physico-chemical potential at the indifferent electrode. Oki (23) reported that the lower was the concentration of KCl-solution, in which the electrode was placed, the higher was the tip-potential. Its value obtained in Ringer's solution could not be used for the correction of the measurement in sucrose solution (non-electrolyte). In the latter solution, however, the consistent value of tip-potential could not be obtained. This might be because of that the low electrolyte concentration resulted an instability of potential measurement. Therefore, the not-corrected value of resting potential was used in drawing Fig. 2. In spite of this difficulty, it could be concluded that the preparation had the tendency to hyperpolarize in the isotonic sucrose solution, as the corrected value was higher than the not-corrected value.

It was assumed, also in Fig. 1, that there was no tip-potential when the electrode-tip was impaled inside the cell. This was the case in Oki's report (23).

The intracellular concentration of potassium, however, was assumed to be about 68.5 mM; and the tip potential would exist in the inside of cell, although it was smaller than that in the extracellular fluid. There was, unfortunately, no means to measure directly the tip potential in the cell-interior. This might be, partly, the cause of flatness in the histograms of Fig. 2 or Fig. 1.

Another difficulty in the correction was an uncertainty of junction potential at the indifferent electrode in sucrose solution. For its estimation, the constant potential from saturated calomel-electrode was fed to the grid of preamplifier and 3M-KCl-agar-electrode, in which the Ag-AgCl wire was embedded, and Ag-AgCl electrode was connected to the ground side. The calomel-electrode was placed in the 3M-KCl solution and the other electrode, in the test solution (Ringer's or sucrose). The two solutions were connected with 3M-KCl agar-bridge. When an indifferent electrode was 3M-KCl-agar, the established potential was stable and the potential at the indifferent electrode was negligible in both solutions. In the case where the Ag-AgCl electrode was indifferent, it could not be neglected, especially in sucrose solution. If it were stable, however, the value of resting potential would not be affected; because, in practice, 0-level of the potential is a relative and not an absolute value. In this experiment, although the stability was not so good as that with KCl-agar electrode, it seemed that one could estimate the resting potential without the correction of this potential.

Although the corrections were incomplete from the above difficulties, it could be observed that the resting potential was, at least qualitatively, increased in sucrose solution. The result could be expected from the Goldman's equation; i. e. $[K]_0$ is lower in sucrose solution than that in Ringer's solution. And, a contribution of potassium ion to the membrane potential is greater than that of sodium, even if the restrictive applicability of the equation in the solution of low electrolyte-concentration has been reported (24). It is also reported in the frog's skeletal muscle that the membrane potential did not change when the NaCl in Ringer's solution was reduced by changing it with isotonic sucrose solution (22).

Effects of some ions or drugs.

The concentration of KCl or $CaCl_2$ in the bathing fluid was changed and the resting potential was measured. In the other experiments, a small amount of $BaCl_2$, acetylcholine or adrenaline was added to the bathing Ringer's solution. There were no corrections for the hypotonicity (see below) and for the tip potentials.

The relationship between the resting potential (ordinate) and the logarithm of KCl-concentration in external Ringer's solution (abscissa) is illustrated in Fig. 3. It shows that the resting potential decreases in the fluid of high potas-

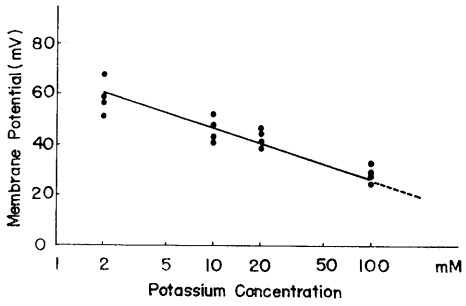

Fig. 3 Relationship between the corrected resting potential and the logarithm of KCl-concentration in external Ringer's solution.

sium concentration.

It is well known that the Nernst's equation is applicable in the physiologically excitable cells and the linear relationship can be seen between the two parameters. The applicability was already shown and discussed in the *Nitella* (23), taenia coli of guinea pig (13, 20) or in the rat uterus (16, 8). This was also seen, although qualitatively, in Fig. 3, and the quantitative study was laid aside. The deviation from the linear relationship could be explained by the high sodium-permeability of the preparation and/or by the technical difficulties for the correction.

Table

Outer solution	Rest. Potential* (mV. mean)	Standard Deviation	Samples (N)	Preparation (portion)
Ringer's Solution	50.5	5.6	65	pyloric
Ringer's Solution	45.0	4.1	18	Body
isotonic Sucrose	82.4	9.0	41	pyloric
isotonic Sucrose	75.2	19.6	27	Body
half-isotonic Sucrose	78.2	9.4	26	pyloric
7.4 mM $BaCl_2$**	44.1	8.3	26	pyloric
10^{-5} Acetylcholine**	41.0	4.8	12	pyloric
10^{-5} Adrenaline**	51.5	6.3	17	pyloric
$CaCl_2$-free Ringer	49.3	3.95	15	pyloric

* tip potential of microelectrode was not corrected, for a comparison.
** final concentration in Ringer's solution. 10^{-5} means g/ml.

The effects of other ions or drugs are tabulated together with the results described before. It can be seen in the table that acetylcholine has a depolarizing effect, as already reported by other investigators on the other smooth muscles (9, 5), and that barium chloride has a similar effect. The administration of adrenaline (10^{-5}) or deprivation of calcium had no appreciable effect on the resting potential, although the hyperpolarizing action concerning the former (5) and the depolarizing effect of calcium-free solution have been reported in the taenia coli of guinea pig (2, 6, 13).

Addendum: 1. The impalement of electrode was easier in the sucrose solution than in Ringer's solution. This might be caused by the efflux of electrolyte from the cell-interior to the sucrose solution and, secondly, by the swelling of the cell following the entrance of water (3, 7). In the present experiment, the distribution of resting potential in 120 mM (half-isotonic) sucrose solution was almost the same as that in the isotonic solution. The entrance of water into the cell might have no appreciable effect on the resting potential values, as Tobias (22) reported in frog's skeletal muscle. The effect of osmotic pressure or the "efflux (influx)" of ions, however, was not studies in the present experiment.

2. The action potential, reported by Kolodny & van der Kloot (18) in the stomach muscle of *Rana pipiens* or by Hoyle and Lowy (15) in the adductor muscle of *Mytilus* (extracellular lead) was not recorded throughout this experiment. In a few cases, a slow potential change lasting about 10 sec was seen simultaneously with a spontaneous rhythmic contraction.

SUMMARY

1. The membrane potential was studied with microelectrode techniques on muscle cells of an isolated strip of toad's stomach. The solutions in which the muscle strip was immersed were Ringer's or sucrose solution, and the effects of a few autonomic stimulating agents were observed.

2. The potential values were statistically different between the pyloric portion and gastric body in both Ringer's and sucrose solution. In the former, they were 50.5 ± 0.7 (standard error) mV at pyloric portion and 45.0 ± 0.9 mV at gastric body. If the tip potentials were corrected, these membrane potential would be 58.3 ± 1.7 mV at pyloric portion and 52.9 ± 1.5 mV at gastric body, respectively. It was likely that the junction potential at an indifferent electrode is negligible.

3. In isotonic sucrose solution, the measured potential was higher (membrane was hyperpolarized) than in Ringer's, and the factors concerning this phenomenon were discussed.

4. The effect of acetylcholine or barium chloride was responsible for the depolarization of muscle membrane. The effect of adrenaline on the muscle membrane was minor.

5. Although the muscle strip showed rhythmical contraction by adding acetylcholine (10^{-5}) or barium chloride (4.4 mM$\sim$7.4 mM) to the bathing Ringer's solution, the evident action potentials could not be recorded.

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