changes in peripheral vasomotor activity may be primary causes of the various temperature changes observed during PS (*4*, *5*). The CNS regulator of T_b has a strong influence on peripheral vasomotor activity. Releasing peripheral vasomotor activity from thermoregulatory control during PS should result in changes in skin temperature, hence, changes in heat exchange between animal and environment and changes in core T_b's. The direction of these changes will depend on whether the peripheral vascular beds were dilated or constricted relative to the thermoneutral condition before the onset of PS.

The fact that proportional regulation of T_b is retained during SWS but not during PS offers an adaptive explanation for the relationship between ambient temperature and proportion of time spent in different sleep states observed in cats and rats. At low ambient temperatures the animals spend a greater proportion of their total sleep time in SWS and a lesser proportion in PS than at thermoneutral temperatures (*11*). The relative increase in SWS at low ambient temperatures enables the maintenance of active thermoregulatory defenses against the cold without drastically curtailing total sleep time.

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Dopaminergic Agents: Influence on Serotonin in the Molluscan Nervous System

Abstract. *Treatment of the mussel* Mytilus edulis *with 6-hydroxydopamine or with α-methyl-p-tyrosine decreased dopamine and increased serotonin in the nervous system. Treatment with dopamine decreased serotonin concentrations and prevented the effect of 6-hydroxydopamine. The serotonin concentration appears to be determined in part by the concentration of dopamine.*

The regulation of the rate of synthesis and turnover of neurotransmitters is of great interest (*1*). Regarding the synthesis of monoamines in the mammalian brain, it was observed that 5-hydroxytryptophan, a precursor of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), decreased the rate of synthesis of norepinephrine (NE) (*2*), whereas norepinephrine decreased the rate of synthesis of 5-HT (*3*). It was suggested that, in situations where dopamine (DA)–containing and 5-HT–containing neurons interact, each type might inhibit the synthesis of the other's transmitter (*3*). The central nervous systems of molluscs have neurons that contain predominantly dopamine (DA) or 5-HT, but the possibility that the rates of synthesis or neuronal content of DA and 5-HT might be interdependent in these animals does not seem to have been studied. In various species the DA and 5-HT content of different ganglia or neurons is influenced by several drugs, such as 6-hydroxydopamine (6-OHDA), dihydroxyphenylalanine, and α-methyl-*p*-tyrosine (α-M*p*T) (*4*, *5*). We have altered, by selective drug treatments, the DA content of neurons in a representative mollusc, *Mytilus edulis*, and have observed changes in the concentrations of 5-HT and DA. The 5-HT content of the ganglia appears to be determined, at least in part, by the concentration of DA.

The marine mussel *Mytilus edulis* was obtained from Woods Hole (Northeast Marine Specimen Company), or collected locally from Long Island Sound and kept in artificial seawater (ASW) at *p*H 7.0 to 7.2 and at 18° to 20°C. The drugs we used were DA, 6-OHDA, α-M*p*T, and 5-HT (all from Sigma Chemical Company). Each drug (1 mg) was dissolved in ASW (1 ml) containing ascorbic acid (0.1 percent). We injected 10 μl of fresh solution into the posterior adductor muscle through a small notch cut in the posterior dorsal rim of the shell near the posterior adductor. Control animals were injected with 10 μl of ASW with ascorbic acid. Each experiment was performed on mussels obtained from the same place at the same time and having the same shell length. Experiments lasted 6 days, each mussel receiving three injections given on alternate days. After treatment, animals were opened and tissue was dissected out; this was either extracted for chemical analysis or freeze-dried for histofluorescence studies. In initial experiments with 6-OHDA we used the same procedure except that we added the drug (4.8 mg) to the ASW (300 ml) bathing an individual mussel. The changes in fluorescence (*6*) were similar to those we report here.

For the histofluorescence studies, excised tissue was freeze-dried and treated with hot paraformaldehyde gas as described by Falck and co-workers (*7*). We used a Reichert Zetopan fluorescence mi-

croscope fitted with a mercury lamp (HBO 200W) and a bright-field or dark-field Reichert condenser. The excitation filter BG-12 permitted activation at 410 to 440 nm, and the filters GG9 and GG1, which excluded ultraviolet-blue, permitted the passage of green fluorescence from DA and yellow fluorescence from 5-HT reaction products. Autofluorescence and nonspecific fluorescence were identified by the standard procedures, including borohydride reduction and reactivation with paraformaldehyde (*8*).

A single lens reflex camera (Minolta SRT 101) was used for photomicroscopy. Black-and-white photography was done with Tri-X film (ASA 400) developed in Kodak D76 and printed on Kodak Polycontrast F or Kodabromide F-2 paper. Color slides were taken with Kodak high-speed Ektachrome and processed by Kodak (ESP-1 processing). The photographs in Fig. 1 were made from color slides.

For extraction and quantitative estimation of tissue serotonin we used the method of Snyder and Taylor (*9*). Fluorescence of the serotonin-ninhydrin reaction product was read in an Aminco-Bowman recording spectrofluorometer with activation at 385 nm and fluorescence at 490 nm, the uncorrected peaks. Specific serotonin fluorescence was identified by the presence of a peak at 490 nm; no such peak occurred in the control extract heated in the absence of ninhydrin. Serotonin creatinine sulfate was used as the internal standard; we estimated the extraction efficiency to be 84 percent.

We examined by histofluorescence the cerebral, pedal, and visceral ganglia, the cerebrovisceral connectives, the branchial nerves, and the gills of treated and control animals. Control animals exhibited the same specific fluorescence as described (*5*, *6*). Cerebral ganglia contained yellow fluorescing nerve cell bodies that contained 5-HT and were arranged in clusters throughout the cortex in close approximation to the connective tissue sheath. Green fluorescing nerve cell bodies containing DA were fewer in number and were arranged closer to the neuropil in small groups. The neuropil consisted mainly of homogeneous green DA fibers and a few 5-HT fibers containing yellow granules. After treatment with 6-OHDA, green nerve cell bodies disappeared from the cortex, and the remaining yellow nerve cell bodies appeared brighter and more numerous. The neuropil lost most of its green fluorescence and contained numerous yellow fibers and granules. Phase contrast microscopy revealed that the nonfluorescing areas in the control animals were filled with cells and fibers, whereas in the 6-OHDA treated animals there were often empty spaces, which we attribute to sites formerly occupied by DA neurons, now degenerated.

The effect of 6-OHDA on the visceral ganglion was similar but even more dramatic because in the control animals the cortex and neuropil were both predominantly green, whereas after treat-

Table 1. Serotonin content of pedal ganglia. Results are expressed as nanograms of serotonin per ganglion pair. Treated mussels (injected with 10 μg of drug in 10 μl of ASW containing 0.1 percent of ascorbic acid) and control mussels (injected with ASW and ascorbic acid) were kept under identical conditions from the time they were collected until the time the tissue was analyzed. The drugs used were 6-hydroxydopamine (6-OHDA) and dopamine (DA). For each treatment, the significance of the difference between treated and control concentrations was determined by Student's *t*-test. Differences in the concentrations among control ganglia are naturally occurring (*13*). *N*, number of pairs; S.D., standard deviation.

Treatment	Treated		Control	
	N	Mean ± S.D.	*N*	Mean ± S.D.
None			3	123 ± 12
None			3	252 ± 34
6-OHDA	2	287 ± 45	2	145 ± 20
6-OHDA	3	445 ± 17	4	263 ± 85
6-OHDA	4	213 ± 18*	4	139 ± 6
6-OHDA + DA	4	150 ± 34*	4	227 ± 35
DA	3	94 ± 11*	4	177 ± 25

*$P < .01$.

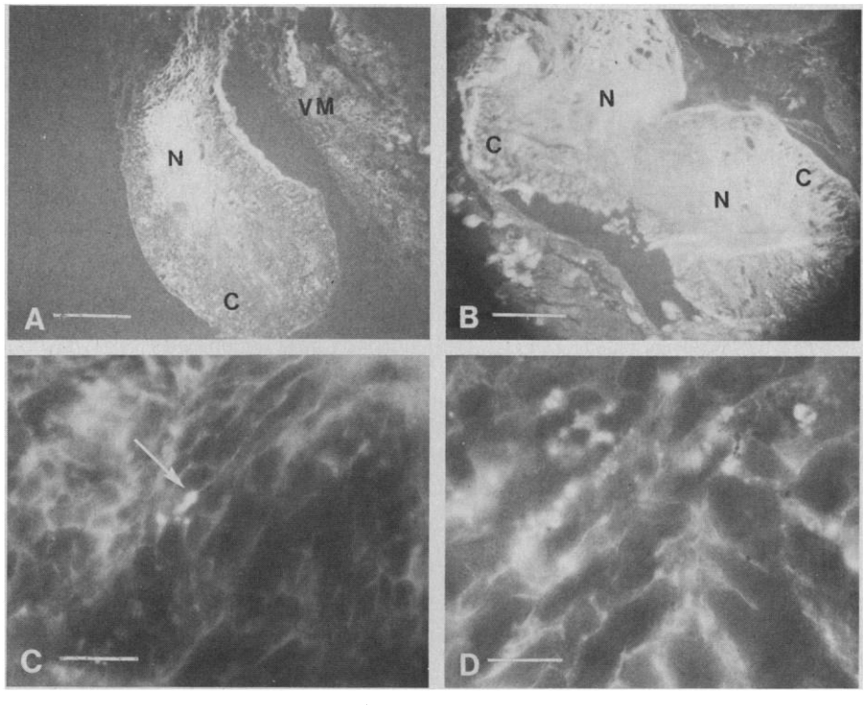

Fig. 1. Fluorescence photomicrographs of 8-μm sections of formaldehyde-treated pedal ganglion tissue of *Mytilus edulis*, reprinted from original color slides. Specific yellow fluorescence (serotonin) and specific green fluorescence (catecholamine) was obtained by exposing the dry tissue to hot formaldehyde gas and activating the reaction product with ultraviolet light. Specific yellow fluorescence decayed visibly within minutes during exposure to ultraviolet light. Specific fluorescence of both types was removed by treatment with borohydride and restored by a second exposure to formaldehyde. Autofluorescence occurred without formaldehyde treatment, and nonspecific fluorescence appeared after formaldehyde treatment but did not meet the above criteria. In all instances, a color photograph was taken, the distribution, intensity, and decay of fluorescent colors was noted, and the appropriate tests performed. (A) Control mussel. A mixture of specific yellow and green fluorescing cells is present in the cortex (*C*), and there is a predominance of green fluorescing fibers in the neuropil (*N*); some attached tissue of the visceral mass (*VM*) has autofluorescence and nonspecific fluorescence (scale, 50 μm). (B) Mussel treated with 6-OHDA. There is a greatly enhanced yellow and almost total loss of green fluorescence (scale, 50 μm). (C) Neuropil of control mussel, showing green fluorescing cells and smooth fiber (arrow) (scale, 20 μm). (D) Neuropil of 6-OHDA treated mussel, showing little green fluorescence and a great increase in yellow fluorescing granules and varicose fibers (scale, 20 μm).

ment they were both predominantly yellow. The neuropil lost its smooth green fibers and instead contained a large number of varicose yellow fibers. We presume that the control neuropil has all these 5-HT fibers but that under normal conditions the 5-HT content is too low to give detectable fluorescence. A comparable loss of green and increase in yellow occurred in the cerebrovisceral connective and in the branchial nerve and gill.

The pedal ganglia, not previously described for this species, are similar to the cerebral ganglia, having a mixture of yellow and green fluorescing cells in the cortex and a predominance of green fibers in the neuropil (Fig. 1A). Treatment of the animal with 6-OHDA removed most of the green and greatly enhanced the yellow (Fig. 1B). In the neuropil, the smooth, green fibers (Fig. 1C) were largely replaced by varicose, yellow fibers and granules (Fig. 1D).

Within certain limits, fluorescence intensity is proportional to the concentration of monoamines present (*10*). Furthermore, the concentration of a monoamine may change without causing an observable change in fluorescence, but an observable change in fluorescence certainly indicates a significant change in monoamine concentration (*10*). This notion has been extended to include the intensity of the photographic image of fluorescent structures (*11*). We are confident, therefore, that the amount of yellow fluorophore in the tissue increased in the 6-OHDA treated animals.

In mammals, 6-OHDA specifically damages catecholaminergic neurons and markedly lowers their neurotransmitter content, while the concentration of 5-HT in serotonin-containing neurons remains the same or decreases slightly (*12*). Because we found in *M. edulis* that 6-OHDA increases the yellow fluorescence, we repeated our experiment and determined by chemical analysis the 5-HT content of the pedal ganglia. These ganglia can be excised quickly and uniformly (*13*). Comparing the amounts of 5-HT per pair of pedal ganglia, we found that although the average 5-HT content of different control groups differed considerably, the variation within each group was reasonably small (Table 1). We therefore compared each drug treated group with a control group of mussels collected, injected, dissected, and analyzed at the same time. In each of three separate experiments there was an increase in the 5-HT content of the 6-OHDA treated group compared with the control group receiving ASW alone (Table 1).

It was possible that the effect we obtained was due to the removal of DA. We therefore repeated the experiment, injecting the 6-OHDA treated animals with 10 μg of DA on alternate days. This prevented both the increase in yellow histofluorescence and the increase in 5-HT (Table 1); the green fluorescing cells were not destroyed. It appears that DA, 6-OHDA, and 5-HT may interact as follows. First, DA may compete with 6-OHDA for uptake by the dopaminergic neurons thereby affecting both the dopaminergic and serotonergic cells. However, DA may also act directly on the 5-HT neurons (*5*). When we injected DA or ASW on alternate days to two groups of mussels for 6 days, the 5-HT content of the pedal ganglia was depressed by the DA (Table 1) which is known to accumulate in the ganglia (*5*).

The drug α-M*p*T inhibits the synthesis and reduces the concentration of catecholamines, including dopamine, in the mammalian brain but only slightly increases or has no effect on the concentration of 5-HT (*14*). All the ganglia from five mussels injected with 10 μg of α-M*p*T on three alternate days showed a decrease in green fluorescence and an increase in yellow fluorescence compared with the ganglia of five mussels given ASW. There was no indication of degenerating neurons. These data support the hypothesis that the concentration of 5-HT in the tissue is strongly influenced by the concentration of DA in catecholaminergic neurons. The data also suggest that the biosynthesis of 5-HT may not be subject to end-product inhibition, because there can be a two- to threefold increase in the concentration of 5-HT under experimental conditions.

In summarizing the evidence for the end-product inhibition of 5-HT synthesis in the mammalian brain, Glowinski (*15*) noted that some evidence supports a contrary conclusion. Most authors agree that the concentration of 5-HT may stay constant in the presence of marked changes in turnover (synthesis and release). Evidence is accumulating that 5-HT turnover is accelerated by a decrease in the activity of catecholamine-containing neurons as, for example, that brought about by treatment with α-M*p*T (*14*), 6-OHDA (*16*), localized lesions of DA neurons (*16*), or by selectively blocking norepinephrine receptors with methiopin (*17*). Conversely, 5-HT turnover has been decreased by treatment with dihydroxyphenylalanine, which increases the concentration of catecholamine transmitter (*17*). In general, the activity of catecholamine neurons in the mammalian brain appears to regulate the turnover of 5-HT in associated serotonergic neurons, the 5-HT levels remaining relatively constant. Our data do not include turnover rates but they indicate that in molluscs the activity of catecholaminergic neurons regulates the level of 5-HT in serotonergic neurons. Neither the concentration nor the turnover rate of 5-HT in the central nervous systems of mammals necessarily indicates the level of functional activity of the neurons (*18*). The marked changes in 5-HT concentrations in molluscan ganglia under various conditions may represent changes in a functionally inactive pool of 5-HT. However, the concentration of 5-HT in the molluscan nervous system appears to be more variable than that in the mammalian brain, as exemplified by the natural variation in different batches of mussels, and by the experimentally induced changes.

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