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Models of Neurodegenerative Disease

by Dr. Maxine Warren

The continuous, targeted administration of compounds attainable using ALZET® osmotic pumps has led to their use in many studies investigating the effects of diverse agents upon brain biochemistry and behavioral responses in live animals. In addition to these applications, the ability to deliver neurotoxic compounds or neuromodulators to defined regions of the brain has allowed the development of animal models of neurodegenerative disease states.

Slow Infusion Improves Parkinson's Disease Model

Parkinson's disease, caused by the protracted degeneration of dopaminergic neurons in the nigrostriatal pathway, is associated with characteristic motor and behavioral deficits which increase as the disease progresses. The commonly used animal model of this disorder involves acute injection of 6-hydroxydopamine (6-OHDA) into the substantia nigra of rats. This results in a rapid and near-complete loss of nigrostriatal dopaminergic neurons. Unfortunately, this is only truly representative of end-stage disease. Recognizing the limitations of this model, Jones *et al*. (of Regeneron Pharmaceuticals, Tarrytown, New York), investigated whether a slow, sustained infusion of 6-OHDA into the striatum would produce a more gradual degeneration of nigrostriatal dopaminergic neurons, similar to that seen in Parkinson's disease in man.[1]

Subcutaneously implanted ALZET pumps, Model 2001, were connected to cannulae inserted into the right striata of rats. The pumps contained either 6-OHDA in an ascorbate-rich vehicle (for improved stability) or vehicle alone. Various biochemical parameters and amphetamine-induced ipsilateral rotation, a behavioral indicator of unilateral dopamine depletion, were monitored throughout the seven day infusion period. Continuous infusion of 6-OHDA produced a 55% decrease in dopamine uptake and a dose-related reduction in striatal dopamine and DOPAC levels, but did not affect the uptake of other neurotransmitters nor the level of various monoamines, thus confirming that the treatment selectively affected dopaminergic neurons. Rotational behavior and [^{3}H]mazindol autoradiography of dopamine uptake sites were compared in animals receiving chronic infusion to those subjected to a single high dose striatal injection of 6-OHDA. Increased ipsilateral rotation and a reduction in dopamine uptake sites occurred within 1.5 days following acute treatment, but did not appear until four days after initiation of continuous infusion. Furthermore, the reduction in the dopamine transporter sites was diffuse and widespread in acutely treated rats, whereas the lesion in continuously infused rats was more complete and restricted to a smaller area (Figure 1).

The data confirm that a continuous infusion of 6-OHDA results in a selective and dose-dependent loss of dopaminergic nerve terminals. The topographically limited terminal axotomy of the dopamine neurons and

INSIDE

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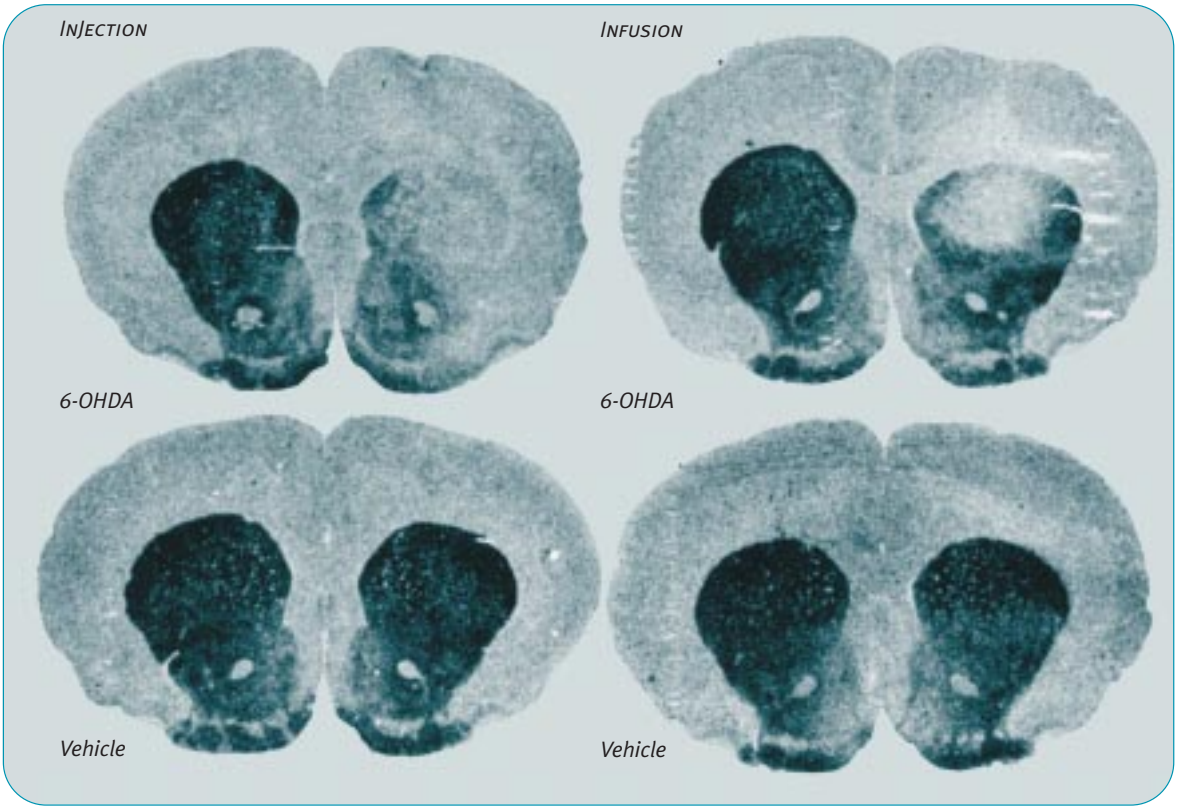
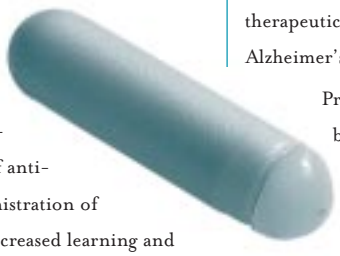


Figure 1. [^{3}H]Mazindol autoradiography of dopamine uptake sites in the neostriatum of rats. Autoradiography was performed seven days after an acute injection (30 µg) or continuous infusion (0.2 µg/hr) by ALZET pump of 6-hydroxydopamine (6-OHDA) or vehicle into the right striata. Loss of dopamine uptake sites was diffuse and over a larger area in acutely treated rats (upper left), whereas the lesion was more complete and restricted to a smaller well-demarcated area in continuously infused rats (upper right).
(Autoradiographs kindly provided by, and reproduced with permission from, Dr. C. Anthony Altar, now Director for Global Neuroscience Research, Otsuka America Pharmaceutical, Inc., Rockville, MD.)

the protracted behavioral impairment mimic the slow degradation associated with Parkinson's disease, indicating that this model may be a valuable tool when studying the early stages of this disorder.

Dementia Models

A model of dementia based on the depletion of endogenous nerve growth factor (NGF) has been used to determine whether propentofylline, a stimulator of NGF synthesis, may be of value in the treatment of Alzheimer's disease. Nitta *et al.*, of Nagoya University, Japan, infused anti-NGF monoclonal antibody (MoAb) directly into the septum of rats via cannulae attached to subcutaneously implanted ALZET pumps.[2,3] In the test groups, treatment with propentofylline commenced three days prior to, and continued throughout, the sixteen day infusion of anti-NGF MoAb. Administration of anti-NGF MoAb decreased learning and memory capacity and reduced hippocampal choline acetyltransferase and cholinesterase activities to 76% and 33% of control levels respectively. Treatment with propentofylline ameliorated the behavioral deficits and prevented the decrease in cholinergic activity observed in this dementia model, leading Nitta *et al.* to conclude that stimulators of NGF synthesis may provide a new therapeutic approach for the treatment of Alzheimer's disease.



Progressive excitotoxicity, caused by the central administration of quinolinic acid (QUIN), an N-methyl-D-aspartate (NMDA) receptor agonist, results in biochemical and behavioral changes in rats which have been likened to those occurring in Huntington's disease in man. In particular, Maeda *et al.*, of the University of Maryland, have shown that continuous intracerebroventricular (ICV)

infusion of QUIN by ALZET pump, Model 2002, over 14 days, affects cholinergic systems and somatostatin levels in specific regions of the brain.[4]

This Huntington's disease model has been used by Misztal *et al*. (of Merz & Co., Frankfurt, Germany) to investigate the potentially protective effects of NMDA receptor antagonists in progressive neurodegenerative disorders.[5] ICV infusion of QUIN was maintained for fourteen days via cannulae attached to subcutaneously implanted ALZET pumps, Model 2002. Test groups received concurrent systemic treatment with memantine or MK-801, both NMDA antagonists, by subcutaneously implanted ALZET pumps, Models 2ML2 or 2002. Assessment of short-term working memory commenced three days after pump removal. QUIN produced a significant deficit ($p < 0.05$) of reinforced alternation in the T-maze test compared to non-infused controls; parallel administration of either memantine or MK-801 prevented the development of this learning impairment. Furthermore, memantine attenuated the decrease in choline uptake sites produced by QUIN infusion. The results led Misztal *et al*. to conclude that both of the NMDA receptor antagonists studied exerted neuroprotective effects in this Huntington's disease-like dementia model.

Damage Control

The ability to deliver a compound directly into the brain avoids many of the complications related to bioavailability, side effects, toxicity and possible biological feedback mechanisms inherent with systemic delivery. Furthermore, limiting drug presence to a discrete area of the brain is advantageous when neurotoxic compounds are administered with the express purpose of generating a specific type of lesion, or a localized modulation of neuronal function. The use of ALZET pumps, as outlined above, has enabled the development of animal models which display biochemical, behavioral and morphological traits characteristic of neurodegenerative disease states. The availability of such models will facilitate identification of neuroprotective agents and the development of therapeutics for the treatment of these disorders.

If you would like more information on pump applications in this or other areas, please contact us as outlined on page 8.

1 Jones BE, Boylan CB, Fritsche M, Juhasz M, Jackson C, Wiegand SJ, Hyman C, Lindsay RM and Altar CA. *Brain Res* 1996;709:275-284.

2 Nitta A, Ogihara Y, Onishi J, Hasegawa T, Furukawa S and Nabeshima T. *Eur J Pharmacol* 1996;307:1-6.

3 Nitta A, Ogihara Y, Onishi J, Hasegawa T, Furukawa S and Nabeshima T. *Behav Brain Res* 1997;83:201-204.

4 Maeda K, Kaneda H, Whetsell WO and Tamminga CA. *Neurosci Res* 1997;29:303-309.

5 Misztal M, Frankiewicz T, Parsons CG and Danysz W. *Eur J Pharmacol* 1996;296:1-8.

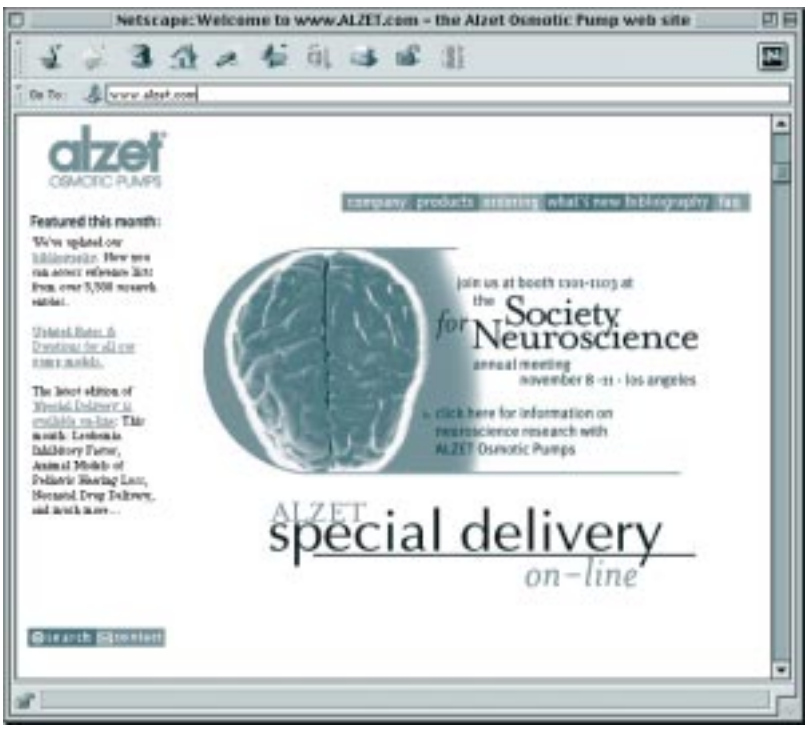


Figure 2: The ALZET web site has undergone recent renovations to provide more up-to-date information which changes more frequently. Visit our web site soon, and e-mail your comments to alzet@alza.com.

Nicotine as a Neuroteratogen

By Laura Whitman

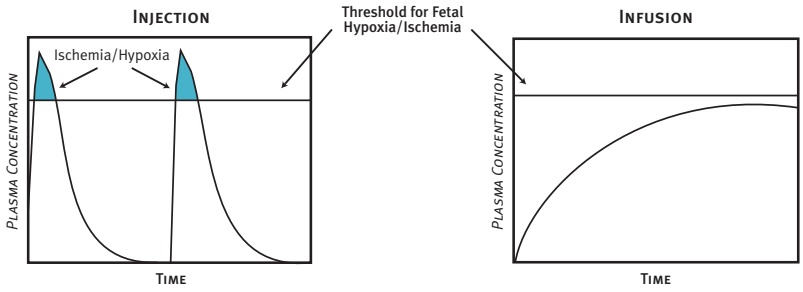


Figure 3: Representational comparison of injection vs. continuous infusion of nicotine in pregnant rats. Injections lead to peak plasma levels which produce fetal hypoxia-ischemia. Continuous infusion via ALZET pumps produces a steady-state plasma level of nicotine.
(Recreated with permission from Slotkin TA. *J Pharmacol Exp Ther* 1998;285(3):931-945.)

Tobacco use occurs during 25% of all pregnancies in the United States despite educational efforts to curtail its use. It may contribute to low infant birth weight, perinatal morbidity and mortality, and Sudden Infant Death Syndrome (SIDS). Parental smoking is strongly correlated with SIDS, which is the major cause of death in young, apparently healthy infants.[1] Early animal research on the teratogenic effects of nicotine was performed via nicotine injections to pregnant rats throughout gestation. Although this simulated episodic smoking of cigarettes by pregnant women, it did little to elucidate direct nicotine effects, and did not mimic the nicotine exposure seen in constant smokers.

A recent article by Theodore A. Slotkin of Duke University (Durham, North Carolina) reviewed his work in the area of fetal nicotine exposure.[2] Dr. Slotkin and his group performed extensive fetal nicotine research by using ALZET osmotic pumps to deliver nicotine to pregnant rats. "By administering nicotine on a continuous basis, we can avoid peak levels that elicit hypoxia-ischemia and can deliver a clearly identifiable, fixed dose of drug … Steady-state plasma levels can be assessed and the dose rate adjusted to simulate human exposure levels."[2] (See Figure 3.) By using this animal model of fetal nicotine exposure, this group and others have shown that nicotine is a neuroteratogen which causes fetal resorption and brain cell damage.

Slotkin *et al*. used ALZET pump models 2ML1, 2ML2 and 2ML4 to deliver nicotine bitartrate continuously to pregnant rats for periods of 8 to 35 days. Fetal nicotine exposure up-regulated nicotinic receptor binding sites, and correspondingly increased the sensitivity of cholinergic trophic responses of the targeted cells. Noradrenergic and dopaminergic synaptic transmissions were determined to be adversely affected by fetal nicotine exposure. Defects in fetal and neonatal postsynaptic signaling mechanisms such as up-regulation of adenylyl cyclase expression, which may contribute to adverse behavioral outcomes, were also seen with continuous nicotine administration. Lastly, nicotine was found to initiate an alteration in the timeline for cell development, leading to cell death. Slotkin *et al*. found that "…protooncogene c-*fos*…is constitutively activated in postnatal brain regions after prenatal nicotine exposure…[which] elicits apoptosis."[2]

This group also determined that "doses of nicotine [in rats] that simulate plasma levels found in moderate smokers and that do not cause growth retardation are nevertheless fully capable of affecting brain development as indicated by markers for cell damage and loss and synaptic dysfunction."[2] Slotkin deduced that nicotine is a neuroteratogen which elicits many of its effects at thresholds below those necessary for growth impairment; therefore, the use of intrauterine growth retardation in humans as an index of fetal damage due to nicotine was inappropriate. Fetal nicotine exposure in rats led to changes in central respiratory control mechanisms, providing the first mechanistic evidence of the association between maternal smoking and perinatal morbidity and mortality. In comparing nicotine to other teratogens, Dr. Slotkin also discussed fetal cocaine exposure. He noted that cocaine exposure has a much lower occurrence and a smaller and shorter range of effects than fetal nicotine exposure. He found that cocaine affected synaptic and behavioral performance but without the damage induced by nicotine.

The ALZET bibliography contains seventeen references in which Dr. Slotkin used ALZET pumps, as well as numerous nicotine references by other researchers. As seen in Dr. Slotkin's work, ALZET osmotic pumps provide a unique advantage in their ability to maintain continuous plasma levels of drug in animals, thus avoiding the peaks and troughs associated with conventional dosing methods. To obtain a bibliography of references on the continuous administration of nicotine using ALZET pumps, on teratology studies, or on the use of the pumps in pregnant animals, please contact us as outlined on page 8.

1 Cornwell AC, Feigenbaum P and Kim A. *Neuropediatrics* 1998;29(2):72-79.

2 Slotkin TA. *J Pharmacol Exp Ther* 1998;285(3):931-945.

Very Special Delivery

By Dr. Maxine Warren

Targeted Protein Delivery Allows Demonstration of Novel Effects

Conventional oral or parenteral dosing of animals is associated with numerous factors which can complicate evaluation of agent effects. These include wide fluctuations in plasma levels and the influence poor bioavailability and short half-life exert upon drug concentration at the site of action. In addition, potential toxicity, side-effects, or activation of biofeedback mechanisms, may all contribute to masking or inhibiting the direct drug-related response. ALZET pumps provide a means of overcoming these factors, enabling continuous administration of low doses, maintenance of effective levels and, if required, direct delivery to a target organ or tissue. The researchers in the following investigations capitalized on these benefits by successfully utilizing ALZET pumps, rather than conventional dosing regimens, for targeted agent delivery.

Localized Peptide Infusion Stimulates Testicular Descent

Normal testicular descent occurs in stages, each of which is associated with different morphological characteristics and hormonal influences. The inguinoscrotal phase of descent involves migration of the gubernaculum across the pubis into the scrotum, an event which may be controlled by the release of calcitonin gene-related peptide (CGRP) from the genitofemoral nerve. To evaluate the importance of this peptide Hutson *et al.*, of the F. Douglas Stephens Surgical Research Laboratory in Melbourne, Australia, administered CGRP to neonatal pigs with congenital cryptorchidism.[1]

Two-week old piglets were implanted with an ALZET pump, Model 2002, containing CGRP or phosphate buffered saline. Pumps were placed in a subcutaneous pocket in the superficial abdominal fascia of Scarpa and oriented such that the exit port pointed toward the undescended testis. The position of the piglets' testes, pump and anatomical landmarks were documented at implantation and after 14 days of treatment, following which the animals were sacrificed and the type of cryptorchidism diagnosed. CGRP administration had no effect upon intra-abdominal or ectopic testes, but did produce a significant, though slight, descent of inguinal testes toward the implanted pumps.

Hutson *et al.* noted that these results conflicted with previous findings in which intrascrotal injections of CGRP inhibited testicular descent in neonatal rodents. Hutson's group suggested that the high level of peptide occurring throughout the scrotum following injection effectively removed the local concentration gradient of endogenous CGRP necessary to stimulate gubernacular migration. Conversely, "the osmotic pump allowed precise, continuous release of low levels of CGRP", concentrating the peptide in a defined area. Hutson *et al.* concluded that a slow release depot preparation of CGRP may provide a suitable treatment for inguinal undescended testes in man.

Hepatocyte Growth Factor and Short Bowel Syndrome

Following extensive bowel resection, the remaining intestine undergoes an adaptive process including elongation, dilation, and epithelial hyperplasia, all of which increase the absorptive surface area. Unfortunately, this response is not always sufficient in children, and reliance on enteral feeding may restrict growth and development, resulting in short bowel syndrome. Thus researchers sought ways to increase the absorptive capacity of the remaining bowel. Various

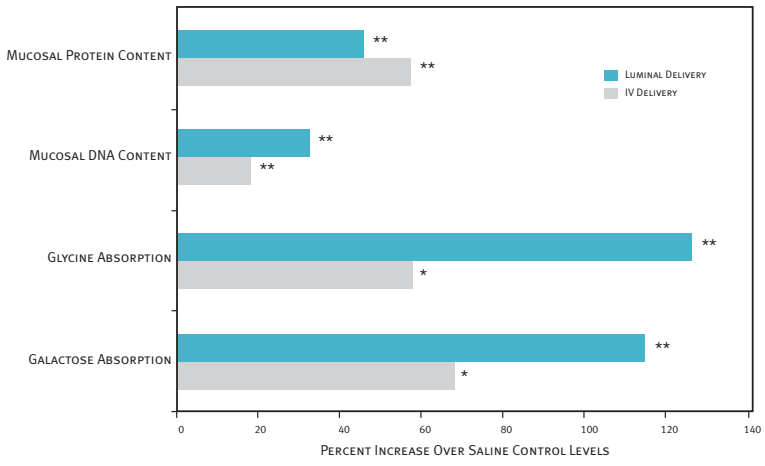


Figure 4: Hepatocyte growth factor, administered over 14 days by ALZET pumps connected to either a jugular catheter or jejunostomy tube, significantly increased various indicators of intestinal function compared to saline delivered by the same route ($p < 0.05$, ** $p < 0.001$).* (Graphic representation of results presented in Kato Y et al. *J Pediatric Surg* 1998;33(2):235-239.)

(Continued)

gastrointestinal peptides, including hepatocyte growth factor (HGF), have been demonstrated to increase mucosal substrate absorption and investigations have suggested they may be of benefit in the treatment of this disorder. In a study by Kato *et al.*, of the Dupont Hospital for Children, Wilmington, Delaware, rats underwent an 80% small bowel resection and end-to-end jejunoileal anastomosis, with concurrent placement of either a jugular catheter or jejunostomy tube.[2] Seven days later, each rat was subcutaneously implanted with an ALZET pump, Model 2002, containing either recombinant human HGF or saline, which was connected to the previously placed venous catheter or J-tube. After 14 days of HGF infusion, [C^{14}]galactose and [C^{14}]glycine absorption by the remaining small intestine was determined, followed by measurement of protein and DNA content of intestinal mucosal biopsies.

HGF significantly improved galactose and glycine absorption, and increased mucosal DNA and protein levels compared to saline controls, as summarized in Figure 4. Furthermore, localized delivery of HGF by J-tube appeared more effective than IV infusion, significantly so with regard to galactose absorption ($p < 0.05$) and mucosal DNA content ($p < 0.01$).

The apparent increase in mucosal mass, evidenced by DNA and protein content, together with improved carbohydrate and amino acid absorption, led Kato *et al.* to conclude that HGF elicits both hyperplasia and enhancement of function of individual enterocytes and, as such, may be useful in patients with short bowel syndrome. Furthermore, in recognition of the effectiveness of locally administered HGF, the authors suggested local application would be advantageous in the clinical setting as "a smaller dose could be used [compared to that which would be required if given systemically], and any risk of systemic exposure would be diminished".

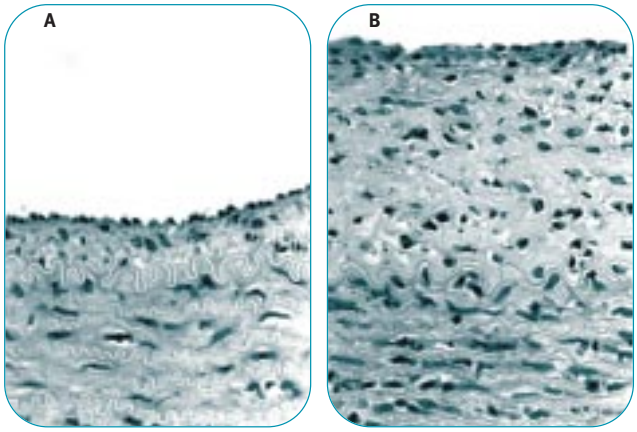


Figure 5: Intimal thickening of collared carotid arteries. Perivascular delivery of vehicle or oxidized low density lipoprotein (oxLDL) to the collared region of the arteries was maintained via ALZET pump over 14 days. Collaring and vehicle resulted in discrete intimal thickening (A) whereas administration of oxLDL produced a much larger intima and pronounced collagen deposition (B). (Photographs provided by Dr. Katelijne E. Matthys, University of Antwerp, Belgium.)

Oxidized Low-Density Lipoproteins and Atherogenesis

With age, human arteries undergo adaptive changes in response to typical mechanical stresses placed upon the arterial walls. In particular, accumulation of smooth muscle cells, connective tissue and isolated macrophages, results in intimal thickening. These areas of intimal thickening are the primary location for fat deposition. The resultant fatty streaks, considered to be the first manifestation of atherosclerosis, may develop into atherosclerotic lesions later in life in response to atherogenic stimuli such as hypercholesterolemia. Also implicated in the formation of such lesions is oxidized low-density lipoprotein (oxLDL), which may chemically promote lesion formation and alter vascular reactivity.

To investigate the role of oxLDL, Matthys *et al.*, of the University of Antwerp, Belgium, adapted a rabbit model of intimal thickening to permit perivascular infusion of oxLDL.[3] In this study, non-occlusive silicone collars were placed around the carotid arteries of rabbits. The interior of each collar was connected to a subcutaneously implanted ALZET pump. Continuous, local delivery of oxLDL or vehicle (PBS) was maintained for 3 or 14 days, after which the arteries were dissected. Segments taken from collared- and proximal non-collared-sections of the vessels were compared for intimal thickening and vascular reactivity.

The presence of a collar for 14 days caused discrete intimal thickening; perivascular delivery of oxLDL significantly enhanced this thickening compared to collaring alone ($p < 0.01$), resulting in a 9-fold increase in intima:media ratio (see Figure 5). Collaring reduced contractile force and increased sensitivity to vasoconstrictors; both responses were augmented by concurrent oxLDL application. Endothelium-dependent and independent relaxation was unaffected by collaring; concurrent oxLDL administration reduced endothelium-dependent relaxation, but had no effect on endothelium-independent relaxation.

Collaring of the rabbit carotid generates some intimal thickening and resembles features of a pre-atherosclerotic stage, but is not representative of human atherosclerotic arteries. The pronounced intimal thickening and modulation of contractile reactivity induced by collaring together with perivascular delivery of oxLDL by ALZET pump does, however, closely resemble that of atherosclerotic blood vessels. The morphological and functional changes observed in this study led Matthys *et al*. to conclude that oxLDL plays an active role in this disease state.

The success of the above studies depended upon the investigators' ability to control the delivery of agents. In one case, very low doses of peptide were essential if a concentration gradient was to be established within a tissue. Another required maintained presence of growth factor, and the final example required precise delivery of lipoprotein to a segment of vessel wall. The use of ALZET pumps provided a means of achieving these aims, thus facilitating performance of the studies. If you would like further information on the varied applications of ALZET pumps in targeted delivery or other areas, please contact us as outlined on page 8.

1 Hutson JM, Watts LM and Farmer PJ. *J Urology* 1998;159:1025-1028.

2 Kato Y, Yu D and Schwartz MZ. *J Pediatric Surg* 1998;33(2):235-239.

3 Matthys KE, Van Hove CE, Kockx MM, Andries LJ, Osselaer NV, Herman AG and Bult H. *Cardiovasc Res* 1998;37:239-246.

Rats and Cardiac Glycoside Sensitivity

By Dr. Maxine Warren

The supposition that rats are insensitive to the positive inotropic and anti-arrhythmic properties of cardiac glycosides was challenged in a recent study by Nelissen-Vrancken *et al*. of Maastricht University, The Netherlands.[1] Recognizing that the beneficial cardiac effects of such drugs in humans may be dependent upon the pre-existence of the pathophysiological condition (or extent of heart failure), they examined the hemodynamic and cardiac effects of ouabain in healthy rats, and rats suffering from heart failure due to myocardial infarction.

Rats underwent sham surgery (controls) or ligation of the left coronary artery to cause myocardial infarction (MI). Chronic ouabain treatment commenced three weeks later by daily SC injections over two weeks, or by continuous infusion via subcutaneously implanted ALZET pumps, Model 2ML1. To achieve two weeks of infusion, pumps were removed and replaced after seven days. Acute treatment was achieved via a single IV infusion of ouabain, lasting 45-60 minutes, administered five weeks after surgery.

Induction of MI significantly increased total peripheral resistance (TPR) and decreased basal and maximal cardiac output (CO) compared to sham-operated controls; these effects were not improved, and to some extent were aggravated, by acute IV infusion or chronic daily injection of ouabain. Conversely, continuous infusion of ouabain normalized TPR and significantly improved CO in MI rats, although it had no apparent effects in sham-operated controls.

These results support past observations that healthy rats are insensitive to cardiac glycosides, but clearly demonstrate profound beneficial cardiac and hemodynamic effects in MI rats. Although the mechanism of action is unclear, and may be related to changes in Na^+,K^+-ATPase or neurohumoral conditions, it was apparent that the delivery regimen greatly influenced the response to this drug. Nelissen-Vrancken *et al*. suggested this is related to the very short half-life of ouabain (45 minutes in rats as opposed to 22 hours in humans). Its beneficial actions became apparent only when effective levels were maintained for prolonged periods, as was achieved using ALZET pumps in this study. Nelissen-Vrancken *et al*. also concluded that, contrary to popular belief, this rat model is suitable for investigation of the effects of cardiac glycosides in heart failure.

1 Nelissen-Vrancken HJMG, Wang J-F, Struijker-Boudier HAJ, Schoemaker RG and Smits JFM. *Naunyn-Schmiedeberg's Arch Pharmacol* 1997;356:203-209.

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New Agents in the ALZET Literature

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Agent	Therapeutic Category
Goralatide	Hemopoiesis Regulator
606A	Angiotensin AT_1-Receptor Antagonist
PDC	Glutamate Uptake Inhibitor
Oxidized Low-density Lipoprotein	Lipoprotein
CHF-1024	D_2-dopaminergic receptor/ α_2-adrenoceptor Agonist
5-Thioglucose	Glucose Analog, Glucoprivation Inducer
Antibody, anti-interferon gamma	Immunologic
Moxonidine	Antihypertensive
Capsaicin	Topical Analgesic
Doxazocin	Antihypertensive
Felodipine	Antihypertensive; Antianginal
GDNF	Neurotrophic Factor
Interleukin-12	Cytokine
Mesulergine	5-HT_{2C} Receptor Antagonist

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