CHANGES IN POLYPHOSPHOINOSITIDE METABOLISM IN GLOBODERA ROSTOCHIENSIS FOLLOWING STIMULATION TO HATCH BY POTATO ROOT DIFFUSATE

BY

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Eggs of Globodera rostochiensis were incubated for 2.5 h in 13.3 mM myo-[2-3H] inositol to allow incorporation of the labelled compound into phosphoinositides. The subsequent in vivo metabolism of these phospholipids released the water soluble products glycerophosphoinositol, (GPIns), inositol 1-phosphate (IP1); inositol 1,4-biphosphate (IP2) and inositol 1,4,5-triphosphate (IP3) which were separated by ion exchange chromatography before the radiolabel incorporated into each was quantified using a scintillation counter. Exposure of eggs to labelled myo-inositol in either active or heat-inactivated hatching factor in potato root diffusate resulted in a significantly higher recovery of the three inositol phosphates from individuals receiving the stimulus to hatch.

The results substantiate earlier work indicating a rapid initial action of hatching factor on unhatched juveniles of G. rostochiensis.

Keywords: myo-[2-3H] inositol, uptake, phospholipids, anion exchange chromatography, nematode hatching, physiology.

Several changes have been reported in eggs of Globodera rostochiensis following their stimulation to hatch by hatching factor(s) in potato root diffusate. There is an alteration in cAMP levels by 2.5 h after the addition of the hatching stimulus (Atkinson et al., 1987) followed by a change in adenylate energy charge at 8-24 h (Atkinson & Ballantyne, 1977a) and an increase in oxygen consumption by 24 h (Atkinson & Ballantyne, 1977b). The latter is probably coincident with the increase in utilisation of endogenous lipid reserves (Robinson et al., 1985) and locomotor activity (Doncaster & Shepherd, 1967). Changes in water content also occur by 48 h (Ellenby & Perry, 1976) and this has been correlated with a release in an osmotic restraint imposed on the nematode by trehalose or other solutes in the perivitelline fluid (Clarke et al., 1978). Sometime later, the juvenile cuts a polar slit in the eggshell with its stomatostylet and hatches (Doncaster & Shepherd, 1967).

Hatching factor may act at the eggshell in one of several possible ways including a change in eggshell permeability (Clarke et al., 1978; Clarke & Perry, 1985) but some recent evidence suggests that the primary action may be at the juvenile. Potato root diffusate influences the locomotor activity of hatched juveniles (Clarke & Hennessey, 1984) and the diameter of the nucleolus of the dorsal pharyngeal gland increases prior to hatching providing juveniles
are expressed from eggs into either potato root diffusate or partially purified hatching factor rather than water (Atkinson et al., 1987a, b; Perry et al., 1989).

Hatching factor may act in a calcium dependent manner (Atkinson & Ballantyne, 1979) and calcium is a ubiquitous second messenger (Kretsinger, 1977). An integral part of the receptor-transduction mechanism that generates Ca\(^{2+}\) and other secondary messenger signals involves changes in metabolism of inositol phospholipids. This involves their hydrolysis to water soluble phosphoinositides that can be separated by anion exchange chromatography and quantified by prior labelling with myo-[2-\(^3\)H] inositol (Berridge et al., 1982). Agonist-dependant hydrolysis of inositol phospholipids particularly with the release of IP3 would be anticipated if calcium was involved in stimulation of the juveniles following addition of potato root diffusate. This work measures inositol phosphates released from eggs and juveniles following their prior incubation for 2.5 h in potato root diffusate containing myo-[2-\(^3\)H] inositol. The principal objective in choosing a time point concurrent with the earliest known changes in stimulated eggs was to help define the first response of the eggs to a hatching stimulus.

**MATERIAL AND METHODS**

Cysts of *G. rostochiensis* were collected from infected soil of pot grown potatoes cv Pentland Crown in 1983 using a Fenwick can (Shepherd, 1970) and stored air-dried in bulk at 4°C until used. The potato root diffusate stimulated hatch from eggs before but not after heat-inactivation using an autoclave at 103 kPa for 1 h as before (Atkinson et al., 1980). Eggs were released from c100 mg of cysts previously soaked in tap-water for 7 days at 20°C. The eggs were mixed and added in two 250 µl aliquots to 200 µl of 13.4 mM myo-[2-\(^3\)H] inositol (3.5 Ci/mmol; Amersham International) plus either potato root diffusate that had been heat-inactivated by autoclaving or active potato root diffusate which had been passed through a 0.47 µm bacterial filter (Millipore). After 30 min, the individuals were centrifuged at 10,000 g and resuspended in further 200 µl aliquots of the two labelled solutions for a further 2 h. The incubation of eggs of both treatments for 2.5 h in 9.4 mCi was terminated by repeated centrifugation and washing of eggs before their homogenisation in 1 ml 15% trichloroacetic acid (TCA) followed by a further 4 ml of the TCA solution. The homogenate was centrifuged and the protein-free supernatant was collected. TCA was removed by five extractions in diethyl ether and the pH of the water extracts neutralised before their use in ion exchange column chromatography (Dowex AG-1 resin).

The anion exchange resin was in its formate form and pre-equilibrated with water. The water soluble extracts were applied to 1 ml columns of the resin. The series of solvents used for elution consisted of: (i) water (ii) 5 mM disodium tetraborate and 60 mM sodium formate (iii) 0.1 mM formic acid