Excessive sympathetic activity is responsible for, and/or contributes to, the morbidity and mortality associated with cardiovascular diseases (e.g. hypertension, stroke, heart failure, ischemic heart disease, ventricular arrhythmias). For example, myocardial ischemia provokes a powerful reflex increase in cardiac sympathetic efferent activity that directly promotes ventricular arrhythmias. Similarly, spinal cord injuries above thoracic level 6 (T6) are associated with episodic bouts of life-threatening hypertension as part of a condition known as autonomic dysreflexia (AD). Physiologically, AD is caused by a massive reflex sympathetic discharge triggered by a stimulus originating below the level of the spinal cord injury.

Importantly, interventions that reduce sympathetic activity protect against ventricular arrhythmias and AD. Accordingly, efforts to reduce sympathetic activity are the first-line therapy for these cardiovascular disorders. However, despite favorable effects, adverse complications (due to generalized sympatho-inhibition, e.g. fatigue, impotence; or specific sympatho-inhibition, e.g. Horner's syndrome, paraesthesia, disruption of sexual, bladder or bowel function) limit compliance and patient satisfaction with these treatments.

However, targeted ablation of cardiac sympathetic neurons reduced the susceptibility to ventricular arrhythmias (2) and targeted ablation of mesenteric projecting sympathetic neurons reduced AD (1) while avoiding these complications.

Specifically CTB-Saporin, injected into the stellate ganglia, reduced the number of left ventricular sympathetic fibers (Figure 1), the number of sympathetic post-ganglionic neurons in the stellate ganglia and virtually eliminated sympathetic pre-ganglionic neurons (SPNs) of spinal cord segments T1-T5 without altering afferent neurons. These structural neuroplastic changes were (continued on page 6)

Figure 1 presents tyrosine hydroxylase-immunoreactive sympathetic nerve fibers from the left ventricular free wall of rats that had CTB (left panel) or CTB-saporin (right panel) injected into both stellate ganglia. The CTB-saporin group showed a significant reduction in sympathetic nerve fibers compared to the CTB group.
Advanced Targeting Systems, the company that pioneered the targeting of specific cell types to manipulate them for the treatment of diseases and for research into the function of biological systems, has been awarded $3 million from the National Cancer Institute (NCI). Representatives from the NCI stated that the Advanced Targeting Systems proposal was ranked #1 for funding out of a nationwide program with hundreds of applicants. Advanced Targeting Systems will use the funds to advance its patented drug, SP-SAP, over the next two years to initiate clinical trials for cancer pain.

SP-SAP is a conjugate between the pain-processing peptide Substance P and the ribosome-inactivating protein saporin. The first publications of SP-SAP in the journal Science demonstrated a new direction for the understanding and treatment of pain pathology such as that which accompanies cancer. These have been followed by numerous publications from top-level scientists around the world delineating the activity of SP-SAP. The mechanism of action of SP-SAP is well characterized, a rarity in pain therapeutics: a small number of cells that process pathological pain signals are removed causing relief that appears to be permanent. Normal acute pain is unaffected.

The Food and Drug Administration has recommended that the first population to undergo treatment with SP-SAP is terminal cancer patients who are resistant to opioids such as morphine. Pain due to cancer is a great fear, at times greater than even the fear of death, in the progression of the disease. To make matters worse, many times this pain is unresponsive to the last stand treatment: opioids. There is also a common fear among terminal patients that current pain treatments will leave them unable to function normally at a time when it is personally extremely important for them. In preclinical tests, a single treatment with SP-SAP alleviated pathologic pain perception without affecting other sensory signal pathways.

Advanced Targeting Systems has assembled a team of experts to carry out the goals of the BRDG-SPAN project. Foremost among these are: Dr. Art Frankel from the Scott & White Cancer Research Center, Dr. Allen Burton from the MD Anderson Cancer Center, and Dr. Dorothy Brown of the University of Pennsylvania. All regulatory aspects of the project will be spear-headed by the professional team at Cato Research.

“I am gratified that the National Cancer Institute and the peer reviewers recognize the strength and quality of a ‘Dream Team’ of physicians, researchers and regulatory specialists that we have put together for this project,” stated Dr. Douglas Lappi, Principal Investigator and President/Chief Scientific Officer of Advanced Targeting Systems.

About The BRDG-SPAN Program

The National Institutes of Health BRDG-SPAN Pilot Program (the Biomedical Research, Development, and Growth to Spur the Acceleration of New Technologies Pilot Program (RC3) is supported by funds provided to the NIH under the American Recovery & Reinvestment Act of 2009, a component of the Federal Stimulus Package. The purpose of this pilot program is to accelerate the transition of research innovations and technologies toward the development of products or services that will improve

(continued on page 6)
**Targeting Topics: Recent Scientific References**

**Reviewed by Matthew Kohls**

**Substance P modulation of hypoglossal motoneuron excitability during development: changing balance between conductances**
Adachi T, Huxtable AG, Fang X, Funk GD

This work examined how neuromuscular networks that are immature, but functional, at birth move through development while remaining operational. The authors focused on hypoglossal motoneurons involved in behaviors such as swallowing, sucking, and breathing. Immunohistochemistry was performed using an anti-NK-1 receptor antibody (Cat. #AB-N04). The data show that although NK-1 receptor density decreases as the animal matures, substance P (the NK-1 receptor ligand) remains an important part of these networks.

**Mu and delta opioid receptors on nociceptors attenuate mechanical hyperalgesia in rat**
Joseph EK, Levine JD
*Neuroscience* Epub, 2010.

In this work the authors analyzed nociceptor populations mediating mechanical hyperalgesia in the rat. Rats received 3.2 µg of IB4-SAP (Cat. #IT-10) into the subarachnoid space between the L4 and L5 vertebrae. Hyperalgesia due to the administration of NGF was inhibited by DAMGO and SNC even in lesioned animals. These data indicate that most nociceptor populations are involved in mechanical hyperalgesia, and that the mu opioid and delta opioid receptors are co-expressed on some TrkA-positive nociceptors.

**Induction of CD4(+)/CD25(+) T regulatory cells with CD103 depletion**
Zikri NN, Schumer E, Wang JJ, Gaughan A, Hadley GA, Moffatt-Bruce SD

CD8+ T cells expressing CD103 have been shown to play a key role in the rejection of renal allografts. Use of M290-SAP (a custom saporin conjugation) allows allograft tolerance even in a completely mismatched islet cell transplant model. Use of 1 mg M290-SAP/kg body weight in mice allowed the authors to characterize the kinetics of M290-SAP and its induction of CD4 CD25 regulatory T cells.

**Orexin-B-saporin lesions in the lateral hypothalamus enhance photic masking of rapid eye movement sleep in the albino rat**
Ocampo-Garcés A, Ibáñez F, Perdomo G, Torrealba F

Photic masking occurs when photic input to the retina interferes with REM sleep. Rats that received 200 ng of orexin-SAP (discontinued) into the lateral hypothalamus experienced dramatically less REM sleep during normal light cycles. Placing them in a skeleton photoperiod (brief pulses of light, one in the morning and one in the evening), however, caused REM sleep during the rest phase to return to normal. This data suggests that photic masking may explain some effects of narcolepsy and cataplexy.

**Saporin toxin-conjugated monoclonal antibody targeting prostate-specific membrane antigen has potent anticancer activity**

Current treatments for prostate cancer are only moderately effective. In this work the authors examined the cytotoxic efficacy of a prostate-specific membrane antigen (PMSA) antibody conjugated to saporin on PMSA-positive cell lines. hJ591, a humanized PMSA antibody, was biotinylated and combined with streptavidin-ZAP (Cat. #IT-27). The hJ591-streptavidin-ZAP complex was specifically cytotoxic to PMSA-positive cell lines, and had anti-cancer activity in a xenograft model. This work demonstrates the anti-cancer potential of targeting PMSA.

**Septohippocampal pathways contribute to system consolidation of a spatial memory: Sequential implication of gabaergic and cholinergic neurons**
Lecourtier L, de Vasconcelos AP, Leroux E, Cosquer B, Geiger K, Lithfous S, Cassel JC

There have been few studies examining the role of GABAergic septohippocampal projections in memory consolidation. The authors administered 192-IgG-SAP (Cat. #IT-01) and orexin-SAP (discontinued) to the medial septum/vertical limb of the diagonal band of Broca of rats. The animals received 400 ng of 192-IgG-SAP, or 70 ng of orexin-SAP, or both. Spatial memory tests were then administered over several weeks. The data indicate that both GABAergic and cholinergic septohippocampal systems contribute to memory stabilization, possibly in a sequential manner.

**A new oxytocin-saporin cytotoxin for lesioning oxytocin-receptive neurons in the rat hindbrain**
*Endocrinology* 151(9):4207-4213, 2010.

There is evidence to suggest that release of oxytocin in the nucleus tractus solitarius (NTS) of the hindbrain can inhibit food intake by augmenting the cholecystokinin satiety response. In order to add support to this hypothesis the authors used oxytocin-SAP (Cat. #IT-46) to eliminate oxytocin receptive cells in the NTS. Blank-SAP (Cat. #IT-21) was used as a control. 0.5 µl-injections of oxytocin-SAP into the NTS caused reduced satiation effect of CCK-8 and blocked the stimulation of food intake by an oxytocin receptor antagonist.

**Contribution of limbic norepinephrine to cannabinoid-induced aversion**
Carvalho AF, Reyes AR, Sterling RC, Unterwald E, Van Bockstaele EJ

The authors used bilateral injections of anti-DBH-SAP (Cat. #IT-03) into the nucleus accumbens and the bed nucleus of the stria terminalis to investigate the role of...
Targeting Topics: Recent Scientific References

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neuropeptide in cannabinoid-induced aversion and anxiety. Lesioned animals received bilateral 52.5 ng-injections of anti-DBH-SAP into the nucleus accumbens or 63 ng into the bed nucleus of the stria terminalis. Saporin (Cat. #PR-01) was used as a control. Lesioned animals displayed reversed aversive behavior, but no change in anxiety-like behavior.

Noradrenergic Nuclei that Receive Sensory Input During Mating and Project to the Ventromedial Hypothalamus Play a Role in Mating-Induced Pseudopregnancy in the Female Rat

Northrop LE, Polston ER, Erskine MS

Maintenance of pregnancy or pseudopregnancy in rats is maintained by bicircadian prolactin surges induced by vaginal-cervical stimulation. In order to test the hypothesis that medullary noradrenergic cell groups are involved in this process the authors infused rats with either 2 ng or 60 ng anti-DBH-SAP (Cat. #IT-03) into the ventrolateral division of the ventromedial hypothalamus and the posterodorsal medial amygdala. Mouse IgG-Sap (Cat. #IT-18) was used as a control. The data confirm that noradrenergic neurons are involved in the maintenance of pregnancy or pseudopregnancy.

Decrease in membrane phospholipid unsaturation induces unfolded protein response

Ariyama H, Kono N, Matsuda S, Inoue T, Arai H

Properties of the cell membrane can be influenced by the degree of fatty acid unsaturation in membrane phospholipids. Alteration of this unsaturation has been implicated in many disease states. Using an anti-SCD1 antibody (Cat. #AB-259) to visualize SCD-1 levels by western blot, the authors determined that there are several genetic factors that affect the level of saturated fatty acid in systems modulating insulin resistance, type 2 diabetes, and cardiovascular disease.

NK-1-receptor-mediated lesion of spinal post-synaptic dorsal column neurons might improve intractable visceral pain of cancer origin


There is evidence that spinal post-synaptic dorsal column neurons begin to express neurokin-1 receptors after visceral stimulation. The authors discuss using this expression profile to target SP-SAP to these neurons and eliminate them. This use of ‘molecular neurosurgery’ may be a replacement for traditional neurosurgery for the treatment of cancer-related visceral pain.

Effect of applying p75NTR saporin to a punctured intervertebral disc on calcitonin gene-related peptide expression in rat dorsal root ganglion neurons


Lumbar intervertebral discs are suspected to be a source of low back pain, in part because of the innervation of these discs by neurons containing substance P and CGRP receptors. Rats received 2.5 µg of 192-IgG-SAP (Cat. #IT-01) into the L5/6 vertebral disc after the disc was punctured. While half of the dorsal root ganglion neurons innervating the disc were positive for CGRP post-puncture, animals receiving 192-IgG-SAP displayed reduced CGRP expression, indicating a role for the p75 receptor in discogenic pain.

Photochemical internalization (PCI): a technology for drug delivery


This review discusses photochemical internalization (PCI), which is a method used to overcome some of the intracellular barriers to introducing molecules into cancer cells. Some difficulties for such therapies include a low rate of release from endocytic vesicles and degradation of the therapeutic molecule by lysosomal enzymes. The use of streptavidin-ZAP (Cat. #IT-27) with a biotinylated EGF receptor antibody is discussed.

Postnatal development and functional adaptations of the melanopsin photoreceptive system in the albino mouse retina

Gonzalez-Menendez I, Contreras F, Cernuda-Cernuda R, Provencio I, Garcia-Fernandez JM

Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) adjust the circadian pacemaker of mammals by detecting light. The authors tracked the development of ipRGCs in postnatal mice under varying light conditions. Immunohistochemistry for these experiments was done using an anti-mouse melanopsin polyclonal antibody (Cat. #AB-N38). Alteration of the standard light/dark cycle clearly affected the development of ipRGCs.

Noradrenergic neurons of the area postrema mediate amylin's hypophagic action

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, Lutz TA

The neuronal pathways used to process the physiological response to amylin were
Q: In the Targeting Trends Newsletter, Oct-Nov-Dec 2006 you mentioned mixing anti-DBH-SAP with a tracer, which tracer would you recommend? We were thinking of using FluoroGold. If we do not use a tracer, we were thinking of using a neutral red solution to dilute the stock of anti-DBH-SAP in order to be able to visibly see the toxin being injected into the spinal cord. Could there be an issue of pH if we used neutral red with anti-DBH-SAP? Our concern is that the toxin is not being ejected from the pipette tip or that it is not being taken up into the pipette tip as we can not see it (it's the same color as the mineral oil). We are confident in the targeting of the spinal area for injection as we have previously used FluorGold only and then were able to visualize it in the area of interest.

A: Our Scientific Advisor, Dr. Ronald G. Wiley, uses Fast Green dye (0.01-0.1% w/v) in the toxin injection solutions. He originally chose Fast Green because intracellular electrophysiologists had long used it while doing intracellular recordings and shown it was non-toxic. Fast Green has more contrast than Neutral Red (easier to see) and does not affect pH significantly. He has used it with many saporin-containing toxins with success.

Dr. Wiley says, “There are two issues when you talk about using "tracers" with targeted toxins: 1) tracing the acute injection volume to be sure it goes into the animal correctly, and 2) tracing the neurons that projected to the injection site and were therefore susceptible to being killed by the toxin.

Dr. Wiley does not use separate anatomic tracers for the immunotoxins, the only agents taken up and retrogradely transported efficiently. Since ATS immunotoxins are so efficient you have to use a high efficiency tracer such as cholera toxin B (but not WGA since it may not play well with saporin).

Dr. Wiley does not favor FluoroGold (a tin compound) because he has seen some local toxicity at FluoroG injection sites which might impair uptake and/or transport of a targeted toxin, and it is not clear if it is compatible with saporin-containing toxins.

investigated using 50 ng-injections of anti-DBH-SAP (Cat. #IT-03) into the area postrema (AP) or 25 ng into the lateral parabrachial nucleus. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The response to amylin administration (reduction of food intake) was significantly reduced in lesioned animals, indicating that noradrenergic neurons in the AP control at least part of this pathway.

Distinct neural pathways mediate alpha7 nicotinic acetylcholine receptor-dependent activation of the forebrain
Thomsen MS, Hay-Schmidt A, Hansen HH, Mikkelsen JD

In this work the authors examine the systems controlling cognitive function in the medial prefrontal cortex (mPFC) and nucleus accumbens shell (ACCshell). Rats received 30 ng-injections of 192-IgG-SAP (Cat. #IT-01) into the horizontal limb of the diagonal band of Broca, eliminating the cortically projecting cholinergic neurons. Deficits in the basal forebrain and the mPFC are shown to be involved in attentional function, while deficits in the ACCshell are shown to be involved in the beneficial effects of antipsychotics on schizophrenia.
Targeted Ablation of Sympathetic Neurons Reduces Ventricular Arrhythmias and Autonomic Dysreflexia

(continued from page 1)

associated with a decreased susceptibility to ischemia-induced sustained ventricular tachycardia.

Furthermore, CTB-Saporin injected into the celiac ganglion reduced the number of sympathetic post-ganglionic neurons in the celiac ganglia and virtually eliminated sympathetic pre-ganglionic neurons of spinal cord segments T5-T12 without altering afferent function. Similarly, these neuroplastic changes were associated with a reduced AD.

Thus CTB-Saporin retrogradely transported from the peripheral ganglia is effective at ablating specific sympathetic neurons and reducing the susceptibility to ventricular arrhythmias and AD. Additional studies are required to further characterize the physiological responses to this procedure as well as determine if this new approach is safe and efficacious for the treatment of conditions associated with excess sympathetic activity.

References


GAT1-SAP targets GAT-1, the GABA transporter

GAT-1 is a sodium-coupled neurotransmitter transporter responsible for moving g-aminobutyric acid (GABA) across cell membranes. GABA is the predominant inhibitory neurotransmitter in the mammalian central nervous system. GAT-1 is widely distributed in both the central and peripheral nervous systems. GAT-1 and GABA are present in numerous neuronal pathways, some of which are implicated in epilepsy, sleep disorders, neuropathic pain, and attention deficit disorders.

We have constructed a conjugate between an antibody to an extracellular domain of GAT1 and saporin, the ribosome-inactivating protein. This construct, GAT-1-SAP (Cat. #IT-32) has been used to specifically remove GABAergic neurons of the anterior bed nucleus of the stria terminalis (1) and the medial septum and diagonal band of rats (2). Figure 1 shows the specificity of the targeted toxin, with parvalbumin-positive neurons being drastically reduced, while sparing most cholinergic neurons cholinergic neurons.

Coming soon is our vesicular GABA transporter-saporin construct to increase the ability to eliminate specific populations of GABAergic neurons.

References

![Immunocytochemistry following sham surgery (top row) or administration of GAT1-saporin (bottom row, 325 ng/µl) into the medial septum-diagonal band of Broca (MSDB). Staining of parvalbumin-immunoreactive neurons in the MSDB was dramatically reduced following GAT1-saporin (left). Parvalbumin-ir neurons in the MSDB are GABAergic septohippocampal neurons. In contrast to parvalbumin-ir neurons, neither cholinergic neurons (ChAT-ir, middle) nor calbindin-ir neurons (right) were altered following GAT1-saporin in the MSDB. Scale bar - 200 µm. Figure provided by KCH Pang et al. (2008) Targeting Trends 9(1).]

GAT-1-SAP, Cat. #IT-32

available in these sizes:
25 micrograms, 100 micrograms, and 250 micrograms

Kit includes the following controls in equal amounts:
Saporin, Cat. #PR-01
GAT-1 Rabbit Polyclonal, Cat. #AB-N37
Rabbit IgG-SAP, Cat. #IT-35

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