Application of kainic acid to the detection of neuronal projections from area CA3 of the dorsal hippocampus of the rat

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ABSTRACT
The neurotoxic property of kainic acid was exploited in an investigation of neuronal projections from the rat hippocampus. Extra- and intrahippocampal projections from the pyramidal cells of area CA3 of the dorsal hippocampus were identified after selective destruction of the pyramidal cells by an intraventricular injection of kainic acid. Subsequent silver impregnation using the Fink-Heimer method showed mainly that the dorsal part of the bilateral lateral septal nucleus, the bilateral cingulargyri and the bilateral subicular area were extrahippocampal projection sites, and that the stratum oriens of areas CA1, CA2 and CA3 of the bilateral dorsal and caudal hippocampus together with the stratum radiatum of area CA1 of the ipsilateral dorsal hippocampus were intrahippocampal projection sites.

KEY WORDS hippocampal projections / pyramidal cells of the CA3 / kainic acid / Fink-Heimer silver impregnation

Efferent projections from the hippocampal formation have been demonstrated by several authors using a variety of experimental procedures (5, 7, 10–12). However, the projection sites from a small and restricted area such as CA3 have not yet been identified due to the difficulty of producing small localized lesions and of injecting substances such as tritiated amino acid for tracing neuronal pathways into such a restricted part.

Kainic acid, a neuro-excitant agent and an analogue to glutamate, has been employed as a tool in neurobiological research (4, 6, 9). The hippocampus, particularly the pyramidal neurons of area CA3, are extremely sensitive to the neurotoxic effects of kainic acid. We have previously confirmed that the smallest dose of kainic acid injected into the lateral ventricle selectively destroys the pyramidal cells of area CA3 of the dorsal hippocampus of the rat, but does not affect any other areas of the brain (8).

The present study has been undertaken to reveal the efferent projections from area CA3 of the dorsal hippocampus by exploiting the susceptibility of their neurons to kainic acid.

Twenty-eight male Wistar rats weighing about 250 g were used. They were stereotaxically injected with 0.3 μg (1.5 nmol) of kainic acid (Sigma Chem. Co.), dissolved in 0.3 μl of phosphate-buffered saline adjusted to pH 7.4, into the lateral ventricle. After a survival period of 72 hr, which was considered to be adequate to detect the degenerating terminals, twenty were sacrificed by perfusion with 10% neutralized formalin and their entire brains were postfixed in the same fixative. Frozen sections 20 μm in thickness were then made, alternate sections being subjected to Fink-Heimer silver impregnation for degenerating terminals (2), or stained with hematoxylin eosin. Five rats were sacrificed by perfusion with Palay's fixative which contained 1% paraformaldehyde and 1% glutaraldehyde adjusted to pH 7.4 with phosphate buffer and processed for electron microscopic examination for degenerating terminals. Three control animals were injected with 0.3 μl of phosphate-buffered saline adjusted to pH 7.4 into the lateral ventricle and subjected to Fink-Heimer
silver impregnation and hematoxylin eosin staining. No degenerating neuronal perikarya except those of the pyramidal cells of area CA3 of the dorsal hippocampus were detected in the preparations of Fink-Heimer silver impregnation and hematoxylin eosin staining. Degenerating neuronal perikarya of the pyramidal cells are shown in Fig. 1a. No degenerating terminals were found in the sections from control animals after Fink-Heimer silver impregnation.

In the sections treated by Fink-Heimer silver impregnation, degenerating terminals were seen as black or dark brown dots or as bead-like configurations on a light brown background. The areas in which degenerating terminals were clearly found by Fink-Heimer silver impregnation were the dorsal part of the bilateral lateral septal nuclei, the bilateral cingular gyrus and the bilateral subicular area (Fig. 1, b and c). The cingular gyrus and the subicular area were particularly well supplied with black dots. In the latter two locations, the number of degenerating terminals in the contralateral site was less than in the ipsilateral site. In the ipsilateral anterior and lateral thalamic nuclei a very small number of degenerating terminals were observed, and a small number of degenerating terminals were also in the ipsilateral entorhinal cortex. The degenerating dark nerve endings, some of which clearly retained their synaptic specialization, were found in the above regions by electron microscopy (Fig. 1d). Also present were several degenerating terminals engulfed by glial processes.

The above observations enabled us to define the efferent projections from the pyramidal cells.
in area CA3 of the dorsal hippocampus to the various regions described above. Swanson and Cowan (12) investigated the efferent pathway of the hippocampal formation including the subicular area by a localized injection of $[^3]$Hproline and obtained results similar to ours, except that they failed to find any silver grains of $[^3]$Hprotein in the thalamic nuclei. Our observations agree with those of Meibach and Siegel (5) who, by inducing retrograde transport of horseradish peroxidase, showed that the lateral septal nucleus receives efferent innervation from the pyramidal cells of area CA3 of the dorsal hippocampus. We were unable to find any degenerating terminals in other areas, such as the preoptic area, the hypothalamic nuclei and the mammillary body, in which several degenerating terminals were observed by Nauta (7) using classical neuroanatomical procedures.

In respect to the intrahippocampal projections from area CA3 of the dorsal hippocampus, degenerating terminals were detected in the stratum oriens of areas CA1, CA2, and CA3 of the bilateral dorsal and caudal hippocampus. However, the number of degenerating terminals was greater in the contralateral site than in the ipsilateral one. The contralateral projection through the hippocampal commissure is therefore considered to be predominant. These observations agree well with those of Gottlieb and Cowan (1) and Hjorth-Simonsen (3). Degenerating terminals were also found in the stratum radiatum of area CA1 of the ipsilateral dorsal hippocampus. These degenerating nerve endings may be ascribed to Schaffer’s collateral. The appearance and distribution of degenerating terminals which demonstrate the pattern of the efferent neuronal projection from area CA3 of the dorsal hippocampus are presented as a schematic drawing (Fig. 2).

We believe that a more precise determination of the extra- and intrahippocampal projections of the pyramidal cells of area CA3 of the dorsal hippocampus has been made by the present study, since this technique has an advantage over conventional lesioning methods of directly destroying fibers of passage through the CA3 area and it also eliminates the background problems of autoradiography.

We conclude that kainic acid can primarily be used to scrutinize precise areas of the efferent projection from pyramidal cells of area CA3 of
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the dorsal hippocampus.

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REFERENCES


