any biochemical action attributable to citrinin. However, it is necessary to note that citrinin causes a definite inhibition of both respiration and photosynthesis in the blue green alga, *Anacystis nidulans*[5].

To sum up, there seems to be a positive indication that citrinin is a phytotoxin; the details are yet to be worked out.

Summary. Observations from preliminary experiments to discover the phytotoxicity, if any, of the fungal metabolite, citrinin, are presented. There seems to be a positive indication, warranting further investigation.

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Inhibitory Effect of β-Hydroxyglutamic Acid on a Molluscan Giant Neurone

Hayashi[1] reported in 1959 that β-hydroxy glutamic acid caused convulsions when injected into the cerebrospinal fluid or when applied to the motor cortex of mammals. At the cellular level, Curtis and Watkins[2] demonstrated the excitatory effect of this substance on spinal neurones, but its effect was weaker than that of glutamic acid.

We observed in the present study that erythro-β-hydroxy-L-glutamic acid (erythro-L-BHGA) had an inhibitory effect on the electrical activity of a spontaneously firing giant neurone, periodically oscillating neurone (PON) identified in the subesophageal ganglia of an African giant snail (*Achatina fulica* Férussac). We observed also some decrease of the neuromembrane electrical resistance under this substance. This neurone was excited by 5-hydroxytryptamine and dopamine, inhibited by L-homocysteic acid and L-homocysteine sulfinic acid, and not affected by acetylcholine, glutamic acid or glycine[3–6].

One or two glass micropipettes filled with 2 *M* potassium acetate were implanted in the neurone. The biopotential was recorded by a pen-writing galvanometer, and the spike discharges were recorded by a spike counter. As an indicator of the neuromembrane resistance, the current-voltage relationships (I–V curve) of the neuromembrane were determined by applying a triangular current (hyperpolarizing, depolarizing and hyperpolarizing) into the soma via the implanted micropipette. In this circuit, a resistor of 70 MΩ was connected in series. Substances to be examined were applied to the ganglia in 2 ways: the bath application and the microdrop application. In the former, we applied to the ganglia the substances dissolved in the physiological solution of the snail[7]. In the latter, a micropipette was filled with the substance to be examined; a microdrop of the solution (100 μm in diameter) was made in the open air at the tip of the micropipette by oil pressure; and this microdrop was placed on the surface of the examined neurone. We avoided the iontophoretic application because of its current effect[8].

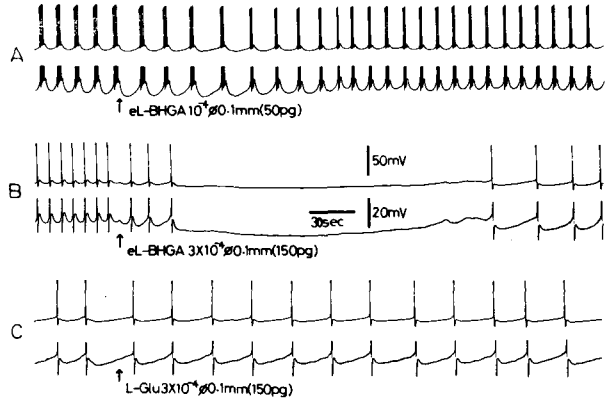

Fig. 1. Inhibitory effect of erythro-β-hydroxy-L-glutamic acid (eL-BHGA, microdrop application) on the electrical activity of the periodically oscillating neurone (PON). The upper traces of A, B, C, are the full-spike recordings of the pen-writing galvanometer. The lower traces of A, B, C, are the recordings in high amplification of the same biopotentials as the upper traces. (The spike peaks have been cut off by an electronic voltage clipper.) In A), a microdrop of eL-BHGA solution (100 μm in diameter) at 10^{-4} g/ml (50 pg) was applied on the surface of the examined neurone (arrow). In B), a microdrop of the same substance (the same diameter) at 3×10^{-4} (150 pg) was applied (arrow). In C), a microdrop of L-glutamic acid (150 pg) was applied (arrow). Note that a microdrop of even 50 pg of eL-BHGA produced some hyperpolarization of the PON membrane, inspite of the ineffectiveness of L-glutamic acid (150 pg) on the same neurone.

The bath application of erythro-L-BHGA (donated by Ono Pharmaceutical Co. Ltd., Osaka; Anal. C 36.56%, H 5.65%, N 8.57%, Calc. for $C_5H_9O_5N$ C 38.81%, H 5.56%, N 8.59%, $[\alpha]_D^{20}$ + 31.91°(C = 1.99 in 20% HCl), confirmed by NMR and IR) in a concentration of $10^{-6} \sim 3\times10^{-6}$ g/ml hyperpolarized the PON membrane and reduced the number of spontaneous spike discharges. Both erythro-D-BHGA and threo-D,L-BHGA also showed some inhibitory effect on the PON. But the effect of the latter two substances was much weaker than erythro-L-BHGA. The PON was completely insensitive to L- and D-glutamic acid, L-aspartic acid, glycine and β-alanine.

Figure 1 shows the biopotential change of the PON caused by the microdrop application of erythro-L-BHGA. A slight hyperpolarization of the PON membrane was produced by the application of 50 pg of this substance dissolved in the microdrop (Figure 1, A). The application of 150 pg erythro-L-BHGA produced a strong hyperpolarization, and no spike discharges of the PON were

[1] T. Hayashi, *Neurophysiology and Neurochemistry of Convulsion* (Dainihon-Tosho Co. Ltd., Tokyo 1959).
[2] D. R. Curtis and J. C. Watkins, J. Neurochem. *6*, 117 (1960).
[3] H. Takeuchi, A. Mori and M. Kohsaka, C. r. Soc. Biol., Paris *167*, 602 (1973).
[4] H. Takeuchi, A. Mori, M. Kohsaka and S. Ohmori, Brain Res. *67*, 342 (1974).
[5] H. Takeuchi, A. Mori and M. Kohsaka, C. r. Soc. Biol., Paris *168*, 653 (1974).
[6] H. Takeuchi, A. Mori and M. Kohsaka, C. r. Soc. Biol., Paris *168*, 658 (1974).
[7] H. Takeuchi, T. Morimasa, M. Kohsaka, J. Kobayashi and F. Morii, C. r. Soc. Biol., Paris *167*, 598 (1973).
[8] H. Takeuchi and N. Chalazonitis, C. r. Soc. Biol., Paris *161*, 1098 (1967).

observed for several minutes (Figure 1, B). In spite of the effect of erythro-L-BHGA, 150 pg L-glutamic acid applied in the same manner did not produce any effect on the PON. We could conclude by the microdrop application experiment that the inhibition produced by erythro-L-BHGA was due to the hyperpolarization of the PON membrane, and not due to synaptic noises.

The neuromembrane rectification was previously reported[9,10]. In the present study, we measured the I-V curve of the neuromembrane as an indicator of membrane resistance by applying a triangular current (hyperpolarizing, depolarizing and hyperpolarizing; almost 2 min a cycle). Figure 2 shows the change in the I-V curve of PON membrane caused by the bath application of erythro-L-BHGA. In this figure, the I-V curve obtained in the application of this substance at 3×10^{-5} g/ml (b) was superimposed on the I-V curve measured in the physiological state (a); in both of these I-V curves, the initial biopotential level (just before the transmembrane current application) was used as the common standard point. In the artificially (by the transmembrane current) hyperpolarized state of the neuromembrane, a clear decrease of membrane resistance was observed in the case of erythro-L-BHGA. In the artificially depolarized state, an elevation of firing level was observed under this substance.

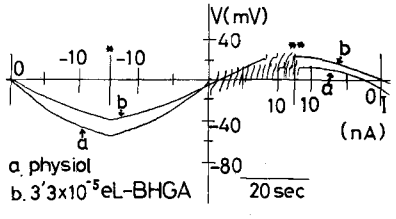

Fig. 2. Change of the current-voltage relationships (I-V curve) of the PON membrane caused by the application of erythro-β-hydroxy-L-glutamic acid (eL-BHGA). A triangular current (hyperpolarizing, depolarizing and hyperpolarizing) was applied to the soma to obtain the I-V curve. Ordinate: Biopotential shift produced by the transmembrane current (mV). Abscissa: Intensity of the transmembrane current (nA). From zero, the current was applied gradually until reaching 15 nA in the hyperpolarizing direction (asterisk). Then the current was adjusted to the depolarizing direction. After reaching about 12 nA in the depolarizing direction (2 asterisks), the current was adjusted again to the hyperpolarizing direction. a) I-V curve measured in the physiological state. b) I-V curve obtained 3 min after the bath application of eL-BHGA at 3×10^{-5} g/ml. The two I-V curves were superimposed, using the initial biopotential level (just before current application) as the common standard point. Note that the lower membrane resistance and the higher firing level were caused by the application of eL-BHGA in comparison with the physiological state.

SCHLEIFER et al.[11] reported the presence of threo-BHGA in the cell wall of a bacterium. OHARA et al.[12] also demonstrated the presence of threo-BHGA in the mammalian brain. We assume that erythro-L-BHGA is probably an inhibitory transmitter of the PON, or a partial agonist of an unknown transmitter which is not glutamic acid or aspartic acid. Previously we reported[4] the inhibitory effect of L-homocysteic acid and L-homocysteine sulfinic acid on the PON. If erythro-L-BHGA is a partial agonist of an inhibitory transmitter of the PON, there arises the question of whether erythro-L-BHGA and the two sulfur-containing amino acids are agonists of the same inhibitory transmitter.

Summary. We found a spontaneously firing neurone, inhibited by β-hydroxy(erythro)-L-glutamic acid, identified in the subesophageal ganglia of an African giant snail (*Achatina fulica* Férussac), although this neurone is not sensitive to L-glutamic acid. We suggest that β-hydroxy(erythro)-L-glutamic acid may be a putative inhibitory synaptic transmitter of the identified molluscan neurone.

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[9] E. R. KANDEL and L. TAUC, J. Physiol., Lond. *183*, 287 (1966).
[10] A. ARVANITAKI, G. ROMEY and H. TAKEUCHI, C. r. Soc. Biol., Paris *161*, 1629 (1967).
[11] K. H. SCHLEIFER, R. PLAPP and O. KANDLER, Biochem. biophys. Res. Commun. *28*, 566 (1967).
[12] K. OHARA, K. NISHINUMA and S. KANEKO, J. Chromat. *7*, 411 (1962).
[13] Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Use of a Tissue Culture Medium for in vitro Studies on the Ion Transport Capacity of Amphibian Epithelia

In most studies on the physiological role of the transport of ions across isolated epithelial membranes, simple electrolyte solutions have been used to incubate the preparations, to keep the concentration of the principal 'physiological' ions, such as Na^+, K^+, Ca^{++} and Cl^-, the total osmolality and pH within the range of values for the extracellular fluids of the species under study. In some cases, especially when mammalian tissues are used, a nutrient substance such as glucose is added to the basic electrolyte solution. These so-called Ringer solutions have proved satisfactory for most physiological experiments, provided that they are carried out within a short time of the isolation of the tissue. However, a decay of the transport capacities of isolated epithelial membranes is an almost constant finding, so that one can argue that such experiments are carried out in dying preparations. This argument is especially valid when applied to mammalian tissues, such as the small intestine, where the rate of decay may be very high from the mounting of the experimental set-up[1]. Although amphibian tissues are much less rapidly affected by the isolation procedures and keep their transport properties reasonably well, at least for a few hours, we decided to see whether the use of a more

[1] S. G. SCHULTZ and R. ZALUSKY, J. gen. Physiol. *47*, 567 (1964).