lated spleen lymphocytes 2 to 6 hours after culturing was shown to be an effect of serum on B lymphocytes: a low serum concentration (0.5 percent) eliminates the effect (Fig. 1B); purified B lymphocytes show this effect, but purified T lymphocytes do not (*8*).

Other experiments demonstrated that intracellular alkalinization occurs only in the mitotically responding cells. Populations of spleen lymphocytes containing only functional B cells (that is, lymphocytes from nude mice or preparations in which T cells were removed by monoclonal α-θ antibody and complement) incorporated thymidine and became alkaline in response to LPS, but not to Con A. In purified T lymphocytes and the lymphocytes from C3H/HeJ mice, which are unresponsive to LPS (*9*), mitogenesis and alkalinization were found only after Con A administration (*4*). In all cases the time course and the magnitudes of the intracellular *p*H changes closely resembled the results shown in Fig. 1.

The rate of DNA synthesis is strongly correlated with intracellular *p*H from the time at which growth begins (12 to 24 hours after stimulation) until senescence of the cells (*4*). This correlation could arise either from independent increases in DNA synthesis and intracellular *p*H, from an effect of DNA synthesis on intracellular *p*H, or from an effect of intracellular *p*H on DNA synthesis. To distinguish between these possibilities, we need to manipulate intracellular *p*H and DNA synthesis independently.

To study the relation between intracellular *p*H and DNA synthesis rate, two types of experiments were performed: (i) 48-hour Con A blasts were incubated with inhibitors of DNA synthesis (1 or 5 m*M* hydroxyurea, 2.5 or 7.5 m*M* thymidine, or 50 μg of mitomycin C per milliliter) which reduced the rate of DNA synthesis by about 30-fold but did not alter the intracellular *p*H; and (ii) the intracellular *p*H of spleen lymphocytes was changed by transferring cells for 2 hours to fresh media with various *p*H values from 6.7 to 7.9. Each sample was then returned to a medium with *p*H 7.3 (to eliminate effects of growth medium *p*H on uptake rates) for the 1/2 hour needed for measuring both intracellular *p*H and [^{3}H]thymidine incorporation rate. As expected, the average intracellular *p*H of these cells, over the time of measurement, was shifted toward the *p*H of the incubation medium. The rate of DNA synthesis increased with intracellular *p*H in dividing populations (24 or 48 hours) but not in unstimulated populations (Fig. 2). Similar results were obtained either when the cells were not returned to the medium with *p*H 7.3 or when *p*H equilibrium across the cell membrane was altered with lipophilic weak acids or bases (*8*).

The kinetics of lymphocyte blastogenesis have allowed discrimination between alkalinizations associated with the biochemical stimulation of resting cells and with DNA synthesis. The early alkalinization follows the stimulation of resting cells and is associated with a burst of metabolite, RNA, and protein synthesis. As the cells enter the mitotic cycle there is a second alkalinization, which is strongly correlated with the rate of DNA synthesis. In both situations many kinase-dependent reactions occur, and for many of these, higher *p*H not only increases the rate of reaction but also shifts the equilibrium constant toward product formation.

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Inability of Oxytocin to Activate Pyruvate Dehydrogenase in the Brattleboro Rat

Abstract. *Oxytocin has insulin-like activity in that it stimulates lipogenesis and increases pyruvate dehydrogenase activity. However, in adipocytes from homozygous Brattleboro rats oxytocin is incapable of stimulating lipogenesis or pyruvate dehydrogenase activity, although insulin stimulation of both processes is normal and the antilipolytic activity of oxytocin is normal. Thus, the Brattleboro rat provides a new genetic model for the study of oxytocin action, wherein recognition of the chemical mediator is partially defective.*

When glucose enters rat epididymal adipocytes, it is immediately phosphorylated to glucose-6-phosphate (*1*). Subsequently, the sugar may be converted to glycogen or fatty acyl triglycerides, or it may be oxidized to CO_2 (*1*). The rate of formation of these metabolic products increases in the presence of insulin (*2*). Oxytocin, a neurohypophysial peptide structurally unrelated to insulin, has many insulin-like effects in isolated rat adipocytes (*3*). These effects are mediated through the binding of oxytocin to its own receptors on the plasma membrane (*4*).

In adipocytes isolated from the epididymal fat pads of rats homozygous for diabetes insipidus (Brattleboro rats) (*5*), the action of oxytocin [stimulation of glucose oxidation (*6*) or lipogenesis (*7*)] is abolished, whereas insulin-mediated stimulation of these activities is the same as in cells from control animals. Remarkably, in homozygous Brattleboro rats the oxytocin recognition site in adipocytes appears to be intact and the responsiveness of the uterus to oxytocin appears normal (*6*).

These observations suggest to us that the defect in the action of oxytocin in adipocytes from such rats is localized at a biochemical step subsequent to receptor binding but prior to the final biochemical steps involved in glucose oxidation and lipogenesis. Our interest has been focused on pyruvate dehydrogenase, a key lipogenic enzyme that also participates in the oxidation of glucose to CO_2. Insulin stimulates the activity of this enzyme.

We report (i) that oxytocin stimulates pyruvate dehydrogenase activity and lipogenesis in adipocytes from control animals but does not do so in adipocytes

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Table 1. Lipid metabolism in adipocytes from control and homozygous Brattleboro rats. To determine lipogenesis, adipocytes were prepared from fat pads by collagenase digestion (*12*). Fat cells (0.2 ml; ~ 10^5) were incubated for 30 minutes at 37°C with 0.1 ml of Krebs-Ringer bicarbonate buffer (*p*H 7.5) containing 100 n*M* oxytocin, 1.7 n*M* insulin, or no peptide and then with 1.7 ml of Krebs-Ringer bicarbonate buffer containing 0.48 μmole of glucose and [U-^{14}C]glucose (0.2 μCi). After a further 60 minutes' incubation at 37°C, 250-μl portions were transferred to Microfuge tubes containing 100 μl dinonyl phthalate (*13*). The fat cell pellet obtained by centrifugation was transferred to 10 ml of toluene containing 0.5 percent 2,5-diphenyloxazole and 0.03 percent 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene and allowed to sit overnight; in the morning 1 ml of water was added. Radioactivity was determined with ~ 65 percent efficiency in a Beckman LS-250 scintillation system. Lipogenesis is expressed as nanomoles of glucose converted to triglyceride per 10^5 cells per hour. Cell number was estimated from the lipid content of the fat cell suspension (*6*). To determine lipolysis, 10^5 adipocytes prepared as described above were incubated for 10 minutes at 37°C in 1.1 ml of Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing 1.8 n*M* insulin, 110 n*M* oxytocin, or no peptide (control). Then 0.1 ml of 3.76 μ*M* norepinephrine bitartrate was added and the fat cell suspensions were incubated for an additional 45 minutes at 37°C. Adipocyte suspensions (1 ml) were deproteinated with perchloric acid and the supernatant was assayed for glycerol content (*14*). Glycerol dehydrogenase (0.1 U) and 6 mg of β-nicotinic acid dinucleotide in 4 ml of 0.05*M* glycine buffer (*p*H 7.5) were mixed with 1 ml of the deproteinated sample and incubated for 90 minutes at 37°C. Reduced nicotinic acid dinucleotide was measured spectrofluorometrically (excitation 350 nm, emission 465 nm) (*15*). Lipolysis is expressed as nanomoles of glycerol released per 10^5 cells per 45 minutes. All values are means ± standard errors.

Strain	Lipogenesis (nmole/hour per 10^5 cells) (N = 4)			Lipolysis (nmole/45 minutes per 10^5 cells) (N = 3)		
	Basal	Oxytocin	Insulin	Control	Oxytocin	Insulin
Sprague-Dawley	4.1 ± 0.3	10.4 ± 0.3	22.2 ± 0.7	45.8 ± 0.4	32.8 ± 0.1	14.9 ± 0.2
Long-Evans	3.5 ± 0.2	5.4 ± 0.3	14.8 ± 0.5	66.8 ± 1.2	48.1 ± 0.6	30.7 ± 0.5
Homozygous Brattleboro	4.2 ± 0.1	4.2 ± 0.2	18.9 ± 0.7	83.6 ± 0.8	55.4 ± 0.7	44.0 ± 2.1

from Brattleboro rats and (ii) that oxytocin acts as an antilipolytic agent in Brattleboro rat adipocytes despite its lack of effect on lipogenesis and pyruvate dehydrogenase activity. This suggests that, although there is a selective defect in oxytocin action in these adipocytes, at least part of the oxytocin receptor–mediator system is still intact.

The basal level of pyruvate dehydrogenase activity is about the same in adipocytes from two strains of control rats, Sprague-Dawley and Long-Evans: 52 ± 2 and 48 ± 1 nmole per gram of tissue in 2 minutes, respectively (Fig. 1). Basal pyruvate dehydrogenase activity in adipocytes from homozygous Brattleboro rats is much lower at 21 ± 1 nmole/g in 2 minutes. This is somewhat surprising, since the basal rate of lipogenesis is similar in all three strains of rats (Table 1) (*7*). Insulin-stimulated pyruvate dehydrogenase activity varies among the three strains (Fig. 1), as does insulin-stimulated lipogenesis. There does not, however, appear to be a correlation between the degree of insulin-stimulated pyruvate dehydrogenase activity and the magnitude of insulin-stimulated lipogenesis (Table 1) (*7*). In contrast, oxytocin-stimulated pyruvate dehydrogenase activity broadly parallels the hormone's effect on lipogenesis; oxytocin is most effective in Spraque-Dawley rats, less effective in Long-Evans rats, and ineffective in Brattleboro rats (Fig. 1 and Table 1).

Although oxytocin has no effect on glucose oxidation (*6*) and lipogenesis in homozygous Brattleboro rats, it does inhibit catecholamine-stimulated lipolysis. Glycerol was released from 10^5 cells in the presence of 0.3 μ*M* norepinephrine at the following rates for Sprague-Dawley, Long-Evans, and homozygous Brattleboro rats: 45.8 ± 0.4, 66.8 ± 1.2, and 83.6 ± 0.8 nmole in 45 minutes, respectively. When 100 n*M* oxytocin was added to the respective incubation media these values decreased to 32.8 ± 0.1, 48.1 ± 0.6, and 55.4 ± 0.7—equivalent to a 30 percent decrease in lipolysis. Insulin (1.7 n*M*) inhibited lipolysis 50 to 60 percent (Table 1).

It is likely that oxytocin, on combining with its receptor, causes a cellular response by stimulating the formation of one or more chemical mediators. Since oxytocin is able to inhibit lipolysis in Brattleboro rat adipocytes, it can be concluded that at least a portion of the oxytocin receptor–mediator system is intact in these cells. It remains to be determined whether there are other mediators related to the effects of oxytocin on

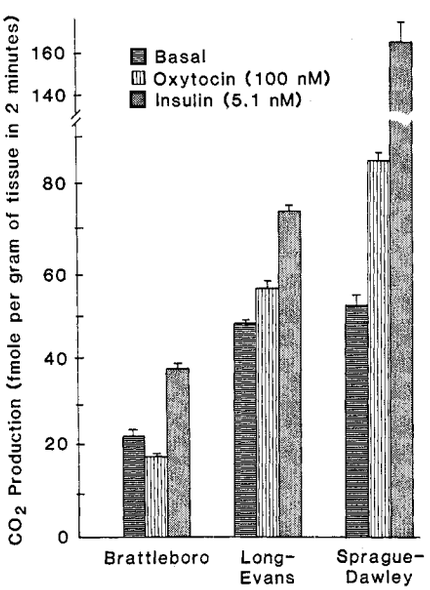

Fig. 1. Pyruvate dehydrogenase activity in rat epididymal fat pads. The data are based on the measurement of $^{14}CO_2$ production in the conversion of [1-^{14}C]pyruvate to acetyl coenzyme A (*11*). Epididymal adipose tissue (100 to 300 mg) was removed from Sprague-Dawley (200 g), homozygous Brattleboro (229 g), and Long-Evans (194 g) rats. The fat pads were incubated for 30 minutes at 37°C in 2 ml of oxygenated Krebs-Ringer bicarbonate buffer (0.5 m*M* $CaCl_2$, 11 m*M* fructose) (*p*H 7.4). This was followed by an additional 30 minutes of incubation in the same buffer containing insulin, oxytocin, or no hormone. The tissue was then homogenized in 0.4 ml of ice-cold phosphate buffer (10 m*M* potassium phosphate, 1 m*M* EDTA, 1 m*M* dithiothreitol, and 1 percent bovine serum albumin) (*p*H 7.4). Portions (0.2 ml) of the homogenized tissue were added to 15-ml Falcon plastic tubes containing 0.3 ml of substrate solution (prewarmed at 37°C for 2 minutes) with a *p*H of 7.4. This solution consisted of 11 m*M* potassium phosphate, 2.8 m*M* $MgCl_2$, 1.6 percent bovine serum albumin, 1.2 m*M* dithiothreitol, 0.16 m*M* coenzyme A, 1.6 m*M* nicotinic acid dinucleotide, 0.08 m*M* cocarboxylase, 0.6 m*M* pyruvate, and [1-^{14}C]pyruvate (final concentration, 0.12 μCi/ml). The assay tubes were immediately sealed with rubber serum stoppers having center wells which contained 1 by 15 cm chromatography paper (Whatman No. 1) impregnated with 0.2 ml of hyamine hydroxide. Less than 5 minutes elapsed between the initiation of homogenization and capping. The tubes were warmed for 2 minutes at 37°C and then 0.8 ml of 0.08*M* citric acid and 0.04*M* Na_2HPO_4 (*p*H 3) was added to the assay solution. Following 30 minutes at room temperature, the filter papers were transferred to scintillation vials containing 15 ml of Bray's scintillation fluid. Radioactivity was determined in a Beckman LS-250 scintillation system. The addition of 75 m*M* fluoride ion to the homogenization buffer did not affect basal or oxytocin-stimulated activity. Values are means ± standard errors (N = 4).

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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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 dopamine 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 m*M* formic acid was added. The animals were then sonicated; disrup-

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