● Translational Vignette

Neuropeptide-Toxin Conjugates in Pain Research and Treatment

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Neuropeptides, including substance P (SP) and calcitonin gene-related peptide (CGRP), are known to be involved in pain perception.[1] Primary nociceptive neurons that release glutamate also cosecrete SP and/or CGRP.[2] Neonatal capsaicin treatment, which selectively destroys SP-secreting primary C nociceptive neurons, produces lifelong decreased pain sensitivity.[3] Transgenic mice with the gene "knocked out" for SP and neurokinin A show decreased pain responses to capsaicin, intraperitoneal acetic acid, and $MgSO_4$, but normal sensitivity to a range of other pain stimuli (thermal, chemical, and mechanical).[4] Mice lacking the receptor for SP (NK-1R) show decreased "wind up" of responses of nociceptive dorsal horn neurons in response to repetitive C fiber stimulation, lack of intensity coding of a hindlimb withdrawal reflex, and less stress-induced analgesia.[5] Mice that overexpress SP are hyperalgesic.[6] These findings, and a number of other lines of evidence, suggest a nociceptive role for dorsal horn neurons that respond to SP, i.e., express the neurokinin-1 receptor (NK-1R).

To assess the role of NK-1R–expressing neurons in pain perception, we developed a toxin that takes advantage of the fact that SP is efficiently and selectively taken up by neurons after binding the NK-1R.[7,8] The toxin, SP-saporin, consists of SP coupled to a ribosome-inactivating protein, saporin.[9,10] Saporin is an enzyme that, if internalized, inactivates ribosomes, thereby inhibiting protein synthesis resulting in cell death. Normally, saporin is not taken up by neurons, but when coupled to SP, it is selectively taken into neurons that express NK-1R (Fig 1). In animals, lumbar intrathecal injection of SP-saporin selectively destroys NK-1R–expressing neurons in the superficial dorsal horn of the spinal cord.[11] Animals treated in this fashion show decreased behavioral responses to subcutaneous capsaicin injection that selectively activates C nociceptor fibers. The effects of the toxin last for at least 200 days in rats and also include decreased hyperalgesia and allodynia in several other experimental pain models, including the following[12]: (1) hindpaw formalin injections (a model of persistent pain); (2) hindpaw carrageenan injection and hindpaw Freud's adjuvant injection (models of inflammatory pain); and (3) sciatic nerve ligation with chromic gut sutures (inflammatory mononeuropathy). More recently, similar inhibition of thermal hyperalgesia after hindpaw mustard oil or capsaicin has been observed in operant pain assays designed to assess the degree of discomfort experienced by rats (Fig 2). These findings suggest an important role in hyperalgesia for lamina I dorsal horn neurons that express NK-1R.

The available animal data point to possible therapeutic usefulness of selectively destroying lamina I NK-1R–expressing neurons in patients with long-term intractable pain. Also, the success of SP-saporin indicates that other neuropeptides, hormones, and growth factors that interact with G-protein–coupled receptors are likely candidates for making additional targeted toxins. Initial studies have begun with a peptide-saporin conjugate targeted at neurons that express the mu opiate receptor. However, another successful approach to selective neural lesions uses immunotoxins, monoclonal antibodies armed with saporin and directed at unique surface molecules, on specific types of neurons. Of particular interest to pain research is anti–DBH-

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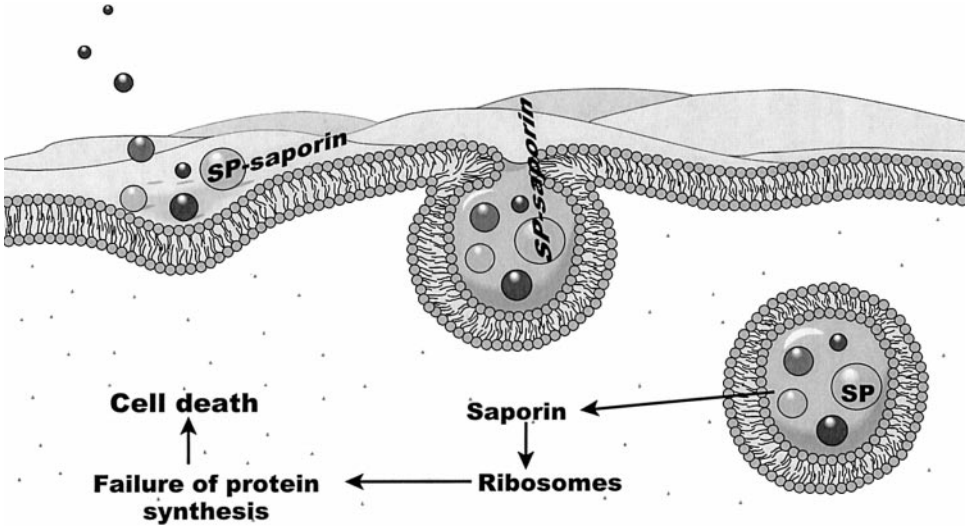

Fig 1. Delivery of saporin into neurons through receptor-mediated endocytosis. SP-saporin binds to the NK-1R on the surface membrane of neurons, which results in endocytosis of the SP-saporin conjugate. Once inside the cell, saporin can escape the endocytic pathway into the cytosol where it catalytically inactivates ribosomes, leading to failure of protein synthesis and cell death.

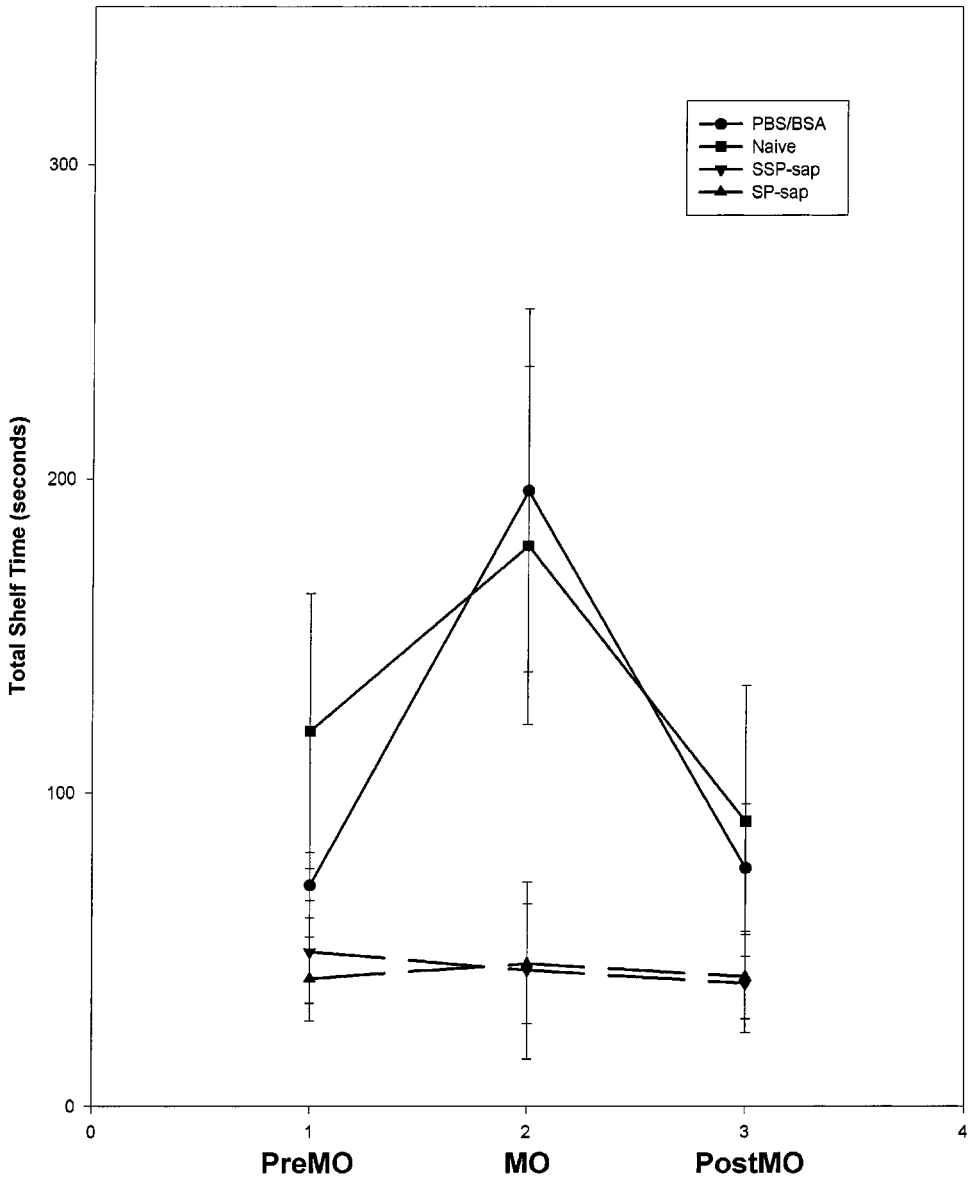

Fig 2. Effects of mustard oil and SP-saporin on thermally induced escape behavior in rats. Rats placed on a dimly illuminated moderately warm surface (44°C) are allowed to escape to a brightly lit room temperature shelf. If pretreated 3 hours earlier with mustard oil (MO) to the dorsal surface of the hindpaws, normal control rats will spend more time on the escape shelf, indicating the presence of secondary thermal hyperalgesia; but SP-saporin–treated rats show no increase in escape after mustard oil, indicating that the toxin prevented development of hyperalgesia. Key: PBS/BSA, 8 rats with lumbar intrathecal injection of saline containing albumin (standard vehicle); Naive, 9 rats never operated; SP-sap, 7 rats with lumbar intrathecal injection of 175 ng of substance P-saporin conjugate; SSP-sap, 8 rats injected with 100 ng of [Sar^{9},$Met(OH)^{11}$]SP-saporin conjugate.

saporin, an immunotoxin directed against dopamine β-hydroxylase that selectively destroys noradrenergic and adrenergic neurons,[13] even when injected into a terminal field.[14,15] Initial experiments using anti–DBH-saporin to selectively destroy pontine noradrenergic neurons show decreased thermal hyperalgesia after mustard oil or capsaicin in operant assays (not shown) and increased intensity of opiate withdrawal.[16] Lumbar intrathecal injections of anti–DBH-saporin selectively destroy the noradrenergic innervation of the spinal cord, resulting in reduced late-phase nociceptive behavior after hindpaw formalin injection and enhanced effect of morphine on early response to formalin.[17] These findings highlight the usefulness of the targeted toxin approach in experiments designed to reveal the role of specific neural systems in pain perception. Both neuropeptide-toxin conjugates and immunotoxins are powerful research tools, and some of these agents may soon prove therapeutically useful.

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