commodate both optical isomers, the bagworm has one type of receptor that is tuned to receive the *R* enantiomer exclusively.

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References and Notes

1. A pheromone is a chemical stimulus emitted by one individual and perceived by another individual of the same species to regulate behavior.
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4. The entire body of the adult female is hirsute but only morphologically distinct hair from membranous lateral and ventral surfaces of the thorax is dispensed from the pupal shell. Scanning electron microscopy and histological study showed that these deciduous hairs occur in association with large nucleated subcuticular cells that are characteristically secretory. Analyses by gas chromatography (GC) of heptane rinses made of the female's abdomen, ovipositor, and head and thorax showed that all but the head and thorax rinses were devoid of pheromone. Replicated analyses of 15 individual females showed that pheromone could be extracted from the head and thorax ($\bar{X}$ = 0.24 μg per female) and from the expelled hair ($\bar{X}$ = 0.53 μg per female). These results indicated the thoracic site of pheromone production and the function of the deciduous hair as a pheromone dispenser.
5. Female pupae were collected in the field, removed from their bags, placed in individual petri dishes, and incubated: 80 percent relative humidity, 16-hour photophase (26°C), and 8-hour scotophase (16°C). Hairs expelled by the females were aspirated onto a plug of glass wool in a pipette and washed with 50 ml of hexane. The extract was concentrated and injected onto a GC column packed with 4 percent SE-30 on 80/100 mesh Gas Chrom Q. The major constituent in the extract was trapped and found to be biologically active. Capillary GC analysis showed that it was > 99 percent pure, and its retention indices [E. Kovats, in *Advances in Chromatography*, J. C. Giddings and R. A. Keller, Eds. (Dekker, New York, 1966), vol. 1] of 1660 (polar column) and 1592 (apolar column) indicated that the compound was weakly polar.
6. Capillary GC was carried out by polar (SP1000) and apolar (SP2100) fused silica columns (60 m by 0.25 mm inside diameter) (J & W Scientific). Low resolution GC–mass spectrometry (MS) showed a molecular ion at mass to charge ratio (*m/e*) 242 ($C_{15}H_{30}O_2$) and intense ions at *m/e* 173 and 155; high-resolution MS established that these ions were $C_{10}H_{21}O_2$ and $C_{10}H_{19}O$, respectively. No reaction occurred on treatment with O_3, $NaBH_4$, or acetic anhydride–pyridine; thus olefin, aldehyde, ketone, or alcohol functionalities for the compound were excluded. The infrared spectrum showed absorption at 1745 cm^{-1}, compatible with C=O absorption of an ester. Reduction of a few micrograms of the compound at 250°C with Pt and $LiAlH_4$ [B. A. Bierl-Leonhardt and E. D. DeVilbiss, *Anal. Chem.* **53**, 936 (1981)] yielded *n*-pentane, according to GC-MS. Without Pt, a similar reduction at 300°C gave *n*-decane and *n*-pentane. The nuclear magnetic resonance spectrum of the compound showed a triplet at 2.3 ppm ($C\underline{H}_2C=O$) and multiplet at 4.9 ppm ($C\underline{H}O$).
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8. The natural ester (~ 1 μg) was reduced with $LiAlH_4$ in CCl_4 and the resulting 2-pentanol was derivatized with Mosher's reagent [J. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.* **34**, 2543 (1969)]. The GC retention time of this diastereomeric derivative was identical to that of the Mosher's derivative of authentic (*R*)-2-pentanol.
9. The GC analysis of the diastereomers of the optically active alcohols used in synthesis of the enantiomers of the pheromone showed that each optical isomer of the alcohol contained 2 percent opposite enantiomer. In a field test similar to but separate from the test described in Table 1, traps baited with 500 μg of *S* enantiomer captured males at about the same rate as traps baited with 10 μg of *R* enantiomer. Thus, the small number of males attracted to the *S* enantiomer were most likely responding to the trace of *R* in the *S* isomer.
10. The 1-methylbutyl decanoate in a crude extract of the females' pheromone-laden hairs was assayed by GC and field-tested against the same amount (5.5 μg per trap) of synthetic pheromone. The number of males captured in traps baited with this extract was not different from those baited with the synthetic pheromone. Thus, the superiority of the synthetic pheromone over females in causing male capture (Table 1) is attributed to the comparatively small quantity of pheromone produced by the female.
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Quinolinic Acid: An Endogenous Metabolite That Produces Axon-Sparing Lesions in Rat Brain

Abstract. *A current hypothesis links the neuroexcitatory properties of certain acidic amino acids to their ability to cause selective neuronal lesions. Intracerebral injection of the neuroexcitatory tryptophan metabolite, quinolinic acid, has behavioral, neurochemical, and neuropathological consequences reminiscent of those of exogenous excitotoxins, such as kainic and ibotenic acids. Its qualities as a neurotoxic agent suggest that quinolinic acid should be considered as a possible pathogenic factor in neurodegenerative disorders.*

Kainate and ibotenate are neuroexcitatory and toxic amino acids of plant and fungal origin, respectively (*1*). Injection of either of these two substances into the brains of experimental animals produces effects that have been construed to provide models of human neurodegenerative disorders. In particular, striatal lesions caused by these agents closely resemble the neuropathologic and neurochemical changes characteristic of Huntington's disease (*2*). Intraventricular (*3*), intrahippocampal (*4*), or systemic (*5*) administration of kainate results in seizures and concomitant nerve cell changes similar to those observed in temporal lobe epilepsy in the human. The structural resemblance of exogenous amino acids, such as kainic and ibotenic acids, to endogenous excitatory amino acids, such as glutamic and aspartic acids, has led to the hypothesis that hyperfunction of the body's own "excitotoxins" may be related to neuronal damage in certain

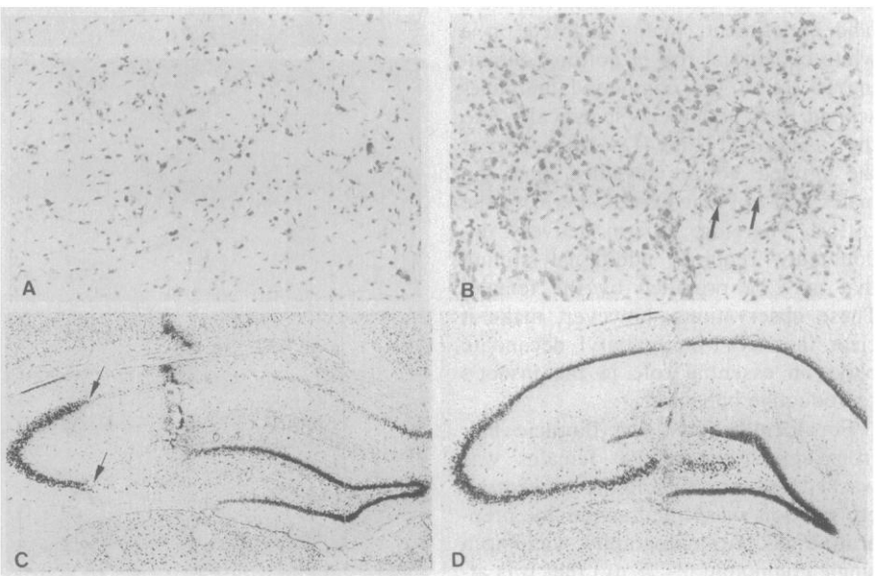

Fig. 1. Light microscopic analysis of 30-μm thionin-stained cryostat sections. (A and B) Micrographs of rat striatum 4 days after intrastriatal infusion of 60 nmole quinolinic (A) or 800 nmole nicotinic (B) acid. Arrows in (B) delineate the track of the injection needle (×50). (C and D) Effects of intrahippocampal administration of 30 nmole of quinolinic acid (C) or 800 nmole of nicotinic acid (D). Rats were killed 4 days after operation. Tracks of the injection needles are clearly identifiable in both micrographs. Arrows in (C) indicate the sharp border between degenerated and intact pyramidal cells (×10).

neuropsychiatric diseases (*6*). Endogenous excitatory amino acids have been shown to exhibit only marginal neurotoxic potency in the mature nervous system when compared to the experimental neurotoxins. We now report that nanomole amounts of the tryptophan metabolite quinolinic acid (2,3-pyridine dicarboxylic acid), known as a mild convulsant and excitant of cortical neurons (*7*), can produce axon-sparing neuronal lesions after intracerebral injection in rats. Neurochemical, neuropathological, and gross behavioral analyses show that quinolinic acid mimics the effects of kainate and ibotenate in both character and intensity.

Unilateral intrastriatal application of $\geq$ 150 nmole of quinolinic acid resulted in tonic-clonic movements of the contralateral forelimb, lasting approximately 4 to 6 hours after the rats awakened from anesthesia (*8*). This behavior, which was dose-dependent and was usually accompanied by episodic barrel-like rotations, was virtually indistinguishable from that seen after identical treatments with nanomole amounts of kainic or ibotenic acids (*2*) and has been related to increased activity of dopamine-containing elements in the striatum (*9*). No abnormal behavior could be observed after the operation.

Neuronal cell loss around the injection site was noticed at doses as low as 12 nmole. The number of glial cells did not appear to be reduced. With the exception of neuronal degeneration, the general appearance of the striatum, including myelinated internal capsule fibers, was unchanged (Fig. 1A). Lesions caused by the toxin radiated from the tip of the needle and increased in size with increase of the amount of quinolinic acid used. Even brains treated with large doses (600 nmole) of the toxin did not reveal "distant" nerve cell loss, such as in limbic structures, which is observed after striatal kainate lesions (*10*). Healthy neurons were abundant close to the injection site of 800 nmole of nicotinic acid, the product of quinolinic acid decarboxylation (*11*) (Fig. 1B).

Ultrastructural analysis of the striatum 4 days after injection of 60 nmole of quinolinic acid revealed disturbance of neuropil and nerve cells (Fig. 2A). The most conspicuous changes were dendritic swelling and decreased numbers of synaptic complexes as compared to controls. When the material was examined at higher magnification, many of the remaining axodendritic synaptic complexes showed swelling and clearing of postsynaptic components while presynaptic components retained a normal appearance (Fig. 2B). Well-preserved axons, both myelinated and unmyelinated, but only very few identifiably normal dendrites, were evident throughout the neuropil.

Neurochemical measurements confirmed the axon-sparing qualities of striatal quinolinic acid lesions. As compared to the contralateral side, the activities of striatal glutamic acid decarboxylase and choline acetyltransferase, marker enzymes for intrinsic GABAergic (GABA, gamma aminobutyric acid) and cholinergic neurons, decreased in a dose-dependent fashion with increasing amounts of quinolinic acid. Tyrosine hydroxylase activity, an indicator of the presence of the dopaminergic terminal network originating from cell bodies in the substantia nigra, remained unchanged (Table 1). At the lower doses, GABAergic neurons appeared to be more susceptible than cholinergic neurons to the toxic actions of quinolinic acid. At our analytical end point (4 days after operation), the protein content on the injected side (600 nmole

Table 1. Effects of intrastriatal quinolinic and nicotinic acid on local neurotransmitter-related enzymes. The amino acids (in 1 μl of phosphate-buffered saline, *p*H 7.4) were injected into the striatum of rats and the animals were killed 4 days later. The striatal tissue was sonicated (1:20, weight to volume) in 50 m*M* tris buffer (*p*H 7.4) containing 2 percent Triton X-100, and enzyme activities (picomoles per minute per milligram of tissue) of the homogenates were determined. Standard errors of the means are indicated (N = 5).

Injected amino acid (nmole)	Glutamic acid decarboxylase		Choline acetyltransferase		Tyrosine hydroxylase	
	Activity	Percent of contralateral striatum	Activity	Percent of contralateral striatum	Activity	Percent of contralateral striatum
			Quinolinic acid			
60	307 ± 15	96	797 ± 40	96	2.5 ± 0.1	102
150	158 ± 13*	55	557 ± 62*	78	2.6 ± 0.3	92
300	85 ± 17*	26	424 ± 56*	50	3.0 ± 0.1	112
600	44 ± 14*	14	116 ± 31*	14	2.9 ± 0.1	86
			Nicotinic acid			
800	298 ± 4	96	790 ±45	95	2.5 ± 0.1	94

*P < .01 by paired t-test as compared to the uninjected contralateral striatum.

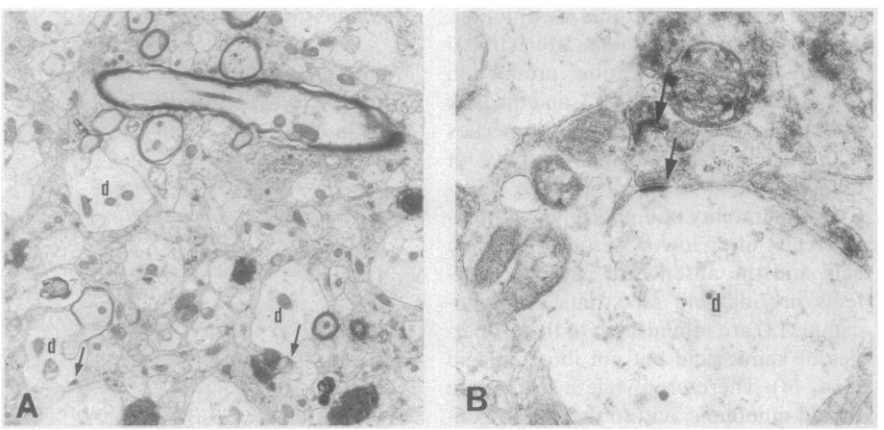

Fig. 2. A. Ultrastructural appearance of rat striatal neuropil 4 days after injection of 60 nmole of quinolinic acid and after perfusion-fixation with 1 percent glutaraldehyde and 1 percent paraformaldehyde in Sorensen's phosphate buffer, *p*H 7.6. Prominent swelling of dendritic processes (*d*) is widespread. Postsynaptic densities can be seen on these dilated dendrites (arrows) in apposition to presynaptic elements, which appear unaffected. Myelinated axons, as seen in this field, appear to be well-preserved (×5000). (B) Enlargement of the ultrastructural appearance of affected synapses in the rat striatum at 4 days after 60 nmole of quinolinic acid. The dilated structure (*d*) represents a swollen dendrite, which is the postsynaptic element in the synapse indicated by the lower arrow. At the upper arrow, another synaptic junction is indicated, but the postsynaptic element may have undergone degeneration and shrinkage (×20,600). Striata from animals receiving intrastriatal injections of up to 800 nmole of nicotinic acid served as controls. In these tissues, there was no appreciable change except for mild tissue disruption immediately around the injection needle track. No abnormalities could be observed in homologous regions from uninjected contralateral striata of either quinolinate- or nicotinate-injected animals.

of quinolinic acid) was only slightly diminished as compared to the contralateral side (1.7 ± 0.2 as compared to 2.1 ± 0.1 mg of protein per striatum).

Intrahippocampal injections of less than 500 nmole of quinolinic acid produced no clinically detectable behavioral consequences. Only doses in excess of 500 nmole reliably resulted in generalized convulsions characterized by intermittent jumping, running fits, ipsilateral turning, and pronounced exophthalmos, which lasted for 2 to 4 hours. Histological analysis demonstrated the degeneration of all hippocampal neuron types when doses more than 60 nmole were used. Such lesions, like the striatal cell loss, were well circumscribed and restricted to the dorsal hippocampus and underlying dorsal thalamic nuclei. Changes resulting from intrahippocampal injections of 30 nmole of quinolinic acid (Fig. 1C) indicated a selective vulnerability of pyramidal cells compared to granule cells. Control injections of 800 nmole of nicotinic acid failed to produce neuronal damage in the hippocampal formation (Fig. 1D).

Stone and Perkins (*7*) have briefly described the neuroexcitatory potency of quinolinic acid on cortical neurons. Our findings demonstrate the neurotoxic properties of quinolinic acid, which in part resemble those of both kainic and ibotenic acids. The endogenous agent is axon-sparing in its toxic effect and, like the exogenous toxins, does not cause an apparent reduction of glial cells in an area of complete neuronal destruction. Absence of distant lesions after striatal or hippocampal application, production of circumscribed lesions (*2*), and the lack of pronounced convulsant properties (*12*) indicate ibotenate-like activity of quinolinic acid. By contrast, the selective vulnerability of hippocampal pyramidal cells after low doses of quinolinic acid and the absence of neurotoxic effects in 7-day-old rat striata or hippocampi (*13*) are reminiscent of the properties of kainic acid but not ibotenic acid (*3, 4, 14*). Therefore, while it seems safe to add quinolinic acid to the list of excitotoxic amino acids originally described by Olney (*15*), it may be premature at this stage to categorize it according to commonly used guidelines, which are based mostly on pharmacological data obtained with electrophysiologically active amino acid antagonists (*16*). The above-mentioned qualities of quinolinic acid are distinct from those of folic acid and its derivatives, which have recently been suggested as endogenous kainate-like substances (*17*).

Morphological, behavioral, and neurochemical observations after striatal quinolinic acid injections revealed a picture similar to that known from intrastriatal infusions of kainic or ibotenic acids. Since those treatments have provided an animal model for Huntington's disease, it is possible that quinolinic acid has a role in the etiology of this disorder. Either increased transport from the periphery or a metabolic defect in the brain may provide an excess of quinolinic acid sufficient to induce neuronal degeneration of the type which we have observed experimentally. While the demonstration of a cerebral pathway for the synthesis of quinolinic acid is incomplete, the biosynthetic mechanisms for 3-hydroxyanthranilate (its immediate metabolic precursor) in the brain have been established (*18*). Our data appear to justify further evaluation of the presence, biochemistry, and physiology of quinolinic acid in the central nervous system.

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19. We thank Deborah Parks, William Zinkand, Madelyn Stolk, Jana Brady, Westelle Sweeney, and Frank Moretta for technical assistance. This work was supported by USPHS grant NS 16941.
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