Phorbol esters enhance glutathione-induced feeding response in Hydra

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Summary. Pharmacological observations on the glutathione-induced feeding response in Hydra demonstrate that phorbol esters, calcium ionophore A 23187 and lithium ions significantly enhance and protract glutathione action, whereas polymixin B induces a dramatic shortening of the feeding response. An involvement of membrane phosphatidylinositol breakdown in the control of the feeding response is suggested.
Key words. Hydra; feeding response; glutathione; phorbol esters; phosphoinositides.

Hydra, a small freshwater coelenterate whose tentacles are armed with peculiar stinging capsules called nematocysts, is a sessile predator lacking sophisticated sensorial systems. When prey accidentally touches a tentacle, it is pierced and poisoned by one or more nematocysts. Only living prey will then be swallowed, because the feeding response, i.e. coordinated tentacle movements and mouth opening, is induced only by the reduced glutathione (GSH) which flows from the wounded prey[1,2]. GSH interacts with a specific receptor, and its action is competitively inhibited by glutamate[3]. Some data suggest that the receptor may be localized on the nematocyte or on the nematocysts. A specific binding of GSH and of glutamate to nematocyst-enriched fractions has been reported[4,5]; moreover, Hydra deprived of nematocysts do not respond to GSH and do not bind labeled glutamate[5]. Dopamine and adenylate cyclase seem to be involved in a negative modulatory pathway of the feeding response[5-7]. No data are available on the transduction mechansims operating in this system. The feeding response does not occur in calcium-deprived Hydra, whereas the GSH receptor binds labeled ligands also in the absence of this ion[5]. These data suggest a possible role for transduction mechanisms operating through calcium release. Some data are reported here on the possible role of membrane phosphoinositide hydrolysis in the control of feeding response. In this transduction mechanism, the activation of specific receptors induces membrane phosphatidylinositol hydrolysis, leading to the production of diacylglycerol and of inositol triphosphate; the former activates protein kinase C, whereas the latter induces calcium release from intracellular stores (reviewed by Berridge[8] and Majerus et al.[9]).

Hydra (*Hydra attenuata* or *Chlorohydra viridissima*) specimens, grown as proposed by Lenhoff and Brown[10] in 'M' solution (1 mM Tris-HCl buffer pH 7.6, 1 mM $NaHCO_3$, 0.1 mM KCl, 0.1 mM $MgCl_2$, 1 mM $CaCl_2$), were kept unfed for at least three days before experiments. For each observation 10 specimens were placed in a Petri dish (3.5 cm diameter) containing 3 ml of 'M' solution. Feeding response was induced with 1×10^{-6} M GSH. The effect of added drugs was studied by recording the number of specimens showing the typical feeding response (tentacles curling and mouth opening) every minute, under a stereo microscope. In each experiment one group treated with GSH alone was used as a control. Statistical analysis was performed according to the sequential trials method[11] for $2\alpha = 0.01$ and $1\text{-}\beta = 0.95$, or using Student's t-test.

The following substances were tested: 12-0-tetradecanoyl-phorbol-13-acetate (TPA), a tumor promoter known to activate protein kinase C, and its inactive analogue 4-α-phorbol-12,13-didecanoate; lithium chloride, as an inhibitor of inositol phosphate breakdown; the calcium ionophore A 23187; and polymyxin B, known to inhibit protein kinase C.

Hydra attenuata and *Chlorohydra viridissima* gave similar responses to GSH and to pharmacological treatments.

In all experiments the same effects are observed either if GSH is added to the pretreatment medium or if GSH alone follows the pretreatment. We did not observe evident differences in the individual intensity of the GSH-induced feeding response of pretreated specimens. The number of specimens showing a typical reaction, and the duration of the response, were on the contrary affected. TPA (1×10^{-8} M)-pretreated specimens show an enhanced response to GSH, and the feeding response lasts longer than in control specimens (fig. 1). The same results are observed in LiCl (10 mM) pretreated animals (fig. 2). On the contrary the phorbol inactive analogue does not modify the GSH action. A significant enhancement of feeding response is also observed in A 23187 (1×10^{-7} M) treated Hydra. In polymyxin B treated specimens (1×10^{-6} M), the onset of the feeding response appears quite normal, but after 2 min tentacles begin to relax and within 4 min the feeding response has ceased in nearly all Hydra (fig. 3). None of the tested drugs modified hydra behavior in the absence of glutathione. The simultaneous treatment with TPA and A 23187, without GSH addition, induces tentacle movements similar to those usually observed with very low doses of GSH (1×10^{-8} M – 1×10^{-9} M) (the so-called 'tentacle concert'[2]) but a typical feeding response is observed only occasionally.

The data reported here suggest that membrane phosphoinositides may be implicated in the control of GSH-induced feeding response. The enhancement of feeding response in-

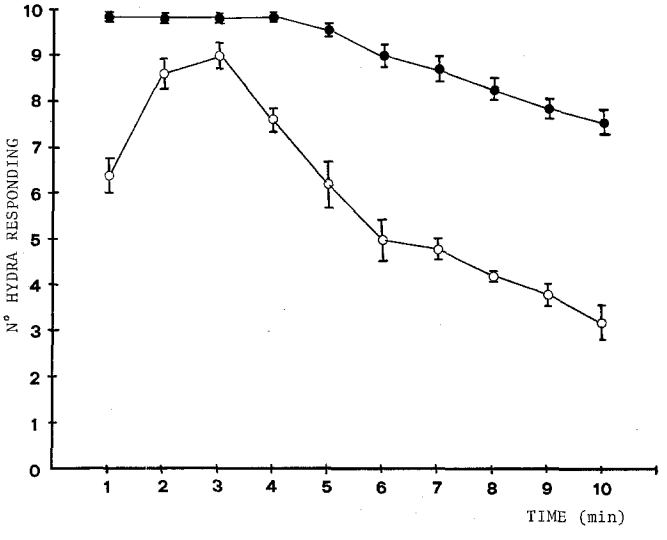

Figure 1. Effects of 12-0-tetradecanoyl phorbol-13-acetate (TPA) (1×10^{-8} M) on glutathione-induced feeding response in *Hydra attenuata*. TPA was applied 10 min before reduced glutathione. Time in min after addition of 1×10^{-6} M glutathione to a Petri dish containing 10 hydra specimens. Ordinate; the number of specimens showing the typical feeding response. Vertical bars = SEM. Open circles = control; closed circles = TPA-treated. TPA-treated significantly different from control ($p < 0 \equiv .01$), n = 20 separate experiments.

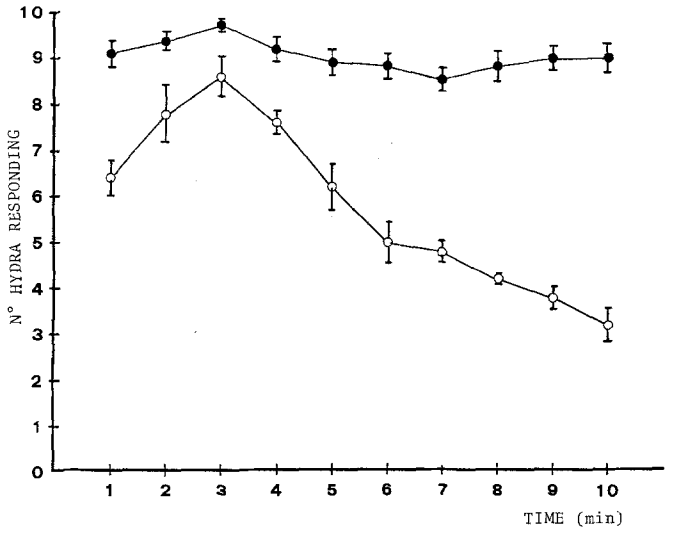

Figure 2. Effects of LiCl (10 mM) on glutathione-induced feeding response in *Hydra attenuata*. LiCl was applied 30 min before reduced glutathione. Time in min after addition of 1×10^{-6} M glutathione to a Petri dish containing 10 Hydra specimens. Ordinate; the number of specimens showing the typical feeding response. Vertical bars = SEM. Open circles = control; closed circles = LiCl-treated. LiCl-treated significantly different from control ($p < 0.01$), n = 20 separate experiments.

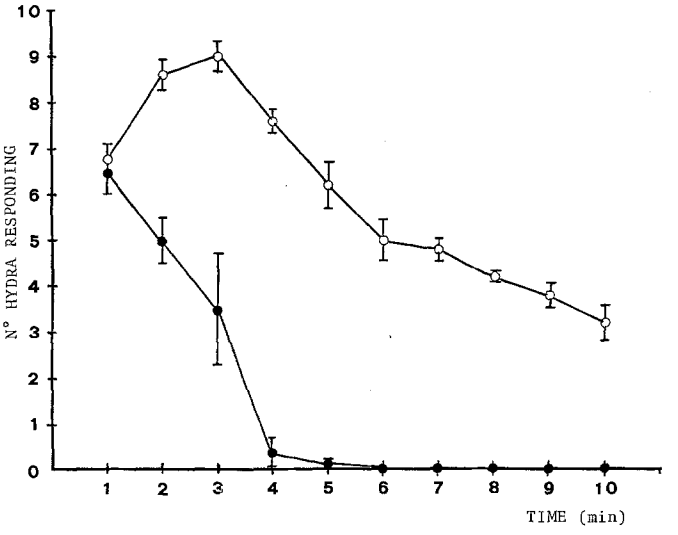

Figure 3. Effects of polymixin B (1×10^{-6} M) on glutathione induced feeding response in *Hydra attenuata*. Polymixin B was applied 10 min before reduced glutathione. Time in min after addition of 1×10^{-6} M glutatione to a Petri dish containing 10 Hydra specimens. Ordinate; the number of specimens showing the typical feeding response. Vertical bars = SEM. Open circles = control; closed circles = polymixin B-treated. Polymixin-B treated significantly different from control ($p < 0.01$), n = 20 separate experiments.

duced by phorbol esters, and by calcium ionophore A 23187, which mimics action of inositol triphosphate on intracellular calcium stores, is also confirmed by the dramatic shortening of feeding response induced by polymixin B, possibly operated through an inhibition of protein kinase C. Of interest are the effects induced by lithium salts. This ion inhibits the dephosphorylation of inositol-1-phosphate, and this inhibition may lead to an increase of inositol polyphosphate levels in Hydra cells, thus increasing and prolonging the feeding response.

Several crucial questions are unanswered: does phosphoinositide breakdown really take place during natural feeding? In this case, does this mechanism represent the first response of the GSH receptor, directly linked to the effector cells, or are one or more nerve cells interposed between these elements? Isolated Hydra tentacles respond to GSH and to added drugs in the same way as whole Hydra, and, in tentacles, nematocytes and musculo-epithelial cells are organized in a 'battery cell complex'[12, 13]. This organization suggests that the nerve cell processes contacting musculo-epithelial cells play a modulatory role in the feeding response, more than providing for transmission of stimulation from the glutathione receptor to the effector cells.

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