Jan-Feb-Mar 2006
Volume 7, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

The Biologically Active Cholecystokinin (26-33) peptide, [Tyr^2-SO$_3$]CCK-8, Retains High Affinity for CCK$_2$ Receptors after Covalent Conjugation to Saporin

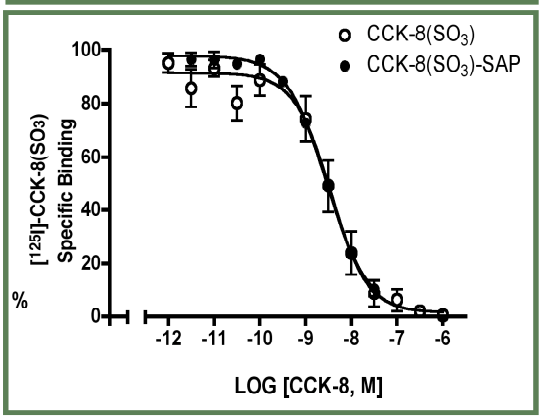
Josephine Lai, Wenjun Zhang, Hamid Badghisi, Victor J. Hruby[1] and Frank Porreca,
Departments of Pharmacology and Chemistry[1], The University of Arizona, Tucson, AZ 85724.

Cholecystokinin (CCK) is widely distributed in the central nervous system and the gastrointestinal tract. The 33-amino acid peptide contains a carboxyl terminal octapeptide sequence Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 which confers the biological activity of CCK, and where the tyrosine residue occurs in sulfated form. This octapeptide, CCK-8(SO$_3$), has high affinity for the two structurally-defined CCK receptor types, CCK$_1$ and CCK$_2$. A covalent conjugate of CCK-8(SO$_3$) to saporin (CCK-8(SO$_3$)-SAP) was synthesized and evaluated for the toxin conjugate's affinity for the human CCK$_2$ receptors in transfected HEK293 cells (Figure 1). The K_i value of CCK-8(SO$_3$) for the CCK$_2$ receptors was 3.6 nM (log K_i = -8.4 ± 0.09, n = 6), and that of CCK-8(SO$_3$)-SAP was 3.2 nM (log K_i = -8.5 ± 0.02, n = 2). Thus, the conjugation of saporin to CCK-8(SO$_3$) does not significantly alter the affinity of CCK-8(SO$_3$) for CCK$_2$ receptors and should be effective in the targeted-lesion of CCK$_2$-expressing cells by CCK mediated internalization of saporin. Furthermore, based on the saturation analysis of [^{125}I]CCK-8(SO$_3$) for the hCCK$_1$ and hCCK$_2$ receptors, the peptide has similar affinity for the two receptor types (Kd values are 1.9 ± 0.2 nM and 1.3 ± 0.4 nM for hCCK$_1$ and hCCK$_2$ receptors, respectively). It is reasonable to predict that CCK-8(SO$_3$)-SAP has similar affinity for both CCK receptor types, while the non-sulfated CCK-8-SAP is likely to be selective for the CCK$_2$ receptors, albeit with lower affinity (56 nM; see *Targeting Trends* 4(3):5). It should be noted also that the sulfated group on CCK-8(SO$_3$) may be hydrolyzed upon storage; thus the affinity of the toxin conjugate should be verified experimentally prior to application.

We evaluated the effect of CCK-8(SO$_3$)-SAP on CCK$_2$ receptor-expressing cells in the rostral ventromedial medulla (RVM) of rats by the stereotaxic microinjection of a single dose of CCK-8(SO$_3$)-SAP (3 pmoles, bilaterally at 1.5 pmoles in 0.5 μL per side) into the RVM of anesthetized rats. Separate groups of rats were given the same dose of either saporin or CCK-8(SO$_3$) as control. All animal use and procedures were reviewed and approved by IACUC. Twenty-eight days after the RVM

(continued on page 6)

Figure 1.
[^{125}I]-CCK-8(SO$_3$) / Ligand competition in transfected HEK 293 cells that express hCCK$_2$ receptors.
Data represent mean % standard error from at least 2 independent experiments. Non-specific binding was defined by the amount of [^{125}I]-CCK-8(SO$_3$) bound in the presence of 1 μM CCK-8(SO$_3$).



Tony Mezzadri Surf Contest

ATS took in the 11th Annual Tony Mezzadri Surf Contest at the Ocean Beach CA pier in October of 2005. This fun-filled, exciting exhibition of top-flight surfing prowess contributes to a great cause: support of spinal cord injury research. Tony was injured in a surfing accident and the good folks of Ocean Beach started this contest to help out. It's so successful that money now goes to support the UCSD lab of Mark Tuszynski and his research on regeneration after spinal cord injury. ATS is proud to be a sponsor.

Brian Russell and Leonardo Ancheta hit the beach to watch these amazing surfers.

A talented surfer hangs five during just one of many thrilling rides.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: RELEASING
PITUITARY
ENDOCRINOLOGY
DIJON
SOMATOSTATIN
ENDORPHINS

Answer: ROGER GUILLEMIN

WINNERS: David Akopian, California State Univ, Northridge * Byran Hudson, Washington State Univ, VCAPP * Kris Preddy, Lakeside, CA * Miriam Burton, Kansas State Univ, Anatomy & Physiology * Andrea Morris, Panacea * Bill Stell, University of Calgary * Tania Bedard, University of Texas Health Center, Pharmacology * Dr. Bruce Pappas, Carleton University, Life Research Ctr * Jindong Ding, UNC, Dept of Cell & Devel Biology * Janice Urban, Rosalind Franklin Univ, Dept of Physiology & Biophysics * Robert Speth, Univ of Mississippi, School of Pharmacy * Seto Chice, SUNY HSC at Brooklyn

Roger Guillemin was born in **Dijon**, France on January 11, 1924. (Happy Birthday!) He won the Nobel Prize in 1977 for discoveries concerning peptide hormone production in the brain. His work brought to light an entire new class of substances shown to be important for the regulation of growth, development, reproduction and responses to stress.

In the early 1950's, he studied experimental **endocrinology** in a program jointly conducted between McGill University and the University of Montreal. During his time in Canada, Guillemin became interested in the problem of the physiological control of the secretion of the **pituitary** gland as it was involved in the acute response to stress.

The impact of the broad expanse of Guillemin's studies has been profound for a variety of diseases and disorders, including thyroid diseases, problems of infertility, diabetes and several types of tumors.

One of the hormones studied, called growth-hormone **releasing** factor, is used to treat growth deficiencies in children; another, called **somatostatin**, is used to control internal bleeding during surgery. Guillemin also was among the first to isolate **endorphins**, brain molecules known to act as natural opiates. Following the isolation of endorphins, his work with cellular growth factors (FGFs), in addition to inhibins and activins, led to the recognition of multiple physiological functions and developmental mechanisms.

Since his retirement from the active pursuit of science in 1989, Guillemin has shifted his long-standing expertise with computers from science to art. He is using the Macintosh computer to create images/paintings that are eventually transferred to paper or canvas. Turn to page 5 for a sample of his work.

Portions of this excerpt along with additional information can be found at: http://www.salk.edu/faculty/faculty/details.php?id=25

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Nicotine-induced switch in the nicotinic cholinergic mechanisms of facilitation of long-term potentiation induction.

Yamazaki Y, Jia Y, Hamaue N, Sumikawa K
Eur J Neurosci 22(4):845-860, 2005

The authors investigated cellular mechanisms underlying improved cognitive function in Alzheimer's disease patients upon the administration of nicotine. To model Alzheimer's disease in rats, 2 μg of 192-IgG-SAP (Cat. #IT-01) was injected into the lateral cerebral ventricle. Examination of the lesioned animals suggests that nicotine promotes the induction of long-term potentiation by enhancing N-methyl-D-aspartate responses, and suppressing acetylcholine-mediated mechanisms in pyramidal cells.

Saporin and ricin A chain follow different intracellular routes to enter the cytosol of intoxicated cells.

Vago R, Marsden CJ, Lord JM, Ippoliti R, Flavell DJ, Flavell SU, Ceriotti A, Fabbrini MS
FEBS J 272(19):4983-4995, 2005

Some bacterial toxins such as Pseudomonas aeruginosa exotoxin A carry a KDEL-like C-terminal peptide sequence, which targets the protein to the endoplasmic reticulum. Saporin (Cat. #PR-01) is a plant ribosome-inactivating protein, which does not contain a KDEL-like sequence. Here the authors examined the intracellular pathways utilized by saporin. Although ricin, another plant ribosome-inactivating protein, could be visualized in the Golgi complex, saporin was not. The data suggest that saporin may utilize endosomes during its journey through the cell.

Selective acetylcholine and dopamine lesions in neonatal rats produce distinct patterns of cortical dendritic atrophy in adulthood.

Sherren N, Pappas BA
Neuroscience 136(2):445-456, 2005

In this work the authors examined lesions of acetylcholine afferents in 7-day-old rat pups, and the effect on dendritic development. 600 ng of 192-IgG-SAP (Cat. #IT-01) were administered to the ventricles of test animals. Various morphological changes in the retrosplenial cortex were observed, including smaller apical tufts and fewer basilar dendritic branches in layer V medial prefrontal cells. The data demonstrate that ascending acetylcholine afferents are very important in the development of cortical cytoarchitecture.

Estrogen contributes to structural recovery after a lesion.

Saenz C, Dominguez R, de Lacalle S
Neurosci Lett 392(3):198-201, 2006

The authors evaluated the trophic effects of 17ß-estradiol (E2) on cholinergic neurons of the basal forebrain after lesioning with 192-IgG-SAP (Cat. #IT-01). Ovariectomized female rats received 200 nl of 0.075 mg/ml 192-IgG-SAP followed by a subcutaneous pellet of E2, which was released over 60 days. Dendritic size in ovariectomized rats receiving the E2 was the same as in control animals, while ovariectomized rats receiving a placebo displayed a significant reduction in dendritic arborization.

Sleep-disordered breathing after targeted ablation of preBotzinger complex neurons.

McKay LC, Janczewski WA, Feldman JL
Nat Neurosci 8(9):1142-1144, 2005

Sleep-disordered breathing is common in elderly humans as well as patients with neurodegenerative disease. The authors investigated the role of preBötzinger complex neurons of rats in respiratory rhythm generation. Using the fact that preBötzinger complex neurons in the ventrolateral medulla express the neurokinin-1 receptor, animals were given bilateral injections of SP-SAP (Cat. #IT-07). Beginning 7 days post-injection, lesioned animals displayed marked respiratory disturbances during both sleep and wakeful periods.

(continued on page 4)

Have you published using an ATS product?

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Targeting Topics: Recent Scientific References

(continued from page 3)

Increased phencyclidine-induced hyperactivity following cortical cholinergic denervation.

Mattsson A, Lindqvist E, Ogren SO, Olson L

Neuroreport 16(16):1815-1819, 2005

A potential contribution to schizophrenia is altered cholinergic function. The authors investigated how lesioning cholinergic corticopetal projections might affect glutaminergic activity. Rats were injected with 0.134 μg of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis. The authors found that cholinergic lesioning of the neocortex led to enhanced sensitivity to phencyclidine, which has been shown to induce clinical symptoms similar to those of schizophrenia. These data suggest that glutaminergic dysfunction may be relevant to schizophrenia pathophysiology.

Selective loss of basal forebrain cholinergic neurons by 192 IgG-saporin is associated with decreased phosphorylation of Ser glycogen synthase kinase-3beta.

Hawkes C, Jhamandas JH, Kar S

J Neurochem 95(1):263-272, 2005

Glycogen synthase kinase-3ß (GSK-3ß) is an enzyme involved in a variety of biological events. In this study the authors examined the potential role of GSK-3ß in degeneration of basal forebrain cholinergic neurons. Rats were treated with 2.0 μg per ventricle injections of 192-IgG-SAP (Cat. #IT-01), then GSK-3ß and other cholinergic marker levels were assayed. The results indicate that increased GSK-3ß activity can provide some protection from 192-IgG-SAP-induced degeneration of forebrain cholinergic neurons.

Insomnia following hypocretin2-saporin lesions of the substantia nigra.

Gerashchenko D, Blanco-Centurion CA, Miller JD, Shiromani PJ

Neuroscience [epub Nov 10], 2005

It is known that orexin (also known as hypocretin) is involved in waking. Here the authors investigate which regions of major arousal areas might be responsible for the changes in sleep-wake architecture upon treatment with orexin-SAP (Cat. #IT-20). Bilateral injection of orexin-SAP into the ventral tegmental (VT) area and the substantia nigra (SN; 92 and 184 ng/µl, 0.25 µl in the VT area and 0.5 µl in the SN) of rats induced insomnia, as well as hyperactivity and stereotypic movements. The results suggest that motor activity is under inhibitory control of the SN.

The septohippocampal cholinergic system and spatial working memory in the Morris water maze.

Frielingsdorf H, Thal LJ, Pizzo DP

Behav Brain Res [epub Dec 2], 2005

The authors examined whether an optimized Morris water maze test could reveal the role of the septohippocampal cholinergic system in spatial working memory. Rats were treated with bilateral 75-ng injections of 192-IgG-SAP (Cat. #IT-01) followed by acquisition of the water maze task, and two independent phases of working memory testing. Test optimization was followed by icv infusion of nerve growth factor in unlesioned animals. The data demonstrate that working memory impairments cannot be revealed by the Morris water maze test.

Cholinergic septo-hippocampal innervation is required for trace eyeblink classical conditioning.

Fontan-Lozano A, Troncoso J, Munera A, Carrion AM, Delgado-Garcia JM

Learn Mem 12(6):557-563, 2005

Classical conditioning of eyeblink responses can be used to evaluate cognitive deficits. The authors lesioned the medial septum/diagonal band of rats with 200 ng of 192-IgG-SAP (Cat. #IT-01), then examined classical and instrumental conditioning paradigms. Lesioned animals displayed a deficit in the acquisition, but not retrieval of eyeblink classical conditioning. The deficit was reversed by carbachol, a cholinergic muscarinic agonist, suggesting a role for the muscarinic system in the acquisition of new motor abilities.

Upcoming Events

Spring Brain Conference
March 15-18, 2006
Sedona, Arizona

Experimental Biology
April 2-4, 2006
San Francisco, CA
Booth #837

Targeting Talk: *LPS Content*

by Dr. Douglas Lappi

Q: In a recent experiment using a saporin-antibody conjugate injected systemically we saw changes in dendritic cells that could be consistent with an LPS effect. Does ATS test for LPS and has this ever been identified as a problem before?

A: Yes, this can happen, but we here at ATS will swear innocence. One of our collaborators just reported the same thing (the first comment like that in several years), so I'll tell you the story.

Generally our materials have a very low endotoxin content, on the order of less than 1 EU/mg protein. We check this occasionally because most of our customers provide an easy assay. That is, these things are often injected into the brains of rats, and they'll die within a few minutes if there is an LPS (lipopolysaccharide) content such as you described. That would be disastrous for us, so we do pay attention to this issue.

The recent situation was with an immunotoxin that was also injected systemically, and gave a response similar to what you're stating. The material had been thawed and used in a set of experiments. Then, because of concerns about the effects of freezing and thawing, it was left in the refrigerator for a considerable period of time. It was then used in the experiments that gave the LPS-consistent result. We believe that this material was no longer sterile and that during the time between uses it grew bacteria. We went back and assayed the original material and it gave the usual less than 1 EU/mg value.

The bottom line is that once the sterility is broken, the material is a decent growth medium for bacteria (a protein in PBS). We filter sterilize all of our targeted toxins and package them in a sterile manner, but we do not add preservative. The thinking there is that in sensitive situations, a preservative can cause its own biological response/effect.

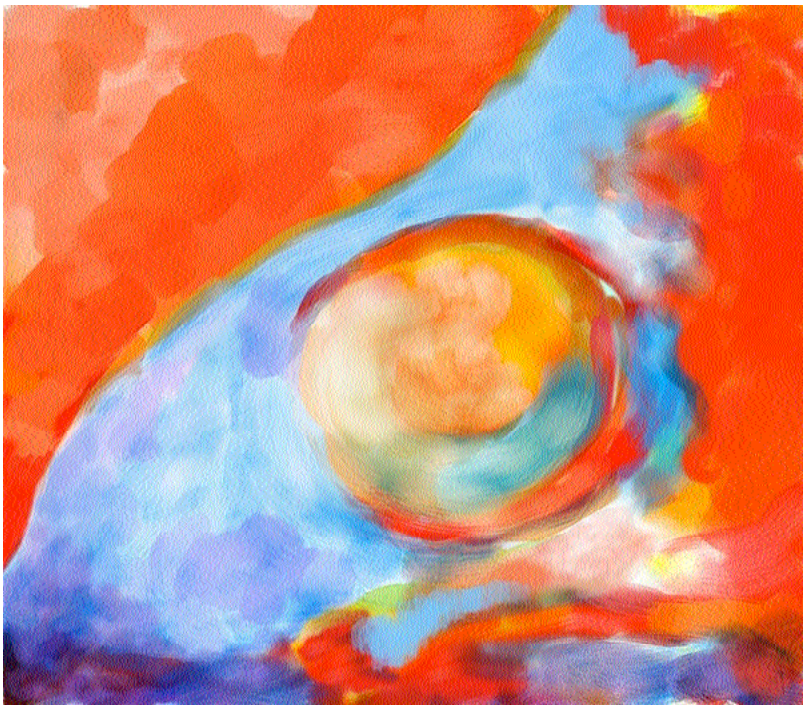
This situation causes us to print rather stringent use instructions: aliquot and store frozen at -20°C.

This brilliant artistic expression is a creation by Dr. Roger Guillemin (see brief bio on page 2). The title of this piece is *Hamadryad*.

When asked about the reason for this title, Dr. Guillemin replied, “Hamadryads, as the name implies, were nymphs of the forest, of trees. Thus, the painting should be more green than red. Why that title? I just don't quite remember. Probably some music (Dryades et hamadryades in Chansons de Galathée by Debussy, if I'm not mistaken) or some poetry that came my way at that time.”

Whatever the inspiration for the title of this piece, the result is indeed an inspiration in and of itself.

For more examples of Dr. Guillemin’s art, visit: holborngallery.com/harwood.html.



[Tyr^2-SO_3]CCK-8, Retains High Affinity for CCK_2 Receptors

(continued from page 1)

injections, rats were evaluated again for their sensory thresholds to both innocuous and noxious stimuli in the hind paw. Coronal sections of the brain stem containing the RVM were processed for the detection of CCK_2 receptor-expressing cells by *in situ* hybridization using a digoxigenin labeled riboprobe for CCK_2. CCK-8(SO_3)-SAP pretreatment did not alter the tactile or thermal sensory thresholds in rats when compared to naïve, CCK-8(SO_3) or saporin pretreated rats. However, CCK-8(SO_3)-SAP pretreatment, but not CCK-8(SO_3) or saporin pretreatment, resulted in a significant reduction in the number of cells expressing CCK_2 transcripts in the RVM (Figure 2). This reduction in the number of CCK_2 immunoreactive cells was seen in serial sections taken throughout the ~1 mm rostral-caudal extent of the RVM, representing a >80% reduction in the total number of labeled cells ($p<0.05$). There was no evidence of necrosis or significant cell loss in the RVM and surrounding regions of the brain stem. These data suggest that the targeted microinjection of a low dose of CCK-8(SO_3)-SAP can significantly reduce the population of RVM cells that express CCK_2 receptors.

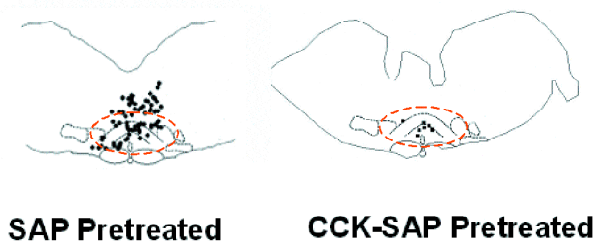


Figure 2. Map of cells that are immunoreactive for CCK2 receptor mRNA on representative coronal sections (20 μm) of the RVM and its adjacent regions of the medulla from rats that have been pretreated with saporin (control, left) or CCK-8(SO3)-SAP (right). CCK2 receptor mRNA were detected by *in situ* hybridization using a digoxigenin labeled riboprobe for CCK2. The riboprobe was detected by an anti-digoxigenin Fab (Roche Diagnostics, Indianapolis, IN) conjugated with alkaline phosphatase. The immunoreactivity was detected chromogenically using fast red as substrate. Computer-assisted mapping was carried out using Neurolucida software (Microbrightfield Inc., Baltimore, MD). Each black dot marks the location of a single cell that was labeled for CCK2. In the left panel, CCK2 immunoreactive cells are localized to the RVM (approximately denoted with dashed red line) and the nucleus gigantocellularis dorsal to the RVM. In the right panel, 28 days after CCK-8(SO3)-SAP treatment, few CCK2 immunoreactive cells are detected.

Acknowledgments

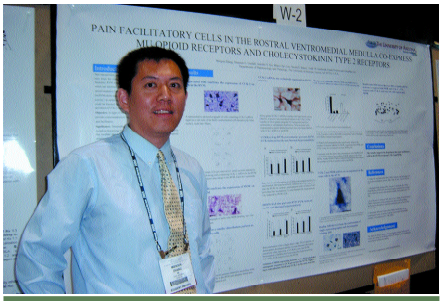
The authors thank Dr. Alan Kopin for the generous gift of the cDNAs for $hCCK_1$ and $hCCK_2$ and Dr. Richard Agnes for the synthesis of CCK-8(SO_3).

17th Annual Spring Brain Conference: *March 15-18, 2006 - Sedona, Arizona*

The Spring Brain Conference (SBC) brings together top neuroscientists with varied backgrounds, interests and approaches to promote the development of new strategies to investigate and stimulate the development of new therapeutic approaches to disorders of the CNS. The conference will consist of a general poster session along with 8-10 plenary sessions each organized around a central theme or topic. For additional information contact Dr. Bob Yezierski (ryezierski@dental.ufl.edu) or visit the SBC website: www.springbrain.org.

2005 Society for Neuroscience Poster Award Winner:

"PAIN FACILITATORY CELLS IN THE ROSTRAL VENTROMEDIAL MEDULLA COEXPRESS OPIOID-μ RECEPTORS AND CHOLECYSTOKININ TYPE 2 RECEPTORS" W. Zhang; S.E. Gardell; Y. Xie; M. Luo; N.E. Rance; T.W. Vanderah; F. Porreca; J. Lai, Univ. Arizona.



Wenjun Zhang at his winning poster at the SfN meeting in Washington DC.

Each year, ATS is pleased to see the interesting ways in which scientists use our products and present their data in abstracts, posters, and presentations. This year we were excited to see the excellent work by Wenjun Zhang and his colleagues at the University of Arizona that was presented in this year's Poster of the Year at the Society for Neuroscience meeting in Washington, Nov 12-16, 2005. Of course we were doubly pleased by the use of both dermorphin-SAP and CCK-SAP (the latter the subject of the cover article in this newsletter), but that was a small part of the nice science that was presented. The use of these reagents to demonstrate the characteristics of "ON cells" was quite original and clever. Congratulations to them!

Doug Lappi presents the awards for best poster -- a copy of the book, "Molecular Neurosurgery With Targeted Toxins," an ATS mug, an ATS ball cap, $500 in product credit, and the opportunity to publish a cover article in *Targeting Trends*. Dr. Josephine Lai accepted the prizes on behalf of Dr. Zhang.

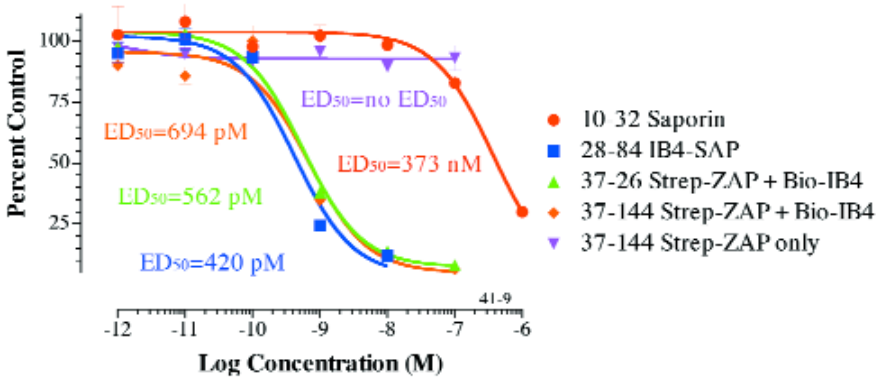
Targeting Tools: Featured Products

Streptavidin-ZAP (Cat. #IT-27)

Saporin conjugated to streptavidin is one of the most useful reagents that we have produced at ATS. It has been used in a wide variety of studies both in academic research and pharmaceutical/biotech labs. Workers are able to easily see if their molecule, that is biotinylated, is internalized by the target cells. The simple read-out of cell death requires only a microscope, but is also easily scaled up for industrial use. The streptavidin-biotin bond is so strong that streptavidin-ZAP can even be used *in vivo*. The technique can be used with all sorts of molecules: growth factors, peptide ligands, cytokines, antibodies—anything that can be biotinylated. Can't biotinylate it? Then let us do it for you!

Streptavidin-ZAP *(conjugate alone)*	
Cat #	Units
IT-27-25	25 micrograms
IT-27-100	100 micrograms
IT-27-250	250 micrograms

Kit: conjugate and control *(saporin)*	
Cat #	Units
KIT-27-25	25 micrograms
KIT-27-100	100 micrograms
KIT-27-250	250 micrograms



KNRK cells are plated at 2500 cells/well and incubated overnight. Streptavidin-ZAP is premixed with Biotinylated-IB4 in equimolar concentrations. Saporin, IB4-SAP, and the Streptavidin-ZAP + Biotinylated-IB4 mixture are then added in 10-µl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 15-30 minutes, then read at 490 nm.

Biotinylation Service

A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with avidin-labeled probes or other detection reagents for use in biological assays. Using streptavidin-ZAP and biotinylated targeting agents, specific cytotoxins can be created JUST BY MIXING!

Step 1: Send ATS your targeting agent (antibody, lectin, etc.).

Step 2: ATS biotinylates your targeting agent and returns it to you with streptavidin-ZAP.

Step 3: You mix together your biotinylated targeting agent and streptavidin-ZAP to specifically eliminate cells that recognize and internalize your targeting agent.

There's nothing better than a nice cozy place to curl up when the weather turns cold.

Please visit our website (www.ATSbio.com) to see a complete list of products.

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

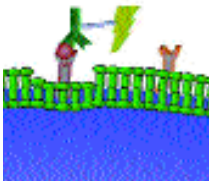
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

[§]or growth factor, peptide, ligand, or cytokine

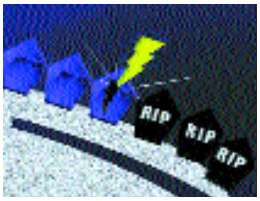
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

LIGGO ◯◯◯◯◯

LANCETRAILRUL ◯◯◯◯◯◯◯◯◯◯◯◯◯

SUNCLUE ◯◯◯◯◯◯◯

TRAINZINEEL ◯◯◯◯◯◯◯◯◯◯

MOODSENE ◯◯◯◯◯◯◯◯

How the Scientist described the process of invention.

Arrange the circled letters to form the answer, as suggested by the above clue.

Answer: ◯◯◯◯◯◯◯◯◯◯◯◯ ◯◯◯◯◯◯

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by February 28, 2006.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

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Targeting Trends

Reporting the latest news in Molecular Surgery

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeted Toxins in Pain

Summary of contribution to "Recontres en toxinologie, 2005" by

Ronald G. Wiley, Neurology Service (127) - VA TVHS, 1310 24th Avenue, South, Nashville, TN 37212

The use of targeted toxins in neuroscience research has evolved over the past twenty-plus years from original suicide transport lesions using ricin to highly selective neuron type-specific lesions made with immunotoxins, such as anti-dopamine beta hyroxylase-saporin (anti-DBH-SAP, Cat. #IT-03), and neuropeptide-toxin conjugates, such as substance P-saporin (SP-SAP, Cat. #IT-07).

Application of these agents to experiments in the neurobiology of pain began about ten years ago with the development of anti-DBH-SAP which selectively destroys adrenergic and noradrenergic neurons, and SP-SAP which destroys neurons that express neurokinin-1 receptor (NK-1r; Figure 1). Anti-DBH-SAP has been used to show the importance of central noradrenergic neurons in withdrawal from chronic opiate administration and in descending regulation of nociception. Intrathecal injection of SP-SAP produces robust decreases in operant responses to noxious thermal stimuli over a wide range of temperatures with preserved innate reflex nocifensive responses to noxious thermal and mechanical stimuli.[1,2] SP-SAP also profoundly decreases operant hyperalgesia and nocifensive hyperreflexia in a variety of animal models, including topical mustard oil or capsaicin, spinal nerve ligation, carrageenan- and Freund's adjuvant-induced inflammation.[3,4] SP-SAP reduces responding in the formalin model of persistent pain (phase II). A targeted toxin using a more stable analog of substance P (SSP-SAP, Cat. #IT-11) produces similar effects at lower doses with better specificity.[5]

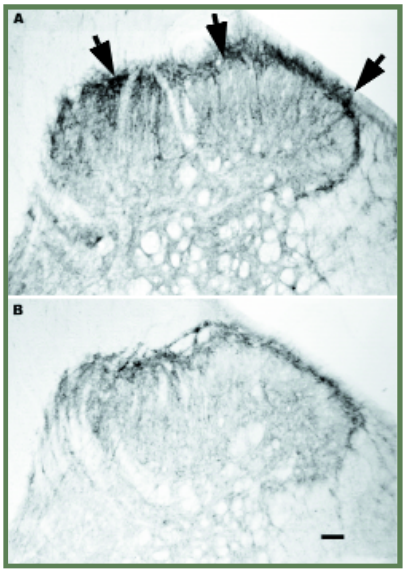


Figure 1.
Effects of intrathecal SP-SAP on NK-1r-expressing rat dorsal horn neurons.
A) Normal immunoperoxidase staining for NK-1r after vehicle injection (arrows).
B) Decreased NK-1r staining after intrathecal administration of SP-SAP (175 ng).
Magnification bar in B indicates 100 µm. Counts of NK-1r-expressing neurons in deeper laminae revealed that cell loss was limited to the superficial laminae (I and II).

A similar construct, dermorphin-SAP (Cat. #IT-12), eliminates mu opiate receptor-expressing neurons from the medulla or the substantia gelatinosa of the spinal cord.[6] Medullary dermorphin-SAP injections produce changes in descending regulation of nociception resulting in decreased hyperalgesia and allodynia in a sciatic nerve constriction injury model of neuropathic pain.[7-9] The successes of SP-SAP, SSP-SAP, and dermorphin-SAP suggest a general strategy for targeting neurons expressing specific G-protein coupled receptors. SP-SAP, and perhaps other neuropeptide-toxin conjugates, may have potential in the treatment of chronic intractable pain (see companion article on Page 2).

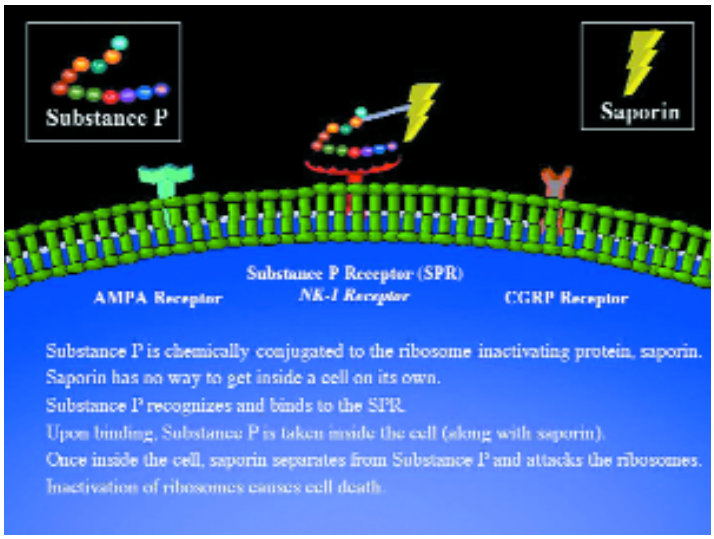
(continued on page 6)

Chronic Pain Drug - Update on SP-SAP Development

ATS continues to make progress toward human clinical trials with SP-SAP. Thanks to the financial support of the National Institutes of Health, National Institute of Mental Health, preclinical studies have been completed, protocols for drug production have been written and the first of two toxicology studies is done. This first study is a GLP toxicology study done in rat. At the time of this printing, the reports are being finalized with the results. The important preclinical work that was published in the journal *Science* was done in rat, but the studies were not GLP, nor did they examine enough parameters to meet the standards set for a toxicology study. The FDA requires one additional toxicology study be completed in another animal model before tests can begin in humans.

Another important part of the drug development process is the careful formulation of the drug itself. ATS is fortunate to have established a relationship with Dr. Arthur Frankel and his team at Scott and White Memorial Hospital, Cancer Research Institute in Temple, Texas. Dr. Frankel has supervised the production of four drugs under GMP (Good Manufacturing Practices) conditions. He is an expert in the field of recombinant toxin drug development. His group is currently working with ATS to improve the production of recombinant saporin (the toxic component of SP-SAP).

For several years ATS has been discussing the possibility of using SP-SAP in veterinary applications, in particular for bone cancer in dogs. Within the next few weeks, the protocol should be completed and the trial set to begin. SP-SAP is expected to provide relief



from the debilitating pain these pets endure. With that relief they should experience a greater freedom of movement and enjoyment of life. Check the next issue of *Targeting Trends* for more details on this exciting project.

And, finally, here is a follow up on the July 2005 article regarding a partnering opportunity for ATS. Meetings have been held with potential candidates, but no deals have been finalized to form a partnership to bring SP-SAP to market for patients in chronic pain. ATS is looking for an experienced pharmaceutical or biotechnology company that can quickly take SP-SAP through the regulatory and clinical phases of development.

If you have a connection with such a company, please contact Denise Higgins, Vice President of Business Development (877-889-2288; denise@atsbio.com). ATS will provide a business plan and other pertinent information to potential partners.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: GOLGI
INTRACELLULAR
NUCLEUS
INTERNALIZE
ENDOSOME

Answer: INTELLIGENT DESIGN

Winners: David Akopian, California State Univ Northridge * Valery Nelson, Panacea Pharmaceuticals Inc * Robert Speth, Univ of Mississippi, School of Pharmacy * Seto Chice, SUNY HSC at Brooklyn

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Estradiol and orexin-2 saporin actions on multiple forms of behavioral arousal in female mice.

Easton A, Dwyer E, Pfaff DW

Behav Neurosci 120(1):1-9, 2006.

Many aspects of female behavioral arousal in response to estrogens are not yet well understood. Here the authors examine the role of orexins as targets for estrogens. Female mice were treated with 10 ng of orexin-SAP (Cat. #IT-20) into each hemisphere of the lateral hypothalamus. The mice were then tested in different modes of behavioral arousal. Mice treated with orexin-SAP displayed decreases in sensory responsiveness and fearfulness concomitant with a reduction in orexin cell number.

Targeting of the receptor protein tyrosine phosphatase beta with a monoclonal antibody delays tumor growth in a glioblastoma model.

Foehr ED, Lorente G, Kuo J, Ram R, Nikolich K, Urfer R

Cancer Res 66(4):2271-2278, 2006.

The receptor protein tyrosine phosphatase ß (RPTPß) is overexpressed in astrocytomas, and is a potential target for tumor therapy. After testing antibodies against an extracellular domain of RPTPß *in vitro* with Mab-ZAP (Cat. #IT-04), two custom conjugates, 7E4B11-SAP and 7A9B5-SAP, were created by Advanced Targeting Systems. The authors tested the custom conjugates, using anti-DAT-SAP (Cat. #IT-25) as a positive control, and mouse IgG-SAP (Cat. #IT-18) as a negative control. The 7E4B11-SAP conjugate displayed significant antitumor activity in mice engrafted with U87 glioma cells.

Photochemically stimulated drug delivery increases the cytotoxicity and specificity of EGF-saporin.

Weyergang A, Selbo PK, Berg K

J Control Release 111(1-2):165-173, 2006.

In this study the authors investigated the use of photosensitizers located in endocytic vesicles that can be induced to release macromolecules upon activation by light. This process is called photochemical internalization, or PCI. Biotinylated EGF was combined with streptavidin-ZAP (Cat. #IT-27), and the compound was applied to various cell lines. The data shows that PCI increases the toxicity of EGF-saporin significantly in EGF receptor-expressing cell lines.

Prenatal glucocorticoid exposure affects learning and vulnerability of cholinergic neurons.

Emgard M, Paradisi M, Pirondi S, Fernandez M, Giardino L, Calza L

Neurobiol Aging [Epub Jan 4] , 2006.

Women at risk of preterm delivery are commonly treated with synthetic glucocorticoids such as dexamethasone and betamethasone. Here the authors examined adult rats that were prenatally exposed to glucocorticoids. After 2.5 μg intracerebroventricular injections of 192-IgG-SAP (Cat. #IT-01) or 0.44 μg of saporin (Cat. #PR-01), the rats were tested in a water maze pool. The evidence suggests that not only do prenatal glucocorticoids affect adult cognitive function, they also make cholinergic neurons more susceptible to challenges later in life.

Catecholamine neurones in rats modulate sleep, breathing, central chemoreception and breathing variability.

Li A, Nattie E

J Physiol 570(Pt 2):385-396, 2006.

Brainstem catecholamine (CA) neurons are thought to modulate the processing of sensory information and participate in the control of breathing. Using a 5 μg injection of anti-DBH-SAP (Cat. #IT-03), or a control injection of mouse-IgG-SAP (Cat. #IT-18) into the fourth ventricle, the authors investigated breathing frequency and wakefulness. The

(continued on page 4)

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Targeting Topics: Recent Scientific References

(continued from page 3)

results suggest that CA neurons promote wakefulness, participate in central respiratory chemoreception, stimulate breathing frequency, and minimize breathing variability during REM sleep.

Effect of N-METHYL-d-aspartate receptor blockade on plasticity of frontal cortex after cholinergic deafferentation in rat.

Garrett JE, Kim I, Wilson RE, Wellman CL
Neuroscience [Epub Mar 7], 2006.

Acetylcholine from the nucleus basalis magnocellularis (NBM) plays roles in neocortical function and plasticity. Here the authors examined whether N-methyl-D-aspartate receptors mediate the increase in the GluR1 subunit of the α-amino-3-

hydroxy-5-methylisoxazole-4-proprionate receptor in the frontal cortex following treatment of the NBM with 0.15 μg of 192-IgG-SAP (Cat. #IT-01). The data indicates that upregulation of GluR1 and spine density after cholinergic deafferentation is regulated by N-methyl-D-aspartate receptors.

Safety evaluation of Intrathecal Substance P-Saporin, a targeted neurotoxin, in dogs.

Allen JW, Mantyh PW, Horais K, Tozier N, Rogers SD, Ghilardi JR, Cizkova D, Grafe MR, Richter P, Lappi DA, Yaksh TL
Toxicol Sci [Epub Feb 24], 2006.

SP-SAP (Cat. #IT-07) has been shown to reverse neuropathic pain behavior in rodents and prevent the formation of hyperalgesia. A safety study was done in beagles to further the use of this molecule as a human therapeutic. Animals received doses from 1.5-150 μg of SP-SAP as bolus intrathecal lumbar injections. Doses of 15 μg and above displayed significant loss of NK1r-expressing cells in lumbar Laminae II and I, but no adverse toxicity was observed at any dose.

American Pain Society
May 3-6, 2006
San Antonio, TX
Booth #339

FENS
July 7-12, 2006
Vienna, Austria
Booth #514

Abstracts from the Experimental Biology Meeting,
April 1-4, San Francisco, CA

Effect of lesions in the nucleus tractus solitarius on hypercapnic ventilatory response in awake rats.

Wilkinson KA, Fu Z, Powell FL
Abstract #480.9

After bilateral injections of 200 nL of SP-SAP (Cat. #IT-07), the authors examined the significance of CO_2-sensitive neurons in the NTS. Blank-SAP (Cat. #IT-21) was injected as control. Results show a physiological role for chemoreceptors in the NTS. Additional studies will determine if these neurons play a unique role in ventilatory acclimitization to hypoxia.

Central baroreflex interruption, cardiac toxicity, and sudden death.

Nayate AP, Moore SA, Weiss RM, Lin L-H, Talman WT. Abstract #467.19

The authors used SSP-SAP (Cat. #IT-11) to eliminate nucleus tractus solitarii neurons that express NK-1 receptors. They hypothesized that cardiac toxicity appears after development of arterial pressure lability. The cardiac changes brought on by this treatment provide a rat model that mimics central lesions in humans.

Brainstem catecholaminergic neurons affect mean arterial pressure and heart rate at rest and during hypercapnic stress in conscious rats.

Emond LA, Li A, Nattie E. Abstract #229.38

The authors used anti-DBH-SAP (Cat. #IT-03) and the control immunotoxin, Mouse IgG-SAP (Cat. #IT-18). They specifically lesioned brainstem catecholaminergic (CA) neurons by injecting anti-DBH-SAP into the fourth ventricle of rats. Control rats produced no neural lesion. The results suggest that brainstem CA neurons lower mean arterial pressure and heart rate at rest.

Targeting Talk: *Retrograde Transport*

by Dr. Douglas Lappi

Q: I spoke with someone from your technical service over the phone and got the impression that your product dermorphin-SAP (Cat. #IT-12) is not a retrograde and will only affect the terminals or the cells that express mu opioid receptors in the injection site in the brain. I have three questions: 1) Do you have any written document on this issue? 2) Will dermorphin-SAP also kill terminals in the injection site or just cell bodies? And 3) If it also kills terminals, will it affect their remote cell bodies?

A: 1) That the peptide-toxins don't undergo retrograde transport is an example of negative data, so people haven't really been publishing too much on that. But two articles deal specifically with it: Lappi and Wiley[1] and Bugarith *et al*.[2] The latter, in particular, presents solid data on the inability of the peptide ligand toxin NPY-SAP (Cat. #IT-28) to undergo retrograde transport.

I don't think we have a single example of a peptide-ligand toxin that undergoes retrograde transport. In order for a peptide-toxin to kill cells, the cell body must have the receptor and the toxin must be injected within reach of the cell body. We've made a mistake in not putting that in the data sheets, and will begin to change that.

2) Let me cite for you: Tokuno *et al*., Efferent projections from the striatal patch compartment: anterograde degeneration after selective ablation of neurons expressing mu-opioid receptor in rats.[3] As the title implies, they address the issue of elimination of processes following cell body destruction.

3) I'm not sure I understand this question, but that won't stop me from trying to answer it: The situation is the contrary, because the destruction of processes comes from the action taking place in the cell body. Our experience is that once the cell body is gone, it's just a matter of time for the process to go away. This makes these toxins a little different than others. In fact, we recommend that you wait two weeks at least to see immunohistological evidence of a toxic effect after injection of a saporin toxin *in vivo*. That's how long it takes the removal process to get rid of all the antigens that you might want to use for evidence of cell loss.

Q: Can I inject NPY-SAP to destroy projections through retrograde transport?

A: Regarding NPY-SAP, a peptide-toxin, see previous response. The antibody-toxins such as 192-IgG-SAP (Cat. #IT-01) or anti-DBH-SAP (Cat. #IT-03) will undergo retrograde transport from terminals to cell bodies. Thus, you can put 192-IgG-SAP into the cortex and it will destroy neurons in the basal forebrain, because the saporin (probably the whole conjugate) is transported from the projection to the cell body. Likewise, anti-DBH-SAP in the spinal cord destroyed hindbrain catecholaminergic neurons by retrograde transport.[4] All the antibody-toxins appear to undergo retrograde transport. (See table on page 6.)

Finally, the lectin-toxins, CTB-SAP (Cat. #IT-14) and IB4-SAP (Cat. #IT-10) undergo retrograde transport, just like the native lectins do. CTB-SAP is well-described in Llewellyn-Smith *et al*.[5] and several others. Please see our website and the references on the CTB-SAP page. For IB4-SAP, Vulchanova *et al*.[6] describe use, along with several other articles on our reference page.

In addition, detailed discussions are available in the book *Molecular Neurosurgery with Targeted Toxins*,[7] available from Humana Press.

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7. Molecular Neurosurgery With Targeted Toxins. Wiley RG, Lappi DA, eds. (2005) *Humana Press*, Totowa NJ.

Targeted toxins in pain

(continued from page 1)

Making selective neural lesions has long been an important experimental strategy in neuroscience. The power of this approach depends in large part on the specificity of the lesions. The term "molecular neurosurgery" refers to the use of targeted toxins to produce specific neural lesions based on targeting surface molecules on the neurons of interest.

This work began with suicide transport agents, such as ricin, that were delivered to target neurons by retrograde axonal transport. Suicide transport agents produce anatomically specific lesions but lack cell type selectivity. In order to selectively destroy specific types of neurons, we developed anti-neuronal immunotoxins such as anti-DBH-SAP, anti-SERT-SAP (Cat. #IT-23), and 192-IgG-SAP (Cat. #IT-01). These agents consist of monoclonal antibodies that recognize molecules expressed on the surface of specific types of neurons; the antibody is armed to kill by coupling to the ribosome-inactivating protein, saporin. (The more recent approach to cell type selective lesioning is neuropeptide-toxin conjugates such as SP-SAP and dermorphin-SAP.) Saporin, by itself, normally enters cells very inefficiently, but when coupled to a carrier that induces receptor-mediated endocytosis, saporin uptake can be highly efficient and limited to cells displaying the target molecule. The table below summarizes the targeted toxins available for the study of pain.

Molecular Neurosurgery Agents of Interest in Pain Research

Agent	Target	Lesioning Use
192-IgG-SAP *Cat. #IT-01*	**P75^{NTR}, low affinity NGFr** (cholinergic basal forebrain, cerebellar Purkinje neurons, postganglionic autonomic neurons and some primary sensory neurons)	Immunolesioning
Anti-DBH-SAP *Cat. #IT-03*	**Dopamine beta hydroxylase** (adrenergic and noradrenergic neurons)	Immunolesioning by application to either dendrites/soma or by retrograde axonal transport
Anti-SERT-SAP *Cat. #IT-23*	**Serotonin transporter** (serotonergic neurons)	Immunolesioning
Dermorphin-SAP *Cat. #IT-12*	**Mu opioid receptor** (striatal neurons, lamina II dorsal horn nociceptive interneurons, ventromedial medulla)	Direct application, spinal intrathecal injection
OX7-SAP *Cat. #IT-02*	**Thy-1** (all rat neurons and some T lymphocytes)	Suicide transport in both peripheral and central nervous systems
SP-SAP and SSP-SAP *Cat. #IT-07 and IT-11*	**Neurokinin-1 receptor (NK-1R)** (striatal cholinergic interneurons, dorsal horn nociceptive projection neurons)	Direct application to dendrites/soma, spinal intrathecal injection

Note: Only antibody-toxin conjugates undergo retrograde transport; see Page 5 for discussion

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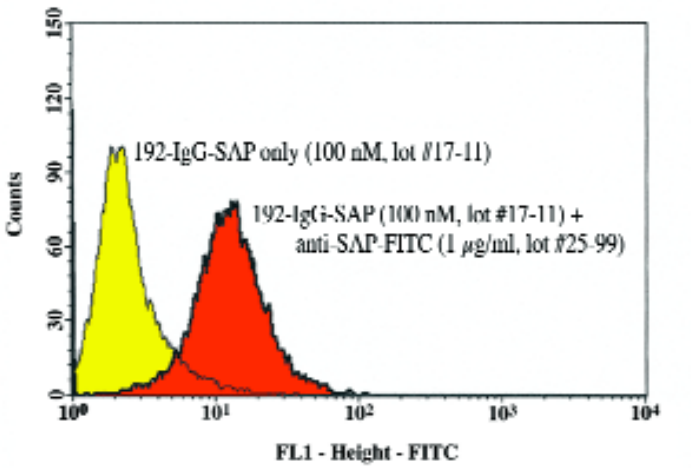
6. Wiley RG, Lappi DA. *Adv Drug Deliv Rev* 55:1043-1054, 2003.
7. Burgess *et al. J Neurosci* 22:5129-5136, 2002.
8. Gardell *et al. J Neurosci* 23:8370-8379, 2003.
9. Porreca *et al. J Neurosci* 21:5281-5288, 2001.

Please visit www.ATSbio.com to see a complete list of references using these targeted toxins.

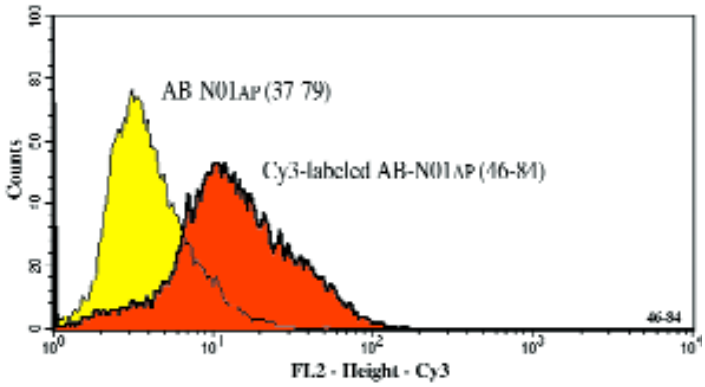
Targeting Tools: Featured Products

Fluorescent Conjugates

Antibodies conjugated to fluorescent dyes are vibrant and vital tools at a scientist's disposal. ATS currently has six fluorescent conjugates in our catalog: Cy3-labeled 192-IgG (Cat. #FL-01), FITC-labeled Anti-Saporin (Cat. #FL-02), Alexa 488-labeled 192-IgG (Cat. #FL-03), FITC-labeled Goat anti-rabbit IgG (Cat. #FL-04), Cy3-labeled anti-NGFr (Cat. #FL-05), and Cy5-labeled anti-NGFr (Cat. #FL-06). ATS also offers custom conjugations. Let us know which dye you want to use and we'll label your antibody.



C6 cells were fixed and incubated with 100 nM 192-IgG-SAP for one hour. After washing, cells were incubated with FITC-labeled Anti-SAP (Cat. #FL-02) at 1 µg/ml for 30 minutes. Samples were run on a FACScan (Becton Dickinson). Data analysis was performed using CellQuest.



NG3 cells, a rat-mouse hybrid neuroblastoma cell line, were incubated with anti-NGFr antibody or Cy3-labeled antibody (Cat. #FL-05) and incubated at 4°C. Cells were analyzed by flow cytometry on a BD FACScan, and data produced using CellQuest software. A concentration of 4 µg of conjugate per one million cells provided a 33% shift compared to the antibody alone.

Fluorescent conjugates can shed new light on your research. For example, FITC-labeled Anti-Saporin (Cat. #FL-02) can be used to verify binding specificity of a targeted toxin to a cell line expressing the target molecule. By first binding the targeted toxin to fixed cells, then binding FITC-labeled Anti-SAP to the targeted toxin, specificity can be confirmed through the use of competing molecules or a control cell line.

Gangsta sighs and settles in, "Alright, I've found a nice comfy position here on your lap. Don't even think about getting up for a snack!"

Flow Cytometry Service

Cytometry Research, LLC, a subsidiary of ATS, provides flow cytometry services (analysis, sorting, and assays) to the research community. Visit them on the web to find out more and to schedule an appointment. Data will be emailed to you within 24 hours of receipt of samples.

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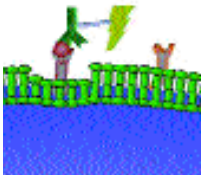
[§]or growth factor, peptide, ligand, or cytokine

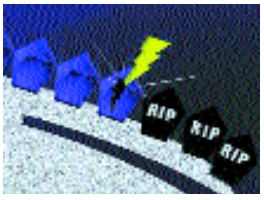
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

GARTERDOER

SIPOON

PRIMESOCCO

TREESLOFUNC

CTHAB

How the Scientist knew she was on the verge of a great discovery.

Arrange the circled letters to form the answer, as suggested by the above clue.

Answer: She understood the...

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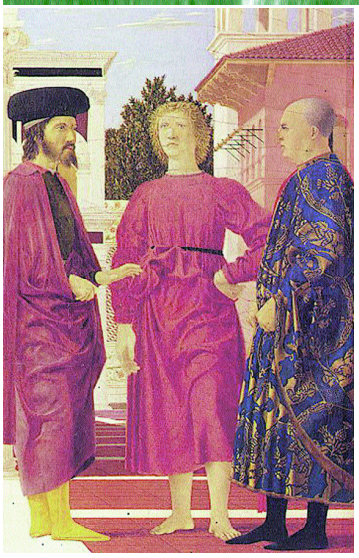

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Safety and Efficacy of Substance P-SAP

by Jeffrey W. Allen, Ph.D.

The author is currently a Senior Scientist in Emerging Therapies at Medtronic Neurological located in Minneapolis, MN. There is no association, financial or otherwise, between Medtronic, Inc. and Advanced Targeting Systems.

Substance P-saporin (SP-SAP, Cat. #IT-07) is a targeted neurotoxin that selectively lesions cells containing the Neurokinin-1 (NK-1) receptor. Previous studies in rodents have shown that a single intrathecal injection can prevent formation of chemically induced thermal hyperalgesia and reverse mechanical allodynia caused by nerve injury without altering normal thermal or tactile function. While these results were very promising, it was unclear if SP-SAP would be effective in larger species given the distance the molecule would need to radially diffuse to its site of action, the spinal dorsal horn. With support provided by the National Institutes of Health a series of studies were performed in beagle dogs, a standard large animal model for intrathecal safety. The primary endpoint of this study was designed to determine intrathecal dosing levels in dogs. Secondary endpoints were to characterize the distribution of SP-SAP in the spinal space and to examine effects of an inadvertent intravenous delivery.

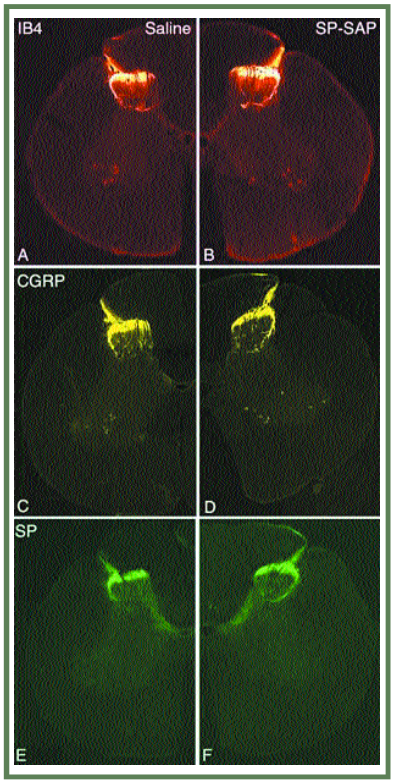


Figure 1.
Staining of coronal spinal cord sections for IB4 (top panel), CGRP (middle panel) and SP (bottom panel). Left panel dog was treated with PBS; right panel dog was treated with SP-SAP.

Purpose-bred laboratory beagles were surgically implanted with an intrathecal catheter that terminated in the upper lumbar space. All animal studies were conducted in the laboratory of Dr. Tony Yaksh, Dept of Anesthesiology, University of California, San Diego. Animals were allowed to recover for approximately 3 days at which time they received a single 0.3 ml intrathecal bolus of SP-SAP. Control animals received either phosphate-buffered saline (PBS) or non-targeted recombinant saporin (SAP). Dogs were dosed with 1.5, 15, 45 or 150 μg SP-SAP while dogs in the control groups received 0.3 ml of PBS or 150 μg SAP. Dogs were fully conscious during dosing and behavior was assessed for at least 8 hours immediately following dosing and twice daily thereafter. Catheters were removed about 3 days after dosing to prevent any effects induced by long-term presence of the catheter.

(continued on page 6)

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: RETROGRADE
POISON
MICROSCOPE
FLUORESCENT
BATCH

Answer: She understood the...
"SCOPE OF THE PROBLEM"

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

Winners: David Akopian- California State University, Northridge CA * Valery Nelson- Panacea Pharmaceuticals Inc, Gaithersburg MD * Robert Speth- University of Mississippi, School of Pharmacy, University MS * Bruce Pappas- Carleton University, Life Research Center, Ottowa Ontario CANADA * Kris Preddy- Lakeside CA * Vivian Yip- Tissuegene Inc, Gaithersburg MD * Joseph Menonna- East Orange VAMC, Bio Medical Research Institute, East Orange NJ * James Fadel- University of South Carolina, Columbia SC * Andrew Johnston- National University Hospital Iceland, Department of Immunology, Reykjavík ICELAND * Elizabeth Rubino- University Texas Health Science Center, Department of Pharmacology, San Antonio TX * Thea Marlinga- Libertyville IL

Upcoming Events

University of Minnesota
Minneapolis, MN
Vendor Showcase
July 20, 2006

University of California San Diego
San Diego, CA
Vendor Showcase
August 31, 2006

Forum of European Neuroscience
Vienna Austria
July 7-12, Booth #514

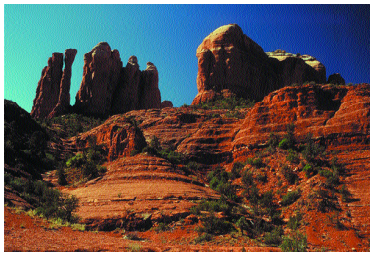
Intl Conference on Alzheimer's Disease and Related Disorders
Madrid, Spain
July 15-20, Booth #510

Spring Brain Conference

The 17th Annual Spring Brain meeting was held March 15-18 in beautiful Sedona AZ, one of the most desired travel destinations in the US. Great science was presented in a beautiful atmosphere. This meeting is exceptional for its broad brush of the Neurosciences in which top-notch scientists in different fields summarize their work.

This year's Keynote Address was given by Allan Basbaum (UCSF), who spoke on *Pain Mechanisms: from Molecules to Circuits*, and his work on transneuronal tracers for circuit definition. Plenary sessions were on topics ranging from presynaptic facilitation of neurotransmission, plasticity in drug abuse, responses to retinal injury and handedness in chimps (see the website www.springbrain.org for the program), among others. Discussions with those outside of your scientific cubicle can be very valuable. Alan Peters (Boston University) gave a Plenary Address on his work that led to his co-authoring the classic *The Fine Structure of the Nervous System*. At the business meeting, Tom Woolsey (WUStL) presented his work with interns for Outreach to the local Navajo population in Flagstaff—it's fun to see a bunch of young people fascinated by the brain.

The Spring Brain meeting is a great event held yearly in Mid-March. Check out the website and come to a great meeting March 14-17, 2007.



Beautiful Sedona, Arizona – one of the most desired travel locations in the United States – is home to the annual Spring Brain Conference.

www.springbrain.org

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Hypotensive hypovolemia and hypoglycemia activate different hindbrain catecholamine neurons with projections to the hypothalamus.

Dinh TT, Flynn FW, Ritter S
Am J Physiol Regul Integr Comp Physiol [Epub May 4], 2006.

Hypovolemia, a decrease in blood plasma volume, results in secretion of arginine vasopressin (AVP). This work investigates the role of hindbrain catecholamine neurons in hypovolemia-induced AVP secretion. Rats were treated with bilateral 42 ng injections of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus of the hypothalamus, and hypovolemia was induced by blood withdrawal. Treated animals displayed severely impaired AVP response, as well as lower food intake and corticosterone secretion in response to insulin.

Aversive stimulus attenuates impairment of acquisition in a delayed match to position T-maze task caused by a selective lesion of septo-hippocampal cholinergic projections.

Fitz NF, Gibbs RB, Johnson DA
Brain Res Bull 69(6):660-665, 2006.

It is known that infusion of 192-IgG-SAP (Cat. #IT-01) into the medial septum of rats impairs acquisition of a delayed matching to position (DMP) T-maze task. Here, the authors evaluated whether introduction of an aversive stimulus 30 minutes prior to training would attenuate this deficit. Treated rats received 0.22 μg of 192-IgG-SAP injected into the medial septum. Data indicate that treated rats receiving an intraperitoneal injection of saline 30 minutes prior to training displayed

less impairment than rats not receiving the aversive stimulus.

Toward better pain control.

Basbaum AI, Julius D
Sci Am 294(6):60-67, 2006.

The authors discuss some of the advances in understanding and treating different types of pain, and specifically outline circuits, receptors, and ligands involved in pain pathways. Several treatments are described, one of which is the use of SP-SAP (Cat. #IT-07) to disrupt the chronic pain pathway in the spinal cord.

Differential responsiveness of dopamine-beta-hydroxylase gene expression to glucoprivation in different catecholamine cell groups.

Li AJ, Wang Q, Ritter S
Endocrinology [Epub Apr 13], 2006.

This work examines how subpopulations of hindbrain catecholaminergic neurons participate in systemic glucoregulation. Rats were treated with bilateral 42 ng infusions of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus of the hypothalamus. Dopamine-beta-hydroxylase (DBH) expression in glucoprivic animals was then analyzed by *in situ* hybridization and immunohistochemistry. The data demonstrate that the ventrolateral medulla contains most of the catecholamine neurons responsive to glucoprivation.

Attenuation of homeostatic responses to hypotension and glucoprivation after destruction of catecholaminergic rostral ventrolateral medulla (RVLM) neurons.

Madden CJ, Stocker SD, Sved AF
Am J Physiol Regul Integr Comp Physiol [Epub Apr 20], 2006.

C1 neurons in the RVLM express dopamine-beta-hydroxylase (DBH). Anti-DBH-SAP (Cat. #IT-03) was used to eliminate these neurons and examine cardiovascular homeostasis in response to a physiological challenge such as hypotension. 21 ng of anti-DBH-SAP was injected into the RVLM of rats. After food and water had been removed from the cage, the lesioned animals were treated with hydralazine to reduce blood pressure. The results demonstrate that RVLM-C1 cells are involved in responses to homeostatic challenges.

(continued on page 4)

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Targeting Topics: Recent Scientific References

(continued from page 3)

Effect of nucleus basalis magnocellularis cholinergic lesions on fear-like and anxiety-like behavior.

Knox D, Berntson GG
Behav Neurosci 120(2):307-312, 2006.

Neurons in the nucleus basalis magnocellularis and substantia innominata (NBM/SI) may play a role in mediating some aspects of aversive states. The authors used 0.1 μg injections of 192-IgG-SAP (Cat. #IT-01) into the NBM/SI of rats to investigate the role these neurons play in elevated maze behavior and fear-conditioned behavioral suppression. The lesions did not affect the elevated maze behavior, but behavioral suppression was attenuated. The results indicate that NBM/SI cholinergic neurons are involved in the mediation of anxiety-like states.

Suppression of natural killer cell activity by morphine is mediated by the nucleus accumbens shell.

Saurer TB, Carrigan KA, Ijames SG, Lysle DT
J Neuroimmunol 173(1-2):3-11, 2006.

In this work the authors investigated the role of dopaminergic projections to the nucleus accumbens in modulation of immune parameters such as morphine-induced suppression of splenic natural killer (NK) cell activity. Studies have indicated that acute exposure to opioids decreases NK cell-mediated cytotoxicity. Rats received bilateral 0.5 μg-injections of anti-DAT-SAP (Cat. #IT-25) into the nucleus accumbens shell. Treated animals showed no immunosuppression upon administration of morphine, indicating that dopaminergic neurons in the nucleus accumbens play a major role in this pathway.

Myeloid precursors and acute myeloid leukemia cells express multiple CD33-related Siglecs.

Nguyen DH, Ball ED, Varki A
Exp Hematol 34(6):728-735, 2006.

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of cell surface receptors which bind to sialic acid. They are found mainly on leukocytes, and also on acute myeloid leukemia (AML) cells. The authors tested several anti-Siglec antibodies against U937 histiocytic lymphoma cells and THP-1 acute monocytic leukemia cells *in vitro*. When these antibodies were combined with Mab-ZAP (Cat. #IT-04), a second immunotoxin, the target cells were eliminated. The data suggest that Siglecs may be a viable target for AML therapy.

Descending facilitation from the rostral ventromedial medulla maintains visceral pain in rats with experimental pancreatitis.

Vera-Portocarrero LP, Yie JX, Kowal J, Ossipov MH, King T, Porreca F
Gastroenterology 130(7):2155-164, 2006.

Here the authors investigated the role of ascending or descending pathways in the mediation of pain caused by pancreatitis. Rats received 1.5 pmol injections of dermorphin-SAP (Cat. #IT-12) into each side of the rostral ventromedial medulla. Abdominal hypersensitivity was tested using von Frey filaments. Although the ablation of mu-opioid receptor-expressing neurons by dermorphin-SAP did not prevent the initial expression of pancreatitis pain, maintenance of this pain was absent. The data link maintenance of pancreatitis pain to descending pathways.

Combined damage to entorhinal cortex and cholinergic basal forebrain neurons, two early neurodegenerative features accompanying Alzheimer's Disease: Effects on locomotor activity and memory functions in rats.

Traissard N, Herbeaux K, Cosquer B, Jeltsch H, Ferry B, Galani R, Pernon A, Majchrzak M, Cassel JC
Neuropsychopharmacology [Epub Jun 7], 2006.

Two characteristics of Alzheimer's disease (AD) are cholinergic dysfunction in the basal forebrain, and neuronal damage in the entorhinal cortex. Using 5 μg intracerebroventricular (icv) injections of 192-IgG-SAP (Cat. #IT-01), and 2.3 μg icv injections of OX7-SAP (Cat. #IT-02), locomotor activity, working, and reference memory of rats were examined. Although 192-IgG-SAP lesions caused limited deficits, rats receiving both lesions exhibited several behaviors associated with AD. The authors suggest that combining these lesions may be a more accurate model for AD than 192-IgG-SAP alone.

Please visit www.ATSbio.com to see a complete list of references.

Targeting Talk: *Retrograde Transport*

by Drs. Douglas Lappi and Ronald G. Wiley

Q: I have a question about the issues raised in the last edition of Targeting Trends *(Volume 7, Issue 2). There is a comment "In fact, we recommend that you wait two weeks at least to see immunohistological evidence of a toxic effect after injection of a saporin toxin in vivo." Are there data to support this recommendation?*

As a researcher who utilizes your toxin products I often get asked about the time course of toxin action. It's difficult to answer because the literature is currently limited with regard to in vivo *toxin application. Any citations, advice, or comments would be greatly appreciated.*

A: Actually there's quite a bit of *in vivo* use. Or is it just that I think the glass is half full? If you search PubMed for the use of the immunotoxin 192-IgG-SAP using the terms '192' and 'saporin,' you'll get 223 hits, and all of these describe *in vivo* use.

As far as the two-week idea, you're right, it's a bit more challenging to pin that down in the literature. In our book, *Suicide Transport and Immunolesioning* (1), Ron Wiley discusses at several points the microglial infiltration that occurs and subsides by 14 days. That is what cleans out the antigens that you probably would use to demonstrate cell death--that is, they aren't there any more. You might want to see if it's in your library; it's a good basic source of info. To get a full list of articles, select the References button on the home page of the ATS website and click on some of the toxins.

As far as the process, Waite *et al*. (2) show the appearance of behavioral effects associated with neuronal loss at day four and plateauing at day 7. This coincides with the time course seen *in vitro* (3). At this point, microglia will infiltrate; this is nicely described in Seeger *et al*. (4). However, they stop at 7 days, which is probably the peak day for infiltration. Once there is complete removal of the detritus, microglia down-regulate and at 14 days, you don't see them, or the antigens that belonged to the cells that were eliminated. So that's the idea behind waiting.

Q: Do you have a product which can be used to produce retrograde lesions WITHOUT killing cells at the site of injection? What I'd like to do is to kill neurons that project to an efferent nucleus without damaging neurons in the efferent nucleus itself.

A: Making a selective retrograde neural lesion based only on the criterion that the cells to be lesioned are afferent to a particular nucleus or population of neurons, is a formidable challenge at present. Conceptually, this would seem to require a targeted toxin that was taken up only by afferent terminals and not by dendrites, cell bodies and/or axonal membranes of the neurons that are to be de-afferented.

There are some instances in which you can avoid local killing, but only in the case in which there are no receptors in that area, except from projections. So for instance, cholinergic neurons will project to the cortex. You can inject 192-IgG-SAP there; it will be taken up and eliminate basal forebrain neurons with little harm to other cortical neurons. Or you can inject anti-DBH-SAP into the spinal cord; it will eliminate brainstem neurons that project to there. Currently, this task is best suited to immunotoxins since there is little data on using neuropeptide toxin conjugates to produce retrograde lesions. If armed to kill, a growth factor such as NGF might also work, if toxin conjugation did not damage binding and intracellular trafficking of the NGF. But these are special cases (which you can find in our reference lists for these products).

Targeting presynaptic antigens that are common to all types of axon terminals would seem a dubious undertaking since success with an immunotoxin requires the target molecule be displayed on the external surface of the terminal and not be present at all on cell bodies or dendrites. I do not know of a suitable target molecule for this purpose.

Send your questions to: ats@atsbio.com

References

1. Wiley RG, Lappi DA (1994) *Suicide Transport and Immunolesioning*. R.G. Landes, Houston.
2. Waite JJ, Wardlow ML, Chen AC, Lappi DA, Wiley RG, Thal LJ (1994) Time course of cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. *Neurosci Lett* 169:154-158.
3. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.
4. Seeger G, Hartig W, Rossner S, Scliebs R, Bruckner G, Bigl V, Brauer K (1997) Electron microscopic evidence for microglial phagocytotic activity and cholinergic cell death after administration of the immunotoxin 192IgG-saporin in rat. J *Neurosci Res* 48:465-476.

Safety and Efficacy of Substance P-SAP

(continued from page 1)

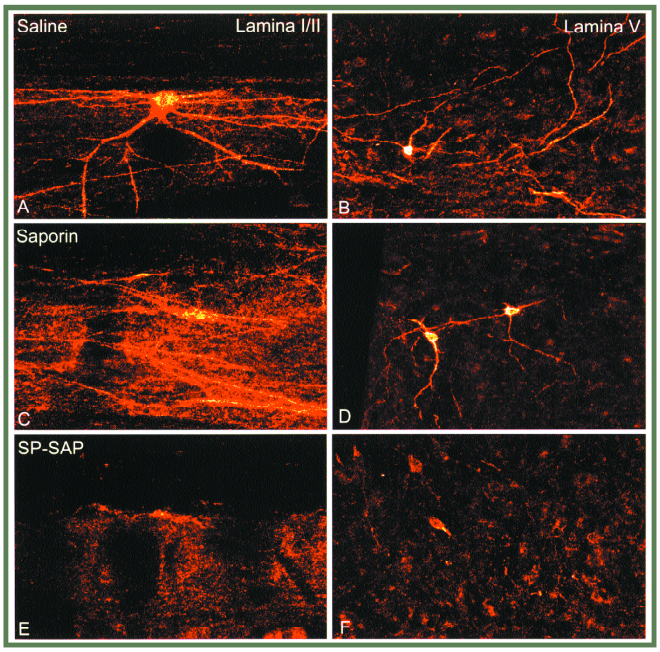

Figure 2. Immunostaining with an antibody to SPR of dog spinal cord after treatment with PBS (panels A and B), SAP (panels C and D), and SP-SAP (panels E and F). Thirty days after injection, animals were sacrificed and cords were removed and processed as described in Mantyh et al.

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

Spinal histopathology was assessed at approximately 7, 28 or 90 days. Spinal cords and brain were processed for hematoxylin and eosin (H&E) staining and were evaluated by Dr. Marjorie Grafe in the Department of Pathology at Oregon Health Science Center in Portland. Immunohistochemical staining of the spinal cord for NK-1 receptors, and various markers for cell types and neuropeptides was performed in the laboratory of Dr. Patrick Mantyh, University of Minnesota, Minneapolis. Additional immunohistochemistry of selected brains was done in the laboratory of Dr. Yaksh.

SP-SAP bolus doses of 15, 45 or 150 μg produced an essentially equivalent and significant reduction in the number of NK-1 receptors in the lumbar spinal cord at 28 or 90 days following dosing. Given previous studies it is believed that this reduction was due to death of these neurons, not simply a loss of the receptor protein. No changes were seen in the total number of neurons or the amount of glial fibrillary acidic protein (GFAP), a marker for astrocytes, suggesting a lack of diffuse neuronal loss. Staining for various neuropeptides, including substance P, was unchanged demonstrating a lack of effect of SP-SAP on primary afferent neurons. H&E staining showed no evidence of any histological damage in any animals. Spinal cord sections from SP-SAP treated dogs were indistinguishable from animals receiving either PBS or SAP.

Importantly, all effects of SP-SAP on NK-1 receptor neurons were limited to the lumbar region near the site of injection. This lack of widespread distribution was also seen when examining SAP levels in spinal cord tissues. This localization of effect may be due not only to the relatively large molecular weight of the molecule (33kDa) but also to the short intrathecal half-life of between 30-60 minutes of the active compound.

There was no sign of pain or discomfort during or immediately following injection. In animals receiving either SP-SAP or the control compound SAP, a transient series of behavioral and physiological signs including an increase in blood pressure, tactile allodynia, neck and truncal rigidity with mild rhythmic tremors and bradykinesia occurred. These signs required butorphanol or buprenorphine analgesia in some animals and were generally dose-related with the time of onset being shortest (3-4 hours) at the 150-μg doses. These signs completely resolved over 1-3 days and by 7 days the dogs were indistinguishable from those who received only PBS. It is hypothesized that this acute constellation of signs was a non-specific reaction to the presence of saporin since it was seen in both the SP-SAP and the SAP groups. Since there were no differences in the total neuronal numbers, GFAP levels or H&E histopathology in any of the SAP dogs, it appears these effects were not due to any permanent cellular toxicity.

In summary, these studies demonstrated that a single intrathecal bolus of 15, 45 or 150 μg SP-SAP produced ablation of greater than 80% of the neurons containing the NK-1 receptor in dogs. This suggests a safety factor of at least 10-fold between a fully effective dose, 15 μg, and a dose, 150 μg, that produced unacceptable acute behavioral signs. Behavior quickly returned to normal, and as evidenced by the lack of pathological signs, there appeared to be no long-term detrimental effects associated with SP-SAP administration at the doses tested. The SP-SAP-induced lesioning was spatially limited to the area of the catheter tip without spread to other spinal regions. These studies, for the first time, demonstrate both safety and efficacy, as defined by loss of NK-1 receptors, on intrathecal SP-SAP in a large animal producing the preliminary data necessary to advance SP-SAP to a clinically available compound.

This article is a summary of material presented by the author in Allen JW, Mantyh PW, Horais K, Tozier N, Rogers SD, Ghilardi JR, Cizkova D, Grafe MR, Richter P, Lappi DA, Yaksh TL (2006) Safety evaluation of Intrathecal Substance P-Saporin, a targeted neurotoxin, in dogs. Toxicol Sci 91(1):286-298.

Targeting Tools: Featured Antibodies

Dopamine anti-idiotype Rabbit Polyclonal (AB-T019)
Immunogen: polyclonal and monoclonal anti-conjugated dopamine antibodies (Ab1)
Targets dopamine receptor, binding site of polyclonal or monoclonal anti-conjugated dopamine antibodies.
Applications include ELISA and immunocytochemistry.

L-Cysteine Rabbit Polyclonal (AB-T034)
Immunogen: synthetic L-cysteine conjugated to protein carrier
Targets conjugated L-cysteine. Applications include ELISA, immunocytochemistry, and immunoblotting.

Pseudomonas aeruginosa Rabbit Polyclonal (AB-T057)
Immunogen: pseudomonas aeruginosa total proteins
Targets pseudomonas aeruginosa total proteins.
Applications include ELISA and immunoblotting.

Stenotrophomonas maltophilia Rat Polyclonal (AB-T062)
Immunogen: Stenotrophomonas maltophilia total proteins
Targets Stenotrophomonas maltophilia total proteins.
Applications include ELISA and immunoblotting.

Pantoea agglomerans Rabbit Polyclonal (AB-T064)
Immunogen: Pantoea agglomerans total proteins
Targets Pantoea agglomerans total proteins - syn. Erwinia herbicola - Enterobacter agglomerans.
Applications include ELISA and immunoblotting.

Acetyl salicylic acid Rat Polyclonal (AB-T074)
Immunogen: synthetic acetyl salicylic acid conjugated to protein carriers
Targets conjugated acetyl salicylic acid.
Applications include ELISA.

Myristic acid Rat Polyclonal (AB-T086)
Immunogen: synthetic Myristic Acid conjugated to bovine serum albumin
Targets conjugated Myristic Acid.
Applications include ELISA and immunohistochemistry.

Acrolein Rabbit Polyclonal (AB-T091)
Immunogen: synthetic acrolein conjugated to bovine serum albumin
Targets acrolein.
Applications include ELISA and immunohistochemistry.

Kynurenic acid Rabbit Polyclonal (AB-T094)
Immunogen: synthetic kynurenic acid conjugated to bovine serum albumin
Targets conjugated kynurenic acid.
Applications include ELISA and immunohistochemistry.

Succinate Rat Polyclonal (AB-T101)
Immunogen: synthetic succinic acid conjugated to bovine serum albumin
Targets succinic acid. Applications include ELISA.

NO-Tyrosine Rat Polyclonal (AB-T114)
Immunogen: synthetic NO-Tyrosine conjugated to protein carrier
Targets conjugated NO-Tyrosine. Applications include ELISA, immunoblotting, and immunocytochemistry.

NO-Glutathione Rat Polyclonal (AB-T124)
Immunogen: synthetic NO-Glutathione conjugated to protein carrier
Targets conjugated NO-Glutathione. Applications include ELISA, immunoblotting, and immunocytochemistry.

NO-L-Cystein Mouse Monoclonal (AB-T125)
Immunogen: synthetic NO-L-Cystein conjugated to protein carrier
Targets conjugated NO-L-Cystein. Applications include immunocytochemistry, ELISA, immunoblotting.

Citrulline Rabbit Polyclonal (AB-T127)
Immunogen: synthetic citrulline conjugated to bovine serum albumin
Targets conjugated citrulline. Applications include ELISA and immunohistochemistry.

L-Kynurenine Rabbit Polyclonal (AB-T152)
Immunogen: synthetic L-Kynurenine conjugated to protein carrier
Targets conjugated L-Kynurenine. Applications include ELISA and immunohistochemistry.

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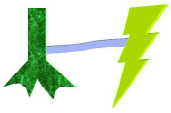
Choose an ANTIBODY[§] specific to your cell type.



SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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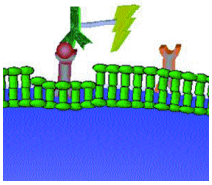
[§]or growth factor, peptide, ligand, or cytokine

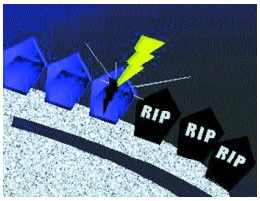
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

ONETICKY

FENTFEAR

TICCULETOYER

YAHWAPT

UFING

How the doctors planned to handle the new operating fee.

Arrange the circled letters to form the answer, as suggested by the above clue.

Answer: They decided to...

WIN $100.00

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Oct-Nov-Dec 2006
Volume 7, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

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- Anti-DBH-SAP Administration *(page 5)*
- Anti-6-His and Anti-SAP-HRP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Basomedial hypothalamic Injections of Neuropeptide Y Conjugated to Saporin Selectively Disrupt Hypothalamic Controls of Food Intake

This article is a summary of data presented in reference #1. Figures 1-4 are taken from that article. This work was funded by NS045520 and DK40498 to S. Ritter.

Neuropeptide Y (NPY) conjugated to saporin (SAP), a ribosomal toxin, is a compound designed to selectively target and lesion NPY receptor-expressing cells. We conducted competitive binding studies using I^{125}-NPY to evaluate the binding of NPY-SAP to rat forebrain homogenates (1). Results indicate that NPY-SAP binds to and has a higher binding affinity than NPY for the NPY receptor (Fig. 1). The binding results, in combination with previous studies demonstrating agonist-driven NPY receptor internalization (2, 3), indicated that this peptide-saporin conjugate would produce effective lesions of NPY receptor-expressing neurons. Accordingly, when we injected NPY-SAP (48 ng in 100 nl) bilaterally into the arcuate nucleus (ARC) of the hypothalamus, we found a profound reduction of NPY Y1 receptor-immunoreactivity (-ir) in the ARC (Fig. 2). We also found a nearly complete loss of NPY, AGRP and CART mRNA expression and α-MSH-ir in the ARC and mediobasal hypothalamus, showing that these NPY receptor-expressing neurons were lesioned by NPY-SAP (Fig. 3).

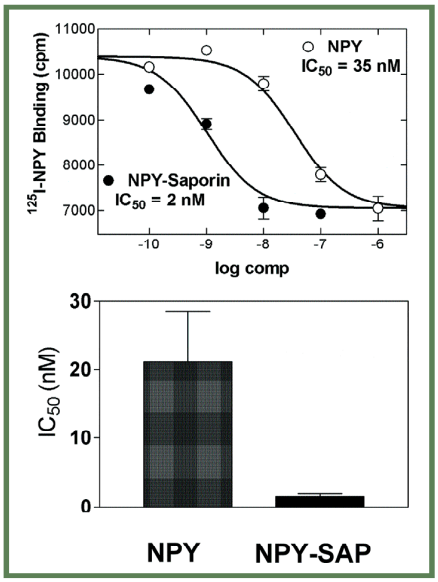

Figure 1. *Top*: Competitive binding of NPY and NPY-SAP with I^{125}-NPY in rat forebrain tissue homogenates. Duplicate determinations were made for each concentration. *Bottom*: Bars show IC50 for NPY and NPY reduced NPY-SAP binding. Data show that NPY-SAP has a binding affinity for NPY receptors that is equal to or greater than NPY at the concentrations examined.

To date, there is no evidence that any of the available peptide-saporin conjugates are retrogradely transported. To determine whether NPY-SAP is retrogradely transported, we injected the conjugate into the ARC and examined catecholamine cell bodies in the A1/C1 region of the ventrolateral medulla. Nearly all of the catecholamine neurons in this area co-express NPY and project to the medial hypothalamus. A1/C1 neurons are almost completely destroyed by medial hypothalamic injections of the retrogradely transported immunotoxin, anti-dopamine-beta-hydroxylase-saporin (anti-DBH-SAP) (4-6). However, there was no loss of cells in this

(continued on page 6)

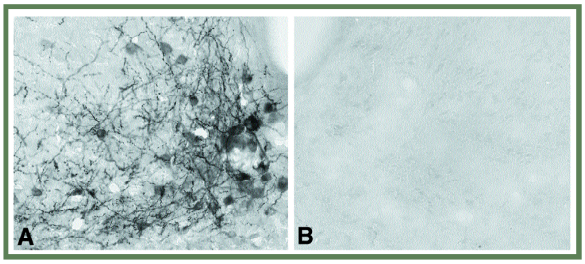

Figure 2. Coronal sections through the arcuate nucleus of the hypothalamus showing effects of Blank-SAP (B-SAP) control (A) and NPY-SAP (B) injections into the arcuate nucleus on NPY-Y1 receptor immunoreactivity.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: CYTOKINE
AFFERENT
RETICULOCYTE
PATHWAY
FUNGI

Answer: They decided to...
"CUT IT OUT"

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

Winners: Shawn McClelland- California State University, Northridge CA * Angela Finney- Panacea Pharmaceuticals Inc, Gaithersburg MD * Robert Speth- University of Mississippi, School of Pharmacy, University MS * Bruce Pappas- Carleton University, Life Research Center, Ottawa Ontario CANADA * Vivian Yip- Tissuegene Inc, Gaithersburg MD * Joseph Menonna- East Orange VAMC, Bio Medical Research Institute, East Orange NJ * Dr. Douglas J. Taatjes- University of Vermont, Dept of Pathology, Burlington VT * Julie Hix- Kansas State University, College of Veterinary Medicine, Manhattan KS * Ed Unsworth- NIH/NCI, Bethesda MD * Kristen Phend- University of North Carolina, Dept of Cell Biology and Anatomy, Chapel Hill NC * Seto Chice- SUNY HSC at Brooklyn, Brooklyn NY

Upcoming Events

Society for Neuroscience
Atlanta, Georgia
October 10-14
Booth #1240

American Society for Cell Biology
San Diego
December 10-13, 2006
Booth #734

ATS Hits the Field

by Kristen Hartman

Advanced Targeting Systems took employees out to the ballgame for our annual employee appreciation event. We had a little extra to watch out for this year! ATS had a banner that flashed on the billboard (see below) and our seats were out in the field in hopes of catching a home run from Barry Bonds. We spent the at-bats rooting for a home run and the in-between field changes watching the billboard. We were all thrilled when the ATS banner flashed not once, but twice during the game. As the game stretched into the 9th inning we had not gotten a chance to catch a ball and the home team Padres were down by 8 runs. At the last moment the Padres hit a grand slam. It wasn't enough to win, but it was very exciting. Another memorable experience at the park.

Doug Lappi, Amalia Dingman, Leonardo Ancheta, Dianna Pinhero, Brian Russell, Kristen Hartman, and Denise Higgins on "the beach" at Petco Stadium.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

High-affinity ligand probes of CD22 overcome the threshold set by cis ligands to allow for binding, endocytosis, and killing of B cells.

Collins BE, Blixt O, Han S, Duong B, Li H, Nathan JK, Bovin N, Paulson JC
J Immunol 177(5):2994-3003, 2006.

CD22, a member of the siglec subgroup of the Ig superfamily, is a potential target for immunotherapy of B cell lymphomas. The authors demonstrate that a biotinylated probe specific for CD22 combined with streptavidin-ZAP (Cat. #IT-27), can eliminate several different lymphoma cell lines.

Ameliorating effect of saporin-conjugated anti-CD11b monoclonal antibody in a murine T-cell-mediated chronic colitis.

Kanai T, Uraushihara K, Totsuka T, Nemoto Y, Fujii R, Kawamura T, Makita S, Sawada D, Yagita H, Okumura K, Watanabe M
J Gastroenterol Hepatol 21(7):1136-1142, 2006.

Using SCID mice, the authors evaluated the effects of Mac-1-SAP (Cat. #IT-06) on the development of chronic colitis. After transfer of T cells to the mice, 12.5 μg of Mac-1-SAP was injected into the intraperitoneal space. The reduction in CD4(+) T-cell infiltration of the colon, and suppression of IFNγ and TNFα production indicate that macrophages play a significant role in the pathogenesis of Crohn's disease.

Immunolesions of glucoresponsive projections to the arcuate nucleus alter glucoprivic-induced alterations in food intake, luteinizing hormone secretion, and GALP mRNA, but not sex behavior in adult male rats.

Fraley GS
Neuroendocrinology 83(2):97-105, 2006.

In this work the author looked at the role hypothalamic glucose may play in reproductive function. 42 ng of anti-DBH-SAP (Cat. #IT-03) was injected dorsal of the arcuate nucleus of rats, which were then given glucoprivic challenges. The data demonstrate the involvement of A1/C1 efferents to the ventromedial hypothalamus in glucostatic regulation of various processes.

CD70 (TNFSF7) is expressed at high prevalence in renal cell carcinomas and is rapidly internalised on antibody binding.

Adam PJ, Terrett JA, Steers G, Stockwin L, Loader JA, Fletcher GC, Lu LS, Leach BI, Mason S, Stamps AC, Boyd RS, Pezzella F, Gatter KC, Harris AL
Br J Cancer 95(3):298-306, 2006.

Renal cell carcinoma (RCC) is usually resistant to chemotherapy. The authors found a potential target for immunotherapy. An antibody against CD70 was combined with Hum-ZAP (Cat. #IT-22). The complex was then added to an RCC-derived cell-line *in vitro*, demonstrating significant killing at several different concentrations.

Cortical choline transporter function measured *in vivo* using choline-sensitive microelectrodes: clearance of endogenous and exogenous choline and effects of removal of cholinergic terminals.

Parikh V, Sarter M
J Neurochem 97(2):488-503, 2006.

The authors investigated the role of high-affinity choline transporters (CHT) in the clearance of exogenous choline, as well as choline from newly released acetylcholine. 0.085 μg of 192-IgG-SAP (Cat. #IT-01) was injected into each hemisphere of the basal forebrain of rats (mouse IgG-SAP, Cat. #IT-18, was used as a control). The results demonstrate that no matter the source, increases in choline concentrations are cleared by CHT's.

Hindbrain catecholamine neurons control multiple glucoregulatory responses.

Ritter S, Dinh TT, Li AJ
Physiol Behav Epub Jul 31, 2006.

The authors focus on mechanisms eliciting glucoregulatory responses; in particular the catecholaminergic neurons in the hindbrain. Rats received injections of anti-DBH-SAP (Cat. #IT-03) into epinephrine (E) and norepinephrine (NE) terminal areas of hypothalamus and spinal cord. The data suggest that E/NE neurons coordinate various components of the behavioral response to glucoprivation.

Lack of neurogenesis in the adult rat cerebellum after Purkinje cell degeneration and growth factor infusion.

Grimaldi P, Rossi F
Eur J Neurosci 23(10):2657-2668, 2006.

Although neurogenesis occurs in very specific areas of the mammalian brain, neural progenitors can be found in many central nervous system sites. Here the authors examined neurogenesis in the rat cerebellum. 2.2 μg of 192-IgG-SAP (Cat. #IT-01) was injected into each lateral ventricle, and some animals were given exogenous EGF, bFGF, or FGF8. In this model, the local environment was not sufficient to direct neuronal differentiation, even with the addition of growth factors.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Secondary hyperalgesia in the monoarthritic rat is mediated by GABA(B) and NK1 receptors of spinal dorsal horn neurons: A behavior and c-fos study.

Castro AR, Pinto M, Lima D, Tavares I
Neuroscience 141(4):2087-2095, 2006.

Hallmarks of secondary hyperalgesia in a rat model of monoarthritic pain are: decreased activation of GABA(B) neurons, and increased activation of NK-1r neurons. Using 10-μl injections of 1-μM SP-SAP (Cat. #IT-07) into T(13)-L(1) the authors looked at the role of each receptor. Results indicate that both GABA(B) and NK-1r are involved in secondary hyperalgesia.

Adenosine and sleep homeostasis in the basal forebrain.

Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR,Shiromani PJ
J Neurosci 26(31):8092-8100, 2006.

The authors investigated whether basal forebrain cholinergic neurons are involved in adenosine regulation of sleep. 6 μg of 192-IgG-SAP (Cat. #IT-01) was administered to the lateral ventricle of rats. In treated animals, adenosine levels did not increase with prolonged waking.

The nuclear DNA repair protein Ku70/80 is a tumor-associated antigen displaying rapid receptor mediated endocytosis.

Fransson J, Borrebaeck CA
Int J Cancer Epub Aug 23, 2006.

In this study, the authors show that Ku70/80 is internalized into pancreatic carcinoma cells upon binding of the antibody INCA-X. INCA-X was combined with Mab-ZAP (Cat. #IT-04) and applied to several pancreatic carcinoma cell lines *in vitro*. Cell death in some of the treated lines demonstrates the potential of Ku70/80 as a therapeutic target.

Neurokinin-1 receptor expressing neurons in the ventral medulla are essential for normal central and peripheral chemoreception in the conscious rat.

Nattie E, Li A
J Appl Physiol Epub Aug 10, 2006.

The authors ask if neurokinin-1 receptor (NK-1r)-positive cells scattered throughout the ventral medulla are involved in central and peripheral chemoreception. Rats received 250-280 ng of SSP-SAP (Cat. #IT-11) into the cisterna magna; mouse IgG-SAP (Cat. #IT-18) was used as a control. The results indicate that NK-1r neurons in the ventral medulla are involved in both central and peripheral chemoreception, during both waking and sleep states.

Purkinje cell loss by OX7-saporin impairs acquisition and extinction of eyeblink conditioning.

Nolan BC, Freeman JH
Learn Mem 13(3):359-365, 2006.

This work examines the effect of a global depletion of Purkinje cells in the cerebellar cortex on delay eyeblink conditioning in rats. 15 μg of OX7-SAP (Cat. #IT-02) was infused into the left lateral ventricle 2 weeks prior to training. Purkinje cell loss in the anterior lobe and lobule HVI correlated with impaired acquisition and extinction of delay eyeblink conditioning.

Descending facilitation from the rostral ventromedial medulla maintains nerve injury-induced central sensitization.

Vera-Portocarrero LP, Zhang ET, Ossipov MH, Xie JY, King T, Lai J, Porreca F
Neuroscience 140(4):1311-1320, 2006.

Rats were treated with 1.5 pmol of dermorphin-SAP (Cat. #IT-12) or saporin (Cat. #PR-01) into each side of the rostral ventromedial medulla, followed by spinal nerve ligation. The data indicate that mu opioid-expressing neurons are necessary to maintain nerve injury-induced central sensitization.

Local and descending circuits regulate long-term potentiation and zif268 expression in spinal neurons.

Rygh LJ, Suzuki R, Rahman W, Wong Y, Vonsy JL, Sandhu H, Webber M, Hunt S, Dickenson AH
Eur J Neurosci 24(3):761-772, 2006.

Long-term potentiation (LTP) has been shown to occur in sensory areas of the spinal cord and may be one of the mechanisms by which acute pain is transformed into chronic pain. 10 μl of 1μM SP-SAP (Cat. #IT-07) or saporin (Cat. #PR-01) were injected into the subarachnoid space (L4-L5) of rats. The authors demonstrate that dorsal horn neuron generation of LTP may transform acute pain into chronic pain.

Please visit www.ATSbio.com to see a complete list of references.

Targeting Talk: *Anti-DBH-SAP Administration*

by Dr. Sue Ritter, Guest Contributor and anti-DBH-SAP expert

Q: We injected anti-DBH-SAP into the hypothalamus of Sprague-Dawley rats and sacrificed them 2 weeks later. We did not see any reduction in the DBH fiber staining.

When the drug arrived, we aliquoted it in 1-µl snap-cap tubes on ice, and stored them at -80°C. For injections, a 1-µl aliquot was diluted to a little over 10 µl so that we had a final concentration of 1 µg/10 µl.

We administered two injections of 100 nl on each side (with 10 ng of anti-DBH-SAP) using a 0.5-µl Hamilton syringe attached to a stereotax. The needle was a 33-gauge with a blunt tip. I tried previously to use glass micropipette tips attached to a Hamilton syringe with the line filled with mineral oil, but found that the actual volume displacement was too unreliable.

A: We have not had any problems related to the stability of anti-DBH-SAP. In our work, failure to lesion is nearly always associated with a misplaced injection. From the information conveyed, I would suggest the following:

(1) It is possible that no drug was actually delivered to the brain. Two things could be done to ensure drug delivery. The first would be to add a tracer to the saporin solution that could be identified histologically. The second would be to visually monitor drug delivery using a calibrated tip. Air bubbles, pressure leaks and compression of the liquid can interfere with accurate delivery.

(2) It is possible that the anti-DBH-SAP was not delivered to the correct site, so that the expected uptake into the targeted terminals did not occur. Again, marking the site so it is clear where the injection was would help evaluate your accuracy. Establishing a reliable set of stereotaxic coordinates that work in your lab, in your rats and with your equipment and then using a dye to estimate the diffusion radius of your selected injection volume are always good ways to start. However, that being said, it should not be difficult to locate the injection site with such a large injector (33 g) - so #1 seems more likely to be the problem in the case you describe. Also, I would add that the larger the injector, the more nonspecific damage there will be. Glass capillary micropipettes are by far preferable to stainless steel cannulas in providing more reliable delivery of small volumes and in producing less nonspecific damage. Chronically implanted cannulas should be avoided, in my opinion, because gliosis at the cannula tip is apt to occur and this may alter the diffusion pattern of the injected substance, as well as interfering with lesion analysis.

(3) Try a different anesthetic. We have not tested a lot of anesthetics, but we have had problems getting a good lesion that we think are attributable to use of a ketamine/xylazine/acepromazine anesthetic cocktail. So we routinely avoid that one.

(4) I assume you are looking at fibers in the area of the injection. If not, it would be important to make sure the fibers being evaluated are associated with the same neurons innervating the terminal field at the injection site. Secondly, the 2-week wait mentioned between toxin injection and histology is critical for evaluating the lesion to assure that immunoreactive products are no longer present. Making sure that tissue processing controls are stringently adhered to so that controls and lesioned animals are run together in the same batch is also important.

(5) You might try injecting only one side and comparing terminal staining with the non-injected side in the same animal. This would not be a good idea, however, if the injection site is too close to the midline, so that both sides might be damaged from a unilateral injection.

Questions about an ATS product or Molecular Surgery technique?
Write to us at ats@ATSbio.com

NPY-SAP Selectively Disrupts Hypothalamic Controls of Food Intake

(continued from page 1)

area in the NPY-SAP injected rats (Fig. 4), indicating that NPY-SAP is not internalized by NPY terminals or that, once internalized, there is no mechanism for retrograde transport of the conjugate. Supporting these findings, we also showed that NPY terminals in the area of cell body loss, though initially reduced, were not obliterated, as they would be if all NPY neurons innervating that area had been retrogradely destroyed.

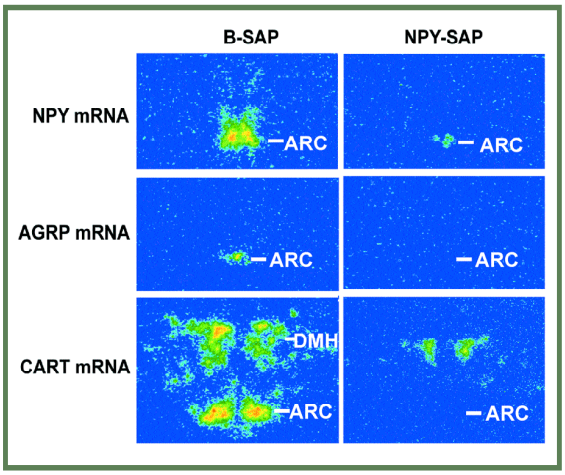

Figure 3. Pseudocolor representation of OD of NPY, AGRP and CART mRNA expression in the arcuate nucleus after bilateral injection of Blank-SAP (B-SAP, left) or NPY-SAP (right) into the arcuate nucleus. NPY-SAP significantly reduced NPY, AGRP and CART hybridization signal at the injection site.

In previous work, we used anti-DBH-SAP to examine the importance of hindbrain catecholamine neurons that innervated the hypothalamus for glucoregulation (7-10). We found that these neurons (many of which co-express NPY) are required for a number of glucoregulatory responses, including feeding, corticosterone secretion and suppression of estrous cycles in response to glucose deficit. In addition, we found that catecholamine neurons with projections to the spinal cord, which are distinct from those that project to the hypothalamus, are required for the adrenal medullary hyperglycemic response to glucoprivation. The goal of our work with NPY-SAP (1) was to determine whether the ARC NPY neurons, which co-express agouti-related protein (AGRP), are required for systemic glucoregulation. Gene knockout studies indicate that the NPY gene is required for glucoprivic feeding (11). However, there are multiple, presumably functionally heterogeneous, NPY populations in the brain. Furthermore, in the medial hypothalamus, the terminals of ARC NPY neurons are co-extensive with those of the hindbrain catecholamine neurons, making it difficult to distinguish the separate functions of these two NPY cell populations. NPY-SAP was useful in addressing this question. To date, we have examined feeding (Fig. 5), hyperglycemic and corticosterone responses to glucoprivation. None of these responses were impaired by ARC NPY-SAP injections that destroyed NPY receptor-expressing neurons, including the NPY/AGRP neurons, in the ARC and basomedial hypothalamus. However, these same lesions severely reduced feeding and body weight responses to leptin and feeding responses to ghrelin, which are known to depend upon ARC NPY receptor-expressing neurons. Thus, using anti-DBH-SAP and NPY-SAP we have been able to functionally differentiate the hindbrain NPY/catecholamine and the ARC NPY/AGRP co-expressing neuronal populations and to establish the primacy of the hindbrain NPY/catecholamine neurons for elicitation of systemic glucoregulatory responses.

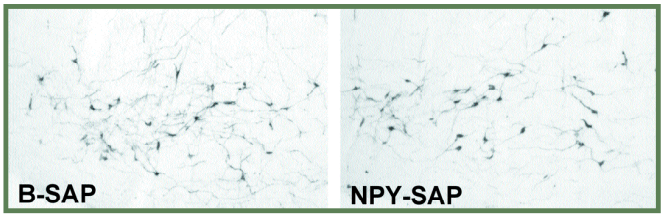

Figure 4. Coronal sections showing tyrosine hydroxylase-ir in the ventrolateral medullary catecholamine cell column ventral to the area postrema in rats injected into the arcuate nucleus with Blank-SAP (B-SAP, control) or NPY-SAP. Nearly all cells in this area co-express NPY and project to the hypothalamus. Arcuate NPY-SAP injections did not cause retrograde destruction of the hindbrain catecholamine/NPY neurons.

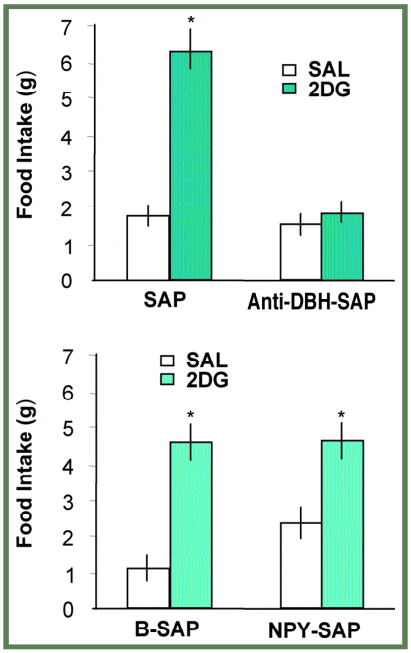

Figure 5. Food intake in response to glucoprivation induced by the antiglycolytic agent, 2-deoxy-D-glucose (2DG, 200 mg/kg, s.c.) in rats injected into the medial hypothalamus with anti-DBH-SAP (top) or NPY-SAP (bottom) or their respective control solutions (unconjugated saporin and Blank-SAP, respectively). Anti-DBH-SAP, but not NPY-SAP, caused impairment of the glucoprivic feeding response. *2DG vs saline for the same group, P<.001

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Targeting Tools: Featured Antibodies

Anti-6-His Mouse Monoclonal, Cat. #AB-20

The use of polyhistidine tags has become a popular method for protein purification, commonly used in the screening process as a tag for your protein or peptide of interest. Whether the material you are screening for is affinity purified or in crude bacterial extract, you will find our antibody suitable to your needs.

This antibody was created as a mouse monoclonal generated to recognize a 6 Histidine (6-His) amino acid sequence, independent of its location. It will recognize C-terminal, N-terminal, or internal 6-His epitopes, with very high sensitivity and low background (Fig. 1).

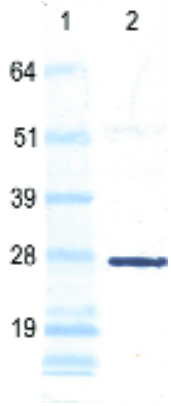

Figure 1: Anti-6-His

Lane 1: Molecular weight standards (See-Blue)

Lane 2: Crude bacterial extract containing a 6-His-tag expressing protein probed with Anti-6-His antibody at a 1:5,000 dilution. Band of interest is visualized at 25-28 kDa.

Gangsta breaks into the catnip.

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HRP-labeled Saporin Goat Polyclonal, Cat. #AB-15HRP

HRP-labeled Anti-SAP can be used to verify binding specificity of a targeted toxin to a cell line expressing the target molecule. By first binding the targeted toxin to protein extract or plate-bound antigen, then binding HRP-labeled Anti-SAP to the targeted toxin, specificity can be confirmed through the use of competing molecules or a control cell line.

This antibody recognizes saporin. Saporin was used as the immunogen. The antibody was coupled to Horseradish Peroxidase (HRP) and dialyzed against PBS.

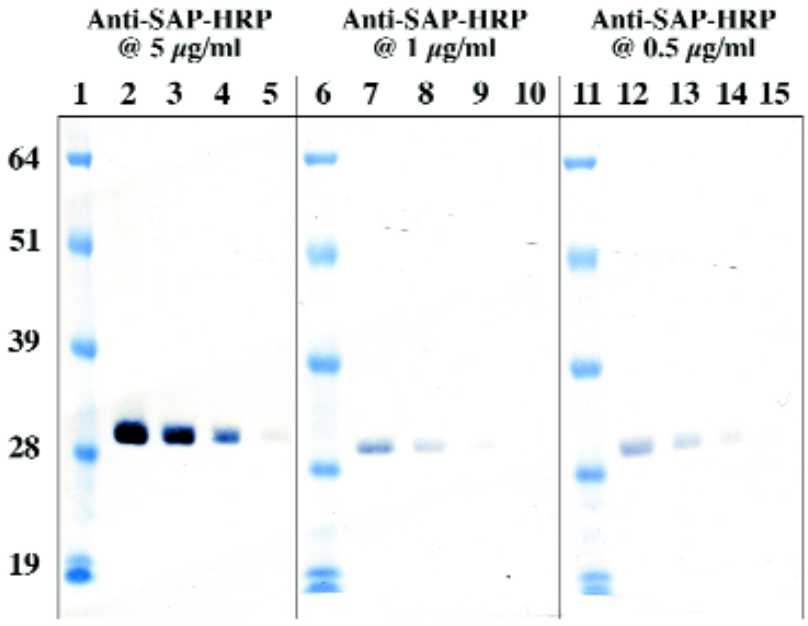

Figure 2: Anti-SAP-HRP

Saporin (200, 100, 50, and 25 ng) was run on a 10% SDS-PAGE gel and transferred to PVDF membrane. The blot was blocked with 4% NFM/TBS, then incubated overnight with 0.5 µg/ml (Lanes 11-15), 1 µg/ml (Lanes 6-10), or 5 µg/ml (Lanes 1-5) of antibody. The blot was washed and developed with 4-chloro-1-naphthol and hydrogen peroxide.

Lane 1, 6, 11: Molecular weight standards (Invitrogen See-Blue)
Lane 2, 7, 12: 200 ng Saporin
Lane 3, 8, 13: 100 ng Saporin
Lane 4, 9, 14: 50 ng Saporin
Lane 5, 10, 15: 25 ng Saporin

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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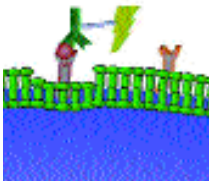
[§]or growth factor, peptide, ligand, or cytokine

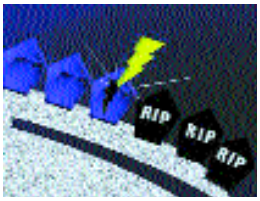
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

LAVTERN

SHOPAMALTHUY

SHARESHORDI

GENEHOSTPASI

STRAPTNOR

Where the Trojan Scientist got the list of ingredients for his latest experiment.

Arrange the circled letters to form the answer, as suggested by the above clue.

Answer: Straight from the... ☐☐☐☐☐'☐ ☐☐☐☐☐

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1. Solve the puzzle.
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