

## SELECTIVE INHIBITORY INTERNEURON LOSS PRODUCES CHRONIC HIPPOCAMPAL HYPEREXCITABILITY

### Focal Inhibitory Interneuron Loss and Principal Cell Hyperexcitability in the Rat Hippocampus After Microinjection of a Neurotoxic Conjugate of Saporin and a Peptidase-Resistant Analog of Substance P

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Episodes of prolonged seizures or head trauma produce chronic hippocampal network hyperexcitability hypothesized to result primarily from inhibitory interneuron loss or dysfunction. The possibly causal role of inhibitory neuron failure in the development of epileptiform pathophysiology remains unclear because global neurologic injuries produce such a multitude of effects. The recent finding that Substance P receptors (SPRs) are expressed exclusively in the rat hippocampus by inhibitory interneurons provided the rationale for attempting to ablate interneurons selectively by using neurotoxic conjugates of SPR ligands and the ribosome inactivating protein saporin that specifically target Substance P receptor-expressing cells. Whereas intrahippocampal microinjection of a conjugate of native SP and saporin produced significant nonspecific damage at concentrations needed to produce even limited selective loss of SPR-positive cells, a conjugate of saporin and the more potent and peptidase-resistant SP analog [Sar(9), Met(O(2))(11)] Substance P (SSP-saporin) caused negligible nonspecific damage at the injection site, and a virtually complete loss of SPR-like immunoreactivity (LI) up to 1 mm from the injection site. Within the SPR depletion zone, immunoreactivities for most GABA-, parvalbumin-, somatostatin-, and cholecystokinin-immunoreactive cells and fibers were eliminated. The few interneurons detectable within the affected zone were devoid of SPR-LI. The apparent loss of interneurons was selective in that calbindin- and glutamate receptor subunit 2 (GluR2)-positive principal cells survived within the affected zone, as did myelinated fibers and the extrinsic calretinin- and

tyrosine hydroxylase—immunoreactive terminals of subcortical afferents. An apparent lack of reactive synaptic reorganization in response to interneuron loss was indicated by zinc transporter-3 (ZnT3)—and beta-synuclein—LI, as well as by Timm staining, all of which revealed relatively normal patterns of excitatory terminal distribution. Control injections produced minor damage at the injection site, but no apparent specific loss of SPR-LI. One to 12 weeks after injection of SSP-saporin, extracellular electrophysiological field responses recorded in the CA1 pyramidal and dentate granule cell layers in response to afferent stimulation were blindly evaluated simultaneously in two sites 1–2 mm apart along the longitudinal hippocampal axis. SSP-saporin-treated rats exhibited relatively normal responses in some sites, whereas disinhibition and hyperexcitability indistinguishable from the pathophysiology produced by experimental status epilepticus were simultaneously recorded at adjacent sites. Anatomic analysis of the recording sites in each animal revealed that epileptiform pathophysiology was consistently observed only within areas of SPR ablation, whereas relatively normal evoked responses were recorded from immediately adjacent and relatively unaffected regions. These data establish the efficacy of [Sar(9), Met(O(2))(11)] Substance P-saporin for producing a selective and spatially extensive ablation of hippocampal inhibitory interneurons in vivo and a highly focal disinhibition that was restricted to the site of interneuron loss. These results also demonstrate that the “epileptic” pathophysiology produced by experimental status epilepticus or head trauma can be replicated by focal interneuron loss per se, without involving principal cell loss and other interpretive confounds inherent in the use of global neurologic injury models.

### COMMENTARY

Methods that selectively kill specific neurons are useful for experimental studies and could potentially have a clinical benefit. Several years ago, Mantyh et al. (1) showed

that a conjugate of Substance P and the ribosome-inactivating protein saporin could be used to target selectively neurons that express Substance P receptors (SPRs). Martin and Sloviter have recently modified this approach in the hippocampus to provide evidence that a microinjection of a conjugate of saporin and a peptidase-resistant analogue of Substance P (SSP-saporin) kills hippocampal neurons with SPRs. Hippocampal interneurons, but not principal cells (i.e., pyramidal cells and granule cells), express SPRs; thus, this treatment protocol led to the death of putative interneurons while sparing principal cells. Primarily using immunohistochemical techniques at the light-microscopic level, Martin and Sloviter described how this "targeted neurotoxin" approach can be used to kill virtually all neurons near the injection site that express SPRs, which they suggest are likely to be interneurons based on the other anatomical data.

This article provides information on how to use this method and describes evidence that SSP-saporin injections kill most or even all of the different types of interneurons, specifically including those neurons that express parvalbumin, somatostatin, and cholecystokinin. Timm staining after SSP-saporin injections revealed no evidence of reorganization of the mossy fibers, which is known to occur after repeated seizures. The authors argue that this approach to inducing selective loss of interneurons could be useful as a method to model the loss of interneurons that is thought to occur after episodes of prolonged seizures or head trauma, independent of other effects on principal neurons. Under some conditions, however, many hippocampal interneurons are preserved in human surgical specimens and in tissue from animal models of temporal lobe

epilepsy. Thus, future anatomical studies will probably address more specifically how the pattern of neurodegeneration after SSP-saporin injections and in models of head injury and status epilepticus are comparable.

The authors also evaluated the electrical correlates of the loss of neurons that express SPRs. Electrophysiological studies were conducted in anesthetized rats after SSP-saporin injections by recording extracellular field potentials from the dentate gyrus and CA1 area in response to electrical stimulation of the angular bundle of the perforant path. The authors did not report evidence of electrographic seizure activity, but this may be because the studies were conducted in anesthetized preparations. The responses to repetitive paired pulses, however, showed hyperexcitability in the areas with SSP-saporin injections and consequent loss of neurons with SPRs (i.e., putative interneurons). The hyperexcitability presumably reflects the loss of interneurons, but it will be interesting in the future to examine the electrophysiological mechanisms with patch recordings in hippocampal slices to determine the properties of inhibitory postsynaptic currents after SSP-saporin injections versus the models of head injury and status epilepticus.

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## References

1. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA. Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the Substance P receptor. *Science* 1997;278:275–279.