Osmundalin (Lactone Glucoside) Stimulates Receptor Cells, Associated with Deterrency, of *Bombyx mori*

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Osmundalin, a glucoside of osmundalactone, isolated from the royal fern, *Osmunda japonica*, stimulated the deterrent cell ("R receptor" cells, associated with deterrency) in the medial sensilla styloconica of *Bombyx mori*. From the dose-response curve, the lower limit of detection was 3.6×10^{-5}–3.6×10^{-4} M. This is the first report that glucoside lactone elicits the impulses from the deterrent cell of *Bombyx mori*.

Osmundalin (Fig. 1), a glucoside of osmundalactone, was first isolated by Hollenbeak and Kuehne (1974) from the royal fern, *Osmunda japonica*. In 1990, Numata *et al.* found that this glucoside has strong feeding inhibition activity (80% inhibition at concentration of 0.2%) for the larvae of the yellow butterfly, *Eurema hecabe mandarina* (Lepidoptera: Pieridae). Very recently, Shimizu *et al.* (1993) reported that compounds purified by high performance liquid chromatography containing osmundalin and three other trace compounds which were isolated from *Osmunda japonica*, prevent feeding of *Bombyx mori*. In these feeding tests, however, it is not clear whether osmundalin alone directly stimulates the deterrent cell ("R receptor" cell) associated with deterrency. The R receptor is stimulated by bitter substances, for example, glucosides, *i.e.*, salicin and phlorizin (Ishikawa, 1966), and there are some reports that some glucosides stimulate the glucoside receptor in the lateral sensilla styloconica (Ss) of *Bombyx mori* (Ishikawa, 1966), *Mamestra brassicae* (Wieczorek, 1976), *Manduca sexta* and *Pieris brassicae* (Schoonhoven, 1969). Therefore, we experimentally determined whether this compound evokes responses of sensory R cells in the medial sensilla styloconica of *Bombyx mori*.

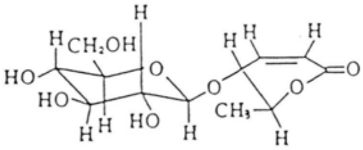

Fig. 1. Chemical structure of osmundalin.

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Materials and Methods

Insects

Larvae of *Bombyx mori* (C 145 × N 140) were reared on an artificial diet (Yakuruto Co. Ltd.) at 25 ± 1 °C under a photoperiod of 12 h light and 12 h dark. Newly molted, unfed, last-instar larvae were used for electrophysiological investigations.

Plants

The dried whole plants of royal fern, *Osmunda japonica*, were used. The extraction and fractionation have previously been described (Numata *et al.*, 1990).

Chemicals

Osmundalin (11 mg) was obtained from Dr. A. Numata, Osaka University of Pharmaceutical Sciences. The purity of osmundalin was determined to be more than 99% by the high performance liquid chromatography (HPLC) and the proton-1 nuclear magnetic resonance (^{1}H NMR) analyses (Numata *et al.*, 1990).

Electrophysiological response

The chemoreceptor tested was the R receptor associated with one of the maxillary sensilla styloconica (called the Ss-II medial hair for ease of reference) (Ishikawa, 1966). From amplitude levels of impulse, we determined as response of the "R cell". The tip-recording method does not allow the stimulation of single receptor cells: the medial sensillum styloconica houses the so-called R cell and the "N cells". The tip-recording method produces a stimulus-onset artifact which causes a delay between contacting the sensillum tip and actual recording.

The electrophysiological methods used in the experiments were the same as described by Ishikawa (1966). The isolated head was fixed on an indifferent, platinum wire electrode. Stimuli were aqueous solutions contained in the stimulating-recording electrode. Stimulation and recording were started simultaneously when the electrode was slipped over the tip of the hair by means of a micromanipulator. The signal was amplified, observed on a cathode ray oscilloscope, and displayed on paper by a thermal array recorder. The period of stimulation was 4 s. Osmundalin was dissolved in 5×10^{-2} M NaCl solution. Concentrations of osmundalin were expressed as ppm in 5×10^{-2} M NaCl solution.

Results and Discussion

Fig. 2 shows the impulses discharged from receptor cells in the Ss-medial of *Bombyx mori* to osmundalin in a NaCl solution (5×10^{-2} M). Osmundalin stimulated sensory R cells in the medial sensilla styloconica of *Bombyx mori* (Figs. 2B–E). The number of impulses per second increased with increasing concentration (Figs. 2B–D, respectively). No response was observed from the lateral sensilla styloconica of *Bombyx mori* to osmundalin containing trace substances (Shimizu *et al.*, 1993). It is not clear that the osmundalin-sensitive cell is the same cell that Ishikawa (1966) named the "R receptor" cell in the present experiment. However, this is indirectly demonstrated mixing sample and salicin in Fig. 3D in Shimizu *et al.* (1993).

Fig. 3 shows the dose-response curve of impulses discharged from R cells in the Ss-medial for osmundalin: The discharge frequency of R impulses increased with increasing concentrations of osmundalin. The sensory input from R receptors, therefore, may be responsible for inhibiting feeding, especially by inhibiting biting behavior (Hirao, 1978). The dose-response curve (10 to 1000 ppm concentrations) presented here very resembles that presented as Fig. 5 in Shimizu *et al.* (1993), suggesting osmundalin is the main deterrent and that the other three unknown substances are not responsible.

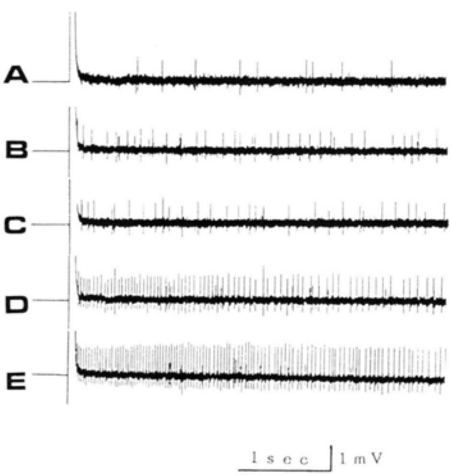

Fig. 2. The impulses discharged from R cells in Ss-medial of *Bombyx mori.* Impulses are measured during the forth second of stimulation with various concentrations of osmundalin in 5×10^{-2} M NaCl solution. A: control (5×10^{-2} M, NaCl solution), B: 1 ppm of osmundalin (3.6×10^{-6} M), C: 10 ppm (3.6×10^{-5} M), D: 100 ppm (3.6×10^{-4} M), E: 1000 ppm (3.6×10^{-3} M).

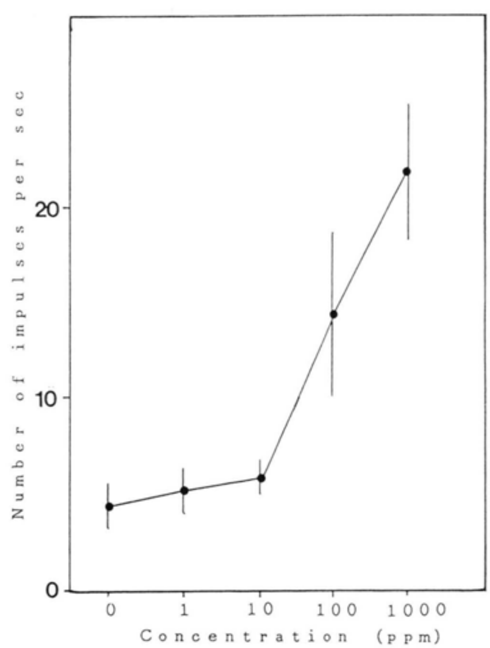

Fig. 3. The relationship between concentration of osmundalin and the number of impulses discharged from R cells in the Ss-medial of *Bombyx mori.* Concentrations of osmundalin are expressed as parts per million in 5×10^{-2} M NaCl solution. Vertical bar of each point indicates S.E.M. (n = 5).

From these results, it was found that the antifeedant action of osmundalin is correlated with taste deterrency. Already it is reported that osmundalin from the royal fern acts as antifeedant for the larvae of the yellow butterfly, *Eurema hecabe mandalina* (Numata *et al.*, 1990). Accordingly, antifeedant effect on this insect is also speculated to be due to taste deterrency. Lower limit of detection of salicin to *Bombyx mori* is 10^{-6} M (Hirao and Arai, 1991), and that of osmundalin is 3.6×10^{-5}–3.6×10^{-4} M (100 ppm) (Fig. 3); therefore osmundalin is an weak antifeedant for *Bombyx mori.*

Although HPLC separation in our previous system (Shimizu *et al.*, 1993) did not yield pure osmundalin, we could conduct electrophysiological investigations using chemically pure osmundalin. By this experiment, it might rule out that the possibility that the three trace compounds were responsible for the behavioural effect.

Acknowledgements

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