approximately 27.5 percent (87 out of 316) of the population if the separation is made at 4.5 units per milliliter of erythrocytes.

When the ratio of enzyme activity after heating (H) to the activity in controls (C) before heating (H/C ratio) is used as a measure of COMT thermostability, there is a highly significant correlation of H/C ratios with basal erythrocyte COMT activities for these 316 subjects ($r = .688$, $P < .001$; Fig. 2C). Of more importance, Fig. 2C shows that the H/C ratios are lower in subjects with erythrocyte COMT activity of less than 8 unit/ml—that is, subjects homozygous for the allele for low enzyme activity—than in the remainder of the population. The average H/C ratio for the 84 subjects with enzyme activity of less than 8 unit/ml is 0.47 ± 0.01 (mean ± S.E.), whereas that for the 232 other subjects studied is 0.65 ± 0.01 ($P < .001$). Therefore, these results confirm those obtained in the initial six subjects and are compatible with the conclusion that there is a difference in at least one biochemical property of COMT in the erythrocytes of subjects with genetically different levels of enzyme activity.

It has not been possible to separate subjects heterozygous for the allele for low COMT activity from subjects homozygous for the alternative allele or alleles on the basis of enzyme activity alone (*5*). It would also be difficult to separate these two groups on the basis of the thermostability of COMT, because most of the enzyme activity in erythrocytes of both groups would be the thermostable form and differences in thermostability between these two groups would be small. Therefore, although the inheritance of the trait of low COMT activity might be autosomal codominant rather than autosomal recessive, this question might best be answered with family studies. In addition, family studies will be necessary to verify that the traits of low erythrocyte COMT activity and of thermolability segregate together.

The existence of differences in the thermostability of erythrocyte COMT in lysates from subjects with low and high enzyme activity, levels of activity that are under genetic control (*4, 5, 12*), suggests inherited differences in the structure of the COMT molecule in the human erythrocyte. We propose that the locus responsible for the genetic regulation of human erythrocyte COMT activity be referred to as *COMT*, and that the alleles for "low" and "high" enzyme activity be designated $COMT^{L}$ and $COMT^{H}$, respectively. These designations conform to the recommendations of the Committee on Nomenclature of the Third International Workshop on Human Gene Mapping (*13*). Although the possibility of genetically determined posttranslational modification of COMT cannot be eliminated, the results of our experiments suggest that the locus *COMT* might represent the structural gene for the human enzyme. However, other genetic or environmental factors might participate in the regulation of COMT activity. It remains to be determined whether genetically mediated differences in COMT activity in the erythrocyte, differences which reflect relative COMT activity in the human lung and kidney, are of importance in individual variations in the metabolism of endogenous catecholamines; of catechol drugs such as isoproterenol, α-methyldopa, and L-dopa; and of hormones such as the catecholestrogens (*1, 2, 14*).

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Octopamine Receptors, Adenosine 3′,5′-Monophosphate, and Neural Control of Firefly Flashing

Abstract. *An adenylate cyclase activated as much as 25-fold by low concentrations of octopamine has been identified in the firefly lantern. The relative potency of octopamine and various other amines in stimulating this enzyme, and effects of antagonists in blocking octopamine activation, correlate well with the known effects of these agents in affecting light production. In addition to suggesting a role for adenosine 3′,5′-monophosphate (or pyrophosphate) in the neural control of firefly flashing, identification of this potent enzyme should facilitate the characterization of phenylethylamine receptors in excitable tissue.*

Octopamine is a naturally occurring phenylethylamine (similar in structure to norepinephrine) found in the tissues of a number of vertebrate and invertebrate species (*1, 2*). In 1973, Nathanson and Greengard (*3*) identified an adenylate cyclase activated specifically by low concentrations of octopamine. The neuronal localization of this enzyme, together with studies of octopamine metabolism (*4*), led to speculation (*1, 3, 5*) that octopamine might function as a neurotransmitter or neuromodulator in the nervous system of invertebrates. Subsequent electrophysiological (*6*) and anatomical (*7*) studies in mollusks, crustaceans, and insects have confirmed the existence of octopaminergic neurons.

Because characterization of hormone-sensitive adenylate cyclases in various tissues has revealed a close similarity between the properties of these enzymes and the known physiological and pharmacological properties of hormone receptors (*8*), efforts have been made to characterize the octopamine receptor by studying octopamine-sensitive adenylate cyclase (*9*). To date, such experiments have been hampered by the lack of a tissue preparation free from other amine receptors. I now report the identification, in the firefly lantern (or "light organ"), of a potent and highly specific octopamine-sensitive adenylate cyclase. The pharmacological characteristics of this enzyme suggest that it may be involved in neural control of lantern flashing.

The timing of firefly light flashes

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(which is species- and sex-specific) is under control of the insect's nervous system (*10*). Thus, periodic flashing is abolished by denervation, mimicked by electrical stimulation of the lantern or its afferent nerves, and preceded (in intact animals) by a volley of afferent nerve impulses. Direct application of catecholamines (but not serotonin, γ-aminobutyric acid, or cholinergic agents) elicits a glow from intact or decentralized light organs (*11*). Amphetamine also initiates light production (*11*), an effect prevented by denervation or prior treatment with reserpine, suggesting the presence of aminergic nerves. Because octopamine is present in the firefly lantern (*12*) and is more potent than norepinephrine in eliciting light production (*13, 14*), several workers (*12-14*) have suggested that octopamine or a related phenylethylamine might serve as a neurotransmitter effecting the neural control of firefly flashing. If such were the case, the existence, in the light organs, of a specific octopamine receptor and, possibly associated with this, an octopamine-sensitive adenylate cyclase might be predicted.

To test this possibility, the effects of octopamine were studied on the enzymatic synthesis of adenosine 3′5′-monophosphate (cyclic AMP) from adenosine triphosphate (ATP) in broken cell preparations from various tissues of the firefly *Photuris* (*15*). Figure 1A shows the effects of octopamine on adenylate cyclase activity in homogenates made from the entire sixth and seventh abdominal segments (which contain the light organs) as compared with the effects on the fourth and fifth segments, which do not contain the lantern. Stimulation by octopamine was noted only in the lantern-containing segments, which responded with as much as a 15-fold activation over basal enzyme activity. Concentrations as low as $10^{-7}M$ caused significant stimulation, and half-maximal activation occurred at a concentration (K_a) of 3 μM. Under the incubation conditions used, phosphodiesterase activity was almost completely inhibited, indicating that octopamine stimulation of cyclic AMP accumulation was due to activation of adenylate cyclase and not inhibition of phosphodiesterase (*16*).

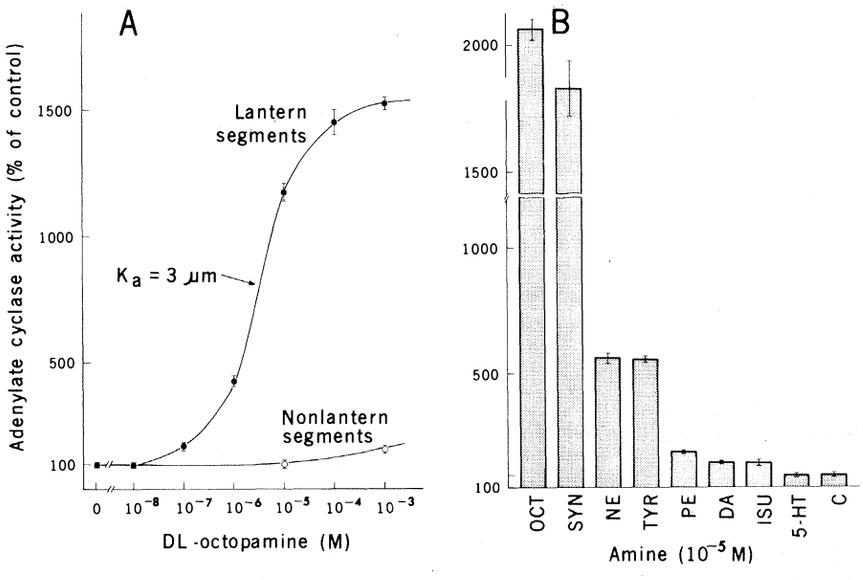

Fig. 1. (A) Effect of (±)-octopamine on adenylate cyclase activity in homogenates of abdominal segments of fireflies that contained lanterns (unstimulated control = 17.3 ± 0.6 pmole per milligram of protein per minute) and those that did not contain lanterns (unstimulated control = 44.2 ± 6.3). (B). Comparative effects of various amines ($10^{-5}M$) on activation of adenylate cyclase in homogenates of isolated light organs dissected free from surrounding tissue (control = 19.8 ± 1.8 pmole per milligram of protein per minute). Values shown are mean (± mean deviation) of replicate samples each assayed in triplicate for cyclic AMP. The K_a for octopamine in homogenates of isolated light organs was similar to that in (A). To measure enzyme activity, tissue [15 mg/ml in (A); 5 mg/ml in (B)] was homogenized in 6 m*M* tris-maleate, *p*H 7.4, 10 m*M* theophylline, 6 m*M* $MgSO_4$, 0.1 m*M* GTP, 1 m*M* EGTA, 1.5 m*M* ATP, and tissue homogenate or washed particulate fraction (0.33 or 1 mg, wet weight), plus or minus amines. The reaction (4 minutes at 30°C) was initiated by addition of ATP, stopped by heating to 90°C for 2 minutes and then centrifuged to remove insoluble material. Cyclic AMP in the supernatant was measured as in (*30*). Under these conditions, basal enzyme activities were comparable to those of adenylate cyclases in other invertebrates (*3, 9*), and octopamine-stimulated activity was linear with respect to time and enzyme concentration. Cyclic AMP produced in the reaction cochromatographed on Dowex AG50X resin with an authentic sample of cyclic AMP. Male and female lanterns showed the same degree of octopamine stimulation, although, because of the female light organ's smaller size, absolute activity was less. Abbreviations are *OCT*, (±-octopamine; *SYN*, (±)-synephrine; *NE*, (−)-norepinephrine; *TYR*, tyramine; *PE*, (−)-phenylephrine; *DA*, dopamine; *ISU*, (−)-isoproterenol; *5-HT*, serotonin; and *C*, control.

To better localize the site of hormonal response, homogenates were made from isolated light organs that had been dissected free from surrounding tissue. In these preparations, $10^{-5}M$ octopamine stimulated basal enzyme activity approximately 20-fold (Fig. 1B), and maximal activation (at $10^{-3}M$) was greater than 25-fold (Table 1). In contrast, octopamine stimulation was less than threefold in the surrounding tissue that did not contain the lantern. These tissues included ganglia, fat, gut, reproductive organs, and cuticle. In washed particulate fractions prepared from the pellet of isolated light organ homogenates that had been centrifuged at 50,000*g*, octopamine stimulation was more than 30-fold (data not shown).

Comparison of the relative effects of various superfused amines on light production in adult and larval firefly lanterns has shown that octopamine is equal to or more potent than norepinephrine, which, in turn, is considerably more potent than either isoproterenol or phenylephrine (*11, 13, 14*). Synephrine, which has not been detected in the firefly (*12*), is the only amine reported to be of equal or greater potency than octopamine (*13, 14*). In my studies, the relative effects of these and other amines on the activation of adenylate cyclase in isolated lantern homogenates were not only consistent with reported physiological studies but also demonstrated structure-activity relationships supporting the activation of an octopamine receptor (Fig. 1B). Thus, synephrine, the *N*-methyl derivative of octopamine, was the only amine nearly as effective as octopamine in stimulating enzyme activity. Both norepinephrine and tyramine, which differ from octopamine by a single hydroxyl group, were of intermediate effect; but dopamine, which differs by two hydroxyl groups, was much less effective (*17*). Serotonin, an indolamine, which is without effect on light production, caused no enzyme stimulation. Further evidence that these amines were activating a single receptor was obtained from additivity experiments (Table 1) in which maximally effective concentrations of each amine were tested alone and in combination. No additive effects were observed.

The α-adrenergic antagonists phentolamine and phenoxybenzamine have been reported to partially or completely block neurally induced lantern flashing in adult and larval fireflies, whereas the β-adrenergic antagonist propranolol ap-

Table 1. Nonadditive effects of synephrine, norepinephrine, and dopamine on the stimulation of adenylate cyclase activity by octopamine in a homogenate of isolated firefly light organs. Other experiments indicated that the concentration ($10^{-3}M$) of each amine used here was that which caused maximal stimulation of enzyme activity when added alone to homogenates of isolated light organs (*31*). Control activity was 20.7 ± 0.9 pmole per milligram of protein per minute, and the values shown here and in Table 2 are the means (± mean deviation) for replicate samples each assayed for cyclic AMP in quadruplicate.

Addition	Enzyme activity (% of control)
Dopamine	420 ± 15
Norepinephrine	2150 ± 75
Synephrine	2510 ± 105
Octopamine	2660 ± 40
Octopamine + dopamine	2695 ± 100
Octopamine + norepinephrine	2705 ± 40
Octopamine + synephrine	2500 ± 10

pears less effective (*14, 18*). Consistent with these physiological studies, phentolamine was also more effective than propranolol in blocking the stimulation of adenylate cyclase activity by octopamine in lantern-containing homogenates (Table 2).

The results of the above experiments support the existence, in the firefly lantern, of an adenylate cyclase activated specifically by octopamine. The K_a of this enzyme for octopamine and the inhibition of hormone stimulation by phentolamine are characteristics similar to those found for octopamine-sensitive adenylate cyclase in invertebrate ganglia (*3, 9*). However, the degree of octopamine stimulation in the light organ is about tenfold greater, and, indeed, more than that of any other adenylate cyclase yet identified in broken cell preparations of excitable tissue (*8, 19*). The great activity of this enzyme may, in part, be related to the redundancy of lantern cellular organization and associated innervation which, in some ways, seems analogous to the arrangement present in the electric organs of certain fishes, which contain high concentrations of cholinergic receptors (*20*).

Fine-structure studies of larval and adult firefly light organs (*21*) show that the afferent axons terminate in varicosities containing large, dense-core vesicles of neurosecretory type, quite similar to those in other invertebrate neurons that are thought to be octopaminergic (*7*). The actual site of innervation of these presumptive octopaminergic terminals in the firefly is of interest in interpreting possible mechanisms of octopaminergic control of light production. Thus, in larval light organs (which glow slowly but are unable to flash), the nerves end directly on the plasma membrane of the photocytes (light-emitting cells). However, in the structurally different adult light organs (which flash), the varicosities terminate between the epithelium of the terminal air tubes (tracheoles) and specialized end cells that surround the tracheolar epithelium. The end cells, which in turn abut the photocyte membrane, are absent from larval light organs, suggesting that, in the adult, these cells may play a role in flash initiation (*10, 21*).

Consistent with the known time course of cyclic nucleotide-mediated events in other excitable cells (*8*), electrophysiological studies (*10*) have shown that the latency between normal nerve stimulation and lantern flashing is relatively long (~70 msec). High-intensity stimulation creates a "quick" flash of shorter latency (18 msec), which is thought to result from direct depolarization of the photocyte membrane, bypassing the nerve-end organ linkage. The latency of the slow flash is temperature-dependent, whereas that of the quick flash is not. This is consistent with an enzymatic process (such as activation of adenylate cyclase) occurring prior to depolarization of the photocyte membrane. Because octopamine is known to both modulate and directly affect synaptic potentials in invertebrate nerve and muscle (*6*), it is possible that this amine, through the generation of cyclic AMP, could depolarize the end cell complex (which might or might not then be capable of a regenerative action potential response). The resulting spread of depolarization would allow the simultaneous activation of surrounding photocytes, triggering a local flash. In larval light organs, which lack end cells, and in which the nerves end directly on the photocyte (*21*), octopamine would be expected to elicit only a slow, local depolarization prior to a gradual onset of glowing. Such depolarization preceding light emission has been observed in larval photocytes, and it has also been reported (*14*) that the phosphodiesterase inhibitor theophylline, known to elevate cyclic AMP in cells, induces glowing in the larval light organ (*22*). Conceivably, depolarization or a change in membrane permeability (or both) in both larvae and adults could play a role in the release or sequestration of some component necessary for the light reaction (*23*).

Other possibilities also exist. (i) Cyclic AMP, via a cyclic AMP-dependent protein kinase, might activate some enzymatic intermediate in either the photocyte or end-organ complex. For instance, stimulation of phosphorylase with resultant glycogen breakdown, as has been reported for octopamine (*24*), would affect energy metabolism (*25*). (ii) Cyclic AMP or cyclic AMP-dependent phosphorylation might also affect the photochemical reaction directly. Thus, Bowie *et al.* (*26*) have noted that cyclic AMP is the only nucleotide which mimicks ATP-Mg^{2+} in altering the emission spectrum of a fluorescent active-site probe bound to firefly luciferase. (ATP-Mg^{24} normally reacts to form the excited enzyme-luciferyl-adenylate complex necessary for light emission.) This observation raises the possibility that cyclic AMP, generated through neural excitation, could play a regulatory role in photocyte light emission reactions (*27*). (iii) Since the photochemical reaction is subject to product (most likely oxyluciferin) inhibition (*28*) that can be released by the addition of pyrophosphate (*29*), it has been postulated (*29*) that neural control of pyrophosphate metabolism might regulate light production, thus raising the possibility that formation of pyrophosphate, and not cyclic AMP, by octopamine-sensitive adenylate cyclase could be involved in receptor-mediated control of light emission. Further studies, including the cellular localization of cyclic AMP accumulation in larval and adult lanterns, should help to differentiate among these possibilities.

Meanwhile, the great activity of octopamine-sensitive adenylate cyclase in the light organ, together with the apparent absence of adenylate cyclases activated by other phenolic amines, should make the firefly lantern an attractive model for studying phenylethylamine receptors.

Table 2. Effects of the α-adrenergic antagonist, phentolamine, and the β-adrenergic antagonist, propranolol, on the stimulation of adenylate cyclase activity by octopamine in a homogenate of lantern-containing abdominal segments. Control activity was 9.6 ± 1.0 pmole per milligram of protein per minute.

Addition	Enzyme activity (% of control)
Octopamine ($10^{-5}M$)	1390 ± 50
Octopamine ($10^{-5}M$) + phentolamine ($10^{-4}M$)	495 ± 15
Octopamine ($10^{-5}M$) + propranolol ($10^{-4}M$)	1005 ± 80
Phentolamine ($10^{-4}M$)	185 ± 20
Propranolol ($10^{-4}M$)	140 ± 10

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Cholecystokinin in the Brains of Obese and Nonobese Mice

Abstract. *Extracts of the cerebral cortex of genetically obese* (ob/ob) *mice with hyperphagia contain 0.05 ± 0.02 microgram (mean ± standard error) of cholecystokinin octapeptide equivalent per gram of wet weight compared to 0.15 ± 0.01 microgram per gram for their nonobese littermates and 0.20 ± 0.01 microgram per gram for normal LAF_1 mice. These findings are suggestive of a causal relation between the diminished brain immunoreactive cholecystokinin content and the unrestrained appetite of the obese mice.*

Over the years work from a number of laboratories has suggested a role for cholecystokinin (CCK) as a satiety factor, perhaps through some type of negative signal from the gastrointestinal tract (*1*). A more direct role for CCK as a neuroregulator has been suggested by the observation that CCK peptides are not restricted to the gut but are found in the brain and appear to be localized in cortical neurons (*2*). Mice that are genetically obese (*ob/ob*) are known to have voracious appetites and seem likely candidates to manifest abnormalities in brain CCK. We now report a comparison of the concentrations of immunoreactive CCK in extracts of the cerebral cortex of *ob/ob* mice, their nonobese littermates, and normal mice of another genetic strain.

A heterozygote breeding pair of the C57BL/6J strain was obtained from Jackson Laboratory, Bar Harbor, Maine. The mice were bred for two successive generations in our laboratory, and the *ob/ob* mice and their nonobese littermates were killed when they were between 6 and 9 months of age. Normal LAF_1 mice had been bred in our laboratory for several years. The mice were killed with lethal doses of sodium pentobarbital. The entire brains were removed, hemisected in the sagittal plane, and immediately frozen on Dry Ice and stored at −70°C until extracted. While still frozen the right half of each brain was added to 0.1*N* HCl to produce a concentration of 0.1 g of tissue (wet weight) per milliliter. The extraction solution was boiled for 3 minutes. The tissue was then homogenized in the extraction solution with a Teflon tissue grinder. The immunoreactive CCK content of the extracts was determined by a radioimmunoassay system described previously (*2*) in which the cross-reactivities of CCK and its COOH-terminal octapeptide (CCK-8) are virtually identical on a molar basis.

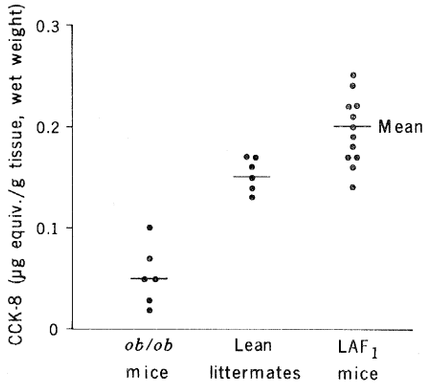

Fig. 1. Scattergram of the concentrations of immunoreactive CCK in extracts of the cerebral cortex of *ob/ob* mice, their nonobese littermates, and normal mice.

The mean body weights (± standard error) of the *ob/ob* mice, their lean littermates, and the LAF_1 mice were 69 ± 2, 30 ± 1, and 28 ± 4 g, respectively. The distribution of the concentrations of immunoreactive CCK in brain extracts of these three groups of animals is shown in Fig. 1. The mean concentrations (± standard error) were 0.05 ± 0.02, 0.15 ± 0.01, and 0.20 ± 0.01 μg of CCK-8, respectively, per gram of tissue (wet weight). There was no difference in brain weight among the three groups. Therefore, the immunoreactive CCK content of the brains of the *ob/ob* mice averaged only about one-third that of their nonobese littermates and one-fourth that of other normal mice.

More than a decade ago Schally and associates (*1*) demonstrated that intravenous and subcutaneous injection of "enterogastrone," a preparation now known to be rich in CCK, caused reduced food intake in mice, whereas glu-

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