lowed to feed freely on carrot leaves with or without angelicin, applied as described earlier. Each day, feces were removed and fresh food plant was supplied. There were 18 caterpillars in each treatment; all were raised under controlled conditions (16:8 light-dark photoperiod, 24.5° and 15.5°C; 45 percent relative humidity). After caterpillars pupated, pupae were sexed and weighed. Upon emergence, female butterflies were mated with males from the same treatment group, placed in a screened cage and given a carrot plant for oviposition. Butterflies were fed daily with a solution of honey and water (1:2). Eggs were removed and counted every 3 days until the butterflies died.

Although there were no significant differences between treatments for larval development and pupal weight for males (Table 1), there were highly significant differences for females. Larvae on leaves with angelicin grew more slowly ($P < .025$) and weighed less at pupation ($P < .05$) than larvae raised on control leaves. Reduced pupal weight is correlated with reduced adult body size, which correlates with reduced fecundity (*16*). In this experiment, we found a 3.5-fold difference in average egg production between the two treatments; individual butterflies in the control treatment laid up to 700 more eggs than did individuals in the experimental treatment.

Individuals feeding on plants containing angelicin are likely to experience a substantial reduction in fitness; the deleterious effect is due to ingestion of angelicin and not to reduced consumption rate. Ability to tolerate linear furanocoumarins does not appear to confer ability to tolerate angular furanocoumarins. The presence of angular furanocoumarins in advanced tribes of the Umbelliferae may thus be an evolutionary response to selective pressures from insects adapted to feeding on umbellifers containing linear furanocoumarins.

Circumstantial evidence suggests, however, that even the angular furanocoumarins are not immune to counteradaptation by insects. While most of the butterflies in the *machaon* complex, the group to which *P. polyxenes* belongs, feed as caterpillars on a wide variety of umbelliferous plants, *P. brevicauda,* the short-tailed swallowtail of Newfoundland, feeds exclusively on plants in the genera *Heracleum, Angelica, Ligusticum,* and *Petroselinum (Apium)* (*8, 17*). Of the four genera, all but *Petroselinum* are reported to contain angular furanocoumarins (*2*). It remains to be seen whether *P. brevicauda* has developed resistance to the toxicological effects of angular furanocoumarins and, as a result, evolved to specialize on umbelliferous hosts that are largely unexploited by its congeners.

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11. Relative growth rate is calculated as milligrams of dry weight gained per milligram of initial dry weight of larva. Initial dry weights of the larvae were estimated by averaging the dry weights of additional freshly molted larvae starved for 24 hours; the average percent dry weight was used as a conversion factor and applied to the known fresh weight of each experimental larva.
12. Relative consumption rate is calculated as milligrams of dry weight of food eaten per milligram of initial dry weight of larva. Dry weights of food eaten were estimated by calculating the average moisture content of additional leaves of each species and using the average as a conversion factor to approximate the dry weight of weighed fresh material given to the larvae. Milligrams of food eaten is equal to the calculated dry weight (in milligrams) of fresh material given to larvae minus the measured dry weight (in milligrams) of uneaten food.
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14. *Papilio polyxenes* raised from eggs on an artificial diet containing 1 percent (wet weight) xanthotoxin showed no significant reduction in larval development time, pupal weight, or survival relative to caterpillars raised on a control diet (M. Berenbaum, *Ecol. Entomol.*, in press).
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Transmitter Sensitivity of Neurons Assayed by Autoradiography

Abstract. *Ionic conductance channels that are opened by activating nicotinic acetylcholine receptors at synapses of sympathetic neurons are permeable to small organic amines. Uptake of a tritium-labeled amine through these channels can be measured by autoradiography. This provides a simple and direct way to assess the sensitivity of individual neurons to acetylcholine without using microelectrodes.*

I have developed a new approach to studying the sensitivity of individual neurons to synaptic transmitters. The approach circumvents the need for microelectrodes and is based on the recent electrophysiological finding that various small, positively charged, organic amines can permeate the acetylcholine (ACh)-activated ionic channels of sympathetic neurons (*1, 2*). These channels resemble ACh-activated channels in skeletal muscles of vertebrates, which are known to be permeable to numerous amines (*3*). The ACh-induced uptake of such amines into a neuron, then, would directly reflect the neuron's sensitivity to ACh. Uptake of an amine by individual neurons can be conveniently assayed by autoradiography, provided the amine is radioactively labeled and has a functional group that allows it to be fixed in situ. In this report I demonstrate the feasibility of this approach through the use of 4-[1-^{3}H](aminobutyl)guanidine (tritium-labeled agmatine) (*4*).

Sympathetic ganglia of frogs consist of clusters of ovoid monopolar neurons with cholinergic synapses directly on their cell somas (*5*). Exposure of a sympathetic ganglion (*6*) to cholinergic agonists stimulates the uptake of [^{3}H]agmatine into the cell bodies of these neurons. Thus, for example, neurons treated with carbachol [carbamylcholine (CCh)] during exposure to [^{3}H]agmatine can be readily distinguished from untreated neurons by autoradiography (Fig. 1). Table 1 presents evidence that the stimulated uptake of agmatine occurs through channels associated with nicotinic receptors. It is evident that (i) uptake is induced by nicotine and ACh as well as CCh, but not by the muscarinic agonist bethanechol (carbamyl-β-methylcho-

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Table 1. Uptake of [^{3}H]agmatine into sympathetic neurons induced by activating nicotinic receptors. Uptake experiments were performed essentially as in Fig. 1. In each experiment, a control ganglion treated like the test ganglion, but not exposed to the test drug, was embedded and sectioned together with the test ganglion. Grain densities from at least 20 neurons from each ganglion were sampled and averaged, and the ratio of grain densities (test : control) is given as mean ± standard deviation.

Drug	Relative grain density	Experiments (No.)
1m*M* CCh	4.6 ± 1.6	13
1 m*M* nicotine	4.3 ± 1.8	4
1 m*M* ACh*	3.9 ± 1.7	5
5 m*M* bethanechol	0.8 ± 0.3	2
1 m*M* CCh + 0.1 m*M* DTC†	1.2 ± 0.5	4
0.1 m*M* DTC†	1.1 ± 0.2	4
1 m*M* CCh in high concentration of K^+‡	1.7 ± 0.1	5

*Ganglia were exposed to the anticholinesterase neostigmine (10 μ*M*) for 1 hour before the uptake experiment. †Ganglia were exposed to 0.1 m*M* DTC for 30 minutes before, as well as during, the uptake experiment. ‡Na-Hepes in normal Ringer solution replaced by K-Hepes (*6*) and NaCl replaced by 113 m*M* KCl (two experiments) or 113 m*M* KCH_3SO_3 (two experiments), or 80 m*M* K_2SO_4 (one experiment). Ganglia were in high K^+ media 30 minutes before, as well as during, the uptake experiment.

line); (ii) the nicotinic antagonist *d*-tubocurarine (DTC) blocks the induced uptake (data shown only for CCh treatment); and (iii) agonist-evoked depolarization alone cannot account for the increased uptake, because CCh stimulates the uptake of agmatine when neurons are already depolarized by high concentrations of potassium ions. However, uptake in both test and control ganglia (data not shown), as well as the ratio of the two, is less here than under normal conditions.

Figure 1 also shows that some [^{3}H]agmatine is present in neurons of the control ganglion not treated with CCh. This background uptake is not significantly affected by DTC (Table 1), is fairly constant from one ganglion to the next, and is not reduced when nonradioactive agmatine (1 m*M*) is present (data not shown). The route of this uptake remains to be elucidated.

For a given agonist, the amount of stimulated uptake of agmatine varies in different experiments (Table 1). These differences may arise from variability in the ACh sensitivities of ganglia from one frog to the next. Preliminary experiments indicate that alteration in ACh sensitivity of neurons upon denervation (*7*) can be detected with the new approach (*2*).

Autoradiographic assessment of [^{3}H]-agmatine uptake provides a straightforward means of identifying sympathetic neurons whose nicotinic receptors have been activated. Since the technique is amenable to electron microscopy, it may be possible to study the ACh sensitivities of small processes, such as axons and nerve terminals. In addition to providing an assay for transmitter sensitivity, the approach can be used to detect individual neurons that are synaptically activated by electrical stimulation of their presynaptic nerves (*2*). Since many neurons can be visualized at one time (Fig. 1), this approach can provide a global, albeit static, display of excitatory synaptic activity in a ganglion. The basic approach, in principle, could be used to study any synapse with ionic channels that are permeable to molecules which can be localized by autoradiography or histochemistry.

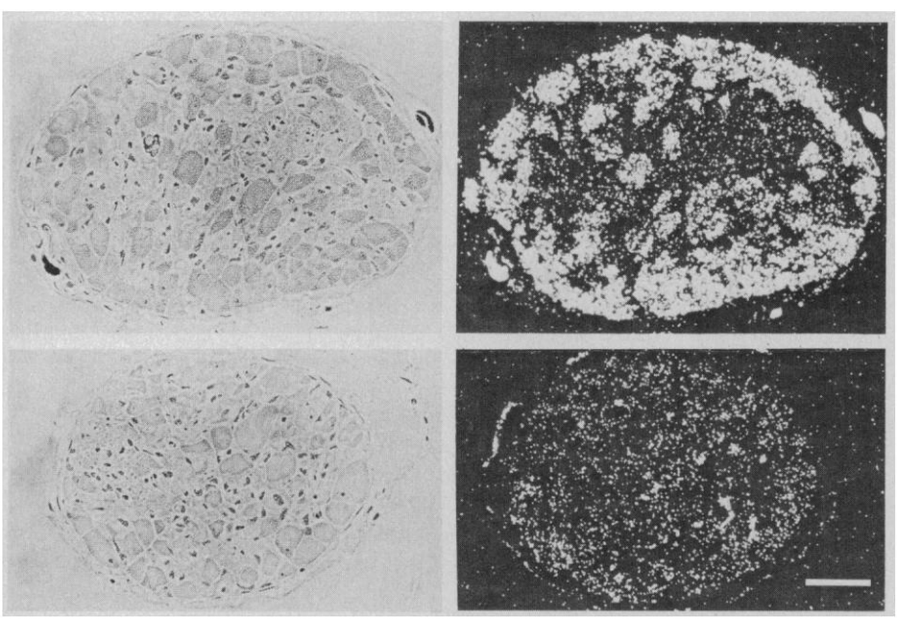

Fig. 1. Light-microscopic autoradiographs of transverse sections through two sympathetic ganglia. Each ganglion was placed in 10 μl of Ringer solution (*6*) containing [^{3}H]agmatine (50 μ*M*, 1 mCi/ml) with (upper ganglion) or without (lower ganglion) carbachol (1 m*M*) for 60 seconds at room temperature (~23°C). Ganglia were then washed for 20 minutes, fixed with 3 percent glutaraldehyde, and processed for autoradiography (*8*). The two ganglia were embedded side by side and sectioned together. Each ganglion was photographed through phase-contrast (left) and darkfield (right) optics. Autoradiographic silver grains appear bright in all photographs. Carbachol treatment increased the average grain density in neurons fourfold. All micrographs are at the same magnification; calibration bar, 50 μm.

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4. Agmatine was selected initially for four reasons. (i) It could permeate the ACh-activated ionic channel (*1, 2*); (ii) it has a primary amino group, which would allow it to be fixed with glutaraldehyde for cellular localization by autoradiography [T. Peters and C. A. Ashley, *J. Cell Biol.* **33**, 53 (1967)]; (iii) it could be readily obtained in high specific radioactivity—[^{3}H]agmatine (99 percent pure) was prepared by enzymatic decarboxylation of L-[5(*n*)-^{3}H]arginine and purified by paper electrophoresis (*2*); and (iv) it was thought that agmatine would not be readily taken up through routes other than ionic channels, since it is not a common metabolite in vertebrates (and a neuron would not be expected to possess a high-affinity uptake system for it) and its lipid solubility would be minimal in view of its two positive charges at neutral *p*H. These considerations do not exclude the possibility that there may be other amines superior to agmatine for this approach.
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6. Ninth and tenth sympathetic ganglia were dissected from 2½-inch *Rana pipiens* and kept in Ringer solution consisting of 113 m*M* NaCl, 1.8 m*M* $CaCl_2$, K-Hepes (2 m*M* KOH, 5 m*M* Hepes), Na-Hepes (2 m*M* NaOH, 5 m*M* Hepes), *p*H 7.2.
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8. Of the radioactivity remaining in the ganglia after washing, more than 90 percent is agmatine as judged by electrophoretic analysis, and about 90 percent is fixed in the ganglion by glutaraldehyde (*2*). After fixation, ganglia were dehydrated, then embedded in Epon. Sections 1 μm thick were dipped in Kodak NTB2 emulsion, and the autoradiographs were developed after exposures of 16 days (Fig. 1) or 1 week (all experiments in Table 1).

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