THE *DUNCE* GENE OF *DROSOPHILA*: ROLES OF Ca^{2+} AND CALMODULIN IN ADENOSINE 3':5'-CYCLIC MONOPHOSPHATE-SPECIFIC PHOSPHODIESTERASE ACTIVITY¹

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Abstract

Two genetically distinct forms of cyclic nucleotide phosphodiesterases are present in adult *Drosophila melanogaster*. Form II, which specifically hydrolyzes adenosine 3':5'-cyclic monophosphate (cAMP), is controlled by the *dunce*⁺ gene. Mutants of this gene either eliminate this enzyme form entirely or alter its kinetic and thermal properties, suggesting that *dunce*⁺ is the structural gene for this enzyme. These mutants are defective in memory formation, habituation, and sensitization and exhibit elevated cAMP levels, implicating cAMP in these neurological processes. The other phosphodiesterase, Form I, which hydrolyzes both cAMP and guanosine 3':5'-cyclic monophosphate (cGMP), is not affected by *dunce* mutations. Because both cAMP and Ca^{2+} serve as intracellular second messengers in mediating the effects of neurotransmitters, the effects of Ca^{2+} on each form of phosphodiesterase have been investigated. Previous work has suggested that Form I is activated by calmodulin in a Ca^{2+} -dependent manner. We confirm this activation and demonstrate that the activation involves the Ca^{2+} -dependent association of two molecules of calmodulin with one Form I molecule. Under conditions permitting activation and association of Form I with calmodulin, we observe no interaction of Ca^{2+} /calmodulin with Form II. Our studies suggest that the primary physiological defect, associated with a defective or absent Form II cAMP-specific phosphodiesterase and leading to the *dunce* neurological phenotype, is due to a direct failure to regulate the cAMP level in nerve cells rather than to a failure to mediate a signal resulting from a cAMP-induced Ca^{2+} influx, associated with presynaptic facilitation.

Drosophila has proven to be a useful organism for the genetic dissection of neurological phenomena. Several genes required for normal learning have been identified by selection of mutants that fail to associate olfactory stimuli with electric shock in the paradigm of Quinn et al. (1974). The first such gene to be discovered in this way was *dunce* (Dudai et al., 1976). Subsequent studies have shown that the normal *dunce*⁺ gene is required for normal habituation and sensitization (Duerr and Quinn, 1982) and normal memory formation (Dudai, 1979; Tempel et al., 1983).

The biochemical defect associated with mutation of the *dunce* gene was shown by Byers et al. (1981) to involve a cAMP-specific phosphodiesterase designated Form II. These studies showed that the *dunce*⁺ gene is

located in chromomere 3D4 of the polytene X chromosome. Previous studies (Kiger and Golanty, 1977, 1979) had shown that the number of doses of chromomere 3D4 was directly correlated with the level of Form II enzyme activity, suggesting that the structural gene for this enzyme resides in chromomere 3D4. These observations have been confirmed and extended by Shotwell (1983). Studies of residual Form II enzyme present in two different mutants of the *dunce* gene have shown that the mutations confer altered kinetics or thermal lability upon the Form II enzyme as would be expected of mutations that alter the amino acid sequence of the enzyme (Davis and Kiger, 1981; Kauvar, 1982). The above observations strongly suggest that *dunce* is the structural gene for the Form II enzyme.

Adult *Drosophila* possess two major forms of phosphodiesterases: a cyclic nucleotide phosphodiesterase which hydrolyzes both cAMP and cGMP, designated Form I; and the cAMP-specific phosphodiesterase, Form II, discussed above (Davis and Kiger, 1980). The existence of a third form specific for cGMP has also been suggested

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by Kauvar (1982). This activity has only been identified in crude homogenates using an assay in which nonradioactive cAMP is employed as a competitive inhibitor of Form I activity and hydrolysis of radioactive cGMP is monitored. The theoretical analyses of Vincent and Thellier (1983) cast doubt on the use of such an assay on crude homogenates to identify new forms of enzymes. It is possible that the activity identified by Kauvar (1982) represents a Form I activity.

Further studies are necessary to determine how complex the pattern of phosphodiesterases may be in *Drosophila* and to relate these enzyme activities to the complex patterns of phosphodiesterase activities that have been described in mammalian tissues, especially brain (Wells and Hardman, 1977; Thompson et al., 1979; for review see Appleman et al., 1982). Further genetic analysis of this enzyme system in *Drosophila* should help to resolve the number of components present and to relate specific enzymes to particular physiological functions.

Ca^{2+} is known to activate several forms of phosphodiesterases in mammalian species through its interaction with calmodulin (Cheung, 1980; Purvis et al., 1981; Kincaid et al., 1981; and review articles listed above). Thus far, studies of the roles of Ca^{2+} and calmodulin in regulating the activities of the *Drosophila* phosphodiesterases have been incomplete. Comparative studies of Ca^{2+} -activated cAMP hydrolysis in crude homogenates of normal *Drosophila* and of *Drosophila* genetically deficient for (lacking) chromomere 3D4 suggest that Form I is activated by Ca^{2+} , whereas Form II is not (Kiger and Golanty, 1979). This hypothesis has been strengthened by the work of Yamanaka and Kelly (1981) which showed that isolated heads of *Drosophila* possess a heat-labile phosphodiesterase that appears not to be activated by Ca^{2+} and a heat-stable phosphodiesterase that is activated by Ca^{2+} . These studies do not definitively demonstrate whether Form II is sensitive to Ca^{2+} activation. Both Form I and Form II are inactivated by heating, albeit at different rates, and heating can alter the properties of phosphodiesterases (Kiger and Golanty, 1979). The studies of Kauvar (1982) made with crude homogenates of normal *Drosophila* indicate that Form I is activated by Ca^{2+} . On the other hand, Davis and Kiger (1980) were unable to demonstrate activation of Form I by Ca^{2+} and a heat-treated extract of *Drosophila*. Moreover, Kauvar (1982) has shown that both Form I and Form II are activated by proteolysis, which is difficult to control; the method of homogenization was found to be important in detecting Ca^{2+} activation. The purpose of the present study is to investigate directly the roles of Ca^{2+} and *Drosophila* calmodulin in the activities of the Form I and Form II phosphodiesterases after physical separation.

Materials and Methods

Reagents for gel electrophoresis and molecular weight protein standards were purchased from BioRad Laboratories. [^3H]cAMP and [^3H]cGMP were obtained from New England Nuclear. DEAE-cellulose was purchased from Whatman, and PEI-cellulose F plates were from EM Labs.

Isolation of calmodulin

Calmodulin was purified following essentially the procedure described for rat testis calmodulin by Dedman et al. (1977). Rat brain, and adults and larvae of *Drosophila melanogaster* were homogenized, and the crude extracts were heated at 95°C for 5 min, followed by a precipitation of most of the proteins with ammonium sulfate (90% of saturation). The precipitate was further purified by ion exchange chromatography on a DEAE-cellulose column (16 cm $\times$ 1.2 cm). Calmodulin was eluted from the column with a linear gradient of NaCl (0.0 to 0.6 M). Fractions were assayed for their ability to activate a standard preparation of calmodulin-depleted phosphodiesterase prepared from rat brain by the method of Teshima and Kakiuchi (1974). Peak fractions were pooled and concentrated 20-fold in an Amicon CS15. Aliquots were stored frozen at -20°C.

Fractionation of phosphodiesterases

Gel filtration. Adult flies were homogenized in buffer A (20 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 1 mM MgCl_2 , 2 mM 2-mercaptoethanol) and centrifuged for 1 hr at 100,000 $\times g$. The supernatant was applied to a Sephadex G-200 column (50 cm $\times$ 3 cm), and the column was eluted with buffer A containing 100 mM NaCl at a flow rate of 8 ml/hr. Each fraction was assayed for phosphodiesterase activity in the presence or absence of calmodulin from *Drosophila*. Peak fractions were pooled, concentrated, and stored at -20°C.

Sucrose density gradient centrifugation. Flies were homogenized at a concentration of 0.1 mg of flies/ml in buffer B (40 mM Tris-HCl, pH 7.2, 10 mM MgCl_2 , 1 mM 2-mercaptoethanol, and either 2 mM EGTA or 1 mM CaCl_2). The homogenates were then centrifuged at 100,000 $\times g$ for 1 hr. An aliquot of the supernatant (0.1 ml) was layered over a 4.8-ml sucrose density gradient (5 to 20%) in buffer B and sedimented in a Beckman SW 50.1 rotor at 50,000 rpm for 12 hr at 4°C. Fractions were collected from the bottom of the gradient and assayed for phosphodiesterase activity in the absence or presence of *Drosophila* calmodulin.

Enzyme assays

Calmodulin activity was assayed by measuring the activation of a calmodulin-depleted phosphodiesterase from rat brain according to the method of Pösch (1971). The assays were carried out in duplicate in a reaction mixture containing 10 mM Tris-HCl, pH 7.4, 0.1 mM CaCl_2 , 1 mM AMP, 0.1 mM cAMP, 20 nCi of [^3H]cAMP, and 35 μg of rat brain phosphodiesterase.

Drosophila phosphodiesterase activities were assayed in a reaction volume of 0.1 ml containing 40 mM Tris-HCl, pH 8.0, 10 mM MgCl_2 , 2 mM 2-mercaptoethanol, and [^3H]cyclic AMP or [^3H]cyclic GMP (0.1 mM; 1.25 μCi). The reaction was started by adding substrate to the enzyme preparation; reaction mixtures were incubated for 15 or 20 min at 30°C followed by heating at 90°C for 2 min. After cooling to room temperature, 0.02 ml of carrier was added (carrier for cAMP phosphodiesterase contained 5 mg/ml of 5'-AMP, cAMP, adenosine, and inosine; for cGMP phosphodiesterase 5 mg/ml of 5'-

GMP, cGMP, and guanosine). A volume of 0.005 ml was spotted on a PEI-cellulose strip and chromatographed in an ascending system for 25 min in 50 mM KCl (Rangel-Aldao et al., 1978). The 5'-nucleotide monophosphate and nucleoside spots were identified under a short wavelength UV lamp, cut from the strips and eluted with 100 mM MgCl₂, 20 mM Tris-HCl, pH 7.4, with agitation for 30 min, and counted in scintillation cocktail as described by Kiger and Golanty (1979).

Protein determination

Protein concentration was determined by the method of Lowry et al. (1951) or the microassay of Bradford (1976).

Gel electrophoresis

Proteins were analyzed on 0.75-mm-thick 12% SDS-polyacrylamide slab gels with a 5% stacking gel. Gels were run for 6 hr, until dye had reached the bottom, and stained with Coomassie blue in methanol/acetic acid. Buffers and stock solutions were prepared as described by O'Farrell (1975).

Results

Characterization of *Drosophila* calmodulin. The existence and purification of calmodulin from adult *D. melanogaster* has been reported by Yamanaka and Kelly (1981). For the present studies, calmodulin has been isolated from both adults and larvae as described under "Materials and Methods" (Dedman et al., 1977) and its authenticity established by comparison with rat brain calmodulin in activation of rat brain phosphodiesterase. Heat treatment of the crude extract from either adults or larvae abolished all intrinsic phosphodiesterase activities. Heated extracts were precipitated with ammonium sulfate and chromatographed on an ion exchange column as described under "Materials and Methods." In order to monitor the purification of *Drosophila* calmodulin, an aliquot of the preparation at each step was tested for activation of calmodulin-depleted rat brain phosphodiesterase; the results are shown in Table I. The elution profile of the DEAE column is shown in Figure 1; the peak fraction shows a 1000-fold increase in its ability to

TABLE I
Partial purification of calmodulin from larvae of *Drosophila melanogaster*

Fraction	Protein mg	Phosphodiesterase Activity ^a		
		+PDE -CaCl ₂	+PDE +CaCl ₂	-PDE +CaCl ₂
Crude homogenate	4680	1.28	0.96	0.42
Crude supernatant	1540	1.48	5.0	0.69
Heat-treated extract	606.2	0.51	11.0	
(NH ₄) ₂ SO ₄ precipitate	115.5	0.76	39.0	
DEAE fractionation	1.35	0.39	684.4	

^a Activation of a calmodulin-depleted phosphodiesterase from rat brain was measured and is represented in the column +PDE. The internal phosphodiesterase activity is measured and shown under -PDE; there was no measurable internal phosphodiesterase activity left after heat treatment. Activities are in nanomoles per minute per milligram of protein.

Figure 1. Chromatography of calmodulin on a DEAE-cellulose column. Extract from *D. melanogaster* described in Table I was chromatographed on a DEAE-cellulose column. The column was eluted with a linear NaCl gradient. Two-milliliter fractions were collected and analyzed for calmodulin in the presence of calmodulin-depleted phosphodiesterase from rat brain with 0.1 mM CaCl₂ present (solid circles) or absent (open circles). Open squares represent protein profile. Inset, SDS-PAGE of calmodulin isolated from *Drosophila* in the presence of 2 mM EGTA or 1 mM CaCl₂. Calmodulin migrates faster in the presence of Ca²⁺ (Ca) than in the presence of EGTA (E). Molecular weight standards are indicated on the right side of the panel.

TABLE II
Activation of calmodulin-depleted phosphodiesterase (PDE) by calmodulin (CAM)

Phosphodiesterase depleted of calmodulin was isolated from rat brain. Activation by calmodulin from either *Drosophila melanogaster* or from homologous tissue was determined in the presence or absence of Ca²⁺. Activities are in nanomoles of cAMP per minute per milligram of protein.

	Phosphodiesterase Activity	
	No Ca ²⁺ present	100 μM Ca ²⁺ present
PDE (rat brain)	0.02	0.08
PDE (rat brain) + CAM (rat brain)	0.13	0.52
PDE (rat brain) + CAM (<i>D. melanogaster</i>)	0.08	0.56

stimulate the rat brain phosphodiesterase activity compared to that activity assayed in the crude homogenate (Table I).

To determine the Ca²⁺ dependency of this activation, phosphodiesterase activity was assayed in the presence and absence of 0.1 mM CaCl₂. The result is represented in Table II. Stimulation of rat brain phosphodiesterase activity 4- to 5-fold by the homologous rat brain calmodulin was observed in the presence of 0.1 mM Ca²⁺. When rat brain calmodulin was replaced by the protein isolated from *Drosophila*, essentially the same degree of activation was obtained.

SDS-polyacrylamide gel electrophoresis of this peak

fraction reveals a low molecular weight protein of about 17,000 that co-migrates with calmodulin from rat brain. To determine the ability of this protein to bind calcium, electrophoresis was performed in the presence of 1 mM CaCl_2 or 2 mM EGTA (Fig. 1, inset). As with calmodulin from other sources, the migration of this protein is faster in the presence of Ca^{2+} than in the presence of EGTA (Burgess et al., 1980). Since this property is characteristic of calmodulin from other sources, we believe that this protein is indeed *Drosophila* calmodulin. Increasing concentrations of *Drosophila* calmodulin increased phosphodiesterase activity in the presence of Ca^{2+} (Fig. 2). Under the conditions employed, no increase could be observed above a protein concentration of 0.15 μg of calmodulin. When calmodulin preparations isolated from either adults or larvae were compared, no differences in activation were observed.

Interaction of *Drosophila* calmodulin and phosphodiesterases. The two major forms of phosphodiesterase in *D. melanogaster* can be separated using either gel filtration or velocity sedimentation through a sucrose gradient (Davis and Kiger, 1980). Form I hydrolyzes both cAMP and cGMP, whereas Form II hydrolyzes only cAMP. To determine possible homologies between these forms and forms described from mammalian sources, the action of *Drosophila* calmodulin on each was investigated.

To obtain *Drosophila* phosphodiesterases free of intrinsic calmodulin, the soluble fraction of an extract from adult flies was fractionated on a G-200 Sephadex column in the presence of EGTA. Aliquots of each fraction were assayed for phosphodiesterase activities in the presence or absence of Ca^{2+} and calmodulin isolated from larvae of *D. melanogaster*. The results are shown in Figure 3. When assayed in the presence of Ca^{2+} and calmodulin, two distinct peaks of cAMP phosphodiesterase activity

could be observed. The peak of cGMP phosphodiesterase activity coincides with the first peak of the cAMP phosphodiesterase activity. This is in agreement with the finding that Form I hydrolyzes both substrates (Davis and Kiger, 1980). When assayed in the absence of calmodulin, Form II cAMP phosphodiesterase activity is undiminished, whereas Form I cAMP hydrolysis is greatly reduced. This reduced activity in the absence of calmodulin is also evident when cGMP is employed as substrate. Moreover, the activity of Form I is not enhanced when calmodulin is present but Ca^{2+} is omitted from the assay. Therefore, Form I phosphodiesterase is activated by calmodulin in a Ca^{2+} -dependent manner.

To test this conclusion further, evidence for physical association of these proteins was sought. To determine the presence of intrinsic calmodulin, two experiments were performed. Adult flies were homogenized in the presence of either 1 mM CaCl_2 or 2 mM EGTA followed by sedimentation through sucrose gradients also containing 1 mM CaCl_2 or 2 mM EGTA. The fractions were then assayed in the presence or absence of extrinsic calmodulin with CaCl_2 present.

Form II activity sedimented identically in both gradients, and its activity was not dependent on either Ca^{2+} or calmodulin as shown in Figure 4 (top).

Form I activity, on the other hand, showed the following behavior. When the fractions from the gradient containing EGTA were assayed in the presence of calmodulin and 1 mM Ca^{2+} , a 40 to 50% activation was observed, compared to the activity when no calmodulin was added (Table III). In contrast to this result, only a slight activation of the Form I phosphodiesterase activity by added calmodulin was observed when 1 mM Ca^{2+} was present throughout preparation. The basal activity of Form I is much higher when Ca^{2+} is present than it is in the presence of EGTA. Therefore, it is likely that in the presence of Ca^{2+} , calmodulin is bound to the Form I phosphodiesterase during preparation and remains bound during sedimentation through the sucrose gradient. Comparison of the phosphodiesterase activity profiles in Figure 4 shows that indeed the peaks of Form I activity sediment differently. When 2 mM EGTA is present throughout preparation, the Form I peak (Fig. 4, top, with cAMP as substrate; Fig. 4, bottom, with cGMP as substrate) sediments more slowly than when 1 mM CaCl_2 is present throughout preparation.

A change in sedimentation rate in the presence of calmodulin has been described for calmodulin-dependent phosphodiesterase from bovine brain (Sharma et al., 1980). The stoichiometry for the binding of calmodulin to phosphodiesterase in these studies was determined to be two molecules of calmodulin to one molecule of phosphodiesterase. To determine whether a similar relationship exists for Form I phosphodiesterase, we calibrated the sucrose gradients employing bovine serum albumin, myoglobin, and Form II phosphodiesterase as molecular weight markers and calculated the molecular weight of Form I in the presence of either EGTA or Ca^{2+} . The apparent molecular weight in the presence of EGTA was 95,000, whereas in the presence of Ca^{2+} it was 126,000. This difference corresponds to 33,000, or approximately two molecules of calmodulin per molecule of Form I phosphodiesterase.

Figure 2. Activation of calmodulin-depleted phosphodiesterase (rat brain) by increasing concentrations of calmodulin from *D. melanogaster*. Solid circles represent calmodulin isolated from adult flies and open circles represent calmodulin from larvae. Phosphodiesterase concentration was 30 μg /assay; 0.1 mM CaCl_2 was present.

Figure 3. Elution profile of a G-200 Sephadex column. The soluble fraction of a fly extract ($100,000 \times g$ supernatant) was applied to the G-200 Sephadex column, and 2.0-ml fractions were collected. These fractions were then assayed for phosphodiesterase activity in the presence or absence of homologous calmodulin. Calmodulin was present at a concentration of $1 \mu\text{g}/\text{assay}$. The open circles represent phosphodiesterase activity in the presence, and the solid circles in the absence, of calmodulin using cAMP as a substrate. The open squares represent phosphodiesterase activity in the presence, and the solid squares in the absence, of calmodulin using cGMP as a substrate.

Discussion

To generalize upon the genetic and biochemical analyses of cyclic nucleotide phosphodiesterases in *D. melanogaster* (Kiger et al., 1981; Kauvar, 1982; Shotwell, 1983), it is desirable to establish homologies that may exist between these enzymes and those that have been investigated in other, predominantly mammalian, species. It has been previously demonstrated that Form I phosphodiesterase of *D. melanogaster* is similar in size to mammalian Ca^{2+} -dependent cyclic nucleotide phosphodiesterase, but differs from it in relative affinities for cAMP and cGMP (Davis and Kiger, 1980). Form I phosphodiesterase exhibits almost identical K_m s for cGMP (3.5×10^{-6}) and cAMP (4.4×10^{-6} M) when partially purified as described, whereas the Ca^{2+} -dependent mammalian cyclic nucleotide phosphodiesterase generally exhibits a K_m for cAMP an order of magnitude or more greater than for cGMP (see, however, Purvis et al., 1981). Several forms of mammalian Ca^{2+} -dependent cyclic nucleotide phosphodiesterases have been described, some of which appear to be related to one another by proteolysis (Kincaid et al., 1981; Purvis et al., 1981). Moreover, Kauvar (1982) has shown that proteolysis can significantly affect the properties of these *Drosophila* enzymes.⁴ The role of

Ca^{2+} in the activities of both Forms I and II has required clarification (Kiger and Golanty, 1979; Davis and Kiger, 1980; Yamanaka and Kelly, 1981; Kauvar, 1982).

The experiments reported here clearly demonstrate that the activity of Form I phosphodiesterase in hydrolyzing both cAMP and cGMP is activated by Ca^{2+} , whose action is mediated by *Drosophila* calmodulin. The activity of Form II, the cAMP-specific phosphodiesterase controlled by the *dunce* gene, is not affected by calmodulin or Ca^{2+} . These observations are consistent when the two phosphodiesterases are separated by gel filtration or by sucrose density gradient centrifugation.

Comparative sedimentation rates of Form I and Form II in the presence of Ca^{2+} or of EGTA show that Form I sediments faster in the presence of Ca^{2+} , presumably because it is physically associated with calmodulin. This presumption is substantiated by the data in Table III showing that Form I activity sedimented in the presence of EGTA is more sensitive to exogenous calmodulin than is that sedimented in the presence of Ca^{2+} . Furthermore, the change in molecular weight upon interaction of Form I phosphodiesterase and calmodulin indicates the binding of two calmodulin molecules to one phosphodiesterase molecule. This stoichiometry has previously been demonstrated for calmodulin-dependent phosphodiesterase from bovine heart using ^{125}I -labeled calmodulin and a cross-linking reagent (LaPorte et al., 1979), and calmodulin-dependent phosphodiesterase from bovine brain using sucrose density gradient centrifugation (Sharma et al., 1980). In contrast to these observations on Form I, the same experiments indicate that Form II does not

⁴ After the manuscript was submitted for publication, Solti et al. (1983) reported further evidence for lability of the Ca^{2+} /calmodulin activation of Form I which they believe not to be due to an irreversible activation of the enzyme as reported by Kauvar (1982). Their studies, made with human calmodulin, also demonstrate that Form II is not activated by human Ca^{2+} /calmodulin.

Figure 4. Sedimentation of soluble phosphodiesterase from *Drosophila* in a linear sucrose gradient. The solid circles represent the activity of phosphodiesterase when 2 mM EGTA was present throughout the preparation, whereas the open circles depict the activity in the presence of 1 mM CaCl_2 . Fractions were assayed for phosphodiesterase activity in the presence of homologous calmodulin with either cAMP (top) or cGMP (bottom) as substrate.

TABLE III

Comparison of the activation of Form I phosphodiesterase (PDE) by calmodulin (CAM) in the presence or absence of calcium

Activity is shown as nanomoles of cyclic nucleotide hydrolyzed per minute. *Drosophila* calmodulin was used in all experiments at the same concentration; 0.1 mg of adult flies was homogenized in the presence of 2 mM EGTA or 1 mM CaCl_2 , and the soluble fraction was applied to a 5 to 20% linear sucrose gradient containing 2 mM EGTA or 1 mM CaCl_2 , respectively. PDE activity was assayed in the presence or absence of calmodulin at a Ca^{2+} concentration of 1 mM, with either cGMP or cAMP as substrate.

	cGMP		cAMP	
	+CAM	-CAM	+CAM	-CAM
Form I PDE EGTA-sucrose gradient	0.15	0.06	0.03	0.015
Form I PDE Ca^{2+} -sucrose gradient	0.15	0.12	0.036	0.029

associate with calmodulin, in agreement with the observations that Ca^{2+} /calmodulin does not activate Form II.

Physiological studies of learning at the cellular level have been carried out in several molluscan systems (Carew et al., 1983; Hawkins et al., 1983; Walters and

Byrne, 1983). These studies have implicated cAMP-dependent phosphorylation of membrane proteins in the presynaptic facilitation observed as a concomitant of learning (Castellucci et al., 1980; Alkon et al., 1983). One result of presynaptic facilitation is an increased cellular influx of Ca^{2+} . Both Ca^{2+} and cAMP have been implicated as second messengers mediating the effects of neurotransmitters, and each mediates the phosphorylation of overlapping sets of proteins in nerve cells (Novak-Hofer and Levitan, 1983).

The studies presented here suggest that the primary physiological defect, associated with a defective or absent Form II cAMP-specific phosphodiesterase and leading to the *dunce* neurological phenotype, is due to a direct failure to regulate the cAMP level in nerve cells rather than to a failure to mediate a signal resulting from a cAMP-induced Ca^{2+} influx, associated with presynaptic facilitation.

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