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Saporin Conjugates and Pain

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INTRODUCTION

Several saporin-containing targeted toxins have been used in studies of nociception/pain. This chapter reviews this exciting area, including some of our most recent work. Certainly, substance P-saporin (SP-sap), the first conjugate used for pain research, has generated the most data and interest, but a number of other saporin conjugates have been introduced, and others are on the way. A review discusses this topic *(1)*.

A large body of evidence has long pointed to a role for SP in nociception (*see*, e.g., refs. *2* and *3*). However, this mass of data has not yet resulted in a comprehensive formulation of the exact role of SP in nociception or led to a useful therapeutic strategy based on SP or SP antagonists. The potential for SP antagonists in clinical management of pain remains controversial. A reasonable distillation of the state of knowledge as of 1994 was the conclusion that some primary nociceptive neurons make and release SP, and dorsal horn neurons with neurokinin-1 receptor (NK-1R) are nociceptive. However, SP-secreting dorsal root ganglion neurons release other transmitters, particularly glutamate, which may explain the limited clinical analgesic efficacy of SP antagonists.

Development of SP-sap began in 1994 and was based on evidence for the roles of NK-1R-expressing dorsal horn neurons in nociception coupled with the evidence for receptor-mediated endocytosis of SP. Mantyh and coworkers *(4)* showed that SP is selectively internalized by cells expressing NK-1R, consistent with the general tendency for G protein-coupled receptors to internalize on binding of appropriate ligand. Subsequent studies have shown internalization of SP in neurons of the superficial dorsal horn in response to noxious stimuli *(5)*.

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Reasoning that dorsal horn cells that internalize SP were important second-order nociceptive neurons, we set out to make and test the properties of SP conjugated to saporin for selectively destroying dorsal horn neurons that express the NK-1R. At that time, there existed a few reports of other neuropeptide–toxin conjugates *(6–10)*, suggesting the approach was feasible. Initial experiments confirmed that SP-sap was selectively toxic to NK-1R-expressing neurons both in vitro and in vivo *(11)*.

NOCICEPTION AND SP-SAP

SP-Sap Effects on Innate Nocifensive Reflex Behavior

Initial studies using lumbar intrathecal injections of SP-sap showed that NK-1R-expressing neurons in the superficial dorsal horn of the spinal cord were ablated, and that within 3 d, the acute nocifensive responses to hindpaw intradermal injection of capsaicin were profoundly inhibited *(12)*. Thermal hyperalgesia and mechanical allodynia induced by intradermal capsaicin also were strikingly reduced. These results implicated NK-1R-expressing neurons of the superficial dorsal horn in both acute nociception and in the development of hyperalgesia and allodynia. A subsequent series of studies *(13)* reinforced and extended the initial scope and duration of observations, showing that intrathecal SP-sap reduced hyperalgesia and allodynia in a number of different models, including neuropathy and inflammation. Intrathecal SP-sap also attenuated phase II of the response to hindpaw formalin injection, a model of persistent pain. These studies showed that the antinociceptive effects of intrathecal SP-sap were persistent (>200 d).

SP-Sap Effects on Dorsal Horn Electrophysiology

Electrophysiological studies of dorsal horn neurons in rats treated with SP-sap have yielded somewhat surprising results. Suzuki and coworkers *(14)* recorded from nociceptive neurons in deeper laminae of the dorsal horn and found that pretreatment with SP-sap reduced excitability of wide dynamic range neurons. In these studies, receptive field sizes were decreased, as was coding of responses to thermal stimuli and central sensitization. They observed decreased expression of c-*fos* by dorsal horn neurons after intradermal formalin injection. Lumbar SP-sap also prevented activation of diffuse noxious inhibitory controls (DNICs) in response to noxious hindpaw stimulation, as revealed by loss of the usual suppression of c-*fos* expression in the cervical dorsal horn from noxious thermal stimulation of forepaws that occurs when similar noxious stimuli are simultaneously applied to hindpaws. The DNIC response to the opposite sequence, noxious forepaw stimulation