near the peak of CAP frequency in the circadian cycle. Interestingly, the reversal point for phase responses to light occurs during the minimum CAP frequency [(*12*), projected night].

In *Acetabularia* (*17*) and *Aplysia* the delays in phase caused by inhibitors of 80*S* protein synthesis occurred at similar times in the circadian cycles (O_2 production at 20°C or CAP activity at 15°C). This common action on such diverse organisms suggests a common mode of action for this class of inhibitors on circadian rhythms generally. The inhibitors (anisomycin, cycloheximide, and puromycin) act on the eukaryotic ribosome (*18*) suggesting that 80*S* protein synthesis is fundamental for the normal functioning of circadian regulatory processes in the cell. There is evidence that synthesis at the ribosome does not itself generate the period of the rhythm in *Acetabularia* (*17*) because the temperature dependence of the period does not match the temperature dependence of the inhibitor effects. Ribosomal protein synthesis as part of the clock is compatible with the membrane model (*3*, *19*) since inhibitors could alter the synthesis or degradation of proteins involved in membrane ionic transport (*19*).

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Selective Destruction of Neurons by a Transmitter Agonist

Abstract. *Microinjection of nanomole amounts of kainic acid, a heterocyclic analog of glutamate, into the cerebellums of adult hamsters and rats causes rapid degeneration of Purkinje, basket, stellate, and Golgi II cells, neurons that receive synaptic input from granule cells, whereas the granule cells themselves are spared. This selectivity is consistent with the evidence that glutamate is the granule cell transmitter and supports the hypothesis that kainic acid exerts its neurotoxic effects through glutamate receptors.*

The selective degeneration of specific groups of neurons with sparing of adjacent, morphologically different groups is characteristic of a number of neurologic disorders. In the cerebellum, granule cells are selectively destroyed by organomercurial poisoning (*1*) and by thiophene (*2*), whereas Purkinje cells degenerate in carcinomatous cerebellar degeneration (*3*) and are destroyed in diphenylhydantoin intoxication (*4*), sodium azide poisioning (*5*), and by an eosinophil extract (*6*). The reasons for this specificity are generally unknown, and it has rarely been possible to relate the selective vulnerability of particular cell groups to their morphologic or chemical properties or functions.

Within the cerebellum, two lines of evidence suggest that glutamate is the granule cell transmitter. First, destruction of the granule cells by virus infection (*7*), radiation (*8*), or genetic mutation (*9*) results in a substantial decline in endogenous glutamate levels without a corresponding decline in the concentrations of other candidate transmitters. Second, destruction of the granule cells is accompanied by a 70 percent decrease in the high-affinity synaptosomal uptake of glutamate (*7*), a process that is thought to be limited to neurons using glutamate as a transmitter.

Systemic administration of monosodium glutamate to immature rodents causes degeneration of neurons in the inner layer of the retina and the hypothalamus. The neurotoxic effects of glutamate appear to be due to its ability to depolarize neurons, since the neurotoxicity of several dicarboxylic and sulfur-containing amino acids structurally related to glutamate is correlated with their neuroexcitatory actions (*10*). A conformationally restricted analog of glutamate, kainic acid, has been shown to be several orders of magnitude more potent than glutamate as a neuronal depolarizer (*11*). Recently, we demonstrated that injection of nanomole amounts of kainate into the striatum produces a selective degeneration of neurons intrinsic to the region (*12*). Therefore, we postulated that if glutamate is the granule cell transmitter and if kainic acid acts on glutamate receptors, the cerebellar neurons that receive granule cell input should have glutamate receptors and should be selectively destroyed by kainate. To test this hypothesis, we injected the cerebellums of rats and hamsters with 2 μg of kainic acid; control animals were similarly injected with 2 μg of α-methylaspartate, a nonneuroexcitatory analog of glutamate (*13*).

Within the immediate vicinity of the injection site there was a small area of necrosis at all periods examined. Thirty minutes after injection, peripheral chromatolysis was already visible in Purkinje cells at the periphery of the injection site, and at 1 hour it was clearly present in basket, stellate, and Golgi II cells as well. Vacuolation was beginning at 1 hour and was much more prominent at 4 and 8 hours, when the area of involvement had a radius of 1.5 to 2 mm. At 24 hours virtually all of the neurons except the granule cells within this radius appeared as pyknotic remnants within large vacuolar spaces (Fig. 1). On electron microscopic examination, peripheral chromatolysis with depolymerization of polyribosomes and cell swelling was present at 30 minutes, and dilatation of the granular endoplasmic reticulum with vacuole formation was extensive by 2 hours (Fig. 2a). These changes pro-

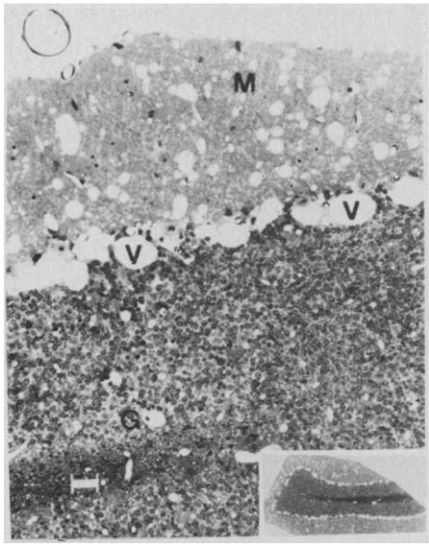

Fig. 1 (left). This micrograph illustrates the appearance of rat cerebellar cortex in a folium adjacent to the injection site 24 hours after injection. The Purkinje cells have disintegrated, leaving behind a series of vacuoles (*V*) in the ganglionic layer. The granule cells in the granular layer (*G*) are well preserved. The vacuoles in the molecular layer (*M*) are the result of Purkinje dendrite, basket, and stellate cell degeneration. Scale bar, 25 μm. (Inset) Micrograph of entire folium showing vacuolation of Purkinje cell layer.

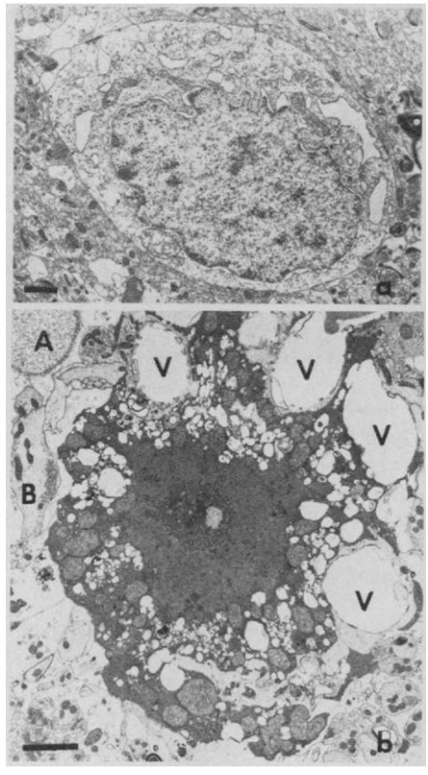

Fig. 2 (right). (a) Electron micrograph of basket cell 2 hours after injection. The endoplasmic reticulum and perinuclear cisternae are dilated, and the ribosomes at the periphery of the cell are depolymerized. (b) Electron micrograph showing a necrotic hamster Purkinje cell 24 hours after kainate injection with early filamentous degeneration of a basket cell process (*B*). Large vacuoles (*V*) are beginning to form at the edge of the cell. The surrounding Bergmann astrocytes (*A*) are markedly swollen. Scale bar, 1 μm.

gressed rapidly, so that by 24 hours all that remained of the affected cells were condensed pyknotic remnants surrounded by vacuoles and basket terminals undergoing filamentous degeneration (Fig. 2b). In the molecular layer, the Purkinje cell dendrites and their spines were collapsed and surrounded by swollen astrocytic processes. Collapsed remnants of the dendritic spines still persisted at 2 weeks, after most other cell debris had been removed. Except for some transient swelling in the first 1 to 2 hours, the granule cells appeared unaffected. The only change seen in control animals was local mechanical injury of the injection site.

The cerebellum contains five types of neurons, the granule cells, Purkinje cells, basket cells, stellate cells, and Golgi II cells. The synaptic circuitry among these component neurons in the cerebellum has been well characterized (*14*). All four neuronal types that are sensitive to the toxicity of kainate receive synaptic contacts from the granule cells; the granule cells themselves, which are resistant to kainate, do not synapse on each other. The neurotransmitter of the granule cells is excitatory and probably is glutamate (*7–9*). The pattern of neuronal sensitivity to kainate in the cerebellum is consistent with the conclusion that kainate exerts its toxicity via its effect on glutamate receptors located on neurons postsynaptic to glutamate-containing terminals.

This is an additional example, along with 6-hydroxydopamine, of selective vulnerability in the mature nervous system where the mechanism is known and can be related to physiologic properties of the affected cells. However, unlike 6-hydroxydopamine, which acts presynaptically, kainate acts on the postsynaptic membrane. It is of particular interest in that it suggests a possible mechanism for a number of toxic and degenerative disorders of the nervous system. The cerebellar degeneration that occurs as a remote effect of carcinoma has a pattern of neuronal loss similar to that caused by kainate injection and could result from the release of a neurotoxic transmitter agonist by the tumor. The syndrome of myoclonus and opsoclonus associated with neuroblastoma is another possible example of a toxic neurologic syndrome associated with a tumor. The tumor cells are capable of synthesizing neurotransmitters in culture (*15*) and could cause these symptoms by excessive production of a neurotoxic transmitter agonist. Some of the highly selective system degenerations such as Huntington's disease and olivopontocerebellar degeneration could also conceivably be due to a metabolic error which results in the accumulation of neurotoxic transmitter agonists.

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