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Response of *Nitella* Internodal Cell to Chemical Stimulation

A Model for Olfactory Receptor System

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Summary. Electrical response of excitable internodal cell of *Nitella* was studied by applying various kinds of odorants to the cell. Changes in membrane potential and resistance during responses induced by odorants were measured intracellularly under a variety of ionic environments in the media. Results were: 1) Some odorants (coumarin, isoamylacetate, methylacetate, 1-octanol, 1-butanol, 1-propanol) produced an all-or-nothing type action potential when the concentration of odorant exceeded a certain threshold. The action potential was followed by a gradual depolarization of the potential whose amplitude depended on the odorant concentration, C. Other odorants (heptanoic acid, β-ionon) induced gradual depolarization of the membrane potential without evoking an action potential. 2) Membrane resistance R_m changed in various ways during depolarization: some odorants led to a temporal or gradual decrease in R_m, and others caused an increase in R_m when the membrane potential was depolarized by the application of odorants. 3) Magnitude of response to odorants OR was found to be represented by the following equation:

$$OR=(\alpha+\beta\sqrt{I})\log(C/C_{\text{th}}) \quad \text{for} \quad C\geqq C_{\text{th}}$$

where α and β are constants for a given odorant, I the ionic strength in the medium, and C_{th} the threshold concentration of the odorant. 4) Plots of olfactory threshold of human and of internodal cell of *Nitella* gave a straight line having slope unity. 5) Local application of odorants on the internodal cell induced impulses which transmitted from the part treated by odorants to the other portion. Physico-chemical and physiological implications of the results obtained were discussed.

In higher vertebrate, olfactory reception takes place at the receptor cell which is the terminal swelling of the olfactory nerve. Application of odorants to the receptor cell is assumed to induce a change in membrane potential of the cell, and to evoke impulses in olfactory nerve. The molecular mechanism of the transduction process from the reception of odorant at the receptor membrane to the production of impulses is not known at the present. This stems partially from the smallness of the olfactory cell, which has made it difficult to record intracellularly

the changes in the potential and resistance of the cell membrane in response to odorants.

It is well known that odorants induce action potentials in various excitable systems; e.g. the trigeminal nerve [3], vomeronasal organ [13], ganglion cell [1], and contact chemoreceptor [5]. These facts imply that an excitable membrane in general has an ability to respond to odorants and that the basic mechanism of reception to chemicals at an excitable membrane might be similar to that at the receptor membrane of olfactory organ. We demonstrated in a previous paper that the excitable protoplasmic droplet isolated from *Nitella* responded to various odorants when the concentration of odorants exceeded their respective thresholds [15].

In the present study, the response of *Nitella* internodal cell to odorants is investigated in the hope that the results obtained here may have some value for the basic understanding of the primary process of olfactory reception. Since the internodal cell of *Nitella* is a giant cell, the changes in electrical properties of a single cell membrane can be measured intracellularly in response to odorants. Furthermore, the length of a single cell of *Nitella* makes it possible to depolarize only one end portion of the cell by applying an odorant, which produces transmitting action potentials at the portion apart from the end portion. In this respect, internodal cell of *Nitella* may afford a model for the primary sensory cell.

Experimental

Materials and Conditions

Internodal cells of *Nitella sp.* used in the present study were collected at Lake Utonai in Hokkaido, Japan, during the summer and were cultured in an artificial pond water containing (mM): 0.025 KCl, 0.05 NaCl, 0.05 NaH_2PO_4, 0.2 $Ca(NO_3)_2$ and 0.1 $MgSO_4$, thermostated at 25 °C. During cultivation, the *Nitella* was illuminated with an incandescent lamp of 40 W at 12-hr intervals. Under this cultivating condition, *Nitella* could grow even in the winter season.

Analytical grade chemicals were used without further purification. Odorants were applied to the internodal cell as an aqueous solution containing 0.5 mM $CaCl_2$ and 300 mM mannitol. This solution suits to change the salt composition in a wide range with no loss of the excitability of internodal cell of *Nitella*, although no essential difference is observed for chemoreception even if we use the artificial pond water as the medium (*see later*).

Experimental Procedures

Membrane potential and resistance of the internodal cell were measured intracellularly by the open vacuolar method [11], which has an advantage that the intracellular recording

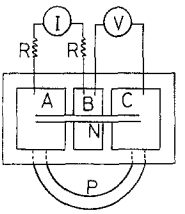

Fig. 1. Schematic diagram of the vessel used for measurements of potential and resistance responses of *Nitella* internode against odorants. *A* and *C* are compartments filled with an artificial sap solution containing 10 mM $CaCl_2$ and 150 mM KCl, and they are connected with a polyethylene tubing *P*. *B* is the compartment where odorant solution is applied to the internodal cell of *Nitella*, *N*. *R*: resistors, *I*: current supply and ammeter, *V*: potentiometer

can be made without inserting a microelectrode into an internodal cell through the hard cell wall. Fig. 1 shows the schematic diagram of experimental arrangement of the open vacuolar method employed. An internodal cell of *Nitella* (ca. 0.5 mm in diameter and 5–10 cm in length) was placed on the narrow ditches in the vessel with three compartments, *A*, *B*, and *C* in the Figure. After wiping off water that adhered to the cell wall, the ditches were filled with white vaseline in order to insulate electrically the separated compartments, *A* and *C*. End portions of the internodal cell in *A* and *C* compartments were dipped in a solution containing 10 mM $CaCl_2$ and 150 mM KCl. This salt composition is almost the same as that of the cell sap of the *Nitella sp.* used. Compartments *A* and *C* were connected with a polyethylene tubing in order to avoid the pressure difference between two compartments because a rapid flow of vacuole often injures the internodal cell. The middle portion of the cell was allowed to stand in the air for a few minutes. Immediately after the internode lost the turgor, two end portions were amputated with scissors, and the middle portion (compartment *B*) was immersed in an isotonic solution containing 0.5 mM $CaCl_2$ and 300 mM mannitol. The internode retains its electrical excitability for several hours in this solution. Hereafter, for the sake of convenience, this solution is referred to as the basal solution. Odorants were applied to the internode by dissolving in the basal solution and flowing the solution gently in compartment *B*. The reason for using this solution as a basal medium had been noted above. The membrane potential was measured by a pair of calomel electrodes through salt bridges (3 M KCl in 3% agar gel) between compartments *B* and *C* as illustrated in Fig. 1, and monitored by a beam of synchroscope (Iwatsu Elec. Co. Tokyo, Model SS-5571) or a channel of a penwriting recorder (Nihon Kohden Co. Tokyo, Model RJG-3024) through a high input impedance d-c preamplifier (Nihon Kohden Co. Tokyo, Model MZ-4). Membrane potential was -120 ± 10 mV in the basal solution mentioned above, and stayed constant for more than 4 hours.

Membrane resistance was measured by delivering inwardly directed current pulse, 0.1 or 1 sec in duration and about 10^{-9} A in strength, which led to a change in transmembrane potential by less than 10 mV. Current pulses were delivered from an electrical stimulator (Nihon Kohden Co. Model MSE-3R) with an isolating unit through high resistors (ca. 120 MΩ) between compartments *A* and *B*, and monitored by the other beam of the synchroscope or a channel of the penwriting recorder. Membrane resistance of *Nitella* internode was between 400 and 700 kΩ cm^2 in the basal solution. Values of the membrane potential and resistance are compared to those reported in the literature for internodal cell of *Nitella* in pond water [12].

All experiments reported here were performed at room temperature, 20 ± 1 °C.

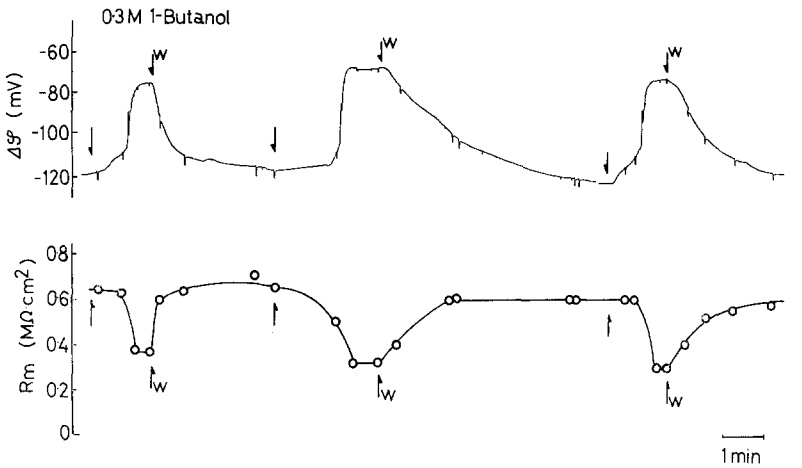

Fig. 2. Reproducibility of potential and resistance responses of *Nitella* internode induced by repeated application of 0.3 M 1-butanol. Arrows show the time when the odorant solution was applied, and the arrows with letter *W* show the time when the odorant solution was washed out by the basal solution (*see text*). Small hyperpolarizing pulses appearing on the potential response curve (upper half) indicate the variations of transmembrane potential induced by electric current pulses (1 sec in duration and ca. 10^{-9}A in strength) delivered externally. Lower half of the Figure shows the calculated resistance R_m during response by using Ohm's law together with the potential data obtained above

Results

Reproducibility of the Responses Induced by Odorants

Application of various odorants to the internodal cell of *Nitella* led to changes in the membrane potential of the cell. Fig. 2 shows the penwriting record of the time-course of the membrane potential of a *Nitella* internode (upper half in Fig. 2) induced by application of 0.3 M 1-butanol dissolved in the basal solution. The solution of 1-butanol was added three times as indicated by arrows. Note that an action potential appeared about one minute after the application of the odorant solution, and followed a gradual depolarization, which approached the steady level. After washing out 1-butanol by the basal solution, the membrane potential returned to the original level. Small hyperpolarizing spikes of 1-sec duration appearing on the potential response represent the change in transmembrane potential induced by the current pulses applied externally. By using these data together with the current strength applied, the membrane resistance during the depolarization is calculated from Ohm's law and the results are presented in the lower half of Fig. 2. The membrane

resistance decreased by about one-half of the original value in the presence of 0.3 M 1-butanol, and recovered to the original level by removal of the odorant from the medium. Successive applications of 0.3 M 1-butanol produced reproducible changes both in the membrane potential and resistance as shown in the Figure. The reproducibility was not lost even by six times application of 0.3 M 1-butanol or more than ten times application of 0.1 M 1-butanol. However, recovery of the membrane potential and resistance became slow by successive applications of the odorant. Similar reproducibility was observed for all odorants examined unless the concentration of odorant applied was too high.

Patterns of Potential Response Induced by Odorants

Application of various odorants to the internodal cell of *Nitella* induced a variety of response patterns in the membrane potential of the cell. The potential responses can be classified into three typical patterns as shown in the upper half of Fig. 3. The trace shown in Fig. 3 *A* illustrates the response pattern induced by coumarin (0.5 mM), which is characteristic in the sense that a repetitive firing of action potentials occurs. With

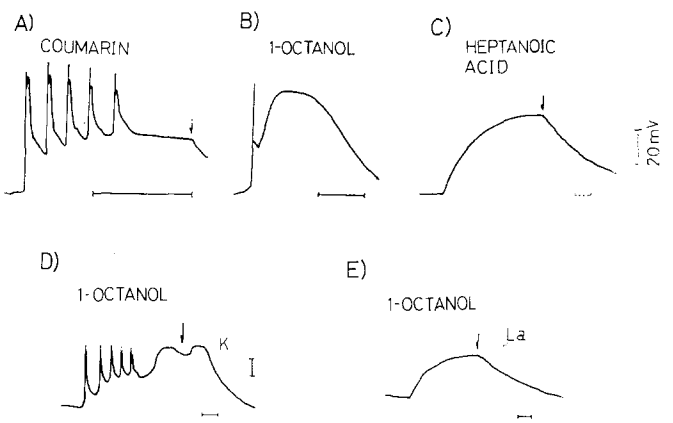

Fig. 3. Various patterns of olfactory response of *Nitella* induced by application of 0.5 mM coumarin (*A*), 1 mM 1-octanol (*B*), and 0.3 mM heptanoic acid (*C*). Lower half of the Figure illustrates the change in response pattern of *Nitella* against 1 mM 1-octanol in different ionic environments in the media. (*D*) shows the case where the medium contains 7.5 mM KCl and 1 mM $CaCl_2$, and (*E*) illustrates the case containing 15 mM $LaCl_3$ in the medium. Note that the response pattern for 1-octanol in the basal solution containing 0.5 mM $CaCl_2$ and 300 mM mannitol is given by Fig. 3 *B*. The lengths of vertical and horizontal bars in the Figure show 20 mV in potential and 1 min in time, respectively

increase of the concentration of coumarin, the plateau level of the depolarized membrane potential becomes high and the action potentials are hidden in the depolarized potential (*see* Fig. 4*C*). Removal of coumarin from the medium leads to reversible return of the membrane potential. Methylacetate also brings about the repetitive firing of action potentials. The second class of the response pattern is represented in Fig. 3*B*, where an action potential is elicited immediately after the application of odorants, which is followed by a gradual depolarization of the potential and approach to a steady value through a maximum. Both the heights of the maximum depolarization and of the steady value depend on the odorant concentration applied. Successive application (more than 5 times) of odorants of high concentration leads to a loss of the action potential and only gradual depolarization occurs. 1-Propanol, 1-butanol, 1-octanol, and isoamyl acetate gave the response pattern similar to that in Fig. 3*B*. The third class of the response pattern is represented in Fig. 3*C*, where no action potential is induced and only a gradual depolarization appears. Heptanoic acid and β-ionone bring about this type of response pattern. The difference in the response patterns shown in Fig. 3*A*, *B* and *C* seems to be attributed to the difference in the effects of odorants on the excitability of the cell membrane. Odorants which belong to the third class (Fig. 3*C*) have a suppressive effect on the excitation, while the odorants belonging to the first class (Fig. 3*A*) enhance the excitability of the membrane. This statement rests on the following experiments. The lower half of Fig. 3 (*D* and *E*) illustrates that the response pattern of a given odorant varies with change of the ionic environment of the internodal cell. 1-Octanol gives the response pattern of Fig. 3*B* type under the external medium containing 0.5 mM $CaCl_2$, but induces repetitive firing of action potentials when the external medium contains 7.5 mM KCl and 1 mM $CaCl_2$ as seen in Fig. 3*D*. On the other hand, no action potential appears under the external medium containing 15 mM $LaCl_3$ as shown in Fig. 3*E*. La^{3+} is known to be a suppressive agent for excitation.

The results shown above indicate that there is a variety of interactions between odorants and the cell membrane which is affected strongly by the ionic composition in the medium.

Changes in Membrane Resistance during Olfactory Response

Fig. 4 shows some typical examples for the changes in membrane resistance during response caused by odorants, where the cases of 1-

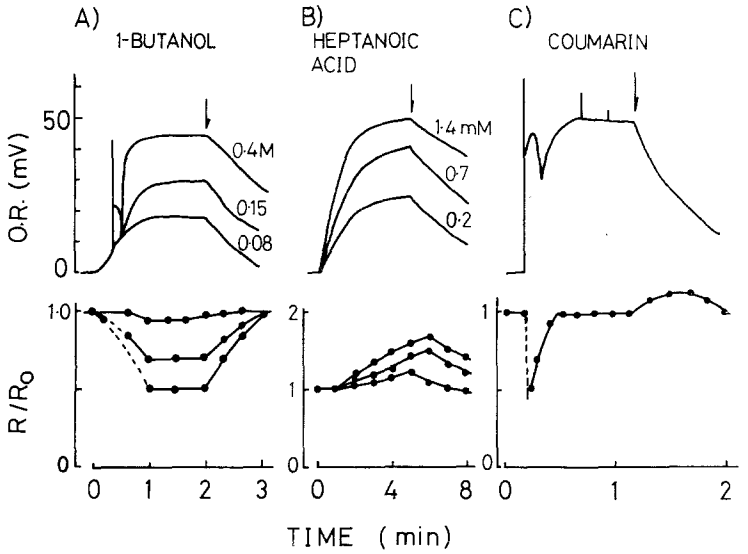

Fig. 4. Time-courses of variations of the membrane potential and the relative value of membrane resistance (R/R_0) during olfactory responses caused by 1-butanol (*A*), heptanoic acid (*B*), and 1 mM coumarin (*C*). Here R_0 stands for the resistance of the cell membrane in the basal solution with no odorant. Concentrations of 1-butanol and heptanoic acid applied are shown in the Figure. Odorants were applied at time zero, and removed at the point shown by the arrow in the top Figures

octanol, heptanoic acid, and coumarin in various concentrations are presented. Application of 1-butanol to the internodal cell led to a decrease in the membrane resistance during depolarization. The absolute values of membrane potential and resistance are shown in Fig. 2. On the other hand, heptanoic acid brought about an increase in membrane resistance. When coumarin was applied to the internodal cell, a remarkable decrease in membrane resistance was associated with the elicitation of the initial action potential. During the gradual depolarization which followed the action potential, however, the membrane resistance returned to the original level. That is, the depolarization occured with no variation in membrane resistance. Isoamylacetate and 1-octanol also gave a temporal change in membrane resistance similar to that observed with coumarin as shown in the previous paper [14].

The results given in Fig. 4 indicate that depolarization of the membrane potential by odorants is not always accompanied with a decrease in membrane resistance [7]. This fact contrasts with the case of the production of an action potential, where a sharp decrease in membrane resistance is always associated with the potential change.

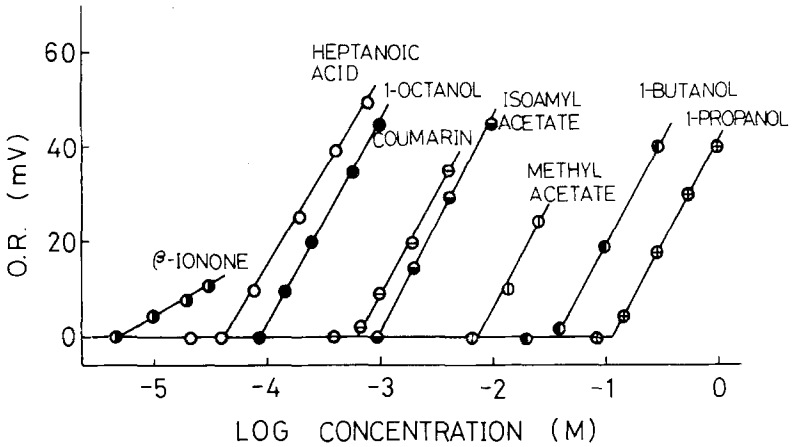

Fig. 5. Olfactory response (*O.R.*) against log *C* plots for various odorants, where *C* stands for the concentration of the odorant in moles/liter in the basal solution containing 0.5 mM $CaCl_2$ and 300 mM mannitol

Comparison of Olfactory Threshold of Human with that of Internodal Cell of Nitella

Membrane potential of the internodal cell does not change until the concentration of odorant exceeds a certain threshold concentration, C_{th}. Magnitude of potential change at the maximum level of the gradual depolarization induced by an odorant is referred to as the response to odorant and is abbreviated by *OR*, hereafter. *OR* increased linearly with logarithm of odorant concentration when the concentration of odorant was higher than the threshold of respective odorant as illustrated in Fig. 5. The linear relation between potential response and log *C* held also for the response to odorants in the naked protoplasmic droplet isolated from *Nitella* [14], and chemoreception of slime mold [15]. As seen in Fig. 5, the threshold varies extensively with odorant species applied. The threshold (C_{th}) obtained from *OR vs.* log *C* relation for various odorants is plotted against the olfactory threshold of human (*T*) in Fig. 6 both in logarithmic scales. The values of *T* were taken from the literature [9]. Data for various odorants fall on a straight line of unit slope as seen in the Figure.

Similarly, a linear relation held for the concentration of odorants giving a surface tension increase of 0.1 dyne/cm in the monolayer of lipids extracted from bovine olfactory epithelium [9]. These results together with the fact that generally the odorants are lipoid soluble material

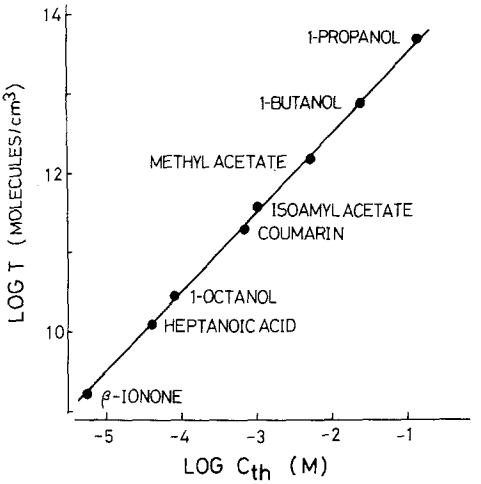

Fig. 6. Linear relation with unit slope between log T and log C_{th}, where T and C_{th} stand for the olfactory threshold for human and the threshold of olfactory response of internodal cell of *Nitella*, respectively

suggest that the primary process of olfactory reception takes place at the hydrophobic parts of the cell membrane, which, in turn, implies that the underlying mechanism of reception to odorant of the internodal cell of *Nitella* is not very different from that of olfactory receptor membrane of a higher vertebrate.

Effect of Ionic Strength on the Olfactory Response

Fig. 7 shows the relation between OR and log C in the case where 1-octanol is applied to a cell under various ionic environments in media. In Fig. 7*a*, the ionic environments were changed by increasing the concentration of $CaCl_2$. Note that an increase of $CaCl_2$ concentration induced a large OR at a given concentration of 1-octanol, and that the threshold concentration C_{th} was not changed by the $CaCl_2$ concentration in the medium. The latter fact is to be compared with the result obtained with the naked protoplasmic droplet of *Nitella*, where the threshold for 1-octanol depended remarkably on the external Ca^{2+} concentration [14]. The difference between the protoplasmic droplet and the internodal cell of *Nitella* may be attributed to different effects of Ca^{2+}: a decrease of external Ca^{2+} concentration brought about an abrupt depolarization in the protoplasmic droplet even in the absence of odorant [6, 14], while

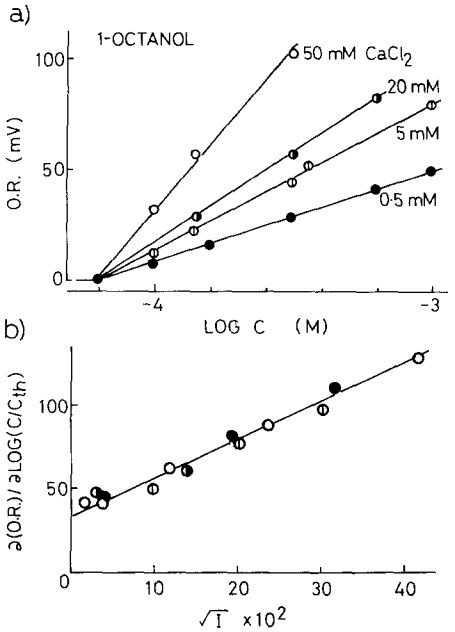

Fig. 7. Dependence of the olfactory response (*O.R.*) for 1-octanol on ionic strength in media. Upper half represents the relation between *O.R.* and log *C*, where *C* is the odorant concentration in moles/liter, in various $CaCl_2$ concentrations in media. The concentrations of $CaCl_2$ are indicated in the Figure, where the osmolarity of solution was adjusted by changing mannitol concentration. The lower half shows the slope of the linear relation between *O.R.* and log *C*, [i.e. $\delta(O.R.)/\delta \log(C/C_{th})$], in various ionic environments as a function of square root of the ionic strength. The ionic strength was varied by changing KCl (◑), NaCl (●), $CaCl_2$ (○), or $LaCl_3$ (⊕) concentrations. The concentration of KCl could be changed until 5 mM at most, since the *Nitella* internode became unstable in high KCl concentration media (*see* Fig. 3*D*)

a decrease of Ca^{2+} concentration did not bring about a depolarization in the internodal cell unless an odorant was added. It is well known that Ca^{2+} bound to the internodal cell membrane of *Nitella* is not easily removed by a decrease of Ca^{2+} concentration in the external medium [8].

The increase in the slope of the linear relation between *OR* and log *C* with increase of ionic strength in medium as observed in Fig. 7*a* is not restricted to $CaCl_2$, but other species of ions induce a similar effect. In Fig. 7*b*, the slopes of the linear relation of *OR vs.* log *C* in various ionic environments are plotted against square root of the ionic strength for 1-octanol. Ionic environment was changed by adding NaCl, $CaCl_2$ and $LaCl_3$ to the external media while keeping the solution isotonic by adjusting mannitol concentration. It is noted that the mem-

brane potential depends on the ionic strength in these salt solutions and is independent of ionic species involved. We can see in Fig. 7*b* that the olfactory response is expressed by the following equation:

$$OR=(\alpha+\beta\sqrt{I})\log(C/C_{\mathrm{th}}) \quad \text{for } C\geqq C_{\mathrm{th}} \tag{1}$$

where α and β are constants which are independent of the ionic strength I and C_{th}. Applicability of Eq.(1) for chemoreception in internodal cell of *Nitella* is examined with 1-octanol, 1-butanol, propanol, coumarin, isoamyl acetate, heptanoic acid, and β-ionone. Results are shown in Fig. 8. It is noted that the values of parameter β depended on the species of odorants and that all alcohols examined in the present study showed nonvanishing value of β. The value of parameter α slightly depended on the odorant species.

Transmission of Impulses Induced by Local Application of Odorants

In the olfactory nerve whose end portion is the receptor cell, action potentials are elicited by a change in membrane potential of the receptor cell. This situation is realized by applying odorants locally to an internodal cell of *Nitella*. Fig. 9*a* shows the schematic diagram of experimental arrangement. *A*, *B*, *C*, and *D* in the Figure denote silver wire electrodes (50 μm in diameter) separated 1 cm from each other. Internodal cell

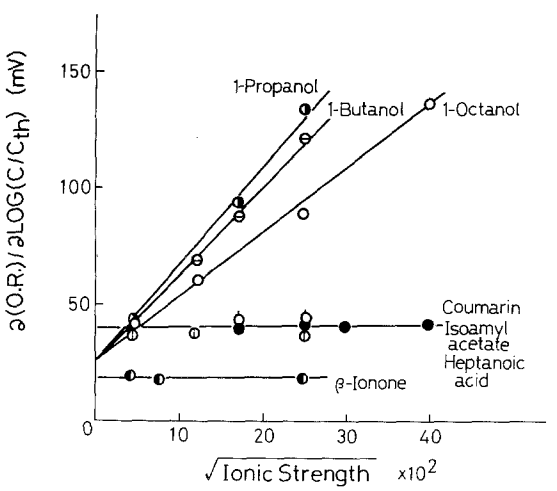

Fig. 8. The slope of the linear relation between *O.R.* and log *C*, [i.e. $\delta(O.R.)/\delta \log(C/C_{\mathrm{th}})$], as a function of square root of the ionic strength for various odorants, where the ionic strength was changed by $CaCl_2$ concentration. Species of odorants examined are shown in the Figure

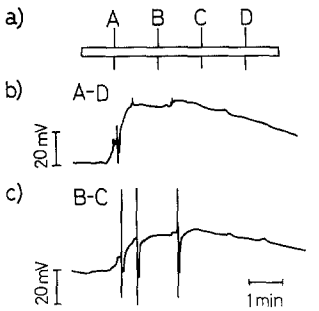

Fig. 9. Schematic diagram of experimental arrangement for observing the transmission of impulses (*a*), and potential patterns recorded extracellularly (*b* and *c*). The odorant (1 mM 1-octanol) was applied to the cell at the portion *A*, and extracellular potential was recorded between two Ag-wire electrodes *A* and *D* (*b*), and between *B* and *C* (*c*), respectively. The odorant was dissolved in the artificial pond water instead of the basal solution (*see text*)

was placed on these wire electrodes as shown in Fig. 9*a* after wiping off the water adhering on the cell wall. The chamber was moistened in order to avoid drying the cell, and an odorant dissolved in the artificial pond water was applied locally to portion *A*. Here, the artificial pond water was used instead of 0.5 mM $CaCl_2$ and 300 mM mannitol solution because the impulses are more easily transmitted to the other portion of the internode in the pond water than in the basal solution. As an example, changes in membrane potential induced by 1 mM 1-octanol are shown in Fig. 9*b* and 9*c*. The trace given in Fig. 9*b* was recorded extracellularly between two electrodes *A* and *D*, while that of Fig. 9*c* was obtained between *B* and *C* electrodes. As the portion *A* is depolarized locally, action potentials are induced and transmitted along the internode from *A* to *D* as demonstrated by the bi-phasic changes of the potential. Note that the response pattern of intracellular potential for 1-octanol in the artificial pond water was the same as that given in Fig. 3*B*. This observation indicates that the local depolarization in an excitable cell is transformed into conducted impulses. An essentially similar fact was demonstrated by Osterhout in studying the mechanism of transmission of an action potential in an internodal cell of *Nitella*, who showed that the local electric current induced transmitting action potentials [10].

Discussion

The internodal cell of *Nitella* was shown to respond to odorants in various ways depending on the quality of odorants applied. The re-

sponse patterns of intracellular potential and of the membrane resistance in response to odorants, which were hard to observe with the intact olfactory cell, were amenable to measurement with use of *Nitella*. Results obtained here quite resemble those of integrated olfactory response observed with olfactory nerve [3] and/or with trigeminal nerve [4]. This fact indicates that at least part of the discrimination of odorant quality may take place at the receptor potential level of a single cell in the olfactory receptor system. Furthermore, results described above, especially the agreement of sequence of thresholds to various odorants between *Nitella* and human (*cf.* Fig. 6), imply that the underlying mechanism of chemoreceptions is more or less similar for all living excitable membranes irrespective of species of animals and plants. Studies of chemoreceptions at the membrane level along this line of thought seem worthy of investigation in more detail.

In the field of gustatory physiology, the Langmuir adsorption isotherm is used frequently for representing the response-stimulus relationship [2]. The experimental formula given by Eq.(1) seems to be quite different from the Langmuir equation. The following analysis will amend the discrepancy between these two expressions. Eq.(1) can be expanded in power series as follows:

$$\begin{aligned} OR &= (\alpha+\beta\sqrt{I})\log(1+\Delta C/C_{\mathrm{th}}) \\ &= 2(\alpha+\beta\sqrt{I})\left[\left(\frac{K\Delta C}{1+K\Delta C}\right)+\frac{1}{3}\left(\frac{K\Delta C}{1+K\Delta C}\right)^3+0(\Delta C)^5\right] \end{aligned} \tag{2}$$

where $K=1/2C_{\mathrm{th}}$, and ΔC is defined by

$$\Delta C=(C-C_{\mathrm{th}}). \tag{3}$$

Neglecting the higher order terms in the right-hand side, Eq.(2) is simplified to give;

$$OR=(OR)_0 K\Delta C/(1+K\Delta C). \tag{4}$$

Here, $(OR)_0$ stands for $(\alpha+\beta\sqrt{I})$. If C_{th} is vanishingly small, Eq.(4) agrees with the Langmuir adsorption isotherm, which is generally referred to as the taste equation of Beidler [2]. As seen in the present study and in the previous paper [14], the chemoreception of *Nitella*, both for the internodal cell and for the naked protoplasmic droplet, can be described satisfactorily by Eq.(1) when the concentration of an odorant applied is not very high in comparison with the threshold of the odorant species. In other words, the critical or threshold concentration C_{th} for the reception is observed clearly in the chemoreception of *Nitella*. This

was also demonstrated in the chemoreception and taxis of true slime mold [15].

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References

1. Arvanitaki, A., Takeuchi, H., Chalazonitis, N. 1967. Specific unitary osmereceptor potentials and spiking patterns from giant nerve cells. *In:* Olfaction and Taste. T. Hayashi, editor. Vol. 2, p. 573. Pergamon Press, Oxford
2. Beidler, L.M. 1954. A theory of taste stimulation. *J. Gen. Physiol.* **38**:133
3. Beidler, L.M. 1965. Comparison of gustatory receptors, olfactory receptors, and free nerve endings. *Cold Spring Harbor Symposia on Quantitative Biology* **30**:191
4. Dowson, W.W. 1962. Chemical stimulation of the peripheral trigeminal nerve. *Nature* **196**:341
5. Dethier, V.G. 1972. Sensitivity of contact chemoreceptors of blowfly to vapors. *Proc. Nat. Acad. Sci. USA.* **69**:2189
6. Inoue, I., Ishida, N., Kobatake, Y. 1973. Studies of excitable membrane formed on the surface of protoplasmic droplets isolated from *Nitella*. 4. Excitability of drop membrane in various compositions of the external salt solution. *Biochim. Biophys. Acta* **330**:27
7. Kamo, N., Miyake, M., Kurihara, K., Kobatake, Y. 1974. Physicochemical studies of taste reception. Parts 1 and 2. *Biochim. Biophys. Acta* **367**:1, 11
8. Kishimoto, U. 1966. Repetitive action potentials in *Nitella* internodes. *Plant Cell Physiol.* **7**:547
9. Koyama, N., Kurihara, K. 1972. Effect of odorants on lipid monolayer from bovine olfactory epithelium. *Nature* **236**:402
10. Osterhout, W.J.V., Hill, S.E. 1930. Negative variations in *Nitella* produced by chloroform and by potassium chloride. *J. Gen. Physiol.* **13**:459
11. Tazawa, M. 1964. Studies on *Nitella* having artificial cell sap. 1. Replacement of the cell sap with artificial solutions. *Plant Cell Physiol.* **3**:33
12. Tazawa, M. 1972. Membrane characteristics as revealed by water and ionic relations of Alogal cells. *Protoplasma* **75**:427
13. Tucker, D. 1971. Nonolfactory responses from the nasal cavity: Jacobson's organ and the trigeminal system. *Handbook of Sensory Physiol.* L.M. Beidler, editor. Vol. 4–1, pp. 151–181. Springer-Verlag, New York
14. Ueda, T., Muratsugu, M., Kurihara, K., Kobatake, Y. 1975. Olfactory response in excitable protoplasmic droplet and internodal cell of *Nitella*. *Nature* **253**:629
15. Ueda, T., Terayama, K., Kurihara, K., Kobatake, Y. 1975. Threshold phenomena in chemoreception and taxis by slime mold *Physarum polycephalum*. *J. Gen. Physiol.* **65**:223