Jan-Feb-Mar 2005
Volume 6, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

IB4-SAP Prevents Axotomy-Induced Sprouting of Aß Fibers

Contributed by ATS's 2004 Society for Neuroscience Poster of the Year Award Winner: Michelle Pearson, Purdue Pharma, Discovery Research, 6 Cedar Brook Drive, Cranbury NJ 08540

Neuropathic pain results in hyperalgesia and allodynia. It has been proposed that sprouting of myelinated touch-responsive Aβ-fibers into the innervation territory of pain-sensitive C-fibers in the spinal cord contributes to these abnormal behaviors.[1] The extent of sprouting has recently been challenged and it has been proposed that C-fibers rather than Aβ-fibers are involved. We have investigated whether selectively ablating a population of small-diameter nociceptors (see Fig. 1) and their associated C-fibers, reduces axotomy-induced sprouting. We ablated this population of sensory neurons by intraneural injection of isolectin B4 conjugated to saporin (IB4-SAP).

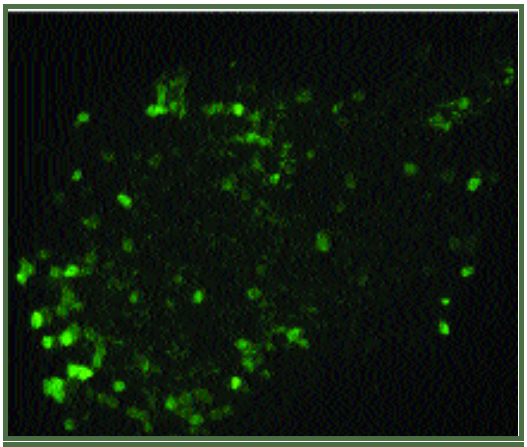


Fig. 1 IB4 labels small diameter nociceptors in the dorsal root ganglia.

Methods: Male, Sprague-Dawley rats received an intraneural injection (directly into the sciatic nerve at mid-thigh level) of either IB4-SAP (2 µl of 0.66 µg/µl) or PBS (2 µl of 0.01 M). Two weeks later, the left sciatic nerve was again exposed and tightly ligated. In the control animals, the nerve was exposed but not ligated. Two weeks following nerve ligation, cholera toxin-β (CTB) subunit conjugated to FITC (2 µl of 20 µg/µl) was administered into the left sciatic nerve in both experimental and control groups. Three days post CTB-FITC injection, animals were perfused with 4% *(continued on page 6)*

ATS Seeks Strategic Partner for Chronic Pain Drug

Toxicology studies with the potential chronic pain therapeutic, SP-SAP (Substance P-Saporin) are continuing successfully. The second study will be initiated in a few months and ATS is diligently pursuing a pharmaceutical partner that can quickly bring the drug to clinical trials in humans.

With the recent news of the undesirable side effects of such pain drugs as Vioxx and Celebrex, the need for a safe, effective chronic pain therapeutic becomes more urgent every day. ATS acknowledges its expertise as a research supply company does not provide the necessary elements for successful drug development. For this reason, an immediate alliance is sought with a partner experienced in bringing a drug to approval and dedicated to the needs of chronic pain patients.

Track progress on SP-SAP drug development on our website: www.ATSbio.com.

Happy New Year from ATS!

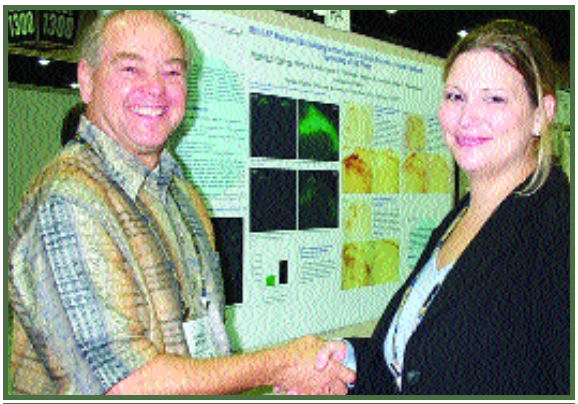
All of us here at Advanced Targeting Systems want to thank you for a great 2004 and we look forward to all the excitement that 2005 will bring.

We are dedicated to meeting your targeting needs and look forward to many thoughtful interactions with scientists around the world. You are our inspiration and motivation to develop and provide innovative targeting tools to keep research on the cutting edge.

Have a great new year! And please don't hesitate to let us know if there's something more we can do for you!

Left: Kristen Hartman, Web and Database Manager
Above: Majid Pajouh, Amalia Dingman, Brian Russell, Denise Higgins, Thea Marlinga, Leonardo Ancheta, Matthew Kohls, Douglas Lappi

Michelle Pearson wins 2004 SfN Poster Award



ATS president, Doug Lappi, congratulates Michelle Pearson in front of her poster at the SfN meeting, October 23-27, 2004 in San Diego.

Once again the choice for Poster of the Year at the Society for Neuroscience meeting was a difficult one with many splendid presentations. This year's award went to a poster from Purdue Pharma presented by Michelle Pearson, *"IB4-SAP Reduces IB4 Staining in the Spinal Cord and Prevents Axotomy Induced Sprouting of Aβ Fibers."* This is the first year that an industrial scientist has won the award.

Last year this same group was in contention for their poster concerning the lack of sprouting after SP-SAP treatment in the spinal cord; a surprising result. This year the Purdue Pharma group continued its work in sprouting, this time using IB4-SAP. The cover article in this issue (Pages 1 & 6) is contributed by Dr. Pearson.

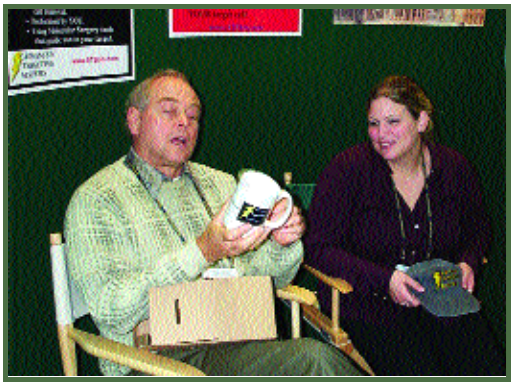
Other contenders for the Poster of the Year award were:

H.J. Ralston, *"The Effects of Chronic Deafferentation and SSP-Saporin on Pain Responses, Spinal Cord Neurons and on the Structure and Function of the Somatosensory Thalamus (VPL) in the Macaque Monkey,"* for his work on SSP-SAP in the spinal cord of macaque monkeys. This excellent multi-disciplinary poster has ATS co-founder Ronald G. Wiley as a co-author and thus was unable to win.

K.H. Bugarith's work with NPY-SAP, *"Injection of the Targeted Toxin, Neuropeptide Y-Saporin (NPY-SAP) into the Basomedial Hypothalamus (BMH) Disrupts Leptin and Ghrelin Signaling,"* which resulted in a publication reviewed on Page 4 of this issue, is the first appearance of results using NPY-SAP.

S.R. Corley from A.E. Butt's group, "192-IgG-Saporin Lesions of the Nucleus Basalis Magnocellularis Impair Biconditional Discrimination Learning in Rats," was an interesting, student-driven presentation on timing behavior tasks and the effect of 192-Saporin.

The Poster of the Year award is given to the presenter and is designed to encourage innovative applications of targeted conjugates in scientific and medical research. The winner receives ATS gear, a $500 product credit and, most importantly, the opportunity to share their work on the cover of *Targeting Trends*.



Dr. Lappi presents Dr. Pearson with her fabulous awards. In addition to the ATS cap and mug, the winner writes the cover article for *Targeting Trends*.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

No facilitation of amphetamine- or cocaine-induced hyperactivity in adult rats after various 192 IgG-saporin lesions in the basal forebrain.

Jeltsch H, Lazarus C, Cosquer B, Galani R, Cassel JC

Brain Res 1029(2): 259-271, 2004

Previous data have indicated that intracerebroventricular (icv) injections of 192-Saporin (Cat. #IT-01) induce a dramatic increase of the locomotor response to amphetamine. The authors of this study examined the locomotor effects of several lesions on the response to amphetamine or cocaine. Rats were injected with 5 μg 192-Saporin icv or 0.4 μg bilaterally into the septal region, or 0.4 μg into the nucleus basalis magnocellularis. While the results did not confirm the amphetamine effect, they did suggest that the effect of cocaine can be altered by these lesions.

The behavioral and neuroanatomical effects of IB(4)-saporin treatment in rat models of nociceptive and neuropathic pain.

Tarpley JW, Kohler MG, Martin WJ

Brain Res 1029(1):65-76, 2004

Using the fact that primary afferent neurons bind isolectin B4 (IB4), the authors injected 5 μg of IB4-SAP (Cat. #IT-10) into the sciatic nerve in the left thigh. After recovery, these animals were then treated with a L5 spinal nerve ligation. Lesioned animals displayed attenuated NGF-induced hyperalgesia, as well as differences in other pain-model markers. The data indicate that IB4-positive C-fibers play a discrete role in NGF-induced hyperalgesia, as well as in the development of neuropathic pain.

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

Spinal neurons that express NK-1 receptors modulate descending controls that project through the dorsolateral funiculus.

Khasabov SG, Ghilardi JR, Mantyh PW, Simone DA

J Neurophysiol [epub] Sep 29, 2004

The involvement of neurokinin-1 receptor-expressing neurons in the spinal cord with the ascending systems of hyperalgesia and central sensitization has been well established. The authors used 10 μl injections of 5 μM SP-SAP (Cat. #IT-07) into the intrathecal space of rats, and examined the descending systems that travel via the dorsolateral funiculus (DLF). While SP-SAP alone had no effect, administration of SP-SAP in conjunction with a DLF transection enhanced neuronal responses to mechanical and heat stimuli.

Medullary noradrenergic neurons release norepinephrine in the medial amygdala in females in response to mating stimulation sufficient for pseudopregnancy.

Cameron NM, Carey P, Erskine MS

Brain Res 1022(1-2):137-147, 2004

Norepinephrine (NE) plays an important role in female reproductive function. While the ventral noradrenergic bundle is known to be necessary for transmitting the pseudopregnancy (PSP) response, the mechanism by which this occurs is not understood. The authors administered 20 ng of Anti-DBH-SAP (Cat. #IT-03) to the left posterodorsal medial amygdala of ovariectomized rats. The results indicate that NE may play an important role in the establishment of PSP.

The medial septum mediates impairment of prepulse inhibition of acoustic startle induced by a hippocampal seizure or phencyclidine.

Ma J, Shen B, Rajakumar N, Leung LS

Behav Brain Res 155(1):153-166, 2004

Deficits in sensorimotor gating, suppression of a motor response by a sensory stimulus are found in schizophrenic patients, as well as laboratory animals after administration of compounds such as phencyclidine (PCP). The authors lesioned the cholinergic system of the medial septum in rats with 0.14-0.21 μg injections of 192-Saporin (Cat. #IT-01) to examine the involvement of these neurons in sensorimotor gating. The authors suggest that GABAergic, but not cholinergic septohippocampal neurons mediate this deficit.

Transient attenuation of CO_2 sensitivity after neurotoxic lesions in the medullary raphe-area in awake goats.

Hodges MR, Opansky C, Qian B, Davis S, Bonis J, Bastasic J, Leekley T, Pan LG, Forster HV

J Appl Physiol 97(6):2236-2247, 2004

The authors wished to investigate the influence medullary raphe-area neurons have on breathing. This control may be through CO_2/H+ chemoreceptors and/or through non-chemoreceptor modulation. 1 or 10 μl of 50 pM SP-SAP (Cat. #IT-07) or Saporin (Cat. #PR-01) was injected into the raphe of goats. Breathing and CO_2 sensitivity were evaluated during different physiologic

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

conditions. The data suggest that SP receptor- and glutamate receptor-expressing neurons in the medullary raphe both influence CO_2 sensitivity, but not altered breathing periods.

Exogenous testosterone prevents motoneuron atrophy induced by contralateral motoneuron depletion.

Fargo KN, Sengelaub DR

J Neurobiol 60(3):348-359, 2004

Gonadal steroids have been shown to supply a variety of neuroprotective and neurotherapeutic effects. Using 1-μl injections of 0.1% CTB-SAP (Cat. #IT-14) into the ipsalateral bulbocavernosus and the levator ani of rats, the authors examined the protective effects of testosterone on motoneuron morphology. After the lesion was induced some rats were castrated, and all animals were treated with exogenous testosterone. The results suggest that high-normal levels of testosterone can prevent motoneuron atrophy induced by contralateral motoneuron depletion.

Involvement of brainstem catecholaminergic inputs to the hypothalamic paraventricular nucleus in estrogen receptor alpha expression in this nucleus during different stress conditions in female rats.

Estacio MA, Tsukamura H, Reyes BA, Uenoyama Y, I'anson H, Maeda K

Endocrinology 145(11):4917-4926, 2004

Norepinephrine release in the paraventricular nucleus (PVN) is increased during periods of metabolic stress. The authors hypothesized that noradrenergic inputs to the PVN may also mediate estrogen receptor a (ERa) expression in the PVN during metabolic stress. 20 ng of Anti-DBH-SAP (Cat. #IT-03) was injected bilaterally into the PVN of rats, and ERa expression was examined in several stress models. Results indicate that during metabolic stress catecholaminergic inputs to the PVN play a major role in mediating the induction of ERa expression.

Small reduction of neurokinin-1 receptor-expressing neurons in the pre-Botzinger complex area induces abnormal breathing periods in awake goats.

Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah T, Davis S, Forster HV

J Appl Physiol 97(5):1620-1628, 2004

Previous work has shown that lesion of the pre-Bötzinger Complex (pre-BötzC) of rats with SP-SAP (Cat. #IT-07) results in hypoventilation and an abnormal breathing pattern. The authors used 1 or 10 μl of 50 pM SP-SAP bilaterally injected into the pre-BötzC area to further investigate this system in goats. The results show transient changes in respiratory rhythm and respiratory muscle activation patterns, indicating that SP receptor-expressing neurons in the pre-BötzC are involved in the regulation of respiration.

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

Large lesions in the pre-Botzinger complex area eliminate eupneic respiratory rhythm in awake goats.

Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah TR, Davis S, Forster HV

J Appl Physiol 97(5):1629-1636, 2004

Previously the authors demonstrated that lesioning the pre-Bötzinger Complex (pre-BötzC) with SP-SAP (Cat. #IT-07) resulted in transient disruptions of normal respiratory muscle activation in goats. The purpose of this study was to examine the effects of a more complete lesion of the pre-BötzC area. The authors treated SP-SAP-lesioned goats with ibotenic acid. The results suggest that the pre-BötzC is critical for generating a normal respiratory rhythm during the awake state.

Basomedial hypothalamic injections of neuropeptide Y-saporin (NPY-SAP) selectively disrupt hypothalamic controls of food intake.

Bugarith K, Dinh TT, Li AJ, Speth RC, Ritter S

Endocrinology [epub] Dec 16, 2004

The authors examined the effect of 48 ng injections of NPY-SAP (Cat. #IT-28) into the basomedial hypothalamus (BMH) on glucoprivic feeding in rats. While there was no evidence of retrograde transport, the lesions inhibited responses to intracerebroventricular leptin and ghrelin. Neither the feeding nor the hyperglycemic response to 2-deoxy-D-glucose was affected by the lesion, indicating that these hindbrain processes do not utilize neurons in the BMH. This work also describes dosing and injection parameter studies for the use of NPY-SAP.

Targeting Talk: *Effective Toxins - continued from last issue*

by Dr. Douglas Lappi

Dear Targeting Talk:

In the most recent issue (Oct-Nov-Dec 2004), you addressed the question of one molecule of saporin killing a cell. Your response overlooked the data on ricin, abrin and modeccin (Eiklid, Olsnes and Pihl, Exp Cell Res, 126:321-326, 1980). In that paper, they showed that these RIP toxins applied to cells in culture produce all-or-none lethality. They used radioactive amino acid uptake and incorporation (as memory serves) and found only two types of cells, those with absolutely no uptake of label or those that were entirely normal - nothing in between.

Also, if the data on ricin-induced apoptosis is correct (numerous authors), and I believe it is, then at low doses, the cells die from triggering apoptosis which seem possible with a single molecule of RIP free in the cytoplasm. To further complete your answer, someone (I haven't found the article yet) showed that it took, on average, about 10,000 molecules of ricin/cell to kill cells in culture. This gives a hint at the efficiency of internalization and translocation in that cell type. I am not aware anyone else has looked at these issues with saporin conjugates.

Fascinated reader

Dear Fascinated,

Overlooking literature is actually a favorite sport of mine, but in this case I would respectfully point out conflicting information. There is a study of something that is quite between an all-or-none phenomenon: Barbieri *et al*. *FEBS Lett*, 2003 Mar 13;538(1-3):178-82. These authors document that ribosome-inactivating proteins have transforming activity on the classic FDA assay cell line: NIH3T3 cells. This would be a non-toxic activity that one presumes is due to internalization, and is somewhat on the none side of all or none, but hey, it's an activity nonetheless.

My personal feeling is that there is material in the literature that can and should be questioned, and that Targeting Talk should actually go back to being done by my clever colleague, Dr. Ronald G. Wiley.

Targeting Talk

Upcoming Events

Experimental Biology
April 2-6, 2005
San Diego, CA
Booth 716

American Assoc for Cancer Research
April 17-20, 2005
Anaheim, CA
Booth 371

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: DIENCEPHALON
WINNER
PSYCHIC
OPHTHALMOLOG
RESPIRATION

Answer: WALTER HESS

WINNERS:

Catheline Gwenaelle, INSERM ERHE Inst. Magendie * Jan Pieter Konsman, INSERM U394 Inst. Magendie * Maria Christensen, Creighton University * Darlene Martineau, Idun Pharmaceuticals * Robert Speth, University of Mississippi * Laura Emond, Dartmouth Medical School * B. Peteri-Brunback, Univ Nice SA * Richard Greene, PerkinElmer Life and Analytical Sci * Valery Nelson, Panacea Pharmaceuticals Inc. * Andrew Johnston, National Univ Hospital, Iceland * Joseph Menonna, E. Orange VA Med. Center

Walter Rudolf Hess was born in Frauenfeld, East Switzerland, on March 17, 1881. Although his aim was to be a physiologist, external reasons first necessitated him to be an assistant in surgery, later in ophthalmology, and finally a practicing ophthalmologist. This detour, however, was by no means a disadvantage, as he learned, particularly in ophthalmology, to investigate and operate with precision.

The scientific interests of Hess were primarily directed towards haemodynamics and the regulation of respiration. Due to his work, a comprehensive picture has emerged of the representation of the vegetative nervous system in the diencephalon, which was accorded distinction when Hess became a winner of the Nobel Prize.

Hess observed that, in the experiments on diencephalic stimulation, modes of behavior were occasionally evident in the experimental animal, which suggested a manifestation of psychic powers. This was the theme of The Biological Aspect of Psychology (1962).

IB4-SAP Prevents Axotomy-Induced Sprouting of Aß Fibers

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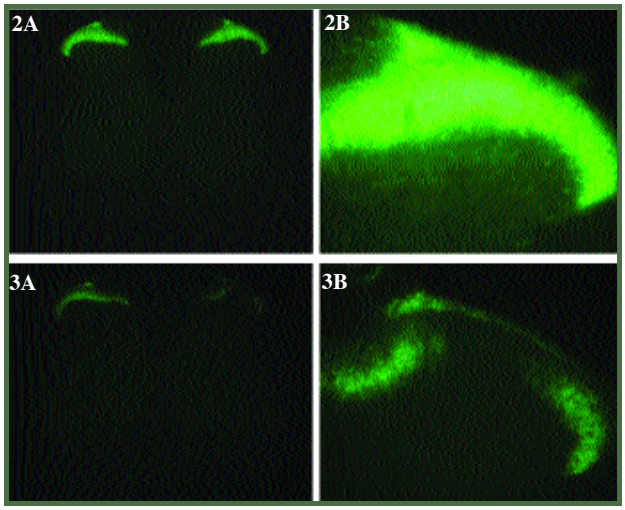
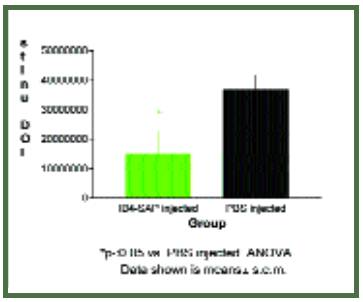


Fig. 2 Isolectin B4 labels the central terminals of small diameter nociceptors in lamina II of the spinal cord.

Fig 3. Intraneural IB4-SAP reduces Isolectin B4 staining in lamina II.

formalin and the relevant tissue was dissected and post-fixed overnight in the same fixative. The spinal cords and dorsal root ganglia were sectioned using a microtome at 60 and 40 µm thickness, respectively. Sections were stained using either IB4-FITC or goat anti-CTB-FITC.



Results: IB4-SAP treatment resulted in a reduction of IB4 staining in lamina II of the spinal cord as compared to PBS-treated animals (Fig. 2 compared to Fig. 3). This result confirms previous work demonstrating that, when injected into the sciatic nerve, IB4-SAP is retrogradely transported to the dorsal root ganglia where it is subsequently toxic to a subpopulation of small diameter nociceptors.[2] Image analysis demonstrated that the decrease in IB4 staining was both large and statistically significant as compared to PBS-injected controls ($p \leq 0.05$; Fig. 4).

As previously described[1] sections from rats that received a sciatic axotomy followed by intraneural CTB displayed an obvious presence of CTB in lamina II in addition to the motor neurons, laminae I and II-IV (Fig. 5). In these sections the borders of lamina II were not discernable. In all cases, lamina II was devoid of stain on the side contralateral to the injection.

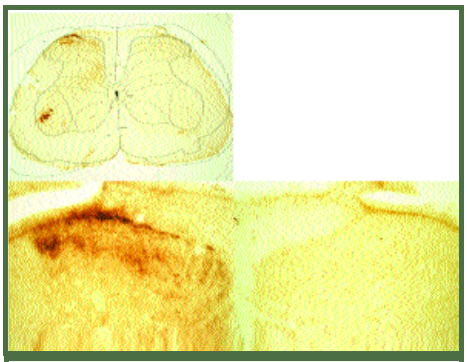


Fig. 5 Evidence of Aβ sprouting in lamina II following peripheral axotomy.

Spinal cord sections from rats that had received sham surgery followed by intraneural injection of CTB displayed prominent CTB immunostaining in the motor neurons, lamina I and lamina III-VI on the side ipsilateral to the injection (Fig. 6). Lamina II was devoid of positive immunostaining for CTB and boundaries between lamina I, II and III (arrows) were easily discernable.

Spinal cord sections from rats that received an intraneural IB4-SAP injection and sciatic axotomy followed by an intraneural injection of CTB (Fig. 7) displayed prominent CTB immunostaining in motor neurons, lamina I and lamina III-VI on the side ipsilateral to the injections. Lamina II was devoid of positive immunostaining for CTB.

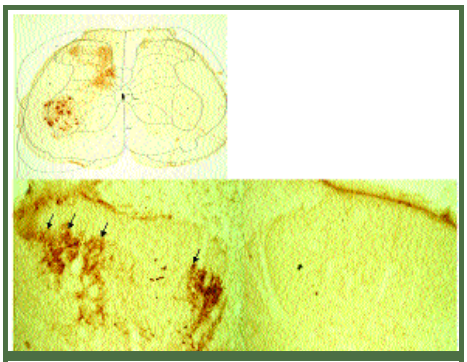


Fig. 6 No evidence of Aβ sprouting in lamina II following sham axotomy.

These data confirm the previous report that intraneural IB4-SAP ablates a population of small diameter nociceptors in the dorsal root ganglion and reduces the quantity of IB4-positive terminals in the spinal cord. Following ablation of this cell population, the extent of axotomy-induced CTB staining in lamina II is reduced as compared to control animals. This study strongly suggests that C-fibers contribute significantly to CTB staining that is observed in the spinal cord following nerve injury.

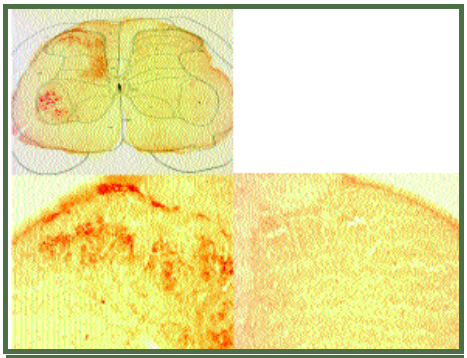


Fig. 7 No evidence of Aβ sprouting in lamina II following IB4-SAPinjection and peripheral axotomy.

References:

1. Woolf, CJ *et al*. (1992) *Nature*, 335:75-78.

2. Honda, CN *et al*. (2001) *Neuroscience*, 108:143-155.

Targeting Tools: Featured Products

anti-ChAT *Cat. #AB-N34*

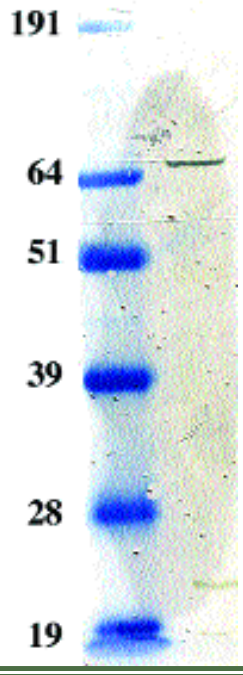


Fig. 1
Lane 1: Molecular weight standards (Novex, See-Blue)
Lane 2: 25 mg of PC12 whole cell extract probed with AB-N34 at 1:2000 dilution.

ATS is pleased to present a polyclonal antibody specific for ChAT protein. Choline acetyltransferase (ChAT) catalyzes the synthesis of the neurotransmitter acetylcholine (ACh) from choline and acetyl-CoA in cholinergic neurons. ChAT serves as a specific marker for cholinergic neurons in both the peripheral and central nervous systems. Dysfunction of cholinergic neurons underlies aspects of clinical symptoms found in neurological and psychiatric disorders such as Alzheimer's disease, Down and Rett syndromes.

The peptide sequence used for immunization has a high degree of homology between rat, human, mouse and pig. We have demonstrated the specificity of anti-ChAT by western blot analysis (Fig. 1) and immunohistochemistry (Figs. 2, 3). Anti-ChAT detects ChAT protein in cholinergic neurons of rat spinal cord, forebrain (Fig. 3) and PC12 cells (Fig. 2). Due to the fact that our ChAT antibody has a very high titer (1:5,000,000 dilution in ELISA and 1:2000-4000 in immuohistochemistry), it is provided as whole sera in 50% glycerol (Cat. #AB-N34). An affinity purified form of anti-ChAT is also available (Cat. #AB-N34AP).

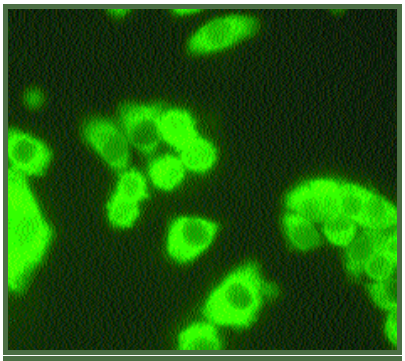


Fig. 2 Fluorescent staining of endogenous ChAT in cytoplasm of PC12 cells. Dr. Majid Pajouh, Advanced Targeting Systems, San Diego, CA.

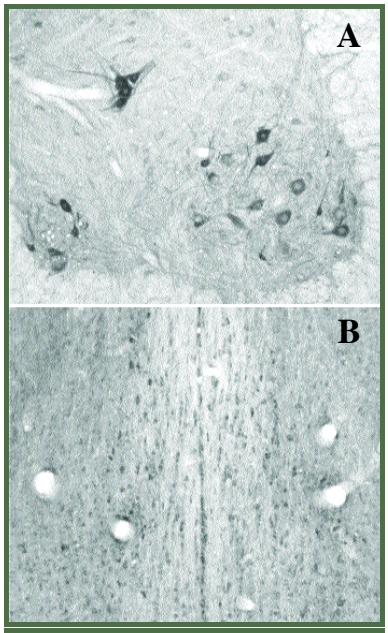


Fig. 3 A fixed section of rat spinal cord (A) and forebrain (B) was stained with our anti-ChAT (1:2000-4000 dilution). Courtesy of Dr. Ronald G. Wiley. and Robert Kline at Vanderbilt University, Nashville, TN.

Visit the ATS website for a complete list of products.

MORE New Antibodies

AB-L001	Muscarinic Acetylcholine Receptor M2	Human; Mouse; Rat
AB-L002	Muscarinic Acetylcholine Receptor M3	Human; Mouse; Rat
AB-L003	Muscarinic Acetylcholine Receptor M4	Human; Mouse; Rat
AB-L004	Muscarinic Acetylcholine Receptor M5	Human; Mouse; Rat
AB-L056	Dopamine Receptor D1	Human; Mouse; Rat
AB-L059	Endothelin A Receptor	Human; Mouse; Rat
AB-L072	GABA(B) Receptor 1	Human; Mouse; Rat
AB-L073	Galanin Receptor GalR1	Human; Mouse; Rat
AB-L074	Galanin Receptor GalR2	Human; Mouse; Rat
AB-L075	Galanin Receptor GalR3	Human; Mouse; Rat
AB-L122	Neuropeptide FF 1 Receptor	Human; Mouse; Rat
AB-L124	Neuropeptide Y Receptor Type 1	Human; Mouse; Rat
AB-L125	Neuropeptide Y Receptor Type 4	Human; Mouse; Rat
AB-L126	Neuropeptide Y Receptor Type 5	Human; Mouse; Rat
AB-L140	Opioid Receptor, Delta 1 (OPRD1)	Human; Mouse; Rat
AB-L141	Opioid Receptor, Mu 1 (OPRM1)	Human; Mouse; Rat

Gangsta. . . the mighty elk!?

Targeting Technology

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

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

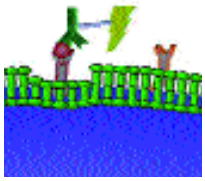
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

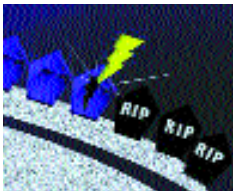
he targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

The conjugate is internalized and APORIN 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 form the name of this award-winning scientist.

ROOMPHENE

TANODOR

TRICUCI

CHOMLOTSK

SHIPSOBCIY

A rose by any other name...

Arrange the circled letters to form the name of this noted scientist, 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, 2005.

Limit one entry per laboratory.

See last quarter's winners, page 5.

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Apr-May-Jun 2005
Volume 6, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- SP-SAP therapeutic update *(page 2)*
- Teaser Winners *(page 2)*
- Control toxins *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Noradrenergic inputs to the medial amygdala originate in the A1 and A2 cells groups and release norepinephrine after mating stimulation sufficient to induce pseudopregnancy

Contributed by Lesley E. Northrop, Nicole Cameron and Mary Erskine, Department of Biology, Boston University

The posterodorsal medial amygdala (MePD) is involved in processing the information from genito-sensory stimuli which is needed for mating-induced pseudopregnancy. The amygdala complex is known to be involved in memory storage and consolidation, processes known to be influenced by amygdalar norepinephrine (1). In addition, previous research has shown the MePD and the A2 noradrenergic cell group show increases in c-fos expression after mating, indicating that cellular activation has occurred in these areas. It is not clear whether there are noradrenergic projections from the A2 nuclei into the MePD.

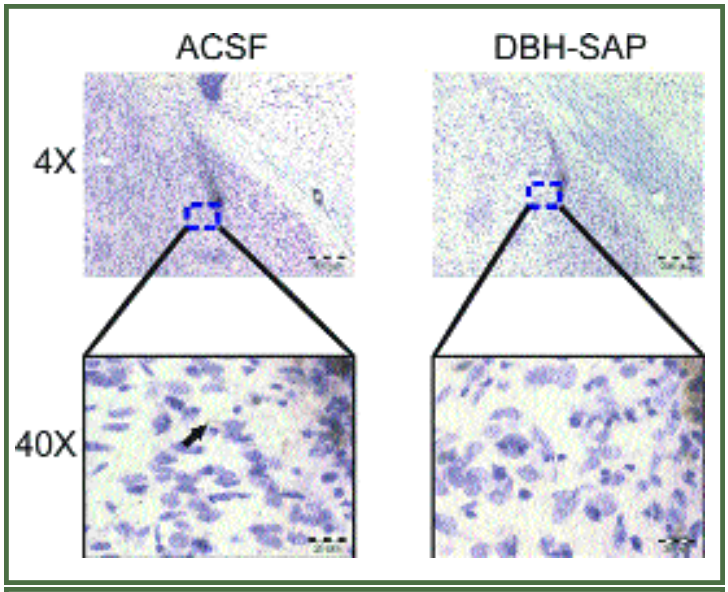


Fig. 1 Location of injection sites and demonstration of lack of damage.

In the first experiment, we infused anti-dopamine-ß-hydroxylase-saporin (anti-DBH-SAP), a ribosome-inactivating neurotoxin which selectively destroys noradrenergic neurons, into the MePD to determine the source of norepinephrine in the MePD. A second experiment examined whether norepinephrine is released in the MePD during and after mating using a microdialysis technique.

Experiment 1: Ovariectomized female rats were infused with 20 ng anti-DBH-SAP in 0.2 μl of aCSF into the left MePD (relative to bregma; AP-2.7 mm, ML 3.5 mm, DV -6.7 mm from dura) according to the atlas of Paxinos and Watson (2). Eight days after infusion, animals were perfused and 30 μm sections through the brainstem were stained immunocytochemically using a DBH antibody. The forebrains were sliced and sections were stained with cresyl violet for injection site verification.

Figure 1 shows the location of the

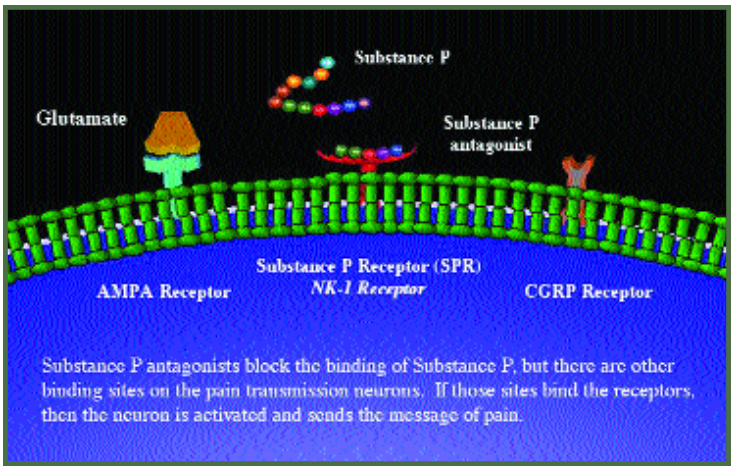
(continued on page 6)

SP-SAP : How It's Different From Substance P Antagonists

As most of you know, SP-SAP is a potential therapeutic that ATS is developing for use in the elimination of chronic pain. For the past seven years, preclinical studies have been carried out in the rat, safety and dosing studies completed in the dog and the first of two toxicology studies has begun in the rat. The second study is due to start later this year.

SP-SAP is a conjugate of the peptide, Substance P, and the ribosome-inactivating protein, saporin. It has been shown in all the recognized animal models of pain to eliminate the chronic pain signal. The acute (normal) pain signal remains intact. The removal of the chronic pain signal is permanent with no discernible side effects.

SP-SAP works by specifically removing the neurons in the spinal cord that express the Substance P (NK-1) receptor. This represents about 5% of the population of neurons in that area. Administration is through intrathecal injection, similar to the epidural used in childbirth.



Years ago, when the role of substance P in the transmission of the signal for pain became clear, it was immediately proposed by pharmaceutical scientists to construct analogs of substance P that would block the ability of it to bind to its receptor (see illustration). It was hypothesized that these antagonists would bring about pain relief. However, these analogs have always been disappointing. The reason for this may be that the pain pathway is very important and there are several redundancies; there are several other neurotransmitters, both peptides such as somatostatin or CGRP and smaller molecules such as glutamate, that are involved in transmission of the signal. Hence, blocking one of them is not sufficient; the others step in and take their place.

ATS is actively meeting with pharmaceutical companies to select the right partner to bring SP-SAP to market. There is a great unmet need among people throughout the world who are suffering from chronic pain. SP-SAP could bring these people a better quality of life. With the right partner, SP-SAP could be in clinical trial in a year.

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: PHEROMONE
ODORANT
CIRCUIT
STOCKHOLM
BIOPHYSICS

Answer: LINDABUCK

WINNERS:

Bryan Hudson, Washington State University * Kim Van Vliet, University of Florida * Michael Lebowitz, Panacea Pharmaceuticals Inc. * Mark Weiss, Kansas State University * Robert Speth, University of Mississippi * Seto Chice, SUNY HSC at Brooklyn * Andreas Lehner, University of Kentucky

Linda Buck was the recipient of the 2004 Nobel prize in physiology or medicine. Dr. Buck is a member of the Fred Hutchinson Cancer Research Center's Basic Sciences Division, investigator for the Howard Hughes Medical Institute, and affiliate professor of physiology and biophysics at the University of Washington School of Medicine. She received the award in Stockholm for her work on odorant receptors and the structure of the olfactory system.

Dr. Buck is honored for discovering the molecular basis of smell: a multigene family that encodes 1,000 different olfactory receptors in the nose. She shared the prize with Dr. Richard Axel of Columbia University. Their work is the first to define the genes and proteins that control the complex odorant response. They were able to carry out this remarkable work by developing a genetic method to visualize a neural circuit.

Buck also discovered and characterized families of receptors for pheromones and tastes, providing insights into the mechanisms underlying pheromone effects and taste perception.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Purkinje cell loss by OX7-saporin impairs excitatory and inhibitory eyeblink conditioning.

Nolan BC, Freeman JH Jr

Behav Neurosci 119(1):190-201, 2005

Although the contributions of the cortical cerebellum to eyeblink-conditioned excitation have been extensively investigated, involvement in inhibition of this reflex is unclear. After intracerebroventricular infusions of 15.0 μg of OX7-SAP (Cat. #IT-02), rats displayed impaired retention and savings of preinfusion excitatory conditioning, indicating that the Purkinje cells that were eliminated by OX7-SAP are essential for maintenance of excitatory eyeblink conditioning. Inhibition is not prevented by loss of these Purkinje cells, suggesting that extracerebellar structures play a critical role in this process.

Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning.

Mohapel P, Leanza G, Kokaia M, Lindvall O

Neurobiol Aging 26:939-946, 2005

New hippocampal neurons that are thought to be involved in memory formation are generated in the dentate gyrus (DG) throughout adulthood. In this study, rats were injected at various sites with 192-Saporin (Cat. #IT-01). The authors found that acetylcholine levels, which are reduced upon administration of 192-Saporin, are linked to proliferation and/or short-term survival of DG neurons, rather than long-term survival or differentiation. Cognitive defects that could be linked to the reduced number of new neurons were also observed.

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Elimination of rat spinal neurons expressing neurokinin 1 receptors reduces bladder overactivity and spinal c-fos expression induced by bladder irritation.

Seki S, Erickson KA, Seki M, Nishizawa O, Igawa Y, Ogawa T, de Groat WC, Chancellor MB, Yoshimura N

Am J Physiol Renal Physiol 288(3):F466-F473, 2005

Substance P is reported to play a role in the micturition reflex as well as in nociceptive responses. The authors investigated the role that neurokinin-1 receptor-expressing cells in the spinal cord play in the micturition reflex of rats. 8 μl of 1.0 or 1.5 μM SSP-SAP (Cat. #IT-11) was injected into the L6-S1 level of the spinal cord, and cystometric parameters were measured before and after capsaicin administration to the bladder. Lesioned animals did not display the bladder overactivity normally seen in the presence of capsaicin.

The effect of central cholinergic and noradrenergic denervation on hippocampal sympathetic ingrowth and apoptosis-like reactivity in the rat.

Harrell LE, Parsons DS, Kolasa K

Brain Res 1033(1):68-77, 2005

Cholinergic denervation of the hippocampus is followed by ingrowth of peripheral sympathetic fibers originating from the superior cervical ganglion. The authors injected 1 μg of 192-Saporin (Cat. #IT-01) into the medial septum of rats along with a noradrenergic fiber neurotoxin to investigate whether the noradrenergic system was involved with this ingrowth as well. The data provide more evidence that hippocampal sympathetic ingrowth can be stimulated by cholinergic denervation alone.

Involvement of GABAergic and cholinergic medial septal neurons in hippocampal theta rhythm.

Yoder RM, Pang KC

Hippocampus Epub Jan, 2005

It is thought that hippocampal theta rhythm (HPCθ) is involved in attention and acquisition of sensory information. The HPCθ circuit includes the medial septum/diagonal band of Broca (MSDB), which projects to the hippocampus through GABAergic and cholinergic neurons. A total of 0.325 μg of 192-Saporin (Cat. #IT-01) was injected into the MSDB of rats. Hippocampal recordings measuring field potential oscillations were taken, indicating that both GABAergic and cholinergic neurons are involved in HPCθ.

Hebb-Williams performance and scopolamine challenge in rats with partial immunotoxic hippocampal cholinergic deafferentation.

Marques Pereira P, Cosquer B, Schimchowitsch S, Cassel JC

Brain Res Bull 64(5):381-394, 2005

Much of the recent work done on the role of cholinergic neurons in the hippocampus has been focused on detecting subtle learning deficits. In this study, the authors investigated the effect of 0.368 μg of 192-Saporin (Cat. #IT-01) administered to the medial septum of rats

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

in four injections. A complex learning task, the Hebb-Williams maze, was used to define small deficits in the learning performance of the lesioned animals prior to, and after the injection of scopolamine.

Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake.

Bugarith K, Dinh TT, Li AJ, Speth RC, Ritter S

Endocrinology 146(3):1179-1191, 2005

Neurons in the arcuate nucleus (ARC) that coexpress neuropeptide-Y (NPY) and Agouti gene-related protein may be involved in glucoprivic feeding. The authors investigated the use of NPY-SAP (Cat. #IT-28) to eliminate neurons expressing NPY receptors. Bilateral injections of 48 ng of NPY-SAP were made into the basomedial hypothalamus dorsal to the ARC in rats. While the NPY-SAP lesions impaired responses to leptin and ghrelin, the data do not support the role of NPY/AGRP neurons as mediators of glucoprivic feeding.

Sexually dimorphic effects of hippocampal cholinergic deafferentation in rats.

Jonasson Z, Cahill JF, Tobey RE, Baxter MG

Eur J Neurosci 20(11):3041-3053, 2004

Studies of cholinergic neuron lesions have been performed almost exclusively in male animals. In this work, the authors examined the differences of cholinergic lesions between males and females. Rats were treated with four injections totaling 0.15 μg of 192-Saporin (Cat. #IT-01) into the medial septum/vertical limb of the diagonal band. The results demonstrate differences in learning and memory processes between male and female rats.

Effects of lesions of the histaminergic tuberomammillary nucleus on spontaneous sleep in rats.

Gerashchenko D, Chou TC, Blanco-Centurion CA, Saper CB, Shiromani PJ

Sleep 27(7):1275-1281, 2004

Although evidence suggests that histaminergic neurons in the tuberomammillary nucleus (TMN) promote wakefulness, this has not been investigated using specific lesioning agents. In this study, the authors utilize the fact that TMN neurons express the orexin-B receptor by eliminating these neurons with an injection of 50 ng of orexin-SAP (Cat. #IT-20) into the posterior hypothalamus. The data indicate that histaminergic neurons are not required for the homeostatic regulation of sleep.

Impairment of skilled forelimb use after ablation of striatal interneurons expressing substance P receptors in rats: an analysis using a pasta matrix reaching task.

Chiken S, Tokuno H

Exp Brain Res 2005 Mar 8; [Epub]

The substance P receptor is expressed by two types of interneurons in the striatum. The authors investigated whether elimination of these neurons would impair motor control by the basal ganglia. Rats were treated with 7.5 ng injections of SP-SAP (Cat. #IT-07) into the dorsolateral part of the striatum. Lesioned animals did not perform as well as controls in a test measuring accurate reaching with the forepaw. The data show that striatal interneurons expressing the substance P receptor are necessary for accurate reaching.

Aging and cholinergic deafferentation alter GluR1 expression in rat frontal cortex.

Kim I, Wilson RE, Wellman CL

Neurobiol Aging 26(7):1073-1081, 2005

Neuronal plasticity is involved in several processes during adulthood, including learning and memory, and recovery from injury. Recent evidence suggests that aging reduces this plasticity. The authors used 0.15 μg injections of 192-Saporin (Cat. #IT-01) into the nucleus basalis magnocellularis of rats to investigate how the loss of cortical plasticity would affect the expression of GluR1. Younger animals displayed a marked increase in the number of GluR1-expressing neurons, a compensatory response not seen in older animals.

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

Targeting Talk: *Cytotoxicity of Unbound Saporin*

by Dr. Douglas Lappi

Q: We are re-examining some data collected using an immunotoxin not prepared by your company (VChAT-sap). Our results in vivo *indicated that there were nonspecific effects although the creators claimed it was specific. We ran a Western Blot and determined that about half the saporin was not bound to the antibody. This may have been the problem but I want to confirm the cytotoxicity of unbound saporin. Can you confirm that? Further, the antibody in question never bound selectively in ferret tissue. Does this suggest a problem as well? Can you give me information on the best way to design and test an immunotoxin.*

A: Yes, saporin at higher concentrations can be cytotoxic. Without specific binding, you will only see non-specific cytotoxicity. The sequence of the immunogen for that antibody is from the rat protein, so I'm not sure if it would target the ferret protein (there usually is good sequence homology among transporters). We worked a lot with this antibody and with an immunotoxin (made by us), but never got any sort of results that would indicate that it was working. We also were greatly concerned that the epitope is an intracellular epitope, and so we have difficulty understanding, from a theoretical standpoint, how it even could work. Because of many concerns, we never commercialized it, and we believe all the effects in the literature were non-specific, but "credible" because of the unusual experimental system that was used.

The best way to design and test an immunotoxin is to talk to us. If your antibody has been tested and shown to be internalized by the cell you are targeting, there should be no problem with the activity. The conjugate will only work as well as your antibody does.

We recommend that you try a second immunotoxin before having a custom conjugation performed. This allows you to use a secondary agent conjugated to saporin that "piggybacks" on your antibody and makes a second immunotoxin for use *in vitro* to test specificity and internalization of your antibody. You can check out the publication that talks about one of the secondary conjugates, Mab-ZAP. We also have Rab-ZAP that works with rabbit polyclonal antibodies, Rat-ZAP that works with rat antibodies, Hum-ZAP that works with human antibodies, anti-M-ZAP that works with IgM antibodies or you can biotinylate your material and use Streptavidin-ZAP.

You can find more information about second immunotoxins on our website. Click on Catalog:Targeted Toxins:Second Immunotoxins.

REFERENCE:

Kohls MD, Lappi DA (2000) Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165.

Second Immunotoxins: *Ready-Made Custom Conjugates*

IT-04	Mab-ZAP	Mouse Monoclonal antibodies	IT-26	Rat-ZAP	Rat Monoclonal antibodies
IT-05	Rab-ZAP	Rabbit Polyclonal antibodies	IT-30	Anti-M-ZAP	Mouse Monoclonal IgM antibodies
IT-22	Hum-ZAP	Human Monoclonal antibodies	IT-27	Streptavidin-ZAP	Biotinylated material

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Norepinephrine Release After Mating Stimulation

(continued from page 1)

injection sites within the MePD at 4X magnification, and the lack of tissue damage around the site of injection at 40X magnification. There was no indication that local infusion of anti-DBH-SAP induced neurotoxic damage around the area of infusion, as similar numbers of neurons were present in anti-DBH-SAP and aCSF groups and since there did not appear to be microglial infiltration around the injection site in either group. Figure 2 shows the effects of anti-DBH-SAP on the number of DBH-stained cells within the A1 and A2 (nucleus tractus solitarius, NTS) cell groups. Significant reductions in DBH-positive cells were observed compared to aCSF controls. Quantification of the results are shown in Figure 3, where it can be seen that decreases in DBH-positive neurons occurred in the A1 and rostral and middle NTS. These data demonstrated that noradrenergic projections to the MePD originate within the latter two cell groups.

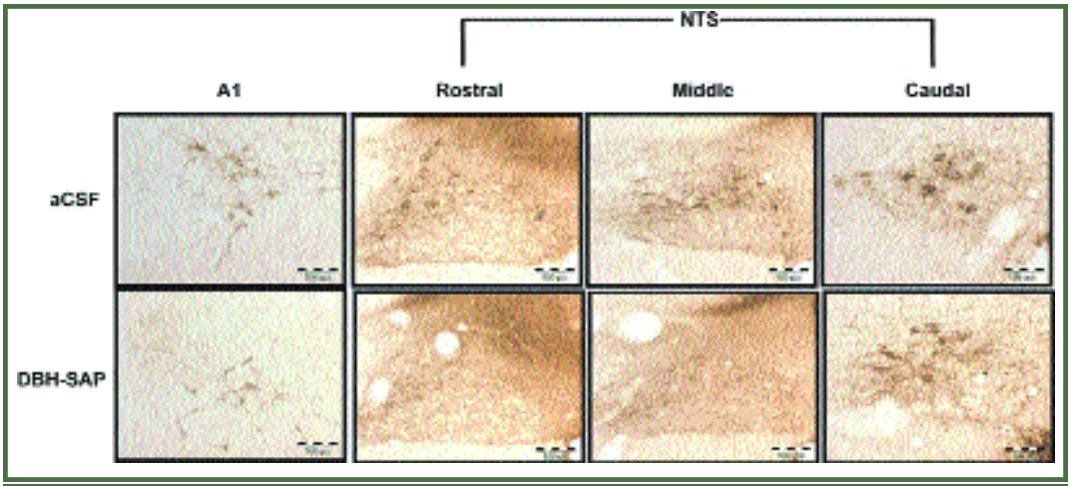


Fig. 2 Anti-DBH-SAP effect compared to aCSF controls.

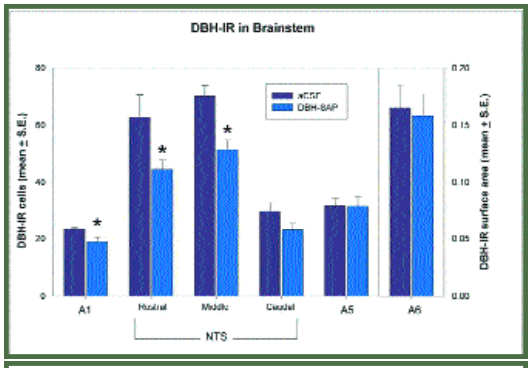


Fig. 3 Decrease in DBH-positve neurons after treatment with anti-DBH-SAP.

Experiment 2:

Ovariectomized female rats were implanted with an intracerebral guide cannula targeting the left MePD, and were treated with estrogen and progesterone to induce sexual receptivity. Fourteen to sixteen hours before mating (36-38 h after injection with estradiole benzoate) a microdialysis probe was inserted through the guide cannula. Microdialysate was collected at 20 min intervals (20 μl/sample) beginning 2 h before to 3 h after mating. Animals received 5 intromissions (5I), 15I or 15 mounts-without-intromission (MO) during mating. The content of monoamines in the dialysis sample was analyzed by HPLC with electrochemical detection. As seen in Figure 4, there was a significant release of norepinephrine (max %) in females who received 15I, a number sufficient to induce the neuroendocrine changes of pseudopregnancy, whereas insufficient mating stimulation (MO and 5I) induced no increase in norepinephrine after or during mating.

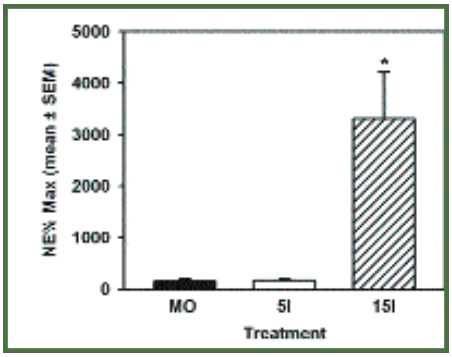


Fig. 4 Neuroendocrine increase after mating stimulation.

These data show that noradrenergic cells within the A1 and A2 cell groups of the brainstem project to the MePD, and suggest that norepinephrine release in response to mating stimulation may be involved in establishment of the neuroendocrine memory of pseudopregnancy.

References

1. Peinado-Manzano MAAmygdala, hippocampus and associative memory in rats. (1994) *Behav Brain Res* 61:175-190.
2. Paxinos, G & Watson, C. *The Rat Brain in Stereotaxic Coordinates. (1986)* Academic, San Diego.

Targeting Tools: Featured Products

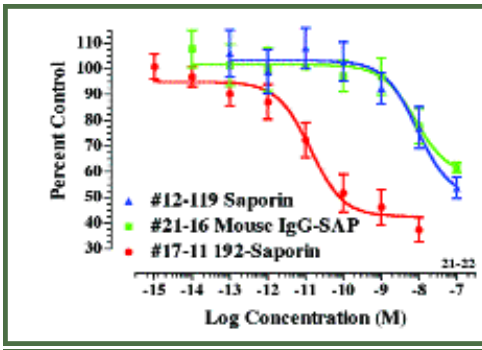
Control Immunotoxins

One of the most important features of the Advanced Targeting Systems product line is the availability of control toxins that allow the researcher to fully evaluate results of the treatment of cells or animals with targeted toxins. For instance, anti-DBH-SAP is targeted with a mouse monoclonal antibody. Its control partner is mouse IgG-SAP, that is, mouse IgG that has no targeting properties. The difference in effect between these two shows the difference between targeted toxins and non-targeted.

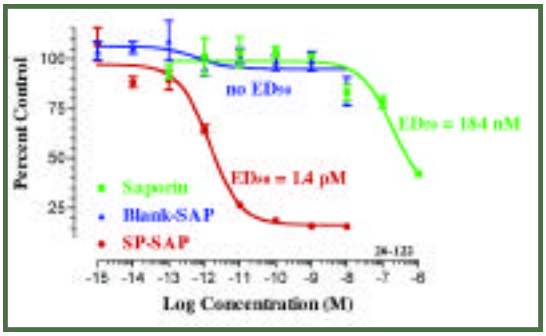
Similarly, Blank-SAP is the appropriate control molecule for the peptide ligand-targeted toxins. Blank-SAP behaves as its name implies; you're shooting blanks because the peptide sequence has no receptor, but the residues in the blank peptide contain the usual amino acids that are often found in ligand sequences.

These materials are provided at a nominal charge in targeted toxin kits. They are a great improvement, we believe, over using just plain saporin as control, or even saporin and its targeting vehicle added together, but not conjugated.

We have recently improved our control toxins by increasing the concentrations such that they are greater than the targeted toxin for which they act as a control. This was suggested to us by one of our clever clients, who asked for an increase in concentration, so that he would not be limited to the dose he used by the concentration of the control immunotoxins. For instance, if, for the 'real' toxin, 4 micrograms of a 2.5 micrograms per microliter concentration were to be injected, 1.6 microliters would be used. But if the control toxin were at a concentration less than 2.5 micrograms per microliter, the control would be injected in a greater volume (let's say at 2 micrograms per microliter, you would need 2 microliters), which by itself could have an effect. Control toxins are now in the range of 4-5 micrograms per microliter so that they can be diluted to the same concentration as the targeted toxin. It's part of our continuing effort to make ATS reagents more user-friendly for you.



Mouse IgG-SAP. PC12 cells, a rat tumor cell line that expresses the Thy 1 receptor, were plated at 5000 cells per well in 90 microliters of medium. After allowing acclimatization overnight, the cells were exposed to the various reagents at the indicated concentrations for 72 hours. MTS (Promega) was added and after two hours, plates were read at 492 nM on a Molecular Diagnostics Spectramax 340 plate reader with SoftMax software. Data were analyzed by Prism 3.0 software.



Blank-SAP. KNRK cells expressing the substance P receptor (NK-1r) were plated at 2500 cells/well in a 96-well plate and incubated overnight at 37°C. The following morning, SP-SAP, unconjugated saporin, or Blank-SAP was added. Plates were incubated for 3 days before being developed with MTS (Promega). The ED_{50}'s show that Blank-SAPhas almost no activity in the tested concentration range.

Control Immunotoxins

Available in targeted toxin kits or individually

IT-21	Blank-SAP	control for peptide-targeted toxins *(SP-SAP, SSP-SAP, Orexin-SAP, Dermorphin-SAP, CRF-SAP, NPY-SAP, and CCK-SAP)*
IT-19	Goat-IgG-SAP	control for goat IgG-targeted toxins *(Mab-ZAP, Rab-ZAP, Hum-ZAP, Rat-ZAP, Anti-M-ZAP)*
IT-18	Mouse IgG-SAP	control for mouse IgG-targeted toxins *(192-Saporin, OX7-SAP, Anti-DBH-SAP, ME20.4-SAP and Anti-SERT-SAP)*
IT-17	Rat-IgG-SAP	control for rat IgG-targeted toxins *(Mac-1-SAP and Anti-DAT-SAP)*

"Let them have their lattes; water is the nectar of life!"
Words of wisdom from Gangsta.

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.

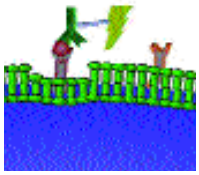
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

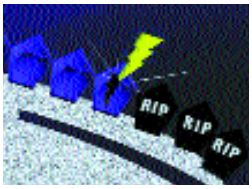
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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 six Jumbles, one letter to each block, to form the name of this award-winning scientist.

RULECUT

GLOINUMOMY

DINTOBESIA

WIZDARTELNS

COLLARUME

BROMICE

He understood how immunity works.

Arrange the circled letters to form the name of this noted scientist, 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 May 31, 2005.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

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Jul-Aug-Sep 2005
Volume 6, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

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- SSTr1, SSTr5 and NK-1r Antibodies *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeted Toxins from Here to There

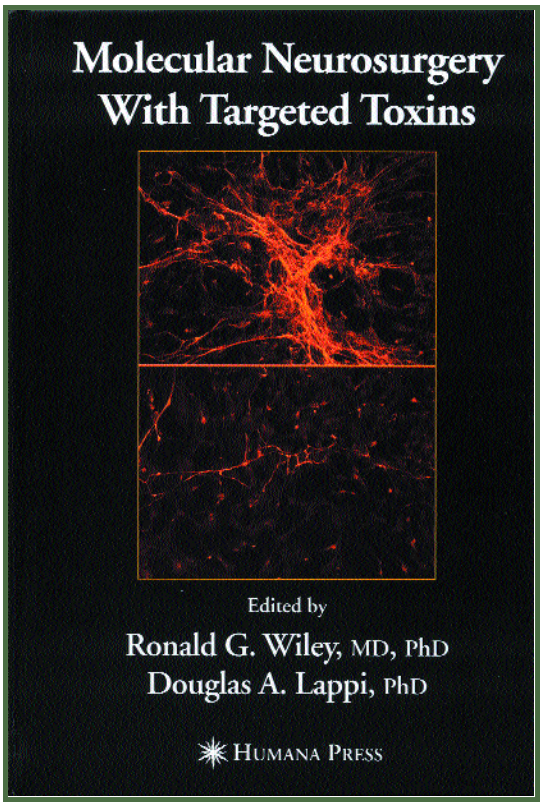
by Dr. Douglas Lappi

Humana Press has released its newest offer to the Neuroscience community, *Molecular Neurosurgery With Targeted Toxins,* with Ronald G. Wiley and myself as editors. This book contains articles by "the best and the brightest" who have used targeted toxins for probing difficult *in vivo* systems biology issues.

The idea behind the book was to provide a road map for the users of Molecular Neurosurgery to see how experienced scientists used these exceptional reagents in their work. Experiments with several targeted toxins are described, and readers can get an idea either specifically about a targeted toxin that they're using, or about how a type of molecule is used and at what dosage, in a paradigm similar to theirs.

The book begins with an Introduction by Dr. Wiley and myself describing the chapters. It is followed by an interesting and very useful article by the discoverer of saporin, Fiorenzo Stirpe of the University of Bologna. We receive many questions from people about the mechanism of action of these targeted toxins that utilize saporin, and this article alone makes the book worthwhile.

The cholinergic toxins 192-Saporin and ME20.4-SAP are the most widely used by the Neuroscience community and there are five articles by such experts as Jerene Waite, Reinhard Schliebs, Martin Sarter and John Bruno for the former, and Rosalind



Ridley and Harold Baker and the Arizona group led by Tom Beach using ME20.4-SAP in primates and rabbits.

Sue Ritter and her colleagues and Patrice Guyenet and his colleagues discuss their work on the use of anti-DBH-SAP in their respective research efforts. It's interesting to see two different groups in two completely different fields tackle their questions with this molecule. These are instructive lessons on problem-solving in science.

(continued on page 6)

ATS visits U.S.S. Asheville

Advanced Targeting Systems was honored to receive a personal tour of the USS Asheville. Thea Marlinga's husband Rick is Master Chief of the Boat and proudly showed us around. Thank you to the brave crew!

USS Asheville (SSN-758) is the 47th Los Angeles Class Fast Attack Nuclear Submarine. Commissioned in 1991, the USS Asheville is a true state-of-the-art sub. Some improved features of the USS Asheville include vertical launch cruise missiles, the Submarine Advanced Combat Control System, and an ESM direction finding capability. In addition to these tactical advances, retractable bow planes, and a hardened sail provide the capability to surface through the ice, allowing USS Asheville to operate freely in any of the world's oceans, including the Arctic basin.

Pictured left to right: Douglas Lappi, Thea Marlinga, Amalia Dingman, Brian Russell, Leonardo Ancheta, Majid Pajouh, Matthew Kohls, and Denise Higgins

The crest of the USS Asheville is made up of a color drawing depicting a surfacing submarine with the mountains of western North Carolina in the background; the words "From the mountains to the seas" complete the crest. USS Asheville is the fourth Navy vessel to bear the name of the North Carolina mountain city with a strong Naval heritage.

Designed for carrier escort, the Los Angeles class submarine combines the most desired attack qualities: speed, silence, and powerful weaponry. The submarine's key attribute has always been and will continue to be its stealth, with nuclear power providing the advantages of sustainability and mission flexibility. Perhaps this is why Master Chief Marlinga suggested the submarine be nick-named the "Ghost of the Coast." Today's SSNs expound the element of surprise and create leverage out of proportion to their size because an adversary does not know whether, or in what number, submarines might be present. These boats are ideally suited for covert surveillance, intelligence gathering and special forces missions.

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: IMMUNOLOGY
ANTIBODIES
SWITZERLAND
MOLECULAR
MICROBE
CULTURE

Answer: SUSUMU TONEGAWA

WINNERS: Geda C. Unabia, Univ of Texas, Med. Branch, Dept of Anatomy * Kris Preddy, Lakeside CA * Ilham Bensmail, Panacea * Rebecca Pearson, Georgetown, Dept of Pharmacology * Vivian Yip, Tissuegene Inc * Genevieve Vazquez, Univ of Miami, The Miami Project * Key Kang, Raven Biotechnologies * Raduwan Dackour, L.I.J Med. Center * Joseph Menonna, VA Bio Med. Center * Laura Emond, Dartmouth Med. School, Dept of Physiology * Marcia McInerney, Univ of Toledo, Dept of Pharmacy * Robert Speth, Univ of Mississippi, School of Pharmacy * Seto Chice, SUNY HSC at Brooklyn * Doug Wallace, Northern Illinois Univ, Dept of Psychology

Susumu Tonegawa was awarded the 1987 Nobel Prize in physiology and medicine for his work on understanding the body's immune system. The Nobel jurors said Tonegawa wrote an influential paper in 1976 resolving questions about how the body fights disease, and that his work had dominated research in the area for two years. Although Tonegawa was cited for his work in immunology, he is a molecular biologist by training.

Dr. Tonegawa's experiments revealed that a body's immune cells during a lifetime reshuffle their genetic makeup to form millions of antibodies against bacteria. This contradicted the prevailing dogma that genes could not change, and helped to explain how the body could resist newly conceived microbes.

Tonegawa was born in Nagoya, Japan. He earned a bachelor of science degree from Kyoto University in Japan in 1963 and at 22 left for the United States. He received a doctorate from the University of California at San Diego. Following postgraduate work at the Salk Institute in San Diego, Tonegawa joined the Basel Institute of Immunology in Switzerland before moving to MIT in 1981. The bulk of the work that led to the Nobel Prize took place in Switzerland. Dr. Tonegawa is also winner of the Order of Culture "Bunkakunsho" from the Emperor of Japan.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat.

Winters BD, Bussey TJ

Eur J Neurosci 21(8):2263-2270, 2005.

The perirhinal cortex of the temporal lobe is crucial to object recognition memory. The authors examined the role of cholinergic input from the basal forebrain in this process. Rats were injected bilaterally with 0.2 µl of 0.02 µg/µl 192-Saporin (Cat. #IT-01) into 3 sites of the perirhinal cortex, and tested in object recognition and spatial working memory tasks. Spatial working memory remained intact, but object recognition was impaired, indicating a specific function for cholinergic input to the perirhinal cortex.

CEACAM6 as a novel target for indirect type 1 immunotoxin-based therapy in pancreatic adenocarcinoma.

Duxbury MS, Ito H, Ashley SW, Whang EE

Biochem Biophys Res Commun 317(3):837-843, 2004.

Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is a cell-surface molecule that is overexpressed in a variety of human cancers. Here, the authors investigate the efficacy of a biotinylated antibody that recognizes CEACAM6 bound to streptavidin-ZAP (Cat. #IT-27) in elimination of tumor cells *in vitro* and *in vivo*. Treatment of cultured tumor cells induced significant specific cytotoxicity, while tumor growth was suppressed in a mouse xenograft model. These results indicate targeting of CEACAM6 may be a viable therapeutic strategy.

Pro- and anti-apoptotic evidence for cholinergic denervation and hippocampal sympathetic ingrowth in rat dorsal hippocampus.

Harrell LE, Parsons DS, Kolasa K

Exp Neurol 194(1):182-190, 2005.

Cholinergic denervation of the hippocampus results in hippocampal sympathetic ingrowth (HSI) of fibers from the superior cervical ganglion; this ingrowth may exert an anti-apoptotic effect. After 1-µg injections of 192-Saporin (Cat. #IT-01) into the medial septum of rats, the authors investigated the levels of apoptotic protein expression and DNA fragmentation. The findings suggest that cholinergic denervation causes pro-apoptotic responses, but HSI exerts a protective effect against programmed cell death.

Evaluation of cholinergic markers in Alzheimer's disease and in a model of cholinergic deficit.

Gil-Bea FJ, Garcia-Alloza M, Dominguez J, Marcos B, Ramirez MJ

Neurosci Lett 375(1):37-41, 2005.

Several markers of cholinergic function may be able to predict cognitive deficits due to disorders such as Alzheimer's disease. The authors compared baseline measurements of acetylcholine, cholinacetyltransferase, and acetylcholinesterase (AChE) of rats against animals treated with 0.067 µg injections of 192-Saporin (Cat. #IT-01) into both hemispheres of the nucleus basalis magnocellularis. The results indicate that measurement of AChE activity is an inexpensive and reliable method to evaluate cholinergic function in rats as well as in humans.

Neonatal lesion of forebrain cholinergic neurons: Further characterization of behavioral effects and permanency.

Pappas BA, Payne KB, Fortin T, Sherren N

Neuroscience 133(2):485-492, 2005.

Neonatal rats treated with bilateral intracerebroventricular injections of 300 ng of 192-Saporin (Cat. #IT-01) showed basal forebrain cholinergic neuron loss that was still evident at 24 months of age. The authors tested the reference memory and attentional processing of these rats in a Morris water maze. The results suggest that impaired performance of the treated animals in complex maze tasks reflects reduced problem solving ability rather than a deficit in attentional processing.

Basal forebrain cholinergic lesions in 7-day-old rats alter ultrasound vocalisations and homing behaviour.

Scattoni ML, Puopolo M, Calamandrei G, Ricceri L

Behav Brain Res 161(1):169-172, 2005.

In this study the authors examined the effects of cholinergic depletion of the basal forebrain on the establishment and maintenance of mother-pup interaction in rats. Post-natal day 7 pups were lesioned with bilateral intracerebroventricular injections of 192-Saporin (0.42 µg, Cat. #IT-01). Treated animals displayed a reduced number of ultrasonic vocalizations, as well as apparent

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

increased difficulty in identifying the nest "boundary." The evidence shows that early damage to basal forebrain cholinergic nuclei can influence behavior as early as the second-postnatal week.

Orexin-saporin lesions of the medial septum impair spatial memory.

Smith HR, Pang KC

Neuroscience 132(2):261-271, 2005.

The medial septum and diagonal band of Broca (MSDB) have been shown to be important for spatial learning and memory. The authors investigated the role orexin-containing neurons from the hypothalamus play in these processes. Rats were treated with three injections of 40-120 ng of orexin-SAP (Cat. #IT-20) into the MSDB. Performance in spatial working and spatial reference memory tasks indicate that orexin innervation of the MSDB may modulate spatial memory through both GABAergic and cholinergic septohippocampal neurons.

Effects of cholinergic deafferentation of the rhinal cortex on visual recognition memory in monkeys.

Turchi J, Saunders RC, Mishkin M

Proc Natl Acad Sci U S A 102(6):2158-2161, 2005.

The rhinal cortex has been shown to play a critical role in recognition memory. The investigators examined the effect of eliminating cholinergic input to the rhinal cortex on the formation of new visual memories in macaques. Animals were given 0.01 μg injections of ME20.4-SAP (Cat. #IT-15) into the perirhinal and entorhinal cortices. The selective cholinergic deafferentation produced a substantial impairment of visual recognition memory, suggesting that cholinergic activation is essential for the formation of new visual memories.

Time course of behavioral changes following basal forebrain cholinergic damage in rats: Environmental enrichment as a therapeutic intervention.

Paban V, Jaffard M, Chambon C, Malafosse M, Alescio-Lautier B

Neuroscience 132(1):13-32, 2005.

In this study the authors examined the effects of 192-Saporin (Cat. #IT-01) administration to the medial septum (37.5 ng/side) and nucleus basalis magnocellularis (75 ng/side) of rats. The results suggest that behavioral deficits immediately after lesioning are due to cholinergic depletion, while deficits later in life may be connected to a gradual degeneration process. Environmental enrichment had a significant positive effect on lesioned rats, indicating a level of cognitive plasticity.

Molecular Neurosurgery With Targeted Toxins.

Wiley RG and Lappi DA, editors

Humana Press, Totowa, New Jersey, 2005.

Chapters included in text are:

Introduction *Wiley RG, Lappi DA*

Ribosome-Inactivating Proteins *Stirpe F*

Biochemical, Physiological, and Behavioral Characterizations of the Cholinergic Basal Forebrain Lesion Produced by 192 IgG-Saporin *Waite JJ*

Basal Forebrain Cholinergic Lesion by 192 IgG-Saporin: A Tool to Assess the Consequences of Cortical Cholinergic Dysfunction in Alzheimer's Disease *Schliebs R*

192-IgG-Saporin-Induced Partial Cortical Cholinergic Deafferentation as a Model for Determining the Interactions Between Brain Aging and Neurodevelopmental Defects in the Cortical Cholinergic Input System *Sarter M, Bruno JP*

Exploring the Role of Acetylcholine in Primate Cognition Using ME20.4 IgG-Saporin *Ridley RM, Baker HF*

Cortical Cholinergic Deafferentation Induces Aβ Deposition: Toward a Physiological Animal Model of Alzheimer's Disease *Beach TG, Walker DG, Potter PE, Sue LI, Scott S, Layne KJ, Newell AJ, Rauschkolb PK, Poston ME, Webster SD, Durham RA, Emmerling MR, Sawada K, Honer WG, Fisher A, Roher AE*

Chemical Dissection of Brain Glucoregulatory Circuitry *Ritter S, Dinh TT, Bugarith K, Salter DM*

Cardiovascular Deficits After Lesions of C1 Adrenergic Neurons With a Saporin-Based Immunotoxin *Guyenet PG, Stornetta RL, Schreihofer AM*

Saporin Conjugates and Pain *Wiley RG, Lappi DA*

The Use of Saporin Conjugates to Dissect Neurons Responsible for Sleep and Wakefulness *Blanco-Centurion C, Gerashchenko D, Murillo-Rodriguez E, Desarnaud F, Shiromani PJ*

Isolectin B4-Mediated Cytotoxic Targeting of Sensory Neurons *Vulchanova L, Honda CN*

B Fragment of Cholera Toxin Conjugated to Saporin *Ohara PT, Kelley K, Jasmin L*

See cover article by Dr. Douglas Lappi for a summary of this new book.

Targeting Talk: *Saporin Safety*

by Dr. Douglas Lappi

Q: You have stated previously in Targeting Trends *that it was unlikely that saporin compounds or constituents would be excreted in urine or feces. However, you acknowledge that experimental data is lacking. Have there been any tests of animal urine or feces for saporin content? My animal care staff are concerned.*

A: One of the reasons that no studies have been done on excretion of saporin is that there isn't much on the theoretical side to cause concern. The primary issue is that the quantity used in mice (and even rabbits) is so small that when looked at in human terms (i.e., an animal 10 to 100-times larger), the dosage becomes insignificant. The LD_{50} for saporin in mice is 4-8 mg/kg;[1] that would translate in humans to more than you'll ever use! The immunotoxins, which contain only about 20% saporin by weight, really do not contain all that much saporin.

Looking at it another way, you need a concentration of about 100 nM to see even a vague hint of toxicity of saporin to cells. In human blood, that would correspond to 24 mg injected systemically into a person. It would be really expensive for anyone to get close to that number.

As far as urine and feces go, the same calculations are appropriate, but there will be considerable degradation – the protein content in urine and feces is quite low and the probability is that you will be dealing with only saporin. Remember, saporin is a plant protein that is related to proteins in foods that we eat (cucumbers, for example).

Reference

1. Stirpe F, Derenzini M, Barbieri L, Farabegoli F, Brown AN, Knowles PP, Thorpe PE (1987) Hepatotoxicity of immunotoxins made with saporin, a ribosome-inactivating protein from *Saponaria officinalis*. *Virchows Arch [B]* 53:259-271.

Q: Are there any studies which indicate what doses of saporin (by itself or compounded with an antibody) would be hazardous if ingested or injected (i.e. systemic dose level resulting in death or organ dysfunction).

A: When there is an antibody that does recognize a human epitope (the human p75-saporin immunotoxin that is used in rabbits, for example), at about 1 pM one sees the slightest bit of toxicity to cells. That translates, if injected by error into a human blood supply, to about 170 micrograms. That also is a gigantic dose. I am using very conservative numbers here, and the bottom line is that you cannot accidentally reach such dangerous levels under normal handling situations.

Having said all this, we still recommend that our customers take excellent care of themselves and we state clearly that precautions should be taken by people handling these materials, just as they should use precautions with all laboratory chemicals. Please refer to the data sheets provided with our products for safety instructions.

Saporin (Cat. # PR-01)
a ribosome-inactivating protein from seeds of the plant
Saponaria officinalis

Saporin is effective as a control in experiments with targeted saporin immunotoxins or ligand toxins.

Also available:
Anti-Saporin (Goat) AB-15
Anti-Saporin (Goat, affinity purified) AB-15AP
Anti-Saporin (Chicken, affinity purified) AB-17AP

Targeted Toxins from Here to There

(continued from page 1)

Ron Wiley and I, the discoverers of the widely used SP-SAP, cover the use of that molecule and the mu opioid receptor-targeted toxin, dermorphin-SAP. This article, and the following one by the group of Peter Shiromani involving the use of orexin-SAP, should leave the reader well-grounded in the use of the peptide-ligand toxins.

The final two chapters of the book discuss the use of the lectin toxins IB4-SAP and CTB-SAP. These articles show the promise of lectins to be carriers of saporin. Others such as wheat germ are in a vein that hasn't been sufficiently mined yet.

This book shows the promise of targeted toxins that will be fulfilled as more targeting agents become available.

Special Offer

Order $500 or more of ATS products during the month of July and receive a free copy of *Molecular Neurosurgery With Targeted Toxins*.

(one book per customer, please)

Upcoming Events

Society for Neuroscience
November 12-16, 2005
Washington DC

Cell Biology
December 10-14, 2005
San Francisco, CA

SP-SAP Partnering Opportunity

Advanced Targeting Systems has received over $3 million in funding from the National Institutes of Health, National Institute of Mental Health for the development of SP-SAP (Substance P-Saporin) as a chronic pain therapeutic. In addition to the cash contribution, the NIH recently established a Commercialization Assistance Program to further enable grantees to bring their products to market.

Denise Higgins, Vice President of Business Development, participated in the program that culminated with the production of a Business Plan and presentation of the Partnering Opportunity at the 11th Annual Venture Forum. This meeting, organized each year by the Larta Institute, showcased seventy of the participating life science companies.

Although the audience was primarily comprised of investors, there were good contacts made with several pharmaceutical companies that are excellent candidates to bring SP-SAP to market to the chronic pain population.

ATS is hopeful that there will be a formal announcement of a partnership in time for the fourth quarter issue of *Targeting Trends*. The sooner a deal can be finalized, the sooner clinical trials can begin and SP-SAP can offer relief to those suffering from chronic pain.

More New Antibodies

Advanced Targeting Systems continues to offer new antibodies. Take a look at our website for a list of more than 350 antibodies for neuroscience, GPCRs, and more.

Cat.#	Antibody Name	Specificity
AB-L005	Adenosine A1 Receptor	Human; Mouse; Rat
AB-L006	Adenosine A2a Receptor	Human; Mouse; Rat
AB-L007	Adenosine A3 Receptor	Human; Mouse; Rat
AB-L356	Brain-Specific Angiogenesis Inhibitor 1	Human
AB-L198	Brain-Specific Angiogenesis Inhibitor 2	Human
AB-L199	Brain-Specific Angiogenesis Inhibitor 3	Human; Mouse
AB-L084	Histamine H1 Receptor	Human; Mouse; Rat
AB-L345	Histamine H2 Receptor	Human; Mouse; Rat
AB-L085	Histamine H3 Receptor	Human; Mouse; Rat
AB-L086	Histamine H4 Receptor	Human; Mouse; Rat
AB-L351	Orexin Receptor 1	Rat
AB-L248	Prokineticin Receptor 1 (GPR73A)	Human; Mouse; Rat
AB-L249	Prokineticin Receptor 2 (GPR73B)	Human; Mouse; Rat
AB-L259	Thrombin Receptor	Human; Mouse; Rat

Targeting Tools: Featured Products

New Antibodies: SSTr1, SSTr5, NK-1r

Advanced Targeting Systems announces three new antibodies to G protein-coupled receptors that will be quite helpful for a number of researchers studying somatostatin and substance P. For somatostatin study, we already market excellent rabbit polyclonals to somatostatin-14 (AB-04) and somatostatin-28 (AB-05). We now announce a new mouse monoclonal antibody to somatostatin receptor 1 (SSTr1, Cat. #AB-N35). Figure 1 shows staining of fixed cells and a distribution of staining typical for a membrane-bound protein. This protein's antigen is to an extracellular domain of the rat sequence and it is an IgM.

We are also releasing a mouse monoclonal to SSTr5 (Cat. #AB-N24). Figure 2 shows staining with this IgM in panel A and normal mouse IgM in panel B, demonstrating specificity. It is also made to an extracellular sequence of the protein.

We have created a rabbit polyclonal to a sequence from the dog substance P (NK-1) receptor (Cat. #AB-N33). Photomicrographs of the use of this antibody are seen in Figure 3. As researchers in this field, we know the difficulty of finding a good, consistent source of this antibody; sequence homologies are very close with rat and human (one amino acid change out of 15), so this antibody should be excellent in other species as well. The antigen for this antibody is a peptide near the C-terminus.

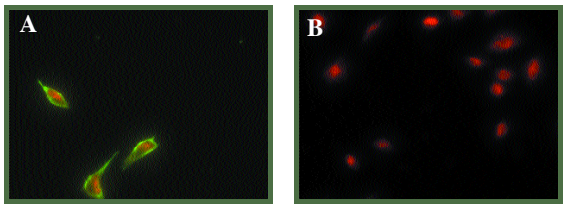


Figure 2. Immunostaining of CHO cells expressing rat SSTr5 using anti-SSTr5 monoclonal antibody. CHO-K1 cells were transfected with rat SSTr5, fixed with paraformaldehyde and A) immunostained with anti-SSTr5 monoclonal antibody (5 μg/ml; green) followed by propidium iodide nuclear staining (red) and B) same as A except that 5 μg/ml of normal mouse IgM was used as control followed by nuclear staining.

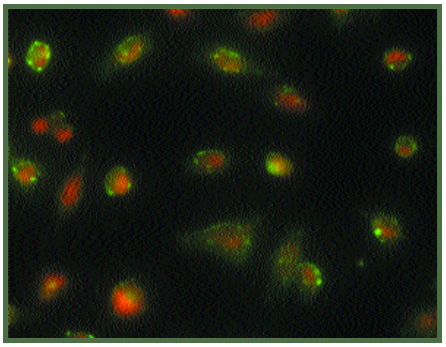


Figure 1. Immunostaining of CHO cells expressing rat SSTr1 using anti-SSTr1 monoclonal antibody. CHO-K1 cells were transfected with rat SSTr1, fixed with paraformaldehyde and immunostained with anti-SSTr1 monoclonal antibody (5 μg/ml; green) followed by propidium iodide nuclear staining (red).

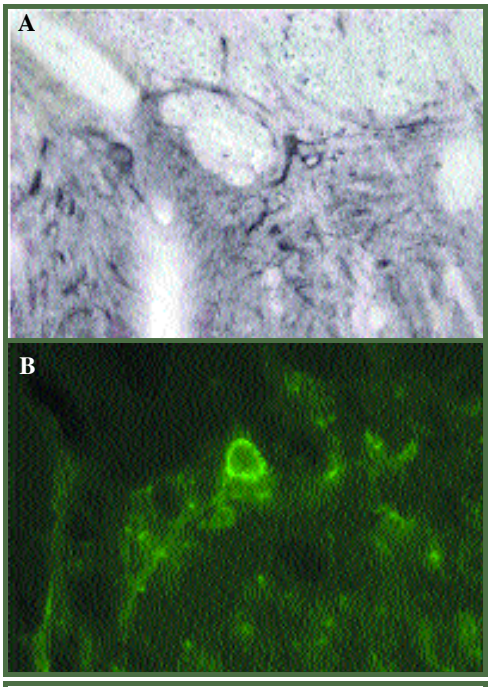


Figure 3. Staining of NK1r-bearing neurons in dog spinal cord. Paraformaldehyde perfused sections of dog spinal cord (cervical dorsal horn) were stained with rabbit anti-dog NK1r antibody by A) enzyme-based immunohistochemistry and B) fluorescent immunohistochemistry.

A cactus by any other name would still stick you in the nose!
Poor Gangsta!

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

Targeting Technology

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

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

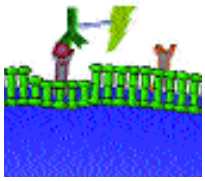
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

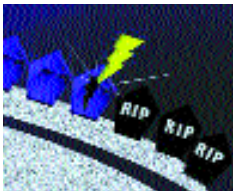
he targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these six Jumbles, one letter to each block, to form the name of this award-winning scientist.

AMAINE

GRAPEU

THREADABROCY

TREES

CLOGNEGY

MOLEYZNYOG

Victory was sweet for this Nobel Prize winner.

Arrange the circled letters to form the name of this noted scientist, 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 August 31, 2005.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

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Oct-Nov-Dec 2005
Volume 6, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Spring Brain Meeting *(page 2)*
- Teaser Winners *(page 2)*
- Take Me Out to the Ballgame *(page 6)*
- Cy3-labeled anti-murine NGFr *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Effects of Intraseptal Orexin-Saporin on Spatial Memory

by Kevin Pang and Heidi Smith, Bowling Green State University, Dept Psychology, Bowling Green, Ohio

Hypocretin (or orexin) neurons are located in the hypothalamus and have been implicated in various functions including sleep, arousal and feeding. One area receiving dense hypocretin projections and expressing hypocretin-2 receptors (H-2r) is the medial septum (MS), an area important in learning and memory. In the medial septum, hypocretin-2 receptors are located on cholinergic and GABAergic projection neurons that innervate the hippocampus and other limbic structures. Previous studies demonstrated that activation of H-2r excites both populations of MS neurons. Our studies investigated whether MS neurons that receive hypocretin innervation are important for spatial memory (Smith and Pang, 2005).

Male Long Evans rats were administered orexin-saporin (orexin-SAP, Cat. #IT-20) into the MS (0.6 µl) and diagonal band of Broca (DB; 0.4 µl into each DB) at three concentrations: 100, 200 and 300 ng/µl. Following a 2-week recovery period, rats were trained on a water maze task. Each daily session consisted of 6 trials. After behavioral testing, brain sections were stained to visualize cholinergic and GABAergic septohippocampal (SH) neurons using immunocytochemistry for choline acetyltransferase (ChAT) and parvalbumin (PV), respectively.

The damage caused by orexin-SAP is shown in Figure 1. Orexin-SAP at 100 ng/µl primarily damaged GABAergic SH neurons, sparing most cholinergic SH neurons. The concentration of 200 ng/µl caused more neuronal damage. At this concentration, a more substantial loss of cholinergic neurons was observed, but GABAergic neurons again showed more damage than cholinergic neurons.

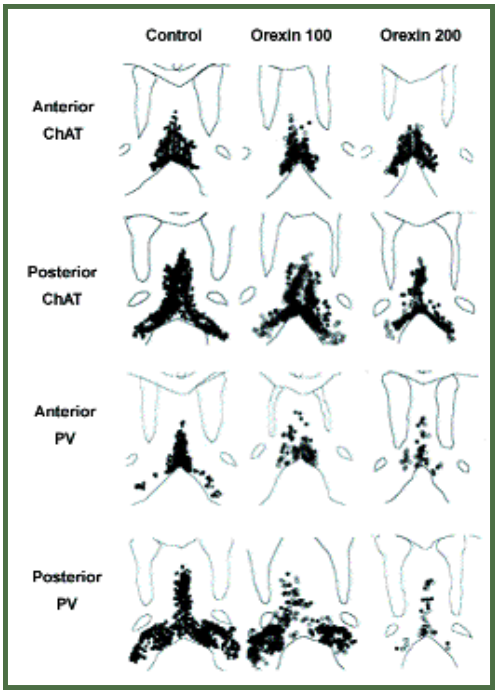

Figure 1. Cholinergic and GABAergic projection neurons in the MSDB region of control rats, and rats treated with 100 ng/µl, or 200 ng/µl of orexin-SAP. The distribution of neurons (closed squares) is drawn for a representative example of the control group. For rats treated with orexin-SAP, open circles represent the distribution of neurons from the rat with the least amount of damage, and the closed circles represent neurons from the rat with the most amount of damage in each treatment group. Originally published in Smith & Pang, 2005.

Finally, the highest concentration of orexin-SAP (300 ng/µl) produced extensive damage to the medial septum, causing shrinkage and non-specific damage of the MS-DB tissue and enlargement of the ventricles. Because of the non-specific tissue damage produced by administration

(continued on page 6)

17th Annual Spring Brain Conference

March 15-18, 2006 Sedona, Arizona

The Spring Brain Conference (SBC) was founded to foster interdisciplinary communication and interactions among scientists studying the many different aspects of brain function. The goal of the conference is to bring 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 informal but focused meeting format promotes direct interactions and discussion. The conference will consist of a general poster session along with 8-10 plenary sessions each organized around a central theme or topic. Opportunities exist for travel awards for post-doctoral and graduate student trainees along with participation in a Neuroscience outreach program for local high school students.

www.springbrain.org

For additional information contact Dr. Bob Yezierski (ryezierski@dental.ufl.edu) or visit the SBC website.

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: ANEMIA
PRAGUE
CARBOHYDRATE
ESTER
GLYCOGEN
ENZYMOLOGY

Answer: GERTRUDE CORI

WINNERS: David Akopian, California State Univ Northridge * Catherine Ulibarri, Washington State Univ, VCAPP * Purna Mukherjee, Boston College * Miriam Burton, Kansas State Univ, Anatomy & Physiology * Angela Finney, Panacea * Gillian Watson, Univ of Oxford, Laboratory of Physiology * Valerie Fritz, Frostberg State Univ, Biology * Lilly Atabekyan, Scripps Laboratories, Operations * Shelly Caltharp, Loma Linda Univ, Pathology and Human Anatomy * Keith Danielson, Thomas Jefferson Univ, Orthopedic Res * Joseph Menonna, VA Bio Med Center * Laura Emond, Dartmouth Med School, Dept of Physiology * Robert Speth, Univ of Mississippi, School of Pharmacy * Seto Chice, SUNY HSC at Brooklyn

Gertrude "Gerty" Cori was born August 15, 1896 in Prague, Czechoslovakia. In 1914, at the age of eighteen, she enrolled in the German branch of the medical school in **Prague**. It was here that she found her two loves: biochemistry and Carl Cori. Carl and Gerty had much in common; they both loved mountain climbing, swimming, skating, and tennis. In 1922, Carl took a job at the New York State Institute for the Study of Malignant Diseases in Buffalo, New York and sent for Gerty six months later after he had secured a position for her as an assistant pathologist at the institute. By 1929, Carl and Gerty could explain how energy moves in a cycle from muscle to the liver and back again to muscle. In 1938 and 1939, Gerty shifted her direction of research towards **enzymology**. Soon after, the Cori's discovered phosphorylase that breaks **glycogen** down into the Cori **ester**. It tears apart the bonds that hold glycogen's sugar molecules together. This was the first time that **carbohydrate** metabolism was studied at the molecular level. By 1944, Gerty was promoted to associate professor and given tenure at Washington University. By 1947, the Cori's lab was alive with the study of enzymes. Their lab produced eight Nobel Prize winners.

In 1947, Gerty was awarded the Nobel Prize along with her husband. They were the first husband and wife team to receive this award and Gerty was the third woman to receive a Nobel Prize in Science. In 1947, Gerty learned that she had a fatal type of **anemia**. After winning the Nobel Prize, she was elected to the National Academy of Science and appointed to the National Science Foundation by Harry S. Truman.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Elimination of neurokinin-1 receptor neurons in caudal nucleus reverses the effects of systemic bicuculline on c-Fos expression in rat trigeminal sensory nucleus: I. High intensity electrical stimulation of the trigeminal ganglion.

Abe T, Ohshita N, Sugiyo S, Moritani M, Kobayashi M, Takemura M

Neuroscience 133(3):739-747, 2005.

The authors investigated the role of NK-1r + neurons in lamina 1 of the trigeminal caudal nucleus (Vc) for ora-facial nociception. After a 5 μl injection of 5 μM SP-SAP (Cat. #IT-07) into the cisterna magna, c-fos expression in the Vc was evaluated. SP-SAP treatment, along with use of bicuculline, a GABA$_A$ receptor antagonist, showed that NK-1r+ neurons in laminae I and III of the Vc are involved in nociceptive processing in the trigeminal sensory nucleus.

Selective cholinergic immunolesioning affects synaptic plasticity in developing visual cortex.

Kuczewski N, Aztiria E, Leanza G, Domenici L

Eur J Neurosci 21(7):1807-1814, 2005.

In this study the authors examined the role of subcortical cholinergic inputs in the regulation of plastic events in the visual cortex during early postnatal development. Four-day-old mouse pups were treated with a total of 0.4 μg of 192-Saporin (Cat. #IT-01), using bilateral injections. Analysis of muscarinic receptor mRNA, long-term potentiation of cortex slices, and theta burst stimulation indicated that synaptic transmission and plasticity of the developing visual cortex depends on cholinergic input.

Autonomic brainstem nuclei are linked to the hippocampus.

Castle M, Comoli E, Loewy AD

Neuroscience 134(2):657-669, 2005.

Stimulation of the vagal nerve has been reported to enhance memory, as well as be an effective treatment for epilepsy. The authors examined the underlying synaptic pathway. The right ventral CA1 hippocampal field of rats was lesioned with 42 ng of either anti-DBH-SAP (Cat. #IT-03), or 192-Saporin (Cat. #IT-01). The results indicate that both noradrenergic and cholinergic neurons are relay sites for this pathway.

Possible role of CRF peptides in burn-induced hypermetabolism.

Chance WT, Dayal R, Friend LA, Sheriff S

Life Sci [Aug 23 Epub], 2005.

Burn trauma has been associated with hypermetabolism and anorexia. Corticotropin releasing factor (CRF) elevates metabolic rate and elicits anorexia, while neuropeptide Y (NPY) reduces metabolic rate while stimulating feeding. After burn treatment, rats were injected with 2.5 μg CRF-SAP (Cat. #IT-13) into the third ventricle. Several parameters, including resting energy expenditure, NPY concentrations in the paraventricular nucleus, and CRFr-2 density were evaluated post-treatment. The results indicate that the CRFr-2 is important in maintaining hypermetabolism resulting from burn trauma.

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Septal innervation regulates the function of alpha7 nicotinic receptors in CA1 hippocampal interneurons.

Thinschmidt JS, Frazier CJ, King MA, Meyer EM, Papke RL

Exp Neurol 195(2):342-352, 2005.

The authors examined whether hippocampal innervation by medial septum/diagonal band of Broca projections is necessary for normal a7 receptor function. 1 μg of 192-Saporin (Cat. #IT-01) was injected into the medial septum of rats. Various methods, including whole-cell patch clamping and immunohistochemistry, were used to evaluate the effects of these lesions. Lesioning with 192-Saporin did not affect a7 receptor currents, indicating that cholinergic neurons are not linked to a7 function.

Compensatory changes in cortical cholinergic innervation in the rat following an immunotoxic lesion.

Hartonian I, de Lacalle S

Restor Neurol Neurosci 23(2):87-96, 2005.

The ability of damaged axons to grow and functionally reinnervate damaged areas of the brain is well documented. Here the authors study this process in the context of rats lesioned with 192-Saporin (Cat. #IT-01). 10.5 ng of the immunotoxin was injected into the right horizontal diagonal band of Broca, and animals were examined from 2 to 24 weeks later. Although the functionality of the neuronal ingrowth was not examined, surviving neurons did extend their terminals into the denervated area.

Origin and immunolesioning of cholinergic basal forebrain innervation of cat primary auditory cortex.

Kamke MR, Brown M, Irvine DR

Hear Res 206(1-2):89-106, 2005.

In this study the authors assessed the use of a cholinergic immunotoxin while

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

examining cholinergic basal forebrain input to the primary auditory cortex in cat. Six 0.5 μg injections of ME20.4-SAP (Cat. #IT-15) were made into the putamen/globus pallidus, and cholinergic cell survival was examined by immunohistochemistry. The injected area showed a large reduction in number of AChE-positive fibers in the primary auditory cortex. This provides the evidence of the efficacy of ME20.4-SAP for investigating plasticity in cat auditory cortex.

Susceptibility to seizure-induced injury and acquired microencephaly following intraventricular injection of saporin-conjugated 192 IgG in developing rat brain.

Koh S, Santos TC, Cole AJ

Exp Neurol 194(2):457-466, 2005.

It is thought that one mechanism for resistance to seizure-induced injury in immature animals is an abundance of neurotrophic growth factors. Rat pups were treated with 2 μg of 192-Saporin (Cat. #IT-01) injected into the left lateral ventricle to examine how cholinergic basal forebrain projections might affect this type of injury. The results indicate that these neurons may be critical for normal brain growth, and that they play a protective role in preventing excitotoxic neuronal injury.

Further analysis of the effects of immunotoxic lesions of the basal nucleus of Meynert reveals substantial impairment on visual discrimination learning in monkeys.

Ridley RM, Baker HF, Leow-Dyke A, Cummings RM

Brain Res Bull 65(5):433-442, 2005.

Several studies in marmoset monkeys indicate that cholinergic projections from the NBM to specific portions of the neocortex are necessary for visual discrimination learning. By combining analysis of studies using a total of 1.4 μg of ME20.4-SAP (Cat. #IT-15) into various areas of the brain, the authors show that degeneration of cholinergic projections contributes to the loss of functions dependent on the neocortex.

Acetylcholine in the orbitofrontal cortex is necessary for the acquisition of a socially transmitted food preference.

Ross RS, McGaughy J, Eichenbaum H

Learn Mem 12(3):302-306, 2005.

Cortical involvement in social transmission of food preference (STFP) has not been established, but the importance of the orbitofrontal cortex (OFC) in odor-guided learning is known. The OFC of rats was injected twice with 192-Saporin (Cat. #IT-01), then the rats were trained in STFP. Depletion of cholinergic neurons in the OFC impaired expression of the odor association, indicating that cholinergic function in the OFC is essential for this form of associative learning.

Ablation of vagal preganglionic neurons innervating the extra-thoracic trachea affects ventilatory responses to hypercapnia and hypoxia.

Wu M, Kc P, Mack SO, Haxhiu MA

Respir Physiol Neurobiol [Aug 10 Epub], 2005.

Hypercapnia, an excess of CO_2 in the blood, is thought to stimulate the release of acetylcholine by airway-related vagal preganglionic neurons (AVPNs). AVPNs in the nucleus ambiguus (NA) were lesioned with ten 1-μl injections of CTB-SAP (Cat. #IT-14) into the trachealis muscle of rats. Treated animals maintained rhythmic breathing patterns, but episodes of increased respiratory rate in response to hypercapnia were significantly reduced.

Mu opioid receptor-containing neurons mediate electroacupuncture-produced anti-hyperalgesia in rats with hind paw inflammation.

Zhang RX, Wang L, Liu B, Qiao JT, Ren K, Berman BM, Lao L

Brain Res 1048(1-2):235-240, 2005.

Electroacupuncture has been shown to significantly reduce inflammatory hyperalgesia. To examine whether this effect is modulated by spinal mu opioid receptors, the authors injected 400 ng of dermorphin-SAP (Cat. #IT-12) into the subarachnoid space at the level of the lumbar spinal cord of rats. The anti-hyperalgesic effect of electroacupuncture was blocked by dermorphin-SAP administration, indicating that mu opioid receptor-containing neurons are involved in this pathway.

Spinal-supraspinal serotonergic circuits regulating neuropathic pain and its treatment with gabapentin.

Suzuki R, Rahman W, Rygh LJ, Webber M, Hunt SP, Dickenson AH

Pain. [Sep 6 Epub], 2005.

The anticonvulsant, gabapentin, is thought to modulate calcium channel function. In animals, it also affects abnormal pain function. 10 μl of 1 μM SP-SAP (Cat. #IT-07) was injected into the subarachnoid space of rats. It was found that the effects of gabapentin were blocked when NK-1r expressing neurons in the dorsal horn were eliminated. The results suggest that not only is the NK-1r pathway a determinant of neuronal and behavioral manifestations of neuropathy, it is also involved in the action of gabapentin.

Targeting Talk: *Dose Ranging*

by Dr. Douglas Lappi

Q: We just completed surgeries where we implanted third ventricular cannulas and temporary bilatera cannulas directed into the nucleus tractus solitarius in the brainstem of animals. We injected either the Blank-SAP control toxin or the experimental material Oxytocin-SAP into the bilateral NTS cannulae over a 30-second period. However, within the next week-two weeks post-surgery, we lost 13 of the 19 animals treated; they appeared not to be able to groom properly and lost over 20% of their body weight. This was apparent in both the Blank-SAP and the Oxytocin-SAP groups. We gave a dose of 40 ng/300 nl for each of the reagents. This dose was determined based on a published article using another of ATS's targeted toxins. I'm very surprised by my results. Can you offer any explanation/advice?

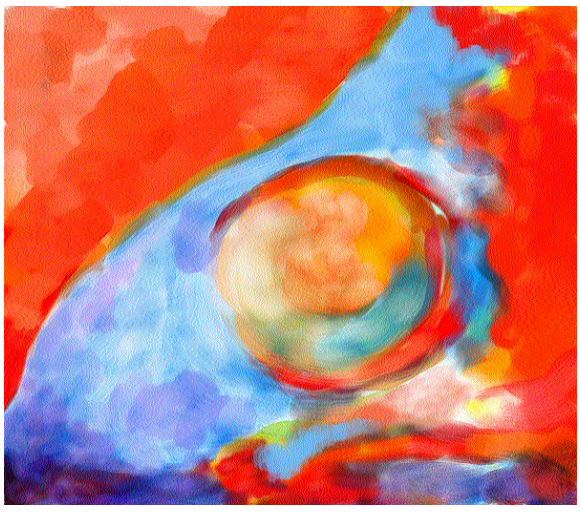
A: This is a particularly disturbing result; it appears that a dose was chosen by comparison to one used with another targeted toxin. Although this can be a good approximating tool to begin a dose-ranging study, it usually doesn't take into account the tissue, system, target molecule — so many parameters that are important to determining the proper dosage.

The literature is quite extensive on targeted toxins, and so there may be a comparable starting dose that has been published. Let's use, for example, 4 μg. Reduce that amount by 20% quantities (4, 3.2, 2.4) and test in a small number of animals to determine a value that is safe and effective. If no trouble is seen at the highest dose, and the effect is minimal, that would indicate a higher dose may be acceptable. You can then test doses in 20% increased increments (4.8, 5.6, 6.4).

The effects you see in your animals should only be reflective of the particular cell type you are eliminating. In the case of control reagents, such as Blank-SAP, no cell type is being targeted, so if you are seeing any kind of result, then you are certainly over-dosing.

Q: Is there some kind of formula that one can use that will help determine a starting point for establishing a range of doses to test in animals prior to initiating a study? For example, if the targeted toxin is administered intravenously, does it take more or less material than when administered directly into tissue?

A: Start with a few animals and do dose-ranging as discussed in the previous question. The various modes of application are really too wide to discuss in any detail here, but I, a biochemist by training, always like the approach of thinking about what sort of concentration will be needed to have a cytotoxic effect. Generally, these molecules have an ED_{50} in the nanomolar to picomolar range. Obviously if you inject systemically, the material from the first becomes greatly diluted, relative to an injection directly into tissue, and so you'll need a lot more. If you inject directly into tissue the local concentration can be quite high.



This brilliant artistic expression is a creation of this issue's Nobel Prize winner as featured in the Targeting Teaser on the back cover. The title of this piece is *Hamadryad*.

Effects of Intraseptal Orexin-SAP on Spatial Memory

(continued from page 1)

of the highest concentration, we restricted our analysis of the behavioral effects of orexin-SAP to the two lower concentrations.

Rats treated with either 100 or 200 ng/µl were impaired at learning the location of the platform in the water maze (Figure 2). Although mean escape latencies decreased across days for all groups, the rate of learning was slower for rats treated with orexin-SAP as compared to control rats (Figure 2A). Analysis of the first trial of each session was used to assess effects on long-term memory (*i.e.*, 24-hr retention). Rats treated with either dose of orexin-SAP demonstrated a pronounced impairment of long-term memory (Figure 2B). Performance on probe trial for day 4 confirmed the impairments observed on platform trials, but performance on probe trial for day 11 was not different between groups. In summary, orexin-SAP administered into the MS-DB resulted in a dose-dependent loss of GABAergic and cholinergic SH neurons with a greater damage occurring to GABAergic neurons than cholinergic neurons. Rats treated with 100 and 200 ng/µl of orexin-SAP were impaired in learning the water maze task with pronounced impairment of long-term retention. These results contrast with our previous studies using intraseptal kainic acid administration, which does not impair performance on the water maze or radial maze. The different behavioral results are especially interesting when one considers that 100 ng/µl orexin-SAP preferentially damages GABAergic SH neurons, similar to the damage produced by kainic acid. So far, the effects of these compounds have only been assessed on cholinergic and GABAergic SH neurons. Current studies are attempting to identify the effects of orexin-SAP and kainic acid on non-cholinergic, non-GABAergic MS-DB neurons. Identifying neuronal populations that are differentially affected by these two toxins will help to highlight other MS-DB neurons that may be involved in learning and memory. In conclusion, these studies demonstrate that orexin afferents to the MS-DB may play a role in modulating memory processes. The results, together with those from previous studies, also suggest an important role of noncholinergic, non-GABAergic MS-DB neurons in spatial memory.

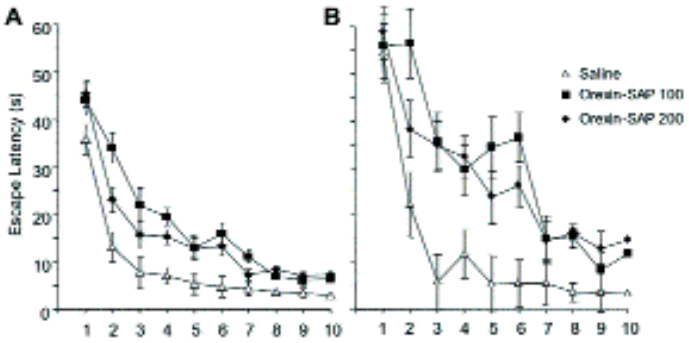


Figure 2. Mean escape latency for daily sessions are shown in Figure 2A. Orexin-SAP significantly impaired the acquisition of a water maze task. No difference was found between the two groups receiving orexin-SAP. **Mean escape latency for the first trial of each daily water maze session is shown in Figure 2B.** Although no difference was observed on the first trial of the first session, rats treated with orexin-SAP took significantly longer than the saline-treated group to reach the hidden platform on the first trial of subsequent sessions. No difference was observed between rats treated with the two concentrations of orexin-SAP.

References:

Gerashchenko *et al*. (2001) *Brain Res* 913:106-155.

Pang *et al*. (2001) *Hippocampus* 11:814-827.

Smith, HR and Pang KCH (2005) *Neuroscience* 132:261-271.

Wu *et al* (2002) *J Neurosci* 22(17):7754-7765.

Wu *et al*.(2004) *J Neurosci* 24(14):3527-3536.

Upcoming Events

Society for Neuroscience
November 12-16, 2005
Washington DC
Booth #2829

Cell Biology
December 10-14, 2005
San Francisco, CA
Booth #1814

Buy Me Some Peanuts...

Advanced Targeting Systems took the afternoon off and set out for the Padres ball game. Half the fun of a ball game is all the food, so some (who shall not be named) attempted to consume a different food each inning. Others settled for a hot dog, cracker jacks, or hot coffee to keep away the chills. Yes, even in sunny San Diego it can get a little chilly (okay, we may be weather wimps!) Regardless of weather, food or bone-jarring action, all had a great time at the park.

Pictured left to right: Leonardo Ancheta, Kristen Hartman, Brian Russell, Amalia Dingman, Rick Marlinga (Thea's husband), Thea Marlinga, Darlene Martineau (Doug's wife), and Douglas Lappi. (Photo taken by Denise Higgins)

We would also like to wish a fond farewell to Thea Marlinga. In our last issue of Targeting Trends we shared our experience on Master Chief Rick Marlinga's submarine. The Marlingas, along with their two children, Charlotte and Sam, will be moving in December to Chicago to continue serving and protecting our country. We will miss you, Thea!

Targeting Tools: Featured Products

New Fluorescent Antibody: Cy3-labeled Anti-murine NGFr (Cat. #FL-05)

Advanced Targeting Systems introduces a new help in immunostaining:

The antibody to mouse nerve growth factor receptor (NGFr, low affinity neurotrophin receptor, p75) conjugated to Cy3.

The antibody is our widely-used rabbit polyclonal that has been affinity purified (Cat. #AB-N01AP).

As can be seen in the figure on the right, this antibody readily identifies the receptor expressed on the surface of mouse cells. This antibody is also the targeting agent of our immunotoxin to mouse cells that express NGFr, mu p75-SAP (Cat. #IT-16).

The Cy3-labeled anti-NGFr has been constructed especially for double immunostaining. Use this fluorescent antibody with other labeled antibodies or after primary and secondary for brilliant results!

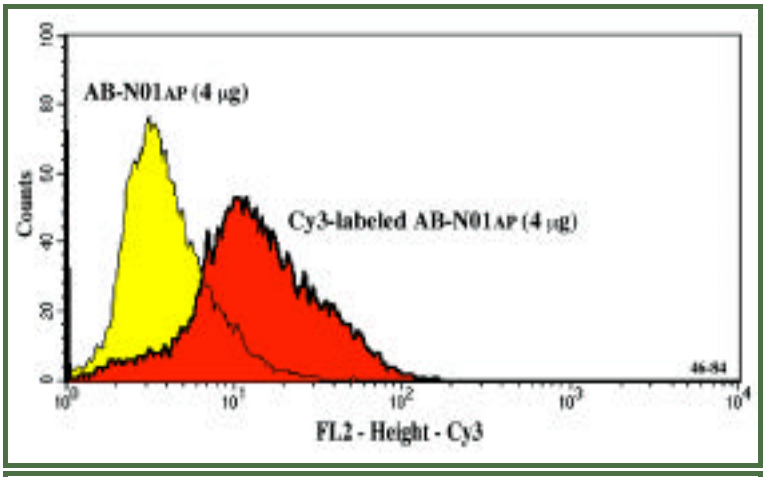


Figure 1. Immunostaining of NG3 cells. NG3 cells, a rat-mouse hybrid neuroblastoma cell line, were incubated with anti-murine NGFr antibody or conjugate and incubated at 4°C. Cells were analyzed by flow cytometry on a BD FACScan, and data produced using CellQuest software. A concentration of 4 µg of conjugate per one million cells provided a 33% shift compared to the antibody alone.

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AB-L104	Melanocortin 1 Receptor	Human; Mouse; Rat
AB-L106	Melanocortin 4 Receptor	Human; Mouse; Rat
AB-L348	Melanocortin 5 Receptor	Human; Mouse; Rat
AB-L132	Olfactory Receptor	Human; Mouse; Rat
AB-L350	Opioid Receptor, Kappa 1	Human; Mouse; Rat
AB-L351	Opioid Receptor 1 (OX1R)	Human; Mouse; Rat
AB-L301	Oxytocin Receptor	Human; Mouse; Rat
AB-L252	Prostaglandin D2 Receptor	Human; Mouse; Rat
AB-L314	Estrogen-Related Receptor Alpha	Human; Mouse; Rat
AB-L365	Steroid/Thyroid Receptor p65	Human; Mouse; Rat
AB-L323	Macrophage-Stimulating 1 Receptor	Human; Mouse; Rat
AB-L335	Protein Tyrosine Phosphatase Alpha	Human; Mouse; Rat
AB-L141	Opioid Receptor, Mu 1 (OPRM1)	Human; Mouse; Rat

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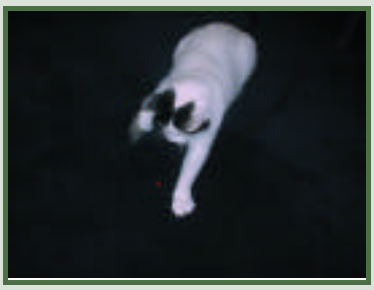
AB-L063	Frizzled-2	Human; Mouse; Rat
AB-L064	Frizzled-3	Human; Mouse; Rat
AB-L065	Frizzled-4	Human; Mouse; Rat
AB-L066	Frizzled-5	Human; Mouse; Rat
AB-L067	Frizzled-6	Human; Mouse; Rat
AB-L068	Frizzled-7	Human; Mouse; Rat
AB-L069	Frizzled-8	Human; Mouse; Rat
AB-L070	Frizzled-9	Human; Mouse; Rat
AB-L062	Frizzled-10	Human; Mouse; Rat



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

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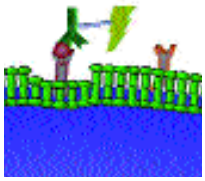
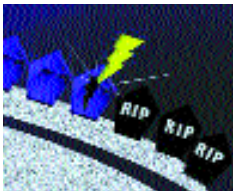


Cells that do not have the receptor will not be affected.

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these six Jumbles, one letter to each block, to form the name of this award-winning scientist.

SELINAGER

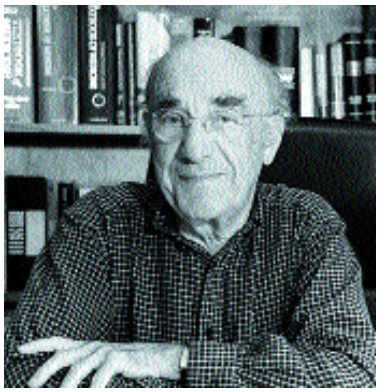
ITRAYPUTI

CORDIGENOLYNO

JOIND

TOMATOSTAINS

POSHINDERN



He is responsible for the growth of many, scientists and non-scientists alike.

Arrange the circled letters to form the name of this noted scientist, as suggested by the above clue.

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