On the Specificity of Kainic Acid

Abstract. *The specificity of the neurotoxic agent, kainic acid, for destroying cell bodies while sparing terminals and fibers of passage was examined by infusing this agent into the axons of the dorsal noradrenergic bundle and measuring the degree of depletion of noradrenaline concentrations and the reduction in noradrenaline uptake in cortex and hippocampus. Extensive neuronal loss and gliosis were observed around the injection site. In addition, a significant and consistent 25 percent depletion of hippocampal-cortical noradrenaline was also obtained. The results suggest that although kainic acid has its greatest destructive action on neuronal perikarya, a significant amount of damage to axons of passage may also occur.*

Kainic acid, a rigid analog of glutamate (*1*), has been reported by two independent laboratories (*2*) to produce a pattern of neurochemical depletion when injected into the striatum of rats, which suggests that it destroys cell bodies without affecting afferent terminals or fibers of passage. Thus, choline acetyltransferase and glutamic acid decarboxylase, enzyme markers for neurons whose cell bodies are intrinsic to the injected area, were severely decreased, while tyrosine hydroxylase, a marker for afferent dopaminergic terminals coming from extrinsic areas, was unaltered or increased (*3*). Histological examination revealed a virtual absence of cell bodies with apparently intact fiber bundles of the internal capsule running through the same area (*3, 4*).

Kainic acid thus appears to offer a unique method to destroy cell perikarya while leaving fibers of passage intact. This would resolve many of the disputes concerning effects of electrolytic lesions in brain regions which may be acting either by the destruction of functional centers at the locus of the lesion or by destroying interconnections between other functional areas, the fibers of which run through the damaged region. As such this technique of intracerebral injection of kainic acid and related compounds has already found widespread application (*4-7*).

We sought to test the specificity of kainic acid toward cell perikarya by injecting it into the dorsal noradrenergic fiber bundle which arises from cell bodies in the locus coeruleus (*8*) and runs through the mesencephalon to innervate wide areas of the forebrain, including the cortex and hippocampus. If kainic acid is specific for cell bodies, injections into this fiber bundle should cause severe local cell loss but no depletion of noradrenaline (NA) concentrations or NA uptake in terminal areas of the bundle.

Male albino Woodlyn rats weighing about 300 g at the time of operation were stereotaxically injected unilaterally with 0.5 μg of kainic acid dissolved in 1 μl of buffered phosphate solution (*9*). The catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) was injected unilaterally into other animals to demonstrate that the injection volume could indeed reach the NA fiber bundle when delivered at these coordinates. After a 2-week period to allow anterograde degeneration of NA terminals in cortex-hippocampus (*10*), the animals were killed by cervical fracture and their brains dissected on ice into cortex-hippocampus as described (*11*). These tissues were then assayed for NA (*12*), and portions were taken for the measurement of [^{3}H]NA uptake (*13*). The mesencephalic region containing the injection site was saved in 10 percent formalin-saline solution for histological examination.

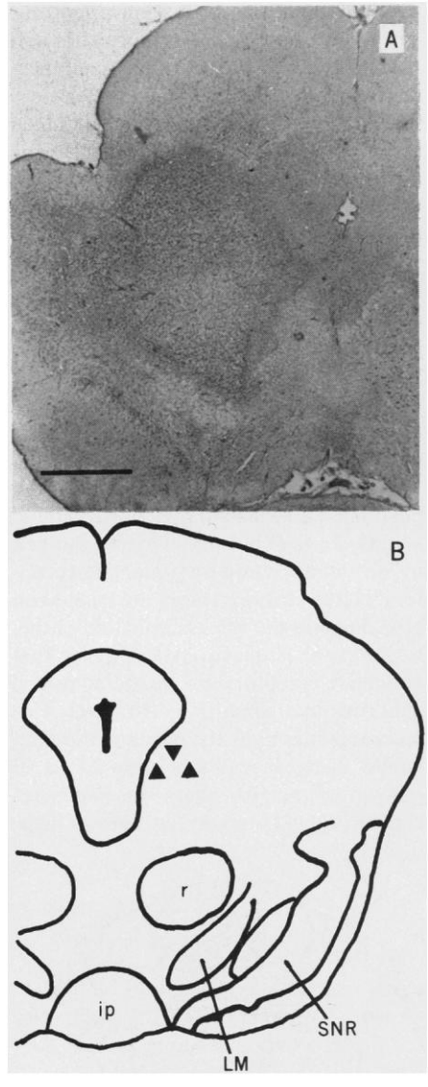

Fig. 1. Histological results on brains of kainic acid-injected animals. (A) Injection site; kainic acid-injected side showing region of gliosis. (B) Diagram showing position of fibers of dorsal noradrenergic bundle (triangles) on frontal coronal section from König and Klippel. Calibration bar, 400 μm. Abbreviations: *r*, nucleus rubra; *ip*, interpeduncular nucleus; *LM*, medial lemniscus; and *SNR*, substantia nigra, pars reticulata.

The results are shown in Table 1. The kainic acid-injected group showed an approximately 25 percent decrease in cortical-hippocampal NA on the injected side. The contralateral side failed to differ from either injected or control sides of animals receiving phosphate vehicle injections. The injection of phosphate buffer produced no significant effect on NA concentrations. 6-OHDA produced an 88 percent depletion, considerably greater than that obtained with the doses of kainic used. The uptake of [^{3}H]NA was reduced by approximately 50 percent on the kainic acid-injected side and was not changed on the contralateral side or by phosphate buffer injections.

The brainstems of the animals on which biochemical data are reported were sectioned on a freezing microtome from the mesencephalic injection site through the locus coeruleus and were stained with cresyl violet. The results are shown in Fig. 1. Massive cell loss occurred in the region of the injection site (Fig. 1A). This cell loss included the area through which the dorsal bundle is known to run (Fig. 1B) and is of roughly the same size as that reported previously for injections of similar volumes and concentrations (*2-4*). The damage did not, however, extend to the locus coeruleus (Fig. 2), the cell bodies of which appeared normal and were indistinguishable between injected and normal sides.

Further confirmation that kainic acid was not having a direct effect on NA cell bodies in the locus coeruleus was obtained by counts of the number of cell bodies in representative 50-μm sections of the kainic acid-injected and contralateral side. In the anterior locus coeruleus section the kainic acid-injected side contained 75 ± 2.5 cells (mean ± standard error), whereas the contralateral, uninjected side contained 73.6 ± 1.7 cells. In the more posterior section containing the main body of locus coeruleus there were 103.6 ± 3.6 cells on the injected side and 105.4 ± 5.8 on the contralateral side. In other animals that had received unilateral kainic acid injections into the

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Table 1. Biochemical and histological measures after unilateral injection of 0.5 μg of kainic acid in 1 μl of phosphate buffer, *p*H 7.2, into the dorsal NA fiber bundle. Values represent means ± 1 standard error as determined in ten animals.

Tissue from	Hippocampal-cortical NA concentration (ng/g)			Hippocampal-cortical NA uptake (nCi/mg protein)		NA content in locus coeruleus (μg/sample)	
	Phosphate buffer	Kainic acid	6-OHDA	Phosphate buffer	Kainic acid	Phosphate buffer	Kainic acid
Injected side	315 ± 14	234 ± 21	51 ± 9	0.651 ± 0.034	0.229 ± 0.009	3.2 ± 0.6	3.5 ± 0.3
Control side	313 ± 17	320 ± 16	309 ± 23	0.673 ± 0.043	0.640 ± 0.048	3.5 ± 0.5	3.2 ± 0.4
Percentage of control side	99	74*	12*	97	45*	91	109

*P = .01; Student's two-tailed t-test.

dorsal bundle we also dissected out a region containing locus coeruleus and measured the NA content by a sensitive radioenzymatic method (*14*). The results (Table 1) indicate that there was no change in NA content between the kainic acid–injected and uninjected side and the phosphate buffer–injected brains. These three lines of evidence—the reconstruction of the lesion indicating that it did not reach locus coeruleus, the unaltered number of cell bodies in locus coeruleus, and the unaltered NA concentrations in locus coeruleus—enable us to exclude the possibility that the kainic acid injection acted directly on the locus coeruleus cell bodies.

The significant and consistent 25 percent decrease in NA concentration and the 50 percent decrease in [^{3}H]NA uptake in the cortex-hippocampus in the kainic acid–injected animals indicates destruction of some of the NA fibers in the injected area. This cannot be an effect of the *p*H since the kainic acid solution was buffered to *p*H 7 and the *p*H was confirmed by testing before and after each injection session. Nor can it be due to the injection procedure since vehicle injections produced no effect on cortical-hippocampal NA. The histological and biochemical evidence rules out spread of the kainic acid back to the cell bodies of origin in the locus coeruleus. One possible explanation might be that there are some NA cell bodies in the mesencephalic region itself which contribute to cortical-hippocampal NA. Noradrenergic cell bodies have been reported along the course of the dorsal periventricular bundle (*8*), but these are believed to contribute to hypothalamic or thalamic NA rather than to the cortical or hippocampal projections (*15*). Further, although some authors report as much as 30 percent of cortical NA remaining after electrolytic lesions to the locus coeruleus (*16*), with well-placed electrolytic lesions in this nucleus it is possible to reduce cortical NA to unmeasurable levels and totally abolish cortical NA fluorescence (*8, 17*). This would seem to make it unlikely that there exist mesencephalic cell bodies which contribute to cortical-hippocampal NA and which could account for the present observations. If such were the case, however, it would require a reordering of classical NA neuroanatomy.

A further possibility, in line with the postulated mechanism of action of kainic acid (*1, 2, 5, 18*) of depolarizing the cell by way of an action on glutamate receptors, is that such receptors are located on NA axons in the mesencephalon. If this is the case, it questions the belief that glutamate receptors are restricted to cell dendrites and soma (*19*). However, this mechanism is unlikely because the data would then suggest that only 25 to 50 percent of the NA fibers have such receptors. Such heterogeneity seems highly improbable. If, however, the 25 to 50 percent loss of cortical-hippocampal NA caused by kainic acid is due to a direct action on NA axons, not requiring the glutamate receptor, it suggests that the susceptibility of brain elements to kainic acid–induced destruction cannot always be used as an indication that they possess receptors for glutamate (*5*).

An indirect mechanism also seems possible. Considerable loss of brain substance was seen in the lesioned area, with cell bodies being almost totally absent. Loss of such supportive elements may render the fine, unmyelinated NA axons more susceptible to mechanical damage during brain or bodily movement.

Whatever the mechanism of the observed effect, our data suggest that the use of kainic acid to dissociate behavioral (*4, 6, 7*) or biochemical (*2, 3*) effects due to destruction of functional nuclei, as distinct from fibers passing through that area, may be limited.

On the other hand, 50 to 75 percent of NA fibers appeared to be spared by our kainic acid lesion and this represents a considerable improvement over electrolytic lesions. It may be that the action of kainic acid varies from one brain region to another. Certainly, no evidence is available for even a 25 percent effect of kainic acid on fibers of passage when injected into the striatum (*2–4*). The fibers of the internal capsule are heavily myelinated and of large diameter and may be less susceptible to the action of kainic acid. However, the dopamine and serotonin terminals are of small diameter and unmyelinated, and they too appear to be completely spared as judged by a number of quantitative biochemical indices (*2–4*). There are conflicting reports, however, about the integrity of afferent glutamate terminals (*20, 21*) and some evidence has recently been found for a possible effect on dopamine terminals within the immediate vicinity of the injection site (*22*) and loss of myelinated fibers of passage (*23*).

From these data it is clear that it will be necessary to show in a quantitative

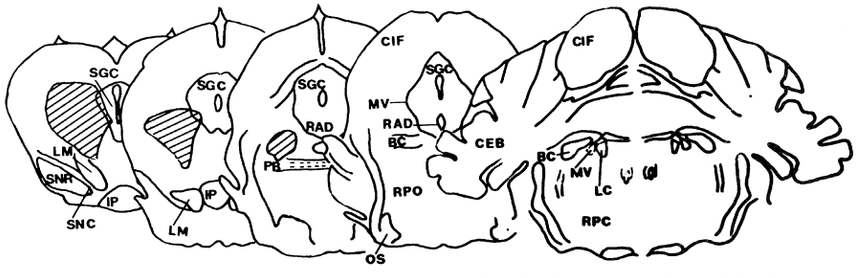

Fig. 2. Reconstruction of posterior extent of lesion showing it stopping short of the locus coeruleus itself. Abbreviations: *LM*, medial lemniscus; *MV*, mesencephalic nucleus of the fifth cranial nerve; *IP*, interpeduncular nucleus; *BC*, brachium conjunctivum; *LC*, locus coeruleus; *OS*, superior olive; *SNR*, substantia nigra, pars reticulata; *SNC*, substantia nigra, pars compacta; *SGC*, substantia grisea centralis; *RAD*, dorsal raphe; *CIF*, inferior colliculus; *CEB*, cerebellum; *RPC*, nucleus reticularis pontis caudalis; and *RPO*, nucleus reticularis pontis oralis.

fashion for every new brain area into which kainic acid is injected that it has indeed spared all fibers of passage. Our results suggest that one type of fiber may be spared while others are not, or that a significant but subtotal percentage of all fibers may be destroyed. Furthermore, this may vary from one brain region to another. Some techniques used to test the integrity of fibers of passage may not be quantitative enough to detect small losses; such techniques include horseradish peroxidase transport through the injected area (*7*) and electrophysiological evocation of a response caused by stimulation of fibers that run through that area (*24*). Conventional histology, either at the light or the electron microscopic level, would not appear to be able to provide an adequately quantitative measure (*2, 3, 25*).

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9. Animals were anesthetized with Nembutal (50 mg/kg, injected intraperitoneally) and positioned in a stereotaxic instrument (David Kopf). Two holes were drilled in the skull. A 34-gauge cannula was lowered to the following coordinates: AP + 2.6 mm from interaural zero, ML ± 1.1 mm from the midline suture at bregma, and DV + 3.7 mm from interaural zero. Kainic acid (0.5 μg) dissolved in 1 μl of 50 m*M* phosphate buffer at *p*H 7.0 was injected at the rate of 1 μl per minute, and the cannula was left in for a further minute to permit diffusion of the drug. Injections were made unilaterally on the left-hand side of the brain. Control animals received injections of 1 μl of phosphate buffer vehicle not containing any kainic acid. 6-Hydroxydopamine hydrobromide (Regis Chemicals) was dissolved in 1 μl of 0.9 percent saline containing ascorbic acid (0.3 mg/ml) as an antioxidant. Concentrations are expressed as the amount injected (6-OHDA as the free base, kainic acid as such) in the total amount of fluid delivered. Kainic acid and 6-OHDA were made up freshly each day, kept on ice, and protected from light between injections.
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Morphological Affinities of *Pan paniscus*

Abstract. *Although the pygmy chimpanzee (*Pan paniscus*) is more similar to man than is the common chimpanzee (*Pan troglodytes*) in some traits, the resemblance is due primarily to the smaller size and concomitant allometric generalization of the former. The two species of chimpanzees are equally good models for the common ancestry of African apes and man.*

The recent discovery of 3- to 4-million-year-old human fossils (*1*) quickens interest in possible living analogs to our common ancestor with apes (*2, 3*). The pygmy chimpanzee (*Pan paniscus*) has been proposed as a good prototype for this common ancestor (*2, 3*). We report our test of the appropriateness of *P. paniscus* as an ancestral prototype by assessing the morphological affinities of *P. paniscus* relative to *P. troglodytes*, *Homo sapiens*, and early hominids, and determining the effects of size and scaling on the morphology of these species.

Morphological similarity was assessed by comparing large sets of measurements (196) that describe body proportions, joint configurations, cranial shape, and dental morphology. Comparisons for each body part were made by calculating the generalized distance among *Homo sapiens*, *Pan paniscus*, *Pan troglodytes*, and the early hominid group of gracile *Australopithecus* in the multidimensional space defined by canonical variates (Table 1). Although the two chimpanzee species are more similar to one another than to either extinct or extant hominids, the affinity between the pygmy chimpanzee and hominids is significantly greater than that between the common chimpanzee and hominids in general body proportions, shoulder, elbow, forearm, premolars, and anterior tooth morphology. Gracile australopithecines are closer to *P. paniscus* than to *P. troglodytes* in proportions, shoulder, and elbow. In all other traits measured, these early hominids significantly converge on *P. paniscus* on the second canonical variate (on which *P. paniscus* is always mutually discriminated from *H. sapiens* and *P. troglodytes*).

Could allometry, the necessary change of proportions with size, explain the differential similarity of *P. paniscus*? While *Pan* species differ less in size than once thought [about 15 percent (*3a*)], the smaller size of *P. paniscus* might still explain some of its more generalized appearance. Static adult allometry often resembles the process of ontogenetic allometry, the earlier (smaller) stages of which usually appear more primitive. Furthermore, the process of neoteny does not necessarily lead to decrease in body size (*4*): differential cessation of growth could still yield a neotenized individual at a larger size. Therefore the differential similarity of pygmy chimpanzees toward humans could be allometric despite the size discrepancy, if it were the result of coincidentally shared juvenile features.

To test the effects of allometry we calculated the correlations between interspecific and intraspecific allometry coefficients for *P. paniscus* and *P. troglodytes* (Table 2). This approximates the angle between the vectors describing the allometric growth pattern within *P. troglodytes* and the vector intersecting the *P. troglodytes* and *P. paniscus* centroids (*5*). The highest correlation in Table 2 is for skeletal proportions resulting from the fact that pygmy chimpanzees differ from common chimpanzees in having shorter arms, longer legs, and

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