

lipolysis and glucose metabolism and whether the oxytocin-related mediators have any relation to the chemical messengers thought to be involved in the action of insulin (8).

Administration of vasopressin does not render Brattleboro rat adipocytes responsive to oxytocin (7). Since the defect in oxytocin activation of pyruvate dehydrogenase cannot be corrected by the missing hormone or by other agents (glucose, diethyl stilbestrol, calcium) which affect oxytocin action (9), it may be assumed that the defect is transmitted genetically along with the defect in vasopressin biosynthesis. To our knowledge, this is the first report to describe oxytocin-mediated stimulation of pyruvate dehydrogenase activity in rat adipose tissue and the first indication that activation of pyruvate dehydrogenase is a metabolic step at which a genetically determined defect in hormone action occurs.

Oxytocin may stimulate the activity of other cellular enzymes, such as glucose-6-phosphate dehydrogenase and glycogen synthetase. The lack of stimulation of pyruvate dehydrogenase in Brattleboro rat adipocytes may, however, be sufficient to explain the lack of oxytocin-stimulated lipogenesis. The inability of these adipocytes to respond to oxytocin in terms of glucose oxidation suggests that activation of glucose-6-phosphate dehydrogenase by oxytocin may also be impaired; oxytocin-stimulated glucose oxidation proceeds largely through the pentose-phosphate pathway (3). It remains to be determined whether the defect we describe is restricted to adipocytes or whether numerous different tissues are involved. Since phosphorylation and dephosphorylation mechanisms govern the activity of pyruvate dehydrogenase (8, 10) it is important to evaluate the role of such reactions in the action of oxytocin. In this respect, the homozygous Brattleboro rat may prove a most useful genetic model, not only for sorting out the mechanisms of oxytocin action but also for uncovering new aspects of the action of insulin.

Note added in proof: A recent report (16) also documents the stimulation of pyruvate dehydrogenase activity by oxytocin.

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References and Notes

1. J. Katz and H. G. Wood, *J. Biol. Chem.* **235**, 2165 (1960).
2. S. Itzhaki and E. Wertheimer, *Endocrinology* **61**, 72 (1957); A. I. Winegrad and A. E. Renold, *J. Biol. Chem.* **233**, 267 (1958).
3. A. Mirsky and G. Perisutti, *Endocrinology* **71**, 158 (1962); D. B. Muchmore, S. A. Little, C. deHaen, *J. Biol. Chem.* **265**, 365 (1981).
4. T. Braun, O. Hechter, J. Rudinger, *Endocrinology* **85**, 1092 (1969); D. Bonne and P. Cohen, *Eur. J. Biochem.* **56**, 295 (1975).
5. Homozygous Brattleboro rats exhibit an autosomal recessive defect in the biosynthesis of vasopressin [H. Valtin and H. A. Schroeder, *Am. J. Physiol.* **206**, 425 (1964)].
6. H. J. Goren, R. M. Geonzon, M. D. Hollenberg, K. Lederis, D. O. Morgan, *J. Supramol. Struct.* **14**, 129 (1980).
7. H. J. Goren, K. Hanif, K. Lederis, M. D. Hollenberg, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 742 (1981); K. Hanif, K. Lederis, H. J. Goren, *Proc. Can. Fed. Biol. Soc.* **24**, 163 (1981).
8. K. Cheng, G. Galasko, L. Huang, J. Kellogg, J. Larner, *Diabetes* **29**, 659 (1980); F. L. Kiechle, L. Jarrett, N. Kotagal, D. A. Popp, *J. Biol. Chem.* **256**, 2945 (1981); J. R. Seals and M. P. Czech, *J. Biol. Chem.* **256**, 2894 (1981).
9. Calcium and glucose concentrations affect the basal rate of glucose oxidation in isolated adipocytes (4). In adipocytes from homozygous Brattleboro rats Ca^{2+} and glucose did not alter oxytocin's inability to stimulate glucose oxidation. Similarly, pretreatment (24 hours prior to assay) of the rats with diethyl stilbestrol had no effect on adipocyte responsiveness (7).
10. W. A. Hughes, R. W. Brownsey, R. M. Denton, *Biochem. J.* **192**, 469 (1980).
11. S. I. Taylor, C. Mukherjee, R. L. Jungas, *J. Biol. Chem.* **248**, 73 (1973).
12. M. Rodbell, *ibid.* **239**, 375 (1964).
13. J. Gliemann, S. Gammeltoft, J. Vintin, *ibid.* **250**, 3368 (1975).
14. W. M. Taylor, M. D'Costa, A. Angel, M. L. Halperin, *Can. J. Biochem.* **55**, 982 (1977).
15. M. B. Davidson and R. Karjala, *J. Lipid Res.* **11**, 609 (1970).
16. S. P. Mukherjee and C. Mukherjee, *Arch. Biochem. Biophys.* **214**, 211 (1982).
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Serotonin and Octopamine in the Nematode

Caenorhabditis elegans

Abstract. *The biogenic amines serotonin and octopamine are present in the nematode Caenorhabditis elegans. Serotonin, detected histochemically in whole mounts, is localized in two pharyngeal neurons that appear to be neurosecretory. Octopamine, identified radioenzymatically in crude extracts, probably is also localized in a few neurons. Exogenous serotonin and octopamine elicit specific and opposite behavioral responses in Caenorhabditis elegans, suggesting that these compounds function physiologically as antagonists.*

The circuitry of the 302-cell nervous system of the free-living nematode *Caenorhabditis elegans* has been established from electron micrographs of serial sections (1-6). In addition, the functions of individual neurons have been determined from experiments in which cells were ablated physically with a laser microbeam (7, 8) or genetically as a result of mutations (8, 9). However, a detailed understanding of the *C. elegans* nervous system requires establishing the identity and function of the chemical signals utilized by each neuron. Dopamine (10) and acetylcholine (11-15) have been implicated as neurotransmitters in *C. elegans*. We now report that the biogenic amines serotonin (5-hydroxytryptamine) and octopamine (*p*-hydroxyphenylethanolamine) are present in *C. elegans* and may act antagonistically.

Serotonin was detected in *C. elegans* by the technique of formaldehyde-induced fluorescence (FIF). Rapidly fading yellow FIF, which is characteristic of serotonin (16), was seen as three chains of varicosities in the pharynx (Fig. 1a). The two cell bodies from which these chains emanated were not visualized by FIF in wild-type animals; however, in the mutant *cat-1(e1111)*, in which dopa-

mine is restricted to the cell bodies (10), two pharyngeal cell bodies with yellow FIF were seen (not shown). The cell bodies as well as their processes were more easily observed in wild-type animals that have been exposed to exogenous serotonin (Fig. 1b), indicating the presence of a serotonin uptake system in these cells.

We used the positions and morphologies of the serotonergic cells to identify them as the two pharyngeal neurosecretory motoneurons (NSM's) previously described by Albertson and Thomson (Fig. 1c) (4). They noted the presence of dense-cored vesicles in the NSM's. Dense-cored vesicles are also found in aminergic neurons in other organisms (17). It has been suggested that serotonin has a neurohumoral role in a number of invertebrate species (18, 19).

Octopamine was identified radioenzymatically in extracts of *C. elegans*. The animals were grown on petri dishes containing NG agar (11) seeded with *Escherichia coli* NA22, removed from the dishes in distilled water (4°C), washed three times in cold water, centrifuged, and weighed. For each 0.1 g of nematodes, 0.6 ml of 10 mM formic acid was added. The animals were then sonicated; disrup-

tion was confirmed with a dissecting microscope. Debris was removed by centrifugation for 2 minutes at 2000 rev/min, and the clear supernatant was heated at 90°C for 3 minutes. The precipitate was removed by centrifugation for 5 minutes at 5000 rev/min and the supernatant was frozen for later assay. Octopamine concentrations were determined biochemically with the method of Evans (20). This assay involves the transfer by phenylethanolamine-*N*-methyltransferase of a radioactive methyl group from [³H]methyl-*S*-adenosyl-*L*-methionine to octopamine to form [³H]synephrine (*N*-methyl-octopamine), which is selectively extracted. The amount of radioactivity is determined by liquid scintillation counting. Identification of the radioactive product as [³H]synephrine was confirmed by subjecting it to chromatography in a variety of solvents (20).

An adult-enriched population of *C. elegans* hermaphrodites grown at 20°C contained $0.13 \pm 0.06 \mu\text{g}$ ($N = 13$) of octopamine per gram of wet weight. This level is similar to that of dopamine (10). Since FIF has shown that dopamine in *C. elegans* hermaphrodites is localized in eight neurons (10), it is plausible that the octopamine is also localized in a few neurons. That mutants with neuronal abnormalities have been found to be octopamine-deficient also supports the hypothesis that octopamine is localized in the nervous system. Octopamine and dopamine are probably synthesized in different cells; otherwise their combined biosynthetic enzymes could generate norepinephrine (18), which is not present in *C. elegans* (10).

Octopamine concentrations were strikingly age-dependent. A population of *C. elegans* was separated into groups of different sizes (and hence different ages) in a gradient of 1 to 5 percent sucrose (21). Fertilized eggs and larvae at all stages had approximately five times less octopamine per gram of wet weight than adults, suggesting that the amine plays a role in adult behavior. Octopamine levels were also dependent on the temperature at which the animals were grown: adults that were grown at 15°, 20°, or 25°C contained 0.3, 0.1, or 0.05 μg of octopamine per gram, respectively.

Exogenous serotonin elicited three distinct behavioral responses by *C. elegans*. First, serotonin depressed locomotion. Serotonin-treated animals were relatively inactive, and moved only briefly when touched with a fine platinum wire. Second, serotonin stimulated pharyngeal pumping. Adult hermaphrodites exposed

to serotonin at 10 mg/ml pumped 267 ± 4 times per minute ($N = 10$), whereas untreated animals pumped only 22 ± 6 times per minute ($N = 10$) (22). Third, in adult hermaphrodites serotonin stimulated the rate at which eggs were released from the uterus (Fig. 2a). Similar effects of exogenous serotonin have been reported by Croll (23). The serotonergic NSM's may modulate pharyngeal pumping, locomotion, and egg laying, since they innervate pharyngeal nerves and muscles and appear to have neurohumoral outputs to the pseudocoelom (4).

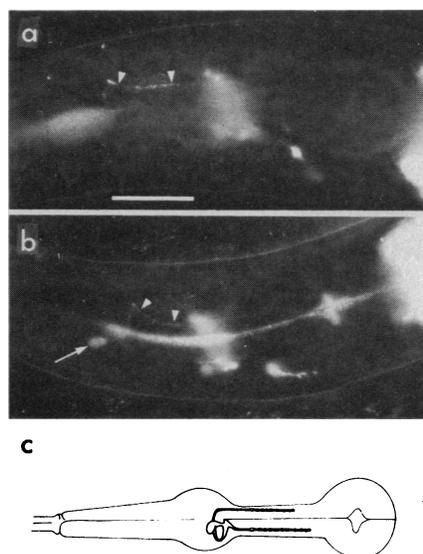


Fig. 1. Serotonergic neurosecretory motoneurons in the *C. elegans* pharynx. (a) Yellow FIF in the NSM processes of an adult hermaphrodite, as visualized by the technique of Sulston *et al.* (10). Focusing through a specimen reveals three distinct chains of varicosities, one each in the left subventral, right subventral, and dorsal pharyngeal nerve cords; the two dorsal processes (one from the left NSM and one from the right NSM) are not resolved and appear as a single chain (arrowheads). FIF is also visible in males and in larvae of all stages. This fluorescence is dependent on treatment with formaldehyde, indicating that it is not autofluorescence. The other visible FIF is green and is located in the dopaminergic processes of the circumpharyngeal nerve ring and in the cell bodies associated with these processes (10). Scale bar, 20 μm . (b) FIF in a wild-type animal exposed to serotonin (1 mg/ml) for 4 hours before being frozen. NSM cell bodies are visible; one is indicated by the arrow. Arrowheads point to the dorsal chain of varicosities. Two phasmidial neurons in the tail (5) and, occasionally, two other cells in the head also accumulate some exogenous serotonin. Exposure to 5-hydroxytryptophan, but not to tryptophan, results in similar loading of the NSM's. (c) Line drawing of one NSM [adapted from Albertson and Thomson (4)].

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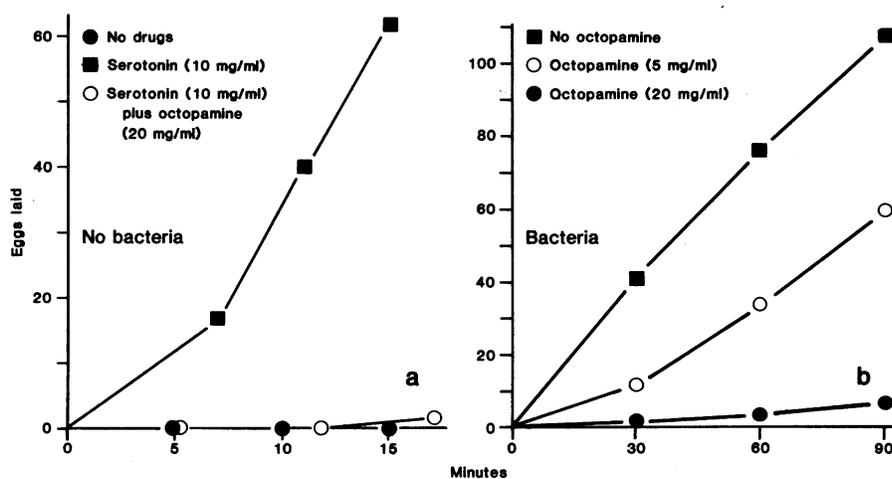


Fig. 2. Effects of serotonin and octopamine on egg laying. Young adult hermaphrodites were transferred to petri dishes with various concentrations of serotonin or octopamine, or both, in NG agar. At various times after transfer, the number of eggs laid was counted. The relatively high concentrations of amine needed to affect egg laying may reflect the general impermeability of *C. elegans* (10, 11, 13). (a) Results for dishes not containing bacteria. Each curve represents data for one petri dish containing ten hermaphrodites. (b) Results for dishes seeded with *E. coli* OP50. Each point represents the mean of four experiments, each involving ten hermaphrodites on one petri dish.

blocks an octopamine receptor in the locust (25), stimulated egg laying in *C. elegans* hermaphrodites not exposed to any other drugs: ten adult hermaphrodites placed in microtiter wells containing phentolamine (10 mg/ml) in M9 buffer (10) released 53 eggs in 30 minutes, whereas untreated control animals released no eggs. This observation suggests that endogenous octopamine also inhibits egg laying. The increased levels of endogenous octopamine seen in adults may reflect the fact that egg laying is an adult behavior.

The finding that serotonin and octopamine affect pharyngeal pumping and egg laying oppositely suggests that these amines function as antagonists physiologically. Consistent with this hypothesis, octopamine depressed serotonin-stimulated egg laying (Fig. 2a). (Octopamine did not, however, prevent serotonin-stimulated pharyngeal pumping.) Antagonistic effects of these amines have been observed in other invertebrates (25, 26).

We have identified mutant strains of *C. elegans* with reduced levels of serotonin or octopamine. The *unc-86* mutants (9) are deficient in yellow FIF of the NSM's. Three mutants with well-characterized neuronal defects—*daf-10(e1387)* (27), *che-3(e1124)* (28), and *osm-3(p802)* (29, 30)—lack detectable levels of octopamine (that is, contain less than 0.02 µg/g). We hope that these and additional mutants may be used to define the roles of serotonin and octopamine in *C. elegans*.

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References and Notes

1. S. Ward, N. Thomson, J. White, S. Brenner, *J. Comp. Neurol.* **160**, 313 (1975).
2. R. Ware, D. Clark, K. Crossland, R. Russell, *ibid.* **162**, 71 (1975).
3. J. White, E. Southgate, N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **275**, 327 (1976).
4. D. Albertson and N. Thomson, *ibid.*, p. 299.
5. J. Sulston, D. Albertson, N. Thomson, *Dev. Biol.* **78**, 542 (1980).
6. J. White, E. Southgate, N. Thomson, S. Brenner, personal communication.

7. J. White and R. Horvitz, *Electro-Opt. Syst. Des.* (August 1979), p. 23.
8. M. Chalfie and J. Sulston, *Dev. Biol.* **82**, 358 (1981).
9. J. Sulston and H. R. Horvitz, *ibid.*, p. 41.
10. J. Sulston, M. Dew, S. Brenner, *J. Comp. Neurol.* **163**, 215 (1975).
11. S. Brenner, *Genetics* **77**, 71 (1974).
12. R. L. Russell, C. D. Johnson, J. B. Rand, S. Scherer, M. S. Zwass, in *Molecular Approaches to Eucaryotic Genetic Systems*, vol. 8, ICN-UCLA Symposia on Molecular and Cellular Biology Series, G. Wilcox, J. Abelson, C. F. Fox, Eds. (Academic Press, New York, 1977), pp. 359–371.
13. J. Lewis, C. Wu, J. Levine, H. Berg, *Neuroscience* **5**, 967 (1980).
14. C. Johnson, J. Druckett, J. Culotti, R. Herman, P. Meneely, R. Russell, *Genetics* **97**, 261 (1981).
15. J. Culotti, G. von Ehrenstein, M. Culotti, R. Russell, *ibid.*, p. 281.
16. T. Caspersson, N. Hillarp, M. Ritzen, *Exp. Cell Res.* **42**, 415 (1966).
17. M. S. Livingstone, S. G. Schaeffer, E. A. Kravitz, *J. Neurobiol.* **12**, 27 (1981).
18. M. J. Berridge and W. T. Prince, *Adv. Insect Physiol.* **9**, 1 (1972).
19. P. D. Evans, *ibid.* **15**, 315 (1980).
20. ———, *J. Neurochem.* **30**, 1009 (1978).
21. J. Sulston and S. Brenner, *Genetics* **77**, 95 (1974).
22. Animals were placed on petri dishes containing various concentrations of serotonin in NG agar.

- After 10 to 20 minutes the rate of pharyngeal pumping was determined by direct observation. The plates did not contain bacterial lawns.
23. N. Croll, *Can. J. Zool.* **53**, 894 (1975).
 24. These experiments were performed as described in (22), except that the plates contained octopamine instead of serotonin and had lawns of *E. coli* OP50.
 25. P. D. Evans and M. O'Shea, *J. Exp. Biol.* **73**, 235 (1978).
 26. M. S. Livingstone, R. M. Harris-Warrick, E. Kravitz, *Science* **208**, 76 (1980).
 27. P. Albert, S. Brown, D. Riddle, *J. Comp. Neurol.* **198**, 435 (1981).
 28. J. Lewis and J. Hodgkin, *ibid.* **172**, 489 (1977).
 29. J. Culotti and R. Russell, *Genetics* **90**, 243 (1978).
 30. E. Hedgecock, personal communication.
 31. We thank D. Moerman for sharing his results with us. Supported by Public Health Service grant GM24663 (H.R.H.), Public Health Service and Muscular Dystrophy Associations of America research fellowships (H.R.H.), a Muscular Dystrophy Association research fellowship and a research fellowship of the American Heart Association and the British Heart Foundation (M.C.), and Public Health Service training grant GM07287 (C.T.).
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Physiological Basis for Swimming Endurance Differences Between LDH-B Genotypes of *Fundulus heteroclitus*

Abstract. Adenosine triphosphate levels in erythrocytes are correlated with LDH-B genotype in *Fundulus heteroclitus*. Adenosine triphosphate is the fish's allosteric modifier of hemoglobin oxygen affinity. Since oxygen delivery to muscle affects swimming performance, fish of each homozygous LDH-B phenotype were swum to exhaustion at 10° or 25°C to determine whether in vitro differences attributed to the LDH-B allelic isozymes were manifest in vivo. At 10°C, the critical swimming speed of the LDH-B^aB^a phenotype was 3.6 body lengths per second, whereas that of the LDH-B^bB^b phenotype was 4.3 body lengths per second. At 25°C there were no differences between LDH-B phenotypes in erythrocyte adenosine triphosphate levels, blood oxygen affinity, or swimming performance.

Adenosine triphosphate (ATP) is the major organic phosphate in the erythrocytes of many fish species (1). Its role as an allosteric modifier of fish hemoglobins is similar to that of 2,3-diphosphoglycerate in mammalian red blood cells in that it decreases the affinity of hemoglobin for oxygen, facilitating oxygen delivery to tissues (2).

Erythrocyte organic phosphate concentrations are heritable traits in humans (3), rats (4), and fish (5). In humans, this trait is correlated with genetic variability of both pyruvate kinase and hexokinase (3). In the fish *Fundulus heteroclitus*, erythrocyte organic phosphate (that is, ATP) levels are correlated with genetic variation at the LDH-B locus (5). Erythrocyte ATP levels are lower in fish with the LDH-B^aB^a phenotype than they are in those with the LDH-B^bB^b phenotype, and concentrations in heterozygotes (LDH-B^aB^b) are intermediate (5). Since ATP is the major organic phosphate in *Fundulus* red cells (1, 6, 7), oxygen affinity differences also exist between LDH-B phenotypes (5). However, the mecha-

nism by which genetic variation at the LDH-B locus affects erythrocyte ATP levels is not known. Either the LDH-B isozyme influences erythrocyte ATP metabolism, or the LDH-B locus is linked to a second locus that affects ATP production.

Kinetic analyses of purified LDH-B allelic isozymes indicated that the greatest catalytic differences between LDH-B^aB^a and LDH-B^bB^b exist at low temperature (10°C), with no significant difference at high temperature (approximately 25°C) (8). Thus, if the LDH-B isozyme has a metabolic influence on erythrocyte ATP concentration, differences in ATP and blood oxygen affinity should exist at acclimation temperatures below 25°C. Furthermore, since organic phosphate amplifies the Bohr effect of *F. heteroclitus* hemoglobins (6, 7), this phenomenon should be exaggerated at low blood pH values, like those produced during swimming performance experiments. We now report that swimming performance is highly correlated with genetic variation at the LDH-B locus for

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