cause retinal RK decreases the cyclic nucleotide response to photon stimulation (*4*), we suspect that pineal RK is involved in the regulation of cyclic nucleotide responsiveness of pinealocytes to adrenergic stimulation (*15, 16*). Finally, because rhodopsin regulates phosphodiesterase activity, we speculate that the pineal adrenoceptor substrate of RK regulates phosphodiesterase. The phosphorylation of β-adrenoceptors associated with modulation of responsiveness has been reported (*17*); the kinase involved might be RK.

The presence of RK activity in diverse tissues suggests that this, or a closely related, enzyme functions in these tissues to phosphorylate related integral membrane receptors, each of which may have evolved from the same ancestral receptor molecule.

The finding of RK in the pineal gland is only one in a series of findings that biochemically link the mammalian eye and pineal gland. First, both the retina and pineal gland contain hydroxyindole-*O*-methyltransferase, an enzyme of limited distribution, which converts *N*-acetylserotonin to melatonin (*18*). Second, as mentioned above, both tissues contain the S antigen (*9*). Third, the occurrence of bilateral retinal blastomas is often followed by the appearance of a pineal tumor—a syndrome described as trilateral blastoma (*19*). Although function may have changed during evolution as the mammalian pineal gland lost its photoreceptor function and became entirely neurosecretory (*11*), both the retina and pineal gland are biochemically more similar in mammals than is generally acknowledged. Recognition of this relation might allow pineal and retinal biochemists to benefit from advances in each other's field.

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References and Notes

1. H. Kuhn and W. J. Dreyer, *FEBS Lett.* **20**, 1 (1972); D. Bownds, J. Dawes, J. Miller, M. Stahlman, *Nature (London) New Biol.* **237**, 125 (1972).
2. H. Shichi and R. L. Somers, *J. Biol. Chem.* **253**, 7040 (1978).
3. M. Weller, N. Virmaux, P. Mandel, *ibid.* **72**, 381 (1975); R. N. Frank and S. M. Buzney, *Biochemistry* **14**, 5110 (1975).
4. A. Sitaramayya, N. Virmaux, P. Mandel, *Neurochem. Res.* **2**, 1 (1977); A. Sitaramayya and P. A. Liebman, *J. Biol. Chem.* **258**, 12106 (1983); H. Shichi, K. Yamanoto, R. L. Somers, *Vision Res.*, in press. Two effects of light on rhodopsin appear to be related to the control of retinal cyclic GMP. One is to initiate events that result in activation of phosphodiesterase. The other is to convert rhodopsin into a form capable of being phosphorylated by RK. The resulting phosphorylation appears to decrease the potency of photolyzed rhodopsin to activate phosphodiesterase.
5. Tissues obtained from 250-g male rats (Sprague-Dawley) were prepared by homogenization (1 mg of wet weight per 20 μl) in 10 m*M* Pipes, containing 4 m*M* dithiothreitol and 1 m*M* EDTA (*p*H 7.0). The kinase assay contained, unless otherwise indicated, 50 m*M* potassium phosphate (*p*H 6.8), containing 1 m*M* $MgCl_2$, 0.1 m*M* [^{32}P]ATP (1×10^6 to 2×10^6 cpm/nmol), 0.5 nmol of rhodopsin in urea-treated bovine rod outer segments [H. Shichi, R. L. Somers, K. Yamamoto, *Methods Enzymol.* **99**, 362 (1983)], and 5 μg of supernatant protein. The final volume of the assay was 50 μl. The assay was stopped by the addition of 200 μl of 0.125*M* potassium phosphate buffer (*p*H 6.8), containing 62.5 m*M* potassium fluoride, 12.5 m*M* EDTA, and 6.25 m*M* ATP. This was centrifuged (10,000*g*), and the supernatant was removed; 2 μl of the supernatant was analyzed for [^{32}P]ATP content by thin-layer chromatography on PEI cellulose (E. Merck), with 1*M* lithium chloride used as the solvent [K. Randerath and E. Randerath, *J. Chromatogr.* **16**, 111 (1964)]. Identification and measurement of the [^{32}P]ATP and $^{32}PO_4$ indicated that less than 20 percent of the ATP originally present was hydrolyzed during the assay.
6. The pellet was dissolved in 40 μl of 10 m*M* tris acetate (*p*H 7.4), containing 2.5 percent sodium dodecyl sulfate (SDS), 2 percent β-mercaptoethanol, and 5 m*M* EDTA. To this was added 10 μl of 50 percent glycerol with 0.05 percent bromophenol blue. The sample was kept at room temperature, and a 25-μl sample was applied to a 10 percent acrylamide-SDS gel [U. K. Laemmli, *Nature (London)* **227**, 680 (1970)]. Electrophoresis was for 30 to 40 minutes at 60 V and then for 3.5 hours at 165 V (constant voltage). The gel was stained, dried, and the [^{32}P]phosphorylated opsin bands were visualized by autoradiography, cut out of the gel, and counted.
7. R. L. Somers and D. C. Klein, unpublished results.
8. To determine the tissue distribution of RK activity, we removed tissues from 250- to 300-g male rats; ovaries were removed from 250-g female rats. The tissues were immediately placed on solid CO_2 and stored (−70°C). Tissues were thawed individually, and small pieces were weighed and homogenized (20 μl per milligram of wet weight) in 10 m*M* Pipes (*p*H 7.0), containing 4 m*M* dithiothreitol, 1 m*M* EDTA, leupeptin (20 μg/ml), and 0.3 m*M* phenylmethylsulfonyl fluoride. The samples were centrifuged (30,000*g*) for 30 minutes, and the supernatant was removed and stored at −70°C. The protein content was measured, and a sample of the supernatant containing 5 μg of protein was assayed as described (*4, 5*), with 0.2 m*M* [^{32}P]ATP and 10 μ*M* rhodopsin.
9. C. M. Kaslow and W. B. Wacker, *Int. Arch. Allergy* **44**, 11 (1973); *Invest. Ophthalmol. Vis. Sci.* **16**, 181 (1977).
10. H. Kuhn, *Biochemistry* **17**, 4389 (1978); H. Shichi, *Jpn. J. Ophthalmol.* **25**, 306 (1981).
11. H. Meissl and E. Dodt, in *The Pineal Organ*, A. Oksche and P. Pevet, Eds. (Elsevier/North-Holland, New York, 1981), pp. 61–80; A. Oksche and H. G. Hartwig, *Prog. Brain Res.* **52**, 113 (1979).
12. B. Vigh and I. Vigh-Teichmann, *Cell Tissue Res.* **221**, 451 (1981); D. W. Eldred and J. Nolte, *Vision Res.* **18**, 29 (1978).
13. R. Y. Moore and D. C. Klein, *Brain Res.* **71**, 1 (1974); D. C. Klein and R. Y. Moore, *ibid.* **174**, 245 (1979).
14. D. C. Klein *et al.*, *Brain Res. Bull.* **10**, 647 (1983); S. M. Reppert and D. C. Klein, in *Endocrine Function of the Brain*, M. Motta, Ed. (Raven, New York, 1980), pp. 327–372.
15. D. C. Klein, in *Catecholamines II*, N. Weiner and U. Trindelinberg, Eds. (Springer-Verlag, New York, in press).
16. ———, D. Sugden, J. L. Weller, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 599 (1983); J. Vanecek, D. Sugden, J. L. Weller, D. C. Klein, unpublished results.
17. J. M. Stadel, P. Nambi, R. G. L. Shorr, D. F. Sawyer, M. G. Caron, R. J. Lefkowitz, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 317 (1983).
18. D. P. Cardinali and J. M. Rosner, *Endocrinolgy* **89**, 301 (1971); D. P. Cardinali and R. J. Wurtman, *ibid.* **91**, 247 (1972); C. A. Nagle, D. P. Cardinali, J. M. Rosner, *ibid.* **92**, 1560 (1973); J. Axelrod and H. Weissbach, *Science* **131**, 1312 (1960); *J. Biol. Chem.* **236**, 211 (1961); J. Axelrod, P. D. Maclean, R. W. Albers, H. Weissbach, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon, New York, 1961), pp. 307–311.
19. J. L. Bader, A. I. Meadows, L. E. Zimmerman, L. B. Rorke, P. A. Voute, L. A. Champion, R. W. Miller, *Cancer Genet. Cytogenet.* **5**, 203 (1982); L. E. Zimmerman, R. P. Burns, G. Wankum, R. Tully, J. A. Esterly, *J. Pediatr. Ophthalmol. Strabismus* **19**, 320 (1982); J. L. Bader *et al.*, *Proc. Am. Soc. Clin. Oncol.* **21**, 323 (1980).

1 May 1984; accepted 2 July 1984

Caffeine and Related Methylxanthines: Possible Naturally Occurring Pesticides

Abstract. *Natural and synthetic methylxanthines inhibit insect feeding and are pesticidal at concentrations known to occur in plants. These effects are due primarily to inhibition of phosphodiesterase activity and to an increase in intracellular cyclic adenosine monophosphate. At lower concentrations, methylxanthines are potent synergists of other pesticides known to activate adenylate cyclase in insects. These data suggest that methylxanthines may function as natural insecticides and that phosphodiesterase inhibitors, alone or in combination with other compounds, may be useful in insect control.*

The methylxanthines, including caffeine and theophylline, are found in the berries, seeds, and leaves of a number of species, including tea, coffee, cocoa, and kola (*1*). Although methylxanthines are frequently used as stimulants by the human population (*2*), little is known about their natural function in plants. It is known, however, that many plants produce endogenous substances which can discourage insect feeding. These include specific toxins, compounds with pheromone-like activity, and bitter-tasting aversive substances (*3*). This study presents evidence that the methylxanthines have pestistatic and pesticidal activity and describes the biochemical mechanisms by which such activity may occur.

In most experiments, tobacco hornworm (*Manduca sexta*) larvae were used to study pestistatic and pesticidal effects,

because this species can be synchronously hatched and easily raised on either artificial or natural food. Before examining the effects of methylxanthines, I first investigated whether tea or coffee has any pestistatic or pesticidal activity. Finely powdered tea leaves (*Camellia sinensis*) or powdered coffee beans (*Coffea arabica*) were added at various concentrations to a liquid artificial medium, which was then placed in small petri dishes and allowed to harden. First instar larvae were then housed in these dishes. At concentrations from 0.3 to 10 percent (by weight) for coffee (Fig. 1A) and from 0.1 to 3 percent for tea, there was a dose-dependent inhibition of feeding associated with hyperactivity, tremors, and stunted growth. At concentrations greater than 10 percent for coffee or 3 percent for tea larvae were killed within 24 hours.

To investigate the possible contribution of endogenous methylxanthines to these effects, I next examined the action of purified methylxanthines on larvae raised on either artificial or natural food. When added to artificial medium, caffeine (the major methylxanthine found in tea and coffee) exerted effects that were qualitatively similar to those described above. In addition, the concentration of purified caffeine required for 50 percent inhibition of weight gain was nearly identical to the concentration of endogenous caffeine in the coffee-medium mixture that caused 50 percent inhibition of weight gain (Fig. 1A). Dried tea leaves, which contain two to three times the caffeine of dried coffee beans, were about two to three times as effective as coffee beans in inhibiting weight gain (data not shown). Furthermore, the concentrations of caffeine found naturally in undried tea leaves (0.68 to 2.1 percent) or coffee beans (0.8 to 1.8 percent) were sufficient to kill most *Manduca* larvae, suggesting that naturally occurring methylxanthines could function as endogenous insecticides (*4, 5*). When applied as a spray to natural feeding substrates such as tomato leaves, caffeine, theophylline, or the synthetic compound 3-isobutylmethylxanthine (IBMX) exerted pestistatic and pesticidal effects that resulted in leaf protection (Fig. 1, B and C).

Certain other insect species in addition to *Manduca sexta* were affected by methylxanthines. IBMX (mixed in appropriate artificial medium) inhibited food consumption by mealworm larvae [*Tenebrio* spp.; median effective dose (ED_{50}), 0.1 to 0.3 percent], butterfly larvae (*Vanessa cardui*; ED_{50}, 0.1 to 0.3 percent), and milkweed bug nymphs (*Oncopeltus fasciatus*; ED_{50}, 0.3 percent); in solution, IBMX killed mosquito larvae (*Culex* spp.; ED_{50}, 0.0007 percent). Adult flour beetles (*Tribolium confusum* and *T. castaneum*) were unaffected by doses of IBMX up to 3 percent however, in long-term tests, IBMX (ED_{50}, 0.2 percent) inhibited reproduction of these two species.

In vertebrate tissues, methylxanthines are known to inhibit phosphodiesterase (PDE) enzymes, which hydrolyze adenosine 3′,5′-monophosphate (cyclic AMP) (*2, 6*). I therefore investigated whether methylxanthines could inhibit PDE activity in *Manduca* nerve cord and, if so, whether the degree of such inhibition was related to observed pestistatic activity. The dose-dependent inhibition of nerve cord PDE activity by the methylxanthines (Fig. 1D) showed a similar pattern of activity (that is, rank-order potency and relative efficacy) to the inhibition of leaf consumption by these same methylxanthines (Fig. 1C).

To determine whether the doses of methylxanthines (see Fig. 1, B and C) ingested by the larvae (causing pestistatic and pesticidal activity in vivo) were actually absorbed by the animals and were sufficient to inhibit PDE in vivo, I performed additional studies to estimate concentrations of methylxanthine present in tissue after 3 days of feeding larvae on various doses of theophylline-treated leaves (*7*). In these studies, larvae feeding on leaves treated with a 1 percent spray (an amount causing about 50 percent inhibition of leaf consumption) were found to contain an internal

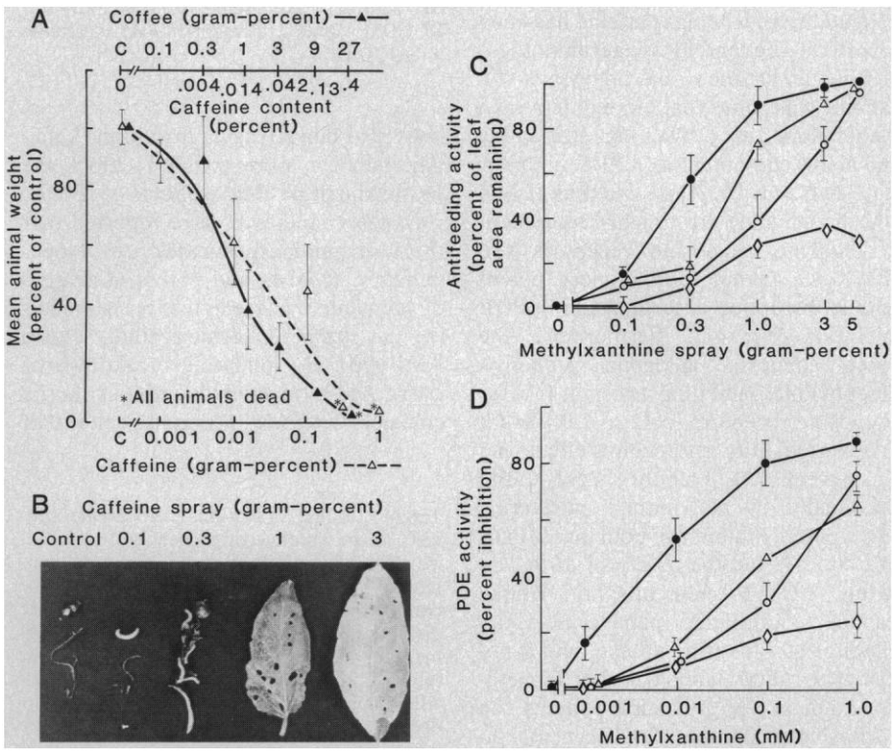

Fig. 1. (A) Effect on tobacco hornworm body weight of powdered coffee beans or caffeine incorporated into artificial medium (Carolina Biological Supply). Weight (mean ± standard error of the mean, $n = 5$) was measured at the end of 7 days. The caffeine content of coffee beans was determined spectrophotometrically after selective extraction and separation by thin-layer chromatography. (B and C) Dose-dependent inhibition of leaf consumption (antifeeding effect) in a typical experiment with caffeine (pictured in B) and a summary of three experiments with IBMX (●), caffeine (△), theophylline (○), and 8-phenyltheophylline (◇) (quantitated in C). Isolated, hydrated tomato leaves were sprayed with the compounds or with vehicle (usually methanol) at the concentrations shown, allowed to dry, and placed in closed plexiglass containers. A group of six 3-day-old tobacco hornworm larvae (initially reared on artificial medium) were then placed on each leaf, and the amount of leaf remaining was measured at the end of 4 days. Values shown are means (± standard error of the mean for three experiments) of leaf area expressed as percent of control area. (D) Effect of the same methylxanthines on inhibition of cyclic AMP phosphodiesterase activity in homogenates of hornworm nerve cord. Nerve cords were dissected from larvae (40 to 60 mm long), cleaned, and homogenized (2mg/ml) in 6 m*M* tris-maleate (*p*H 7.4). Phosphodiesterase activity was measured in an assay system containing (in 0.1-ml total volume) 80 m*M* tris-maleate (*p*H 7.4), 6 m*M* $MgSO_4$, 10^{-7} *M* ^{3}H-labeled cyclic AMP, and 0.02 ml of tissue homogenate with or without drug. The rate of formation of ^{3}H-labeled 5′-AMP was measured (*23*) during a 4-minute incubation at 30°C. Under these conditions, enzyme activity was linear with respect to time and enzyme concentration. Values shown are means ± standard error of the mean for three separate experiments.

theophylline concentration of 4.1 ± 1.1 m*M* (mean ± standard deviation for two groups of six pooled animals). Such a concentration was sufficient to cause more than 80 percent inhibition of PDE activity in hornworm nerve cord in vitro. This observation helps to rule out the possibility of adenosine receptors acting as a mechanism for the inhibition of leaf consumption (antifeeding effects) of the methylxanthines since, in vertebrates, methylxanthines such as theophylline are competitive adenosine receptor antagonists but exert such antagonism at much lower concentrations, typically 1 to 25 μ*M* (*8*). Thus, in the present experiments, a 0.1 percent spray of theophylline, which had little effect on feeding, resulted in concentration of theophylline in tissue (~400 μ*M*) which would have been sufficient virtually completely to block adenosine receptor binding (*8*).

In addition, whereas caffeine has been reported to be tenfold weaker than theophylline as an adenosine antagonist (*8*), caffeine was somewhat more potent than theophylline in preventing leaf-eating and about equipotent as a PDE inhibitor (Fig. 1, C and D). Also, whereas IBMX and theophylline are roughly equipotent in blocking adenosine receptors (*8*), IBMX was about tenfold more potent both in disruption of feeding and in PDE inhibition (Fig. 1D). Furthermore, the potent adenosine antagonist 8-phenyltheophylline [inhibition constant (K_i) for adenosine receptors, 0.12 to 1.0 μ*M* (*8, 9*)] exerted little antifeeding effect and was a weak PDE inhibitor (Fig. 1, C and D). Finally, the nonxanthine papaverine was a potent inhibitor of both insect PDE (K_i, 40 μ*M*) and the ability of *Manduca* to feed (ED_{50}, 0.1 percent spray). Unlike the methylxanthines, papaverine is an inhibitor of adenosine uptake, and it potentiates, rather than blocks (as do methylxanthines), physiological effects on adenosine receptors (*10*). Taken together, these data are more consistent with a mechanism of action related to inhibition of PDE than to blockade of adenosine (*11*).

Furthermore, other experiments suggested that the effects described above were probably not due to the reported calcium-mobilizing effects of the methylxanthines (*12, 13*). Specifically, methylxanthines are known to mobilize calcium from the sarcoplasmic reticulum, an effect blocked by diltiazem or procaine, and in the present experiments neither diltiazem nor procaine reversed the antifeeding effects of IBMX (*12, 14*). Methylxanthines may also affect calcium movement across the plasma membrane (*12, 15*), and in the present studies D-600, verapamil, and nimodipine, which are known to block plasma membrane calcium channels, were unable to block the pestistatic or pesticidal effects of IBMX.

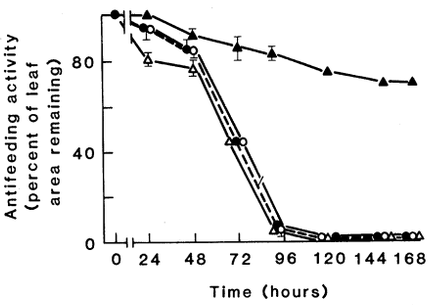

Fig. 2. Time-course experiment showing the synergistic effect of caffeine and didemethylchlordimeform (DDCDM, an octopamine agonist) compared with the effects of either compound alone on consumption of tomato leaves sprayed with the various agents. The experimental procedure was similar to that described in the legend to Fig. 1C. Values shown are the mean ± range of duplicate area measurements expressed as a percent of total (starting) area and are typical of those seen in two to four replicate experiments. Symbols: (●) control; (△) caffeine (0.1 percent); (○) DDCDM (0.01 percent); (▲) caffeine + DDCDM.

In other studies, I have reported that the formamidine pesticides are octopaminergic agonists and potent activators of adenylate cyclase in insects, stimulating the synthesis of intracellular cyclic AMP (*16*). By inhibiting breakdown of cyclic AMP, the methylxanthines should enhance receptor-mediated action of such octopamine agonists. Accordingly, it was of interest to determine whether methylxanthines would enhance the pesticidal activity of the formamidines or of certain other octopamine agonists (*17*).

Table 1. Effect of various octopamine agonists in the absence or presence of isobutylmethylxanthine (IBMX) on cyclic AMP content of *Manduca sexta* nerve cord. Nerve cords from larvae (40 to 60 mm long) were dissected; each cord was cut into 5-mm segments, and 6 to 8 segments were placed per tube in 0.3 ml of oxygenated insect saline (*22*). After incubation for 10 minutes, the drug or drugs listed were added, and the tubes were incubated for 5 minutes at 25°C and then boiled for 2 minutes. The contents of each tube were then homogenized and centrifuged, the supernatant was assayed in duplicate for cyclic AMP content by binding assay (*23*), and the sediment was assayed for protein content. The values (mean ± standard error of the mean for three experiments) show the cyclic AMP content as a percent of control activity (6.25 ± 1.05 pmol per milligram of protein for six replicates).

Compound*	Cyclic AMP (percent of control)	
	Without IBMX	With IBMX
DDCDM	540 ± 380	1110 ± 270
NC7	220 ± 20	1390 ± 300
Octopamine	90 ± 30	640 ± 100
		220 ± 30

*The concentration of all compounds and IBMX was 0.1 m*M*.

Leaves treated with a low dose of caffeine or with a low (minimally toxic) dose of the formamidine pesticide didemethylchlordimeform (DDCDM) were eaten by a hornworm larvae at a rate similar to that for control leaves (Fig. 2). However, when caffeine was combined with an identical amount of DDCDM, marked inhibition of feeding occurred and, by day 6, all larvae were dead. Synergism was also observed when DDCDM was combined with theophylline or IBMX. To quantitate this synergism, I next tested various concentrations of a primary octopaminergic pesticide in the absence or presence of a fixed dose of methylxanthine and measured remaining leaf area at the end of 4 days. In the absence of IBMX, the ED_{50} of DDCDM for inhibition of leaf consumption was about 0.2 gram-percent; in the presence of IBMX (0.1 percent spray), the ED_{50} of DDCDM was 0.003 percent. In other words, IBMX shifted the DDCDM dose-response curve to the left, causing more than a 50-fold increase in potency of the octopamine agonist. Similar enhancements were seen when IBMX was combined with other formamidines, including chlordimeform and monodemethylchlordimeform (*18*). IBMX also enhanced the pesticidal potency of nonformamidine octopamine agonists, such as 2-(2-methyl,4-chlorophenylimino)imidazolidine (NC7) (30-fold increase in potency) and 4-fluorophenylethanolamine (100-fold increase) (*17*). Furthermore, octopamine (0.1 to 1 percent spray), although exerting little antifeeding effect alone, had substantial antifeeding activity when combined with 0.1 percent IBMX.

As a result of the findings from these experiments, I next investigated whether IBMX, alone or combined with a primary octopamine agonist, would actually increase the cyclic AMP content of insect tissues and whether this content would be correlated with observed pesticidal effects. IBMX alone (0.1 m*M*) caused more than a doubling of tissue cyclic AMP content when intact, isolated *Manduca* nerve cords were incubated under physiological conditions (Table 1). Furthermore, when combined with various primary octopamine agonists, 0.1 m*M* IBMX caused synergistic increases in cyclic AMP content. In other experiments with live larvae, the rank-order pesticidal effectiveness of these same combinations (on the basis of the ED_{50}

value of the octopamine agonist for inhibition of leaf consumption) was DDCDM + IBMX = NC7 + IBMX-octopamine + IBMX = DDCDM alone > NC7 alone > octopamine alone (almost inactive). Thus, pesticidal effectiveness of various octopamine agonists, alone or combined with IBMX, agreed well with the ability of these same agents, alone or in combination, to increase tissue cyclic AMP (Table 1).

These data, together with the following additional observations, provide supporting evidence for an involvement of cyclic AMP in the primary and synergistic pesticidal effects of the methylxanthines. (i) Forskolin, a diterpene that appears to activate the catalytic subunit of adenylate cyclase directly (*19*), stimulated cyclic AMP production in hornworm nerve cord in the absence of calcium and, in leaf-eating experiments, caused a disruption of feeding that was enhanced by IBMX. (ii) IBMX did not enhance the pesticidal effects of certain insecticides, including DDT (a chlorinated hydrocarbon), chlorpyriphos (organophosphate), and Karathane, none of which stimulated adenylate cyclase activity in insects in vitro. (iii) The meta-hydroxy isomer of octopamine was at least ten times less potent than octopamine (where the hydroxyl is in the para position) in activating octopamine-sensitive adenylate cyclase in insects; likewise, in the presence of IBMX, the meta isomer of octopamine showed no pestistatic or pesticidal activity whereas octopamine did. (iv) Among several phenyliminoimidazolidine derivatives, there was a rank-order correlation between the ability to activate adenylate cyclase in hornworms and the ability to disrupt their feeding on tomato leaves. (v) Finally, lipid-soluble cyclic AMP analogs such as the *p*-chlorophenylthio derivative of cyclic AMP were observed to disrupt feeding. These analogs, which were found in vitro to undergo significant hydrolysis by PDE activity in nerve cord (an effect blocked by IBMX), had their antifeeding activity in vivo enhanced by IBMX.

Taken together, these data suggest that the pestistatic and pesticidal effects of the methylxanthines are mediated through an alteration of concentrations of cyclic AMP in tissue, most likely secondarily to an inhibition of PDE. These findings also suggest that naturally occurring methylxanthines could function as endogenous insecticides. Finally, these results raise the possibility that methylxanthines or nonxanthine PDE inhibitors may be of some practical use in pest control, either alone or as synergists of certain other primary pesticides. In the latter case, potential toxicity for mammals could be reduced by targeting the primary pesticide at a receptor-associated adenylate cyclase (for example, octopamine) found primarily or exclusively in invertebrates (*20, 21*).

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References and Notes

1. E. Kaplan, J. H. Holmes, N. Sapeika, *S. Afr. J. Nutr.* **10**, 32 (1974); R. W. Schery, *Plants for Man* (Prentice-Hall, Englewood Cliffs, N.J., 1972), p. 583.
2. T. W. Rall, in *Pharmacological Basis of Therapeutics*, A. G. Gilman, L. Goodman, A. Gilman, Eds. (Macmillan, New York, 1980), p. 592.
3. P. A. Hedin, Ed., *Plant Resistance to Insects* (American Chemical Society, Washington, D.C., 1983).
4. Calculated from (*1*) by using caffeine content and known water content of tea plants.
5. Evaluation of the natural insect pests of caffeine-containing crops does not help to assess this hypothesis because insects capable of damaging a plant with a particular defense are also those that have resistance to the defense.
6. E. W. Sutherland and T. W. Rall, *J. Biol. Chem.* **232**, 1077 (1958); R. W. Butcher and E. W. Sutherland, *ibid.* **237**, 1244 (1962).
7. Groups of six larvae were placed on leaves treated with vehicle or theophylline spray. After 3 days, leaf area was recorded and larvae (alive or dead) were rinsed to remove any compound adhering to their cuticle, homogenized whole, and centrifuged and the cell-free supernatant was assayed for theophylline content by immunoenzymatic assay (Emit-aad Theophylline Assay, Syva). This assay shows little cross-reactivity with theophylline metabolites. Mammalian studies indicate that theophylline penetrates freely into all body compartments (*2*).
8. R. Bruns, J. Daly, S. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5547 (1980); M. Williams and E. A. Risley, *ibid.*, p. 6892.
9. J. W. Daly, *J. Med. Chem.* **25**, 197 (1982).
10. M. Huang and J. W. Daly, *Life Sci.* **14**, 489 (1974).
11. Because adenosine receptors can regulate production of cyclic AMP (*9*), these data do not preclude the possibility that compounds primarily affecting adenosine receptors (and subsequently affecting cyclic AMP concentrations in the same direction as PDE inhibition) might also exert antifeeding effects.
12. J. R. Blinks, C. B. Olson, B. Jewell, P. Braveny, *Circ. Res.* **30**, 367 (1972).
13. Because methylxanthines have also been reported to antagonize certain prostaglandin actions [D. Horrobin, M. Manku, D. Franks, P. Hamet, *Prostaglandins* **13**, 33 (1977)], I tested but found no effect of aspirin, either alone or combined with DDCDM, on the ability of *Manduca* to feed on tomato leaves. Furthermore, although methylxanthines may also block catecholamine uptake [S. Kalsner, R. Fran, G. Smith, *Am. J. Phys.* **228**, 1702 (1975)], β-phenylethylamine, a potent octopamine reuptake blocker in insects [P. Evans, *J. Neurochem.* **30**, 1015 (1978)], exerted no antifeeding effects.
14. A. M. Katz, D. I. Repke, W. Hasselbach, *J. Biol. Chem.* **252**, 1938 (1977); T. Ishizuka and M. Endo, *Circ. Res.* **52** (Suppl. 1), 110 (1983).
15. K. Saeki, S. Ikeda, M. Nishibori, *Life Sci.* **32** 2973 (1983).
16. J. A. Nathanson and E. J. Hunnicutt, *Mol. Pharmacol.* **20**, 68 (1981); J. A. Nathanson, *Soc. Neurosci. Abstr.* **7**, 317 (1981).
17. J. A. Nathanson, in *Proceedings of the 17th International Congress of Entomology*, Hamburg, Germany, 20 to 26 August 1984, in press.
18. IBMX also enhanced the ability of chlordimeform to inhibit hatching of *Manduca* larvae from their eggs, a task demanding of motor coordination.
19. K. Seamon, W. Padgett, J. Daly, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3363 (1981).
20. C. Lingle *et al.*, *Handb. Exp. Pharmacol.* **58-2**, 787 (1982).
21. Such a combination might, however, still be toxic to certain beneficial insects.
22. N. Zilber-Gachelin and D. Paupardin, *Comp. Biochem. Physiol* **49**, 441 (1974).
23. B. Brown, P. Elkins, J. Albano, *Adv. Cyclic Nucleotide Res.* **2**, 25 (1972).
24. C. R. Filburn and J. Karn, *Anal. Biochem.* **52**, 505 (1973).
25. Supported in part by the McKnight Foundation and the JLN-Daniels Research Fund. I thank E. J. Hunnicutt and C. J. Owen for technical assistance, W. J. Schwartz for helpful editorial advice, Ciba-Geigy for formamidine compounds, and the Gateways Tomato Farms for botanical specimens.

13 April 1984; accepted 15 June 1984

Regulation of a Hybrid Gene by Glucose and Temperature in Hamster Fibroblasts

Abstract. *A novel eukaryotic hybrid gene has been constructed from the 5′ sequence of a rat gene and the bacterial neomycin-resistance gene. After transfection into hamster fibroblasts, the neo transcripts can be induced to high levels by the absence of glucose. Furthermore, this hybrid gene can be regulated by temperature when it is introduced into a temperature-sensitive mutant cell line.*

The ability to introduce defined DNA segments into mammalian cells is a powerful tool for studying the regulation of eukaryotic gene expression and for identifying specific DNA sequences responsible for such regulation. We have been using DNA-mediated gene transfer techniques to study the regulated expression of a set of "glucose-regulated proteins" (GRP's) in mammalian cells. The GRP's are cellular proteins synthesized constitutively at low but detectable levels under normal tissue culture conditions or in whole organs; yet their synthesis is markedly enhanced in response to glucose starvation or exposure to inhibitors of glycosylation (*1*). The most abundant GRP in chicken, hamster, rat, mouse, and human cells is a 78-kilodalton protein. While a one- to twofold increase in GRP78 can be detected after heat shock of the cultured cells, it is distinct from the major 72- to 73-kilodalton heat shock protein commonly observed in mammalian cells (*1, 2*).

We and others have described a temperature-sensitive (ts) hamster mutant cell line, K12, which overproduces the GRP's by a factor of 20 to 50 when the cells are incubated at the nonpermissive temperature, 40.5°C (*3*). Since the messenger RNA (mRNA) for GRP78 is also