

Discussion Letter

Some properties of adenylate cyclase which might be important for memory formation

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Analysis of the biochemical defects detected in a *Drosophila* memory mutant permits dissection of properties of adenylate cyclase which may play a role in elementary memory mechanisms. Of special relevance appear to be those properties which are related to the intracellular regulation of the enzyme.

Short-term memory Adenylate cyclase Forskolin Ca²⁺ Calmodulin Drosophila conditioning mutant

1. INTRODUCTION

Mutations that disrupt learning and memory without markedly affecting other behavior, can shed light on the molecular processes that enable an organism to acquire, store and retrieve new information. Such mutations have been identified in the fruit fly, *Drosophila melanogaster*. The experimental procedure was usually as follows: wild-type flies were fed with a chemical mutagen, ethylmethanesulfonate, and their progeny bred into lines, each representing a different mutagenized chromosome. The lines were then tested for their learning ability, using a paradigm in which the flies are trained to avoid an odorant by associating it with an electric shock. Mutants unable to pass the test, but otherwise displaying normal motor and sensory behavior, were thus identified. In all the cases tested so far, the effect of the mutation on learning and memory was found to be quite general; i.e., the mutants fail in conditioning paradigms that differ from the one which was used for their isolation, including learning tests that use sensory modalities other than olfaction (reviews [1,2]).

Two of the most studied conditioning mutants

to date are *dunce* (*dnc*) and *rutabaga* (*rut*). Both are actually memory mutants, i.e., they can acquire new information but memory decays very rapidly. Both mutations affect facets of cAMP metabolism. *dnc* appears to be a structural gene for a subpopulation of the enzyme cAMP phosphodiesterase; *rut* affects a subpopulation, or a functional state, of the enzyme adenylate cyclase. Both genes and their products are currently being further characterized by various techniques, including recombinant DNA (reviewed as above). One of these mutants, *rut*, is the subject of the present discussion.

The data on *rut* are not the first line of evidence to suggest that adenylate cyclase plays a role in memory formation. Previous evidence has emerged mainly from studies of the gill-withdrawal reflex in the marine snail *Aplysia* (review [3]). The *Aplysia* studies demonstrated that persistent elevation in the level of cAMP underlies short-term memory of the modified reflex, and that blockage of adenylate cyclase (by using the ligand GDP β S) quickly terminates the alteration in synaptic efficacy that is presumed to encode the memory [3,4].

Studies of memory in *Drosophila* cannot yet

take advantage of the direct electrophysiological and pharmacological approaches which are feasible in the identified networks of *Aplysia*. *Drosophila*, nevertheless, offers mutations that can be used as microscalpels to dissect molecular events. *rut* provides an example for the potential of the genetic dissection approach: the *rut* data can provide a unique opportunity to delineate specific properties of adenylate cyclase which are presumed to be crucial for memory (without yet, of course, revealing the detailed structure and operation of the memory apparatus). This is so because the behavioral experiments suggest that the memory defect which is caused by the *rut* mutation is specific (i.e., other physiological processes and behaviors are apparently normal); because the biochemical and genetic experiments suggest that the normal *rut* gene product is indeed a component of an adenylate cyclase system (and see below); and because the behavioral and the biochemical defects both map to the same chromosomal locus [5]. One is thus in a position to relate the altered or missing properties of adenylate cyclase in *rut* to the abnormal behavior.

The studies to date suggest that the *rut* gene product is either a subpopulation of a catalytic subunit of adenylate cyclase, or a not-yet-identified regulatory subunit that is intimately associated with the catalytic subunit [5–8]. These studies revealed several differences between the activity of adenylate cyclase in mutant tissues and in the corresponding normal tissues. The differences thus represent lesions in an affected component, or properties of a missing component of the enzyme [5–9].

In brief, the adenylate cyclase activity in *rut* is characterized by a lower $V_{\max}$ [5–8], different K_m for the substrate (i.e. the metal-ATP complex) [6], different affinity for allosteric Mg^{2+} [6,8], a decrease in the high-affinity sites for the activator forskolin [8], lack of activator-induced alterations in the affinity for the substrate and for allosteric Mg^{2+} [8], and lack of stimulation by Ca^{2+} -calmodulin [5,9]. In principle, any of the above-mentioned defects might be important for proper operation of a memory apparatus in *Drosophila*. The possible relevance of each of these properties of adenylate cyclase to the memory defect is evaluated below.

2. ON THE HETEROGENEITY OF ADENYLATE CYCLASE

The first clear-cut defect observed in *rut* is a decrease in the $V_{\max}$ of adenylate cyclase, which is especially pronounced in posterior body parts. The $V_{\max}$ in abdominal membrane preparations is less than 50% of normal, and in whole body homogenates is ~70% of normal. This uneven anatomical distribution of the biochemical defect is very probably due to the existence of different proportions of the affected cyclase out of the total cyclase in different body regions [5,6]. As mentioned above, the reduced $V_{\max}$ may be, in principle, due to the absence of a subpopulation of catalytic subunits or to the presence of slower enzyme molecules.

It is thus possible that the molecular mechanisms that are involved in memory formation are defective in *rut* because the mere ability to synthesize cAMP in certain loci is simply missing or much reduced. In such a case the specific kinetic and regulatory properties of the missing subpopulation may not by themselves bear relevance to learning, and any other appropriately active adenylate cyclase in the deprived loci would have been sufficient to ensure proper operation of the learning apparatus. Tissues and cells which are utterly deprived of their adenylate cyclase activity should be expected to function abnormally. Since *rut* cannot remember but can learn, i.e. the network required to form the learnt association is functional, and since memory is expected to reside in the network that learns, the postulated deprived loci are probably not whole tissues or cells but rather subcellular microcompartments.

Differential incorporation of an enzyme into a specific microcompartment might result from specificity in the microenvironment, or in the enzyme molecule, or in both. Recent studies performed on Lubrol-solubilized enzyme preparations have clearly demonstrated that the defect in *rut* is not in the membraneous milieu, but in the enzyme per se [8,10]. One has therefore to assume that there is heterogeneity in the cyclase itself (see also below), and this makes it more tempting to suggest that some specific inherent properties of the missing component, in addition to the mere ability to catalyze the formation of cAMP from ATP, are crucial for memory formation.

3. THE INTERACTION OF THE ENZYME WITH ITS SUBSTRATE

The affinity for MgATP of the residual adenylate cyclase in *rut* is lower than normal; the difference in K_m is not very large (~3-fold) but is highly significant [6]. It is thus possible that for memory to be established in a normal way, an adenylate cyclase form (or functional state) with a high affinity for the metal-ATP complex must be present in the appropriate microcompartment, and a low-affinity form would not do the job appropriately. Indeed, adenylate cyclase in normal *Drosophila* tissues displays kinetic heterogeneity with respect to the K_m for metal-ATP when tested in vitro, suggesting the existence in vivo of several subpopulations or functional states of the enzyme which differ in their affinity for the substrate [6].

It is not however clear yet how much of this kinetic heterogeneity is due to inherent properties of catalytic subunits and how much is contributed by reversible associations of the catalytic subunit with various regulatory subunits and by regulatory ligands. In addition, the intracellular concentration of ATP is not considered a limiting factor in the activation of adenylate cyclase (e.g., see discussion in [11]). Therefore, although the adenylate cyclase that plays a role in memory formation may have a different affinity for the substrate than other adenylate cyclase(s), this property is not very likely to play a crucial role in the process.

4. THE INTERACTION OF THE ENZYME WITH FREE Mg^{2+}

The residual adenylate cyclase in the most affected tissues of *rut* also displays a higher than normal affinity for free Mg^{2+} [6]. Free Mg^{2+} is known to regulate adenylate cyclase activity and plays a crucial role in mediating the interaction of neurotransmitters and hormones with the catalytic subunit of the enzyme via the G regulatory subunits [12,13]. Intracellular concentrations of free Mg^{2+} are in the range expected to play such a role in vivo (e.g., see discussion in [11]). It is thus possible that the adenylate cyclase which is crucial for memory formation requires special affinity states for Mg^{2+} .

The problem, however, is that the active states of adenylate cyclase are characterized by high af-

finity for Mg^{2+} [12], and the *rut* data therefore indicate that the normal counterpart of the *rut* enzyme is in a subactive state. It is not very likely that such a subactive state is crucial for memory. It is more likely that the difference in affinity states for the substrate and an allosteric ligand, that exists between *rut* and normal tissue, reflects a certain mode of association of the catalytic subunit with regulatory subunits, or even molecular rigidity which might be relevant to memory (and see below).

5. THE INTERACTION OF THE ENZYME WITH FORSKOLIN, OR A FORSKOLIN-LIKE LIGAND

The residual adenylate cyclase in the most affected *rut* tissues differs from the normal enzyme in its interaction with the diterpene forskolin [14]. In the membrane-associated state, the mutant's enzyme displays a much reduced proportion of high-affinity forskolin interacting sites [6,8]. In Lubrol-solubilized preparations, the mutant's enzyme displays very little forskolin activation when compared to normal [8]. Forskolin is known to be capable of direct interaction with the catalytic unit of adenylate cyclase, although the presence of a G regulatory unit is required for optimal activation [14]. It is not yet clear what is the physiological role of the forskolin-binding site(s) in adenylate cyclase, and the physiological analogue of the diterpene (if it does exist at all) has not yet been identified [14]. The *rut* results turn attention to the possibility that high-affinity forskolin-binding site(s) play a role in the operation of the learning apparatus, e.g., by interacting appropriately with the not-yet-identified intracellular physiological analogue of forskolin.

6. ALTERNATING STATES OF AFFINITY OF THE ENZYME SYSTEM FOR THE SUBSTRATE AND FOR ALLOSTERIC LIGAND(S)

When forskolin stimulates *Drosophila* adenylate cyclase, it increases the V_{max} of the enzyme, and in addition it also alters the affinity of the enzyme for the metal-ATP complex as well as for free Mg^{2+} [8]. Such activator-induced affinity shifts are not detected in the defective *rut* preparation. If

adenylate cyclase indeed participates in the early steps of memory formation, then an activator-induced new functional state of the enzyme, which might manifest itself in alteration of affinity toward substrate and/or regulators (e.g., operation of Mg^{2+} -switches [12,13]; see also above), could be expected to play a role in the initiation of the molecular cascade. The different affinities for the substrate and for the allosteric metal which characterize the enzyme in *rut* ([6,8], and see above) may be a reflection of the absence, in the mutant, of an enzyme system that can appropriately switch between functional states. In other words, the adenylate cyclase crucial for memory formation may be endowed with molecular plasticity that underlies features of behavioral plasticity.

7. THE INTERACTION WITH Ca^{2+} -CALMODULIN

In some tissues, and especially in tissues of neuronal origin, the response of adenylate cyclase to calcium is biphasic. At low Ca^{2+} concentrations (usually below $0.1 \mu M$), there is a calmodulin-mediated stimulation of activity, and at high Ca^{2+} concentrations there is a calmodulin-independent inhibition of activity [15]. The calmodulin-mediated stimulation is present in normal *Drosophila* preparations but is absent in *rut* [5,9].

Intracellular calcium concentrations are altered when an action potential invades a synaptic terminal, and it has been suggested that a Ca^{2+} -activated adenylate cyclase, that is sensitive to such calcium level modulations, plays a role in the integration of signals during associative conditioning [16,17]. For example, in conditioning of the gill-withdrawal reflex in *Aplysia*, Ca^{2+} may signal the activity of the conditioned stimulus (i.e., mild stimulation of the skin sensory neurons) and a neurotransmitter may signal the unconditioned stimulus (shock to the tail). Both signals interact with adenylate cyclase, and therefore the cyclase system could in principle integrate them [16,17]. Such integration may result in instantaneous elevation in the level of cAMP which is larger than that induced by each of the signals alone [18], and/or in alterations of some sustained properties of the cyclase system (e.g., kinetics, stability).

The *rut* data support a role for the

Ca^{2+} -activation function of the cyclase in memory formation. One should, however, note that the *rut* data do not *prove* that the Ca^{2+} activation of the cyclase is a crucial event in memory formation in general and in signal convergence during associative conditioning in particular. Also, Ca^{2+} has been suggested to play a role in memory formation by activating other neuronal mechanisms (e.g. [19]). The Ca^{2+} hypothesis, nevertheless, suggests experiments to test in vitro those features of the interaction of neurotransmitter and Ca^{2+} , which might underlie some behavioral characteristics of associative learning. For example, can Ca^{2+} and a neurotransmitter (such as a biogenic amine) stimulate additively, or even synergistically, the activity of a subpopulation of adenylate cyclase or a functional state of an adenylate cyclase complex [3,16,17,20]? Are there additional enzymes (e.g., kinases, ribosyltransferases, phospholipases) that participate, together with the Ca^{2+} -stimulated adenylate cyclase, in the postulated convergence of the Ca^{2+} and the neurotransmitter signals (and see [21] and references therein)? And are there any constraints imposed upon the sequence of the interaction of a neurotransmitter and Ca^{2+} , which might explain the temporal specificity of the conditioned and unconditioned stimuli and the failure of backward conditioning, that are observed in behavioral tests? For example, does it matter whether Ca^{2+} acts before or after the neurotransmitter?

The recent purification of a Ca^{2+} -calmodulin sensitive adenylate cyclase from mammalian brain [22] already makes it possible to investigate in detail the direct interaction of Ca^{2+} with the isolated or reconstituted enzyme, and to test directly, whether neurotransmitters, or other extracellular and intracellular factors, accelerate or attenuate the Ca^{2+} effect, and vice versa.

8. PROPERTIES OF ADENYLATE CYCLASE WHICH ARE NOT DEFECTIVE IN THE MEMORY MUTANT

It is of interest to note what properties of the adenylate cyclase system are not affected in *rut*. Firstly, most of the adenylate cyclase activity in *rut* is spared [5-7]. This may explain why the behavioral repertoire, memory excluded, is essentially normal. It also emphasizes the notion that

the adenylate cyclase system is heterogeneous and that genetic dissection could be used to identify the different types of the enzyme.

Secondly, the mutant's enzyme is normally stimulated by putative neurotransmitters [5,6]. And thirdly, the mutation seems also to spare the capability of G regulatory units to interact with guanyl nucleotides and with NaF [5,6].

On the basis of the above-mentioned and other information [1,2,5-9], it is tempting to postulate that the properties of adenylate cyclase which are especially important for memory are associated with the interaction of the enzyme with intracellular signals. *rut* is capable of forming an association between stimuli, but not of storing it normally, whereas another *Drosophila* mutant, *Ddc*, which has a defective aminergic neurotransmission, was reported to be defective in learning but to remember normally once it had learned [23]. It is therefore also tempting to postulate as a working hypothesis that in this system intercellular communication is crucial for learning whereas intracellular communication is more important for memory. Intercellular communication is of course crucial for all operations of the nervous system; one should therefore expect that memory mutants would be more specific, and easier to isolate, than learning mutants. This indeed seems to be the case to date [2].

9. CONCLUDING REMARKS

Adenylate cyclase in the *Drosophila* memory mutant *rut* displays multiple defects in activity. These defects could pinpoint features of the adenylate cyclase complex which might be crucial for memory formation. Of the scenarios briefly outlined above, the one that depicts the Ca^{2+} -calmodulin stimulation of adenylate cyclase as playing a key role in the operation of the memory apparatus has received much attention, and is corroborated by data from *Aplysia* [1,2,5,6,9,16,17]. Other properties of the cyclase which are missing in *rut*, e.g. those related to forskolin stimulation, or a combination of such properties, especially those which are related to intracellular regulation of the enzyme, may also be crucial for memory. Detailed analysis of the molecular defects, comparison with data obtained by cellular research methods in other organisms,

and cloning of the *rut* gene, may together shed light on specific functions of adenylate cyclase that underlie the ability of the nervous system to acquire and to store novel information, and on the evolution of the ability of a ubiquitous enzyme to play specialized functions within the context of the nervous system.

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