Ann. Rev. Pharmacol. Toxicol. 1977 17:243–58

AQUATIC INVERTEBRATES: MODEL SYSTEMS FOR STUDY OF RECEPTOR ACTIVATION AND EVOLUTION OF RECEPTOR PROTEINS

❖6678

Howard M. Lenhoff and Wyrta Heagy
Department of Developmental and Cell Biology and Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

INTRODUCTION

A major objective of research in pharmacology, hormone and neurotransmitter action, olfaction, and chemoreception in general, is to understand how specific cellular receptors are activated. Such a concern is evidenced by the large amount of work being carried out on receptors today (see 1–21). Much of the research on receptors deals with molecules of obvious medical importance, such as insulin (1–3), other peptide hormones (2–6), acetylcholine (7–11), other neurotransmitters (12, 13), and opiates (14, 15). The experimental systems used in most of these investigations are as complex as the organisms, usually vertebrates, housing those receptors.

Yet it may be possible to find and use simpler and experimentally more amenable systems if we focus on the primary event common to all receptor systems: the combination of the activator with the receptor. It seems strange, then, that in the study of such a fundamental cellular process as receptor activation, there have not been many systematic searches for organisms free of the complexity of vertebrates. Some recent notable exceptions are the studies on bacterial chemotaxis of Adler (16) and Koshland (17–19). We suggest that lower aquatic organisms might serve as possible fruitful sources of material for research on receptor activation because the chemical senses, relative to other senses, are highly developed in these organisms.

The objective of this review, therefore, is threefold: (*a*) to survey the large spectrum of biological phenomena initiated by activator molecules in lower aquatic organisms that may prove useful in receptor research; (*b*) to present a summary of results obtained with one such system, the glutathione receptor of hydra, that has

been used to investigate the activation of cellular receptors; and (*c*) to present a view which grew out of studies with a large number of lower animals, and which may provide insight into the evolutionary development of receptor proteins.

CHEMICAL ACTIVATION IN LOWER ORGANISMS

Since the turn of the century biologists have been intrigued by aquatic invertebrate behavior in response to chemicals emanating from food, predators, and other environmental sources (22). Within the aquatic environment, chemical cues are often the major stimulus for initiating biological processes.

This interest has led to numerous investigations in which the phenomena activated by chemicals have been defined, and in recent years, the activating substances have been purified, characterized, and, in some cases, identified and synthesized (see 23, 24). Types of activator molecules implicated in stimulating responses include cations, anions, amino acids, polypeptides, proteins, sugars, glycoproteins, lipoproteins, simple alcohols, and steroids (see 23, 25–27).

The phenomena activated by these molecules are diverse (see 23, 25–27). Listed in Table 1 are the classification of the types of activators, the phenomena controlled by the chemicals, and references to major reviews covering these topics in depth. This table does not attempt to be exhaustive but rather it highlights the major types of events activated by chemicals within the aquatic environment. It should be noted that we have not made a distinction between interspecific communication (i.e. between different species as in item V.B. of Table 1) and intraspecific communication (i.e. between members of the same species as in item V.A. of Table 1). Pheromones fit this latter classification.

The common features of interest to the pharmacologist and cellular biologist in all of the examples listed in Table 1 are the binding of a specific activator by cellular receptors and the activation of those receptors to initiate specific coordinated responses. It would seem likely, therefore, that among the wide spectrum of aquatic organisms in which chemical activation of receptors occurs, there exist some in which the receptors are of such a simple and accessible nature that it is possible to investigate this basic phenomenon. We find that the freshwater hydra and related marine cnidarians offer a number of experimental advantages for study of the activation of cellular receptors.

MECHANISM OF ACTION OF THE GLUTATHIONE RECEPTOR OF HYDRA

Research on feeding chemoreceptors in the Cnidaria has reached a high level of sophistication. Not only have specific activators (incitants) been identified in a large number of species, but also it is possible to make reasonable speculations about the evolution of receptors in general, and, with hydra, to study the mechanism by which the receptor is activated.

In this section we focus primarily on the mechanisms by which the tripeptide reduced glutathione (GSH), activates the GSH receptor of hydra to coordinate the

Table 1 Classification of external chemical activators and their functions in aquatic invertebrates

Classification of activator	Function of activator	References
I. Feeding		27–31
A. Attractant	Stimulates orientation toward food	
B. Arrestant	Arrests locomotion toward food	
C. Incitant	Initiates feeding	
D. Suppressant	Inhibits onset of feeding	
E. Stimulant	Promotes ingestion and continuation of feeding	
F. Deterrent	Prevents or interrupts feeding	
II. Symbiosis[a]		32–36
A. Attractant	Orients host-seeking organism toward potential host	
B. Stimulant	Induces and maintains the association after initial contact	
C. Repellent	Repels host-seeking organism	
III. Habitat-niche selection		23, 37, 38
A. Homing attractant	Orients toward habitat by allowing recognition of previous home or territory	
B. Aggregation and schooling attractant	Initiates forming of clusters of organisms belonging to same species	
C. Larval settlement inducer	Initiates settlement of larvae on appropriate substrate	
IV. Development		23, 38
A. Metamorphosis inducer	Initiates change from larval to subsequent developmental stage	
B. Growth inhibitor	Suppresses growth of members of the same species	
V. Avoidance responses		23, 25, 39–41
A. Alarm activator	Initiates response to signal emitted from members of same species	
B. Escape activator	Initiates movement away from predators or in response to environmental factors (pH, salinity)	
C. Defense activator	Initiates specific defense mechanisms	
VI. Reproduction		23, 24
A. Mate attractant	Orients toward opposite sex	
B. Reproductive incitant	Initiates characteristic mating behavior	
C. Spawning inducer	Induces and/or synchronizes release of gametes	
D. Gamete attractant	Orients sperm toward egg	

[a]Includes interactions between different species, such as commensalism, mutualism, and parasitism (see 42).

animal's movements involved in feeding. This system offers a number of experimental advantages for receptor research. For one, the molecule GSH itself is complex enough, though not too large, to allow us to make a sufficient number of analogues for determining the conformation(s) of GSH necessary to activate the receptor.

A second advantage derives from the very nature of hydra itself: (*a*) it is of simple tissue-level structure (see below), (*b*) the receptor is on the surface of the outer epithelium, (*c*) the biological response can be readily quantified, (*d*) pure clones of the animals can be easily grown in the laboratory in kilogram quantities (wet weight), and (*e*) the fluid environment ("medium") surrounding the receptor can be accurately controlled within a pH range of about 4 to 8 (see 27).

The animal is shaped like a two-ply hollow tube, about 8 mm by 1 mm when extended, made up of both outer (ectodermal) and inner (endodermal) epithelial layers. At the posterior end of the tube is a basal disc with which the hydra usually attaches to a surface, and at the anterior end is a mouth surrounded by a ring of tentacles. The tentacles are armed with many nematocytes, one of the seven cell types of hydra. The nematocytes contain nematocysts ("stinging capsules") which start the feeding process by piercing the prey with long spear-like tubules.

In nature, the feeding behavior is initiated by trace amounts of GSH which are emitted from wounds in the prey caused by the penetrating nematocyst tubule. The key feature of the behavior is that GSH stimulates the tentacles to writhe and the mouth to open. It is the duration of this mouth opening that can be quantified and used as an accurate and reliable bioassay of GSH activity and the activity of its analogues, and of other factors affecting the feeding behavior.

Evidence for a GSH Surface Receptor

Earlier experiments by Loomis (43) showed that GSH was the substance leaking from the wounded prey that activated feeding in *Hydra littoralis.* To eliminate the possibility of the activity being caused by a contaminant in the GSH preparation, he showed that chemically synthesized GSH was also active (43). Later work by Lenhoff (44), investigating the competitive action of GSH analogues, confirmed that it was the GSH in the prey's fluids that activated the response. Other early experiments (45) showed that the duration of the response was dependent upon the concentration of GSH applied to the hydra and that the response would take place only when GSH remained in the solution. Furthermore, the GSH was neither consumed nor metabolized. These experiments suggested that on the surface of the hydra's outer epithelium, there were receptor sites that would become activated only in the presence of GSH.

A saturable receptor was indicated by analysis of plots of the duration of the feeding response against the concentration of GSH to which the hydra was exposed (45). An analysis of the resultant curve with concepts borrowed from enzymology suggests that there is a receptor which is saturated and gives a maximum response at GSH concentrations of 5×10^{-6} M and greater. A maximum response is considered analogous to the maximum velocity of an enzyme catalyzed reaction; both occur during saturation of an active site.

Determination of the Dissociation Constant

The assumptions made in determining the dissociation constant, K_A, between the activator (A) and the receptor (R) have been reported elsewhere (46, 47). The effect of the activation is signified by ϵ and the maximum effect, by ϵ_M. The equation derived

$$(A)/\epsilon = 1/\epsilon_M\,(A) + K_A/\epsilon_M \qquad 1.$$

is analogous to the second form of the Lineweaver-Burk (48) plot, the equation developed by Beidler (49) for mammalian taste chemoreception, and, of course, a form of the Langmuir adsorption isotherm. This equation is useful in analyzing chemoreception phenomena because it minimizes deviations in individual animal responses that occur at very low levels of activator.

Previous data (50) show that this equation can be used to interpret the plot of $(A)/\epsilon$ against (A); we obtain straight lines at most glutathione concentrations. From such plots we can determine, for example, at pH 7, a dissociation constant of 10^{-6} M. Such a low K_A is meaningful from at least three viewpoints: (*a*) the smallness of the constant indicates a high affinity of the receptor for glutathione; (*b*) concentrations around 10^{-6} M are well within the physiological range to be expected under natural conditions of feeding; (*c*) this constant provides a means of characterizing the receptor—that is, the glutathione receptor of *H. littoralis* may be said to have a dissociation constant of 10^{-6} M under the given conditions. The constant is a characteristic of the receptor and remains nearly the same no matter what the nutritional state of the hydra (44, 45). Similarly, experiments in which the buffer anion is varied alter the maximum response, but not the dissociation constant (50).

In Vivo Determination of pH Profile of Receptor

Changes in the K_A with pH can be used to determine the pK's of the ionizable groups on glutathione or at the receptor site which are involved in the combination with glutathione. The pK measurements were made by means analogous to those used by enzymologists in determining the pK's of ionizable groups at the active site of enzymes. For our purposes, we needed an equilibrium equation, like Dixon's (51) for enzymes, which would take into account the influence of pH on the dissociation constant. This modified equation (46, 47, 50) involved the assumption that if the activator, receptor site, or activator-receptor complex ionizes, then, in the expression for equilibrium, each component (A, R, AR) equals its concentration multiplied by a term which is a function of pH. For example, if the activator ionized, then the total concentration of free activator, A_t, would be A times the pH function of A, or f_a (pH). The logarithimic form of the equation is:

$$\mathrm{p}K_A = \mathrm{p}K_A^0 + \log f_{ar}\,(\mathrm{pH}) - \log f_r\,(\mathrm{pH}) - \log f_a\,(\mathrm{pH}). \qquad 2.$$

Here pK_A refers to the negative logarithm of the dissociation constant of AR, while pK_A^0 is the same constant if none of the components has ionic groups; if no component ionizes, then pK_A and pK_A^0 are equal. The derivation of this equation is explained elsewhere (47).

The foregoing equation indicates that a plot of pK_A against pH will consist of a series of straight lines joined by short curved parts, and holds true for the glutathione-hydra system. The results (50) followed almost exactly the predictions from the modified Dixon equations. The following interpretations were made (50): 1. Ionizable groups at the receptor site participated in binding glutathione, because significant variations in pK_A occurred with change in pH. 2. The concave downward inflections at pH's 4.6, 4.8, 6.5, and 7.6 represented pK's of ionizable groups at the receptor site. These pK's probably do not represent ionizable groups of glutathione, which have pK's either below pH 4 (2.1 and 3.5) or above pH 8 (8.7 and 9.6) (52). If the receptor site is protein, then the pK's determined may represent two β-carboxyls of peptide aspartic acid (or γ-carboxyls of peptide glutamic acid), an imidazole group, and a terminal α-amino group, respectively. 3. The horizontal lines indicate pH values that do not affect the combination of glutathione with the receptor site. 4. The quenching of the charges (53) at about pH 4 and 8 indicated that receptor-site groups having pK's of 4.6 and 7.6 may be associated with complementary charged groups of glutathione.

Active Structure of Glutathione

To determine the structure of GSH present at the receptor site, it was necessary to quantify the ability of different structural analogues of GSH either to activate or inhibit a response (54, 55). The aforementioned bioassay experiments were used to determine the effectiveness (K_A and ϵ_M) of synthetic agonists (44, 54, 55). The relative ability of analogues that bind but do not activate, i.e. antagonists, was determined by measuring their ability to inhibit competitively the activity of GSH in eliciting the feeding response (55).

Data from these investigations established that (*a*) the thiol is not required for activation, because ophthalmic acid (γ-glu-abu-gly), norophthalmic acid (γ-glu-ala-gly), and S-methyl glutathione (S-me-GSH) also activated the response (54, 55); (*b*) activation of the response requires the intact tripeptide backbone of glutathione, because the just mentioned analogues activated feeding while amino acids, dipeptides, and a number of tripeptide analogues with large and charged substituents at the sulfhydryl group did not activate (44, 55); (*c*) the receptor has a high affinity for the glutamyl part of the tripeptide because glutamic acid and glutamine were the only amino acids to show competitive inhibition (55) and the tripeptide aspartthione (β-asp-cys-gly) did not initiate the response (43, 55); and (*d*) the α-amino of glutathione is probably required for association of glutathione with
because glutamic but not α-keto glutaric acid competitively inhibited GSH action (44, 55).

Current research on the specificity of the glutathione receptor of *H. attenuata* agrees with data found using *H. littoralis*. New information was uncovered using tripeptide analogues with substitution of the glycyl residue. For example, when either leucine or tyrosine are substituted for glycine, the resultant tripeptide still activated a response (56).

Currently over 70 analogues are being quantified as to their ability to act as either agonists or antagonists. Both the published and unpublished results indicate that the

GSH receptor of hydra has a unique and rigid specificity for the tripeptide structure of GSH.

Other Factors Influencing the Response

The feeding response of hydra is also influenced by a number of external factors, such as the ionic composition of the medium (46) and certain proteases (57). Environmental cations, for example, affect the response activated by GSH in many ways. Without calcium ions, hydra do not respond to reduced glutathione (58). The requirement for calcium is pH-dependent (H. M. Lenhoff, unpublished), and a concentration of about 10^{-4} M was necessary for a maximum response. Strontium was the only ion that could substitute for calcium, and even it was much less effective (58). The chelating agent ethylenediaminetetraacetic acid (EDTA) also inhibited feeding. This inhibition was completely reversed only by calcium ions, and to some degree by strontium ions (58). Magnesium ions were not required; in high concentrations they inhibited the responses by competing with calcium ions (44, 58). Sodium likewise competed with calcium, but less effectively than magnesium (58).

Potassium ions were found to inhibit the feeding response (46), but, unlike magnesium and sodium ions, they did not act by competing with calcium ions. Concentrations of potassium ions as low as 10^{-4} M could lower the response to glutathione significantly, and this inhibition could be reversed by placing the animals in a potassium-free medium for a few hours (H. M. Lenhoff, unpublished). Since potassium ions play an important role in bioelectric potential, it may be that these ions act by affecting the cellular membrane potential of hydra. More recent work using *H. attenuata* shows that the presence of sodium ions in the environment is an absolute requirement for the glutathione-mediated feeding response (59).

Temperature effects on the feeding response are complex (44), but they do indicate that there is a rate-limiting thermochemical step in the effector system that has an activation energy of about 13,000–14,000 calories (H. M. Lenhoff, unpublished). This step is thought to involve the consumption of some regenerable substance, such as ATP.

A number of nontripeptides can activate a feeding response in some coelenterates in the absence of added GSH (57, 60–62). Of special interest are the proteolytic enzymes papain, ficin, and trypsin (57) because it is now recognized that numerous control mechanisms can be activated by proteases (see 63).

Cyclic Nucleotides

As with higher organisms, cyclic nucleotides are thought to play in lower forms a similar function as second messengers. The first report that cyclic AMP functions in such a fashion in cnidarians is that of Gentleman & Mansour (64) in the sea anemone *Anthopleura elegantissima.* They showed that on the addition of GSH the concentration of cAMP in the oral disc and pharynx tissues increased. Preliminary work with hydra shows that on the addition of GSH there are rapid changes in the levels of both cAMP and cGMP (65).

Summary

In summary, the GSH receptor system of hydra has much in common with the receptor systems of higher organisms now under study. The hydra receptor shows a precise specificity for the activator, the combination of the activator with receptor follows "saturation kinetics," and the cyclic nucleotides appear to be involved as secondary messengers.

The hydra GSH receptor system has a number of advantages that are inherent in the nature of the GSH molecule and in the hydra itself (see above). But perhaps the most unique advantage of this system is that it is possible to investigate in vivo properties of the receptor both while it is carrying out a behavioral response (50) and while measurements are being made on the binding of either activators or competitive inhibitors to the whole animal (65).

ON THE EVOLUTIONARY DEVELOPMENT OF RECEPTOR PROTEINS

The following view on the evolutionary development of receptor proteins is derived from our analysis of research carried out mostly on feeding activators in lower forms. We propose that the most primitive of cnidarian receptors responded to a range of amino acids and peptides, and that from these general receptors, more specific ones evolved. Furthermore, we propose that receptors in higher forms, such as those involved in chemoreception and in neurotransmitter and hormone action, evolved from similar primitive general receptors which originally functioned in pinocytosis and phagocytosis.

Inspection of Table 2 shows a number of patterns within the Cnidaria which support our hypotheses. The data show that cnidarians tested from every class and most families elicit a feeding response to either one or a few small molecules. The molecules found most commonly to initiate a feeding response are the tripeptide GSH and the imino acid proline. In the Hydrozoa the feeding response of each organism investigated was induced by a single specific molecule (41, 65, 66). Proline is especially prevalent as an activator among the athecate colonial marine hydroids (61, 67–70). For example, all members of these groups thus far tested, i.e. *Cordylophora* (61), *Pennaria* (67), *Tubularia* (69), and *Proboscidactyla* (68), responded only to proline. All hydras tested responded only to GSH (41, 65, 66). The only other hydrozoans tested also responded to GSH (70, 71).

In the Anthozoa we see three trends. In general, although more than one compound may elicit feeding behaviors, the animals exhibit varying ranges of specificity. For example, the most specificity is seen among the sea anemones: *Boloceroides* responds primarily to valine (72), *Anthopleura* to GSH (73), *Haliplanella* to leucine (74), *Actinia* to glutamic acid (75), and *Calliactis* to GSH (76). The specificity broadens when we consider the colonial anemones: whereas *Zoanthus* responds primarily to GSH (77), *Palythoa* responds to relatively high concentrations of either GSH or proline, or to low amounts of these two activators acting synergistically

Table 2 Chemical activation of feeding in the Cnidaria

Type of organism	Activator	References
I. Hydrozoans		
A. Hydroids		
1. Five species of hydra	GSH	43, 65, 66; H. M. Lenhoff, unpublished; W. Heagy, unpublished
2. Four species of colonial marine hydroids (without theca)	Proline	61, 67–69
3. One species of colonial marine hydroid (with theca)	GSH	70
B. Siphonophores		
Two species (including Portuguese man-of-war)	GSH	70, 71
II. Anthozoans		
A. Sea anemones		
1. *Anthopleura*	GSH	73
2. *Boloceroides*	Valine	72
3. *Actinia*	Glutamate	75
4. *Haliplanella*	Leucine	74
5. *Calliactis*	GSH, proline	76
B. Colonial anemones		
1. *Palythoa*	Proline and/or GSH	78
2. *Zoanthus*	GSH	71
C. Corals		
1. Six species	Proline or GSH	66, 79
2. One species	4 amino acids	80
III. Scyphozoa		
One species (large jellyfish)	20 amino acids, GSH, glycylglycine	81

(78). Lastly, corals seem to respond best to proline alone or GSH alone, as well as to numerous other amino acids at relatively higher concentrations (66, 79, 80).

Chrysaora, the only example of the large jellyfishes tested, seems to respond to GSH and to a wide number of amino acids (81). More kinds of these organisms need to be tested before we can make any generalizations about this group.

Do the Glutathione and Proline Receptors Have a Common Origin?

Fulton (61) has suggested that the evolution of a receptor site for glutathione into one for the α-imino acid proline may have proceeded by means of slight structural changes in the receptor site. His postulate was based on the knowledge that one of

the possible cyclized forms of glutathione in solution is close in structure to an α-imino acid. Because proline is also present in the fluids released from wounded prey organisms, the change in structure of the receptor site was not disadvantageous to *Cordylophora* but, under some circumstances, advantageous, and so persisted.

In support of Fulton's suggestion (61) are a number of cases reported in Table 2. For example, let us assume that the earliest cnidarians responded to a wider range of amino acids and to GSH, as has been reported for *Chrysaora* (81). Possibly from these early cnidarians there evolved one whose receptor was modified so that it would recognize both GSH and proline. Such a receptor might exist in *Palythoa* (78), although it is also possible that *Palythoa* has two distinct receptors, one for each of those activators. Possibly organisms with this proposed "GSH and proline" receptor might then have evolved into organisms having two distinct receptors, one to GSH and another to proline. Such a situation appears to exist in most of the corals listed in Table 2. And, eventually organisms evolved with only GSH receptors, as seen in hydra and siphonophores, or proline receptors, as found in the athecate colonial hydroids.

A similar argument could be constructed regarding the evolution of receptors to amino acids having apolar side chains. For example, the valine receptor of *Bolocer-oides* (72) and the leucine receptor of *Haliplanella* (74) may have evolved from a receptor with a specificity for apolar amino acids in general. Possibly receptors for other amino acids may have followed similar pathways from the original primitive cnidarian receptor which responded to many amino acids.

Evolution of Cellular Receptor Sites in General

Among the earliest receptor sites to evolve were probably those associated with the induction of pinocytosis in single cells. In recent years the chemical induction of pinocytosis has been studied in ameba (82, 83) and in white blood cells (84). Both kinds of cells respond to a range of small charged molecules; of the amino acids, aspartate and glutamate are particularly effective (82, 84). In general, it might be said that single cells depend on external chemical cues that stimulate the uptake of nutrients from their environment; hence, these cells may have evolved receptor sites with broad specificity such as might prove useful to guarantee the cell sufficient food to survive.

It thus seems reasonable to suppose that cnidarian cells utilizing pinocytosis to take up nutrients from their gastrovascular cavity also respond to a broad range of molecules. In accord with this supposition is Slautterback's finding that certain amino acids could stimulate the immediate formation of a large network of microvilli at the apical end of the endodermal digestive cells that line the gut of hydra. Among the most active amino acids were the isomers of tyrosine, with *m*-tyrosine the most active. Phenylalanine was ineffective. Other amino acids showing activity were cysteine and glutamate (D. Slautterback, personal communication).

Slautterback's finding that tyrosine stimulates microvilli formation in hydra endoderm cells takes on particular importance in light of the discovery by Blanquet & Lenhoff (85). They showed that hydra have a receptor on the surface of cells lining the gut. When activated by tyrosine, the hydra exhibited a "neck response," i.e. a constriction of the upper third of the body tube. These neck constrictions apparently

allow hydra to retain previously ingested food in the gut while swallowing newly captured prey. No other natural amino acid, including phenylalanine, could substitute for tyrosine (85). It is interesting to note, however that *m*-tyrosine was more active than the other tyrosine isomers in activating both the neck response and in microvilli formation (85).

Recognizing that in response to tyrosine hydra display these two events, a cellular one and an organismic one, we can pose a number of intriguing questions: Is the same receptor site used to trigger both events? If not, did the receptor for neck formation evolve from the receptor for microvilli formation? It would appear simpler for hydra to use an existing receptor for two functions rather than to evolve another.

This reasoning may be stretched even further to postulate that there exists a direct line of evolution of receptor sites from those found on single cells inducing pinocytosis or phagocytosis, to those coordinating feeding responses in such simple "tissue-level" organisms as cnidarians, and finally to the receptors for neurotransmitters and peptide hormones in higher organisms. For example, because dopamine and norepinephrine are formed directly from tyrosine, it seems simpler and more efficient from an evolutionary standpoint for organisms to retain and utilize modifications of a primitive tyrosine receptor to recognize structurally related compounds rather than to evolve new receptor sites for each of these "analogues."

Would not the same argument apply to the evolution of receptors for such neurotransmitters as glutamic acid or glycine? Are there similar evolutionary relationships between the glutathione receptors involved in activating the cnidarian feeding response, and the glutathione site associated with the γ-glutamyl transpeptidase mechanism of amino acid transport (86) (see below)? In any of these cases, it would seem simpler for organisms during evolution to modify existing receptors to control new tasks rather than to develop completely new receptor-effector systems.

Summary and Future Directions

We feel the current information justifies these speculations on the evolution of receptor proteins because such views are analogous to our ideas of the conservative view of the evolution of proteins in general. The difficulty, of course, lies in proving these speculations. Actually, there are a number of ways in which evidence can be obtained. These ways, however, depend upon the development of methods for investigating receptors. One method, for example, would involve the isolation of receptor proteins and the analysis of their amino acid sequence. Based upon the current status of receptor purification, however, such an approach will not be possible for years. A second approach would consist of a search for different kinds of receptors to amino acids and neurotransmitters among members from all the phyla. From such a comparative study it might be possible to determine at which point in evolution the different receptors arose.

We are using a third approach, that of comparing the relative activities of two proteins that might be related to each other, with analogues. In our case, we are interested in proteins that recognize the tripeptide reduced glutathione. Previously we have reported (see 62) that the GSH receptor of hydra is specific for the γ-glutamyl moiety of GSH and can tolerate some changes in the thiol of the

cysteinyl moiety. Hydra, for example, can respond to GSH which has its thiol methylated. At that time, no other protein combining with GSH was known to have such an unusual specificity for the molecule. Recent work with the enzyme γ-glutamyl transpeptidase ("GTP"), however, has shown a similar specificity of this enzyme for GSH (87).

We were intrigued by this similar specificity of GTP, especially since this enzyme is implied to act in the transport of amino acids into cells (86). Could it be that the GSH receptor of hydra evolved from the GTP thought to be used in amino acid transport? Could they be the same protein? Next we analyzed hydra for this enzyme (56, 88). We found it present in significant concentrations (see also 89), and its requirement for amino acid acceptors similar to that reported for the same enzyme activity as found elsewhere (56, 88).

To answer the question of whether or not the GSH receptor and the GTP from hydra are the same, we decided to look deeper into their relative specificities for GSH. Using analogues synthesized by our colleagues M. H. Cobb and G. Marshall of Washington University, together with J. Danner we were able to distinguish between the two activities. Whereas both proteins had about the same specificity for the γ-glutamyl-cysteinyl part of GSH, they differed with respect to their activities with analogues having substitutions at the glycine moiety; on one hand the GTP reacted poorly with analogues having amino acids with large side chains substituted for the glycine in GSH, while on the other hand, the GSH receptor of hydra was activated by those same analogues (56).

Such experiments show how those two proteins in hydra have similar and yet different specificities for GSH. Possibly the GSH receptor evolved from a mutation in a repeating unit of a gene controlling the synthesis of GTP.

CONCLUSIONS

The above speculations, like most concerning evolution, are difficult to prove. But they may help to make us aware that unifying concepts, tacitly assumed in the case of enzymes and cell organelles, also may apply to the basic aspects of chemoreception. Specifically, such speculations emphasize that the behavioral responses of lower invertebrates to a peptide or an amino acid may have many fundamental features in common with some hormonal and neurotransmitter responses in higher organisms. By focusing on the primary events of the combination of the activator with the receptor to initiate a series of coordinated activities, we may find new approaches and new insights into universal, yet little understood, chemical control mechanisms.

ACKNOWLEDGMENTS

We thank Dr. David Slautterback for his comments and for allowing us to quote from his unpublished material on microvillus formation. The research discussed was supported by grants from the National Institutes of Health and the National Science Foundation.

Literature Cited

1. Pilkis, S. J., Park, C. R. 1974. Mechanism of action of insulin. *Ann. Rev. Pharmacol.* 14:365–88
2. Cuatrecasas, P., Hollenberg, M. D., Chang, K., Bennett, V. 1975. Hormone receptor complexes and their modulation of membrane function. *Recent Prog. Horm. Res.* 31:37–93
3. Roth, J., Kahn, C. R., Lesniak, M. A., Gorden, P., DeMeyts, P., Megyesi, K., Neville, D. M., Gavin, J. R., Soll, A. H., Freychet, P., Goldfine, I. D., Bar, R. S., Archer, J. A. 1975. Receptors for insulin, NSILA-s, and growth hormone: applications to disease states in man. *Recent Prog. Horm. Res.* 31:95–139
4. Roth, J. 1973. Peptide hormone binding to receptors: a review of direct binding studies *in vitro*. *Metab. Clin. Exp.* 22:1059–73
5. Giorgio, N. A., Johnson, C. B., Blecher, M. 1974. Hormone receptors. III. Properties of glucagon-binding proteins isolated from liver plasma membranes. *J. Biol. Chem.* 249:428–37
6. Marshall, G. R., Bosshard, H. E., Vine, W. H., Glickson, J. D., Needleman, P. 1974. Angiotension II: Conformation and interaction with the receptor. In *Recent Advances in Renal Physiology and Pharmacology,* ed. L. G. Wesson, G. M. Fanelli, 215–55. Baltimore: Univ. Park Press. 388 pp.
7. Cohen, J. B., Changeux, J. P. 1975. The cholinergic receptor protein in its membrane environment. *Ann. Rev. Pharmacol.* 15:83–103
8. McNamee, M. G., Mark, G., Weill, C. L., Karlin, A. 1975. Further characterization of purified acetylcholine receptor and its incorporation into phospholipid vesicles. In *Protein-Ligand Interactions,* ed. H. Sund, G. Blauer, 316–27. Berlin: de Gruyter. 486 pp.
9. Hucho, F., Gordon, A., Sund, H. 1975. Subunit structure and binding sites of the acetylcholine receptor. See Ref. 8, pp. 306–15
10. Sugiyama, H., Popot, J. L., Cohen, J. B., Weber, M., Changeux, J. P. 1975. Binding and functional states of the cholinergic receptor protein from *Torpedo marmorata.* See Ref. 8, pp. 289–305
11. Raftery, M. A., Bode, J., Vandlen, R., Michaelson, D., Deutsch, J., Moody, T., Ross, M. J., Stroud, R. M. 1975. Structural and functional studies of an acetylcholine receptor. See Ref. 8, pp. 328–55
12. Snyder, S. H., Young, A. B., Bennett, J. P., Mulder, A. H. 1973. Synaptic biochemistry of amino acids. *Fed. Proc.* 32:2039–47
13. Roberts, P. J. 1974. Glutamate receptors in the rat central nervous system. *Nature* 252:399–401
14. Dole, V. P. 1970. Biochemistry of addiction. *Ann. Rev. Biochem.* 39:821–40
15. Takemori, A. E. 1974. Biochemistry of drug dependence. *Ann. Rev. Biochem.* 43:15–33
16. Adler, J. 1975. Chemotaxis in bacteria. *Ann. Rev. Biochem.* 44:341–57
17. Lovely, P., Dahlquist, F. W., Macnab, R., Koshland, D. E. Jr. 1974. An instrument for recording the motions of microorganisms in chemical gradients. *Rev. Sci. Instrum.* 45:683–86
18. Taylor, B. L., Koshland, D. E. Jr. 1974. Reversal of flagellar rotation in monotrichous and peritrichous bacteria: generation of changes in direction. *J. Bacteriol.* 119:640–42
19. Strange, P. G., Koshland, D. E. Jr. 1976. Receptor interactions in a signalling system: competition between ribose receptor and galactose receptor in the chemotaxis response. *Proc. Natl. Acad. Sci. USA* 73:762–66
20. Beidler, L. M. 1971. Taste receptor stimulation with salts and acids. In *Handbook of Sensory Physiology,* ed. L. M. Beidler, Vol. 4, Part 2:200–20. New York: Springer. 518 pp.
21. Fischer, R. 1971. Gustatory, behavioral and pharmocological manifestations of chemoreception in man. In *Gustation and Olfaction; Food Science and Technology,* ed. G. Ohloff, A. F. Thomas, 187–235. New York: Academic. 275 pp.
22. Jennings, H. S. 1906. *Behavior of the Lower Organisms.* Republished by Indiana Univ. Press, Bloomington, 1962. 366 pp.
23. Mackie, A. M., Grant, P. T. 1974. Interspecies and intraspecies chemoreception by marine invertebrates. In *Chemoreception in Marine Organisms,* ed. P. T. Grant, A. M. Mackie, 105–33. New York: Academic. 295 pp.
24. Kittredge, J. S. 1974. Comparative biochemistry: Marine biochemistry. In *Experimental Marine Biology,* ed. R. N. Mariscal, 226–55. New York: Academic. 373 pp.

25. Kohn, A. J. 1961. Chemoreception in gastropod molluscs. *Am. Zool.* 1:291–308
26. Barber, S. B. 1961. Chemoreception and thermoreception. In *Physiology of Crustacea*, ed. T. H. Waterman, 2:109–31. New York: Academic. 681 pp.
27. Lenhoff, H. M., Lindstedt, K. J. 1974. Chemoreception in aquatic invertebrates with special emphasis on feeding behavior of Coelenterates. See Ref. 23, pp. 143–72
28. Dethier, V. G., Browne, L., Smith, C. N. 1960. The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol.* 53: 134–36
29. Beck, S. D. 1965. Resistance of plants to insects. *Ann. Rev. Entomol.* 10:207–32
30. Lindstedt, K. J. 1971. Chemical control of feeding behavior. *Comp. Biochem. Physiol.* 39:553–81
31. Laverack, M. S. 1974. The structure and function of chemoreceptor cells. See Ref. 23, pp. 1–41
32. Davenport, D. 1966. The experimental analysis of behavior in symbiosis. In *Symbiosis*, ed. S. M. Henry, 1:381–429. New York: Academic. 478 pp.
33. Cheng, T. O. 1967. Marine molluscs as hosts for symbiosis: with a review of known parasites of commercially important species. In *Advances in Marine Biology*, ed. F. S. Russell, 5:1–424. New York: Academic. 424 pp.
34. McCauley, J. 1969. Marine invertebrates, chemical signals and marine products. *Lloydia* 32:425–37
35. Harris, L. 1971. Nudibranch associations as symbioses. In *Aspects of the Biology of Symbiosis*, ed. T. Cheng, 77–90. Baltimore: Univ. Park Press. 327 pp.
36. Ache, B. W. 1974. The experimental analysis of host location in symbiotic marine invertebrates. In *Symbiosis in the Sea*, ed. W. Vernberg, 45–60. Columbia, SC: Univ. South Carolina Press. 276 pp.
37. Kittredge, J. S. 1971. Marine-fouling organisms: natural attractants and repellents. *U. S. Natl. Tech. Inform. Serv. A.D. Rep. 1971, No. 734106*
38. Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. See Ref. 23, pp. 177–253
39. Passano, L. M. 1957. Prey-predator recognition in the invertebrates. In *Recent Advances in Invertebrate Physiology*, ed. B. Y. Scheer, T. H. Bullock, L. H. Kleinholz, A. W. Martin, 37–47. Eugene: Univ. Oregon Press. 304 pp.
40. Baylor, E. R., Smith, E. E. 1957. Diurnal migration of plankton crustaceans. See Ref. 39, pp. 21–35
41. Whittaker, R. H., Feeney, P. P. 1971. Allelochemics: chemical interactions between species. *Science* 171:757–70
42. Starr, M. P. 1975. A generalized scheme for classifying organismic associations. In *Symbiosis*, ed. D. H. Jennings, D. L. Lee, 1–20. Cambridge: Cambridge Univ. Press. 633 pp.
43. Loomis, W. F. 1955. Glutathione control of the specific feeding reactions of hydra. *Ann. NY Acad. Sci.* 62:209–28
44. Lenhoff, H. M. 1961. Activation of the feeding reflex in *Hydra littoralis*. In *The Biology of Hydra and of Some Other Coelenterates*, ed. H. M. Lenhoff, W. F. Loomis, 203–32. Coral Gables: Univ. Miami Press. 467 pp.
45. Lenhoff, H. M. 1961. Activation of the feeding reflex in *Hydra littoralis*. I. Role played by reduced glutathione and quantitative assay of the feeding reflex. *J. Gen. Physiol.* 45:331–44
46. Lenhoff, H. M. 1965. Some physicochemical aspects of the microenvironments surrounding hydra during activation of their feeding behavior. *Am. Zool.* 5:515–24
47. Lenhoff, H. M. 1968. Chemical perspectives on the feeding response, digestion and nutrition of selected coelenterates. In *Chemical Zoology*, ed. M. Florkin, B. Scheer, 2:157–221. New York: Academic. 639 pp.
48. Lineweaver, H., Burk, D. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 56:657–66
49. Beidler, L. M. 1954. A theory of taste stimulation. *J. Gen. Physiol.* 38:133–39
50. Lenhoff, H. M. 1969. pH profile of a peptide receptor. *Comp. Biochem. Physiol.* 28:571–86
51. Dixon, M. 1953. The effect of pH on the affinities of enzymes for substrates and inhibitors. *Biochem. J.* 55:161–70
52. Wieland, T. 1954. Chemistry and properties of glutathione. In *Glutathione*, ed. S. Colowick, A. Lazarow, E. Racker, D. R. Schwarz, 45–59. New York: Academic. 341 pp.
53. Dixon, M., Webb, E. C. 1964. *Enzymes*, 154–65. New York: Academic. 950 pp. 2nd ed.
54. Cliffe, E. E., Waley, S. G. 1958. Effect of analogues of glutathione on the feeding reaction of hydra. *Nature* 183: 804–5

55. Lenhoff, H. M., Bovaird, J. 1961. Action of glutamic acid and glutathione analogues on the hydra glutathione-receptor. *Nature* 189:486–87
56. Danner, J., Lenhoff, H. M., Cobb, M. H., Heagy, W., Marshall, G. R. 1976. *Biochem. Biophys. Res. Commun.* 73: 180–86
57. Lenhoff, H. M., Bovaird, J. 1960. Enzymatic activation of a hormone-like response in hydra by proteases. *Nature* 187:671–73
58. Lenhoff, H. M., Bovaird, J. 1959. Requirement of bound calcium for the action of surface chemoreceptors. *Science* 130:1474–76
59. Asbill, M. 1975. Sodium ion effect on the glutathione induced feeding response of *Hydra attenuata. J. Undergrad. Res. Biol. Sci., Univ. Calif., Irvine* 4:210–28
60. Lenhoff, H. M., Zwisler, J. 1963. Zinc activation of a coordinated response in hydra. *Science* 142:1666–68
61. Fulton, C. 1963. Proline control of the feeding reaction of *Cordylophora. J. Gen. Physiol.* 46:823–37
62. Lenhoff, H. M. 1974. On the mechanism of action and evolution of receptors associated with feeding and digestion. In *Coelenterate Biology: Reviews and New Perspectives,* ed. L. Muscatine, H. M. Lenhoff, 359–89. New York: Academic. 501 pp.
63. Reich, E., Rifkin, D. B., Shaw, E. eds. 1976. *Proteases and Biological Control.* Cold Spring Harbor, NY: Cold Spring Harbor Lab. 1002 pp.
64. Gentleman, S., Mansour, T. E. 1974. Adenylate cyclase in a sea anemone-implication for chemoreception. *Biochim. Biophys. Acta* 343:469–79
65. Cobb, M. H., Marshall, G. R., Heagy, W., Danner, J., Lenhoff, H. M. 1976. On the mechanism of the glutathione induced feeding response in hydra: hormone-receptor interaction. *Fed. Proc.* 35:1617 (Abstr.)
66. Mariscal, R. N. 1971. The chemical control of the feeding behavior in some Hawaiian corals. In *Experimental Coelenterate Biology,* ed. H. M. Lenhoff, L. Muscatine, V. Davis, 100–18. Honolulu: Univ. of Hawaii Press. 281 pp.
67. Pardy, R. L., Lenhoff, H. M. 1968. The feeding biology of the gymnoblastic hydroid, *Pennaria tiarella. J. Exp. Zool.* 168:197–202
68. Spencer, A. N. 1974. Behavior and electrical activity in the hydrozoan *Proboscidactyla flavicirrata* (Brandt). *Biol. Bull.* 146:100–15
69. Rushforth, N. B. 1976. *Electrophysiological correlates of feeding behavior in the Tubularia.* In *Coelenterate Ecology and Behavior,* ed. G. O. Mackie, 729–38. New York: Plenum. 744 pp.
70. Lenhoff, H. M., Schneiderman, H. A. 1959. The chemical control of feeding in the Portuguese man-of-war, *Physalia physalia* L., and its bearing on the evolution of the cnidaria. *Biol. Bull.* 116:452–60
71. Mackie, G. O., Boag, D. A. 1963. Fishing, feeding and digestion in siphonophores. *Publ. Stn. Zool. Napoli* 33: 178–96
72. Lindstedt, K. J., Muscatine, L., Lenhoff, H. M. 1968. Valine activation of feeding in the sea anemone *Bolocerоides. Comp. Biochem. Physiol.* 26: 567–72
73. Lindstedt, K. J. 1971. Biphasic feeding response in a sea anemone: control by asparagine and glutathione. *Science* 173:333–34
74. Lindstedt, K. J. 1971. Chemical control of feeding behavior. *Comp. Biochem. Physiol.* 39:553–81
75. Steiner, G. 1957. Über die chemische nahrungswahl von *Actinia equina* (L). *Naturwissenschaften* 44:70–71
76. Reimer, A. A. 1973. Feeding behavior in the sea anemone *Calliactis polypus* (Forskal, 1775). *Comp. Biochem. Physiol.* 44:1289–1301
77. Reimer, A. A. 1971. Feeding behavior in the Hawaiian zoanthids *Palythoa* and *Zoanthus. Pac. Sci.* 25:512–20
78. Reimer, A. A. 1971. Chemical control of feeding behavior in *Palythoa* (Zoanthidea, Coelenterata). *Comp. Gen. Pharmacol.* 2:383–96
79. Mariscal, R. N, Lenhoff, H. M. 1968. The chemical control of feeding behavior in *Cyphastrea ocella* and some other Hawaiian corals. *J. Exp. Biol.* 49: 689–99
80. Lehman, J. T., Porter, J. W. 1973. Chemical activation of feeding in the Caribbean reef building coral *Montastrea cavernosa. Biol. Bull.* 145:140–49
81. Loeb, M., Blanquet, R. S. 1973. Feeding behavior in polyps of the Chesapeake Bay sea nettle, *Chrysaora quinquecirrha* (Desor, 1848). *Biol. Bull.* 145:150–58
82. Chapman-Anderson, C. 1962. Pinocytosis in amoebae. *C. R. Trav. Lab. Carlsberg* 33:73–264
83. Allen, H. J., Ault, C., Winzler, R. J., Danielli, J. 1974. Chemical characteri-

zation of the isolated cell surface of amoebae. *J. Cell. Biol.* 60:26–38
84. Cohn, Z. A. 1967. The regulation of pinocytosis in mouse microphages. II. Factors inducing vesicle formation. *J. Exp. Med.* 125:213–32
85. Blanquet, R. S., Lenhoff, H. M. 1968. Tyrosine enteroreceptor of hydra: its function in eliciting a behavior modification. *Science* 159:633–34
86. Meister, A. 1973. On the enzymology of amino acid transport. *Science* 180: 33–39
87. Tate, S., Meister, A. 1974. Interaction of γ-glutamyl transpeptidase with amino acids, dipeptides, and derivatives and analogs of glutathione. *J. Biol. Chem.* 249:7593–7602
88. Danner, J., Cobb, M. H., Heagy, W., Marshall, G. R. 1976. γ-Glutamyl transpeptidase in hydra. *Fed. Proc.* 35:1529 (Abstr.)
89. Tate, S., Meister, A. 1976. γ-Glutamyl transpeptidase in *Hydra littoralis*. *Biochem. Biophys. Res. Commun.* 70: 500–5