whole seeds or where the embryo was removed after soaking. The embryo enzyme, however, was not affected by phytic acid. A dose response study showed (table 2) that a minimum of 15–20 ppm of phytic acid is needed for induction of the cotyledon enzyme. After 62 h of germination, however, there was no difference in control or phytic acid treated groups.

Phytic acid has been shown to accumulate in seeds[12] and during germination it decreases as a result of increased phytase (E.C.3.1.3.8) activity to provide the phosphate required for growing tissue[13]. Phytic acid, being a polyanion like nucleic acids, may also form complexes with polyamines. The induction of cotyledon diamine oxidase by phytic acid suggests that the levels of polyamines may be controlled by phytic acid. To investigate whether polyamines have any effect on phytase activity, to maintain a balanced concentration of phytic acid and polyamines, studies were carried out by soaking the seeds in 0.1% putrescine, cadaverine, spermidine and spermine. These compounds had no effect on the phytase activity of the cotyledon or embryo, suggesting that polyamines may not have any control over phytic acid levels. The inducing effect of phytic acid was not due to a higher level of phosphate or inositol since these compounds could not induce the cotyledon enzyme when the seeds were soaked in their presence.

Table 2. Effect of phytic acid on pea cotyledon and embryo diamine oxidase activity

Concentration of phytic acid (ppm)	Enzyme units/g fresh tissue in Cotyledon				Embryo	
	Seeds soaked and germinated with embryo		Embryo removed from seeds after soaking			
	38 h	62 h	38 h	62 h	38 h	62 h
0	0.02	0.43	0.07	0.53	0.25	0.69
5	0.05	0.44	0.08	0.51	0.29	0.66
10	0.09	0.47	0.09	0.56	0.31	0.72
15	0.11	0.49	0.09	0.61	0.29	0.74
20	0.20	0.64	0.21	0.66	0.28	0.77
25	0.25	0.68	0.22	0.76	0.33	0.82

Values are means from duplicate samples.

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Lack of effect of neurotransmitters on cyclic AMP phosphodiesterase activity in an insect CNS[1]

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Summary. The inhibition of cyclic nucleotide phosphodiesterase from the nerve cord of *Manduca sexta* was studied using theophylline as a model compound. 11 putative neurotransmitters had no effect on enzyme activity.

In previous work by our laboratory we have studied the action of putative neurotransmitters on the accumulation of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in intact nerve cords[4] of the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae), in neuronal- and glial-enriched cellular fractions derived from it[5], and on the activity of adenylate cyclase (E.C. 4.6.1.1) in crude homogenates of this tissue[6]. The activity of the cyclic nucleotide phosphodiesterase (PDE, E.C. 3.1.4.1c) in this tissue was thoroughly explored enzymatically in this laboratory[7], but a detailed pharmacological investigation was required to support the hypothesis that neurotransmitters effect elevation of cyclic AMP levels by stimulation of the adenylate cyclase rather than by inhibition of the PDE[4,6,8]. The results of this study have been presented in abstract form[9].

Materials and methods. Nerve cords from 5th instar 'wanderer' larvae[10] were dissected and frozen as previously described[7]. Freshly-thawed nerve cords were homogenized in all-glass tissue grinders in 50 mM Hepes-KOH, pH 7.5, 152 mM NaCl, 4.7 mM KCl, 2.8 mM $CaCl_2$[11], and centrifuged at 34,000 × g for 30 min to remove insoluble debris.

PDE activity was quantitated essentially by assay II[7]. Brie-

Effects of putative neurotransmitters on cyclic AMP phosphodiesterase activity

Addition	Phosphodiesterase activity (pmole cyclic AMP/ µg protein/min)
None	1.83 ± 0.06 (21)
Acetylcholine 50 mM	1.95 ± 0.14 (3)
Serotonin 10 mM	1.52 ± 0.21 (3)
Aspartic acid 500 µM	1.88 ± 0.09 (3)
Glutamic acid 500 µM	1.74 ± 0.16 (5)
Glycine 500 µM	1.97 ± 0.10 (5)
Gamma-aminobutyric acid 500 µM	1.70 ± 0.09 (6)
Epinephrine 250 µM	1.76 ± 0.05 (3)
Norepinephrine 250 µM	1.75 ± 0.07 (3)
Isoproterenol 250 µM	1.62 ± 0.06 (3)
Octopamine 250 µM	1.62 ± 0.03 (5)
Dopamine 250 µM	0.84 ± 0.06 (14)*
Phenylthiourea 20 µM	1.58 ± 0.16 (5)
Phenylthiourea 20 µM + Dopamine 250 µM	1.47 ± 0.14 (5)

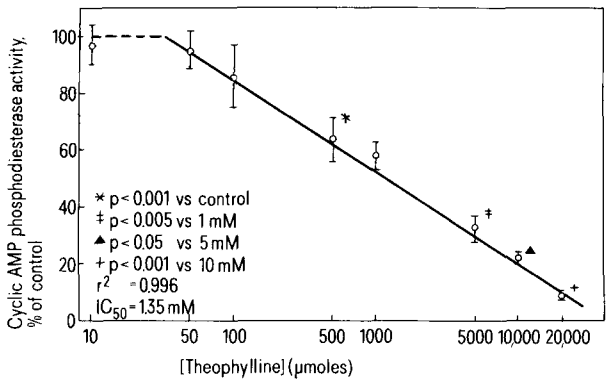
Results are given as mean ± SE. The number of samples is given in parentheses. Statistical significance was determined using Student's t-test. * $p < 0.001$ vs no addition.

fly, 57.5 μM [^{3}H] cyclic AMP was hydrolyzed by enzyme preparations in a buffer containing 50 mM Hepes-KOH, pH 7.5, 152 mM NaCl, 4.7 mM KCl, 2.8 mM $CaCl_2$, 100 μM ascorbic acid. Additional agents were incorporated as noted, the mixture was incubated at 30 °C for 15 min, and hydrolysis terminated by boiling for 5 min. Reaction products were separated by ascending paper chromatography, visualized by UV, and counted by liquid scintillation. Protein was assayed by the method of Lowry et al.[12] using bovine serum albumin as standard.

Results and discussion. Theophylline is the most potent methyl xanthine inhibitor of *M. sexta* nerve cord PDE[7]. The dose-response of PDE to varying concentrations of theophylline was studied as a model of PDE inhibition. Such inhibition in activity becomes significant at 0.5 mM theophylline and is half-maximal at 1.35 mM (IC_{50}, figure).

Of 11 putative neurotransmitters tested, only dopamine had any significant effect on PDE activity ($p < 0.001$, table). During the course of the dopamine incubation, it was noted that a black precipitate was formed in the tube. Because insect hemolymph may contain high levels of phenol oxidase activity which may convert dopamine to a reactive quinone, resulting in protein cross-linking and subsequent enzyme inhibition, the experiment was repeated employing a phenol oxidase inhibitor, phenyl thiourea[13]. In the presence of this inhibitor, dopamine has no significant effect on PDE activity.

Serotonin elevates cyclic AMP levels in intact nerve cords[4], in neuronal-enriched cellular fractions[5], and stimulates adenylate cyclase activity in crude nerve cord homogenates[6]. Acetylcholine, aspartic and glutamic acids, glycine, and gamma-aminobutyric acid elevate cyclic guanosine 3′,5′-monophosphate levels in intact nerve cords[4]. The work presented here is consistent with the hypothesis that the action of several putative neurotransmitters known to occur in the *M. sexta* CNS[4] may be mediated by the activation of appropriate nucleotide cyclases resulting in elevations of cyclic nucleotide levels.



Inhibition of cyclic AMP phosphodiesterase activity by various concentrations of theophylline. Points represent the means ± SE of 6 different determinations. The line was drawn by linear regression analysis (coefficient of variation = 0.996). Statistical significance was assessed using Student's t-test.

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Selective and reversible modification of essential thiol groups of D-glyceraldehyde-3-phosphate dehydrogenase by isothiocyanates

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Summary. Aralkyl and alkyl isothiocyanates, like aryl isothiocyanates, undergo a selective and reversible reaction with essential thiol groups of D-glyceraldehyde-3-phosphate dehydrogenase; the former 2 substances require for a reversible reaction course a more alkaline medium and presence of a thiol.

Thiol enzymes are of special interest in many biochemical processes, and therefore they are suitable model substances in molecular enzymology[2,3]. To study these substances by chemical modification methods, selective thiol reagents are often used. An especially favourable feature of these reagents is the reversibility of reactions with thiol groups. So far, there are many selective thiol reagents; nevertheless, only a few of them satisfy the 2nd criterion[4,5].

These features have been stressed in our preceding model experiments with isothiocyanates (ITC)[6–8], which are believed to be the natural regulators of activity of some plant thiol enzymes[8]. This paper is aimed to verify the afore-mentioned favourable features of these substances with D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a notable thiol enzyme. Rabbit muscle GAPDH is a well characterized tetramer with 4 essential thiol groups ascribed Cys-149[9].

Material and methods. Activity of rabbit muscle D-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (Biochemica Boehringer GmbH, Mannheim) was determined ac-