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Feline Immunodeficiency Virus as a Gene Transfer Vector in the Rat Nucleus Tractus Solitarii

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Abstract

Gene transfer has been used to examine the role of putative neurotransmitters in the nucleus tractus solitarius (NTS). Most such studies used adenovirus vector-mediated gene transfer although adenovirus vector transfects both neuronal and non-neuronal cells. Successful transfection in the NTS has also been reported with lentivirus as the vector. Feline immunodeficiency virus (FIV), a lentivirus, may preferentially transfect neurons and could be a powerful tool to delineate physiological effects produced by altered synthesis of transmitters in neurons. However, it has not been studied in NTS. Therefore, we sought to determine whether FIV transfects rat NTS cells and to define the type of cell transfected. We found that injection of FIV encoding LacZ gene (FIVLacZ) into the NTS led to transfection of numerous NTS cells. Injection of FIVLacZ did not alter immunoreactivity (IR) for neuronal nitric oxide synthase, which we have shown resides in NTS neurons. A majority ($91.7 \pm 3.9\%$) of transfected cells contained IR for neuronal nuclear antigen, a neuronal marker; $2.1 \pm 3.8\%$ of transfected cells contained IR for glial fibrillary acidic protein, a glial marker. No transfected neurons or fibers were observed in the nodose ganglion, which sends afferents to the NTS. We conclude that FIV almost exclusively transfects neurons in the rat NTS from which it is not retrogradely transported. The cell-type specificity of FIV in the NTS may provide a molecular method to study local physiological functions mediated by potential neurotransmitters in the NTS.

Keywords

Feline immunodeficiency viral vector; Gene transfer; Neuronal marker; Nucleus tractus solitarii

Introduction

The nucleus tractus solitarii (NTS) plays an important role in central regulation of cardiovascular, digestive, gustatory, and respiratory functions (Kalia and Sullivan 1982; Kalia and Mesulam 1980). Many potential neurotransmitters are present in the NTS, and some of them are implicated in modulating these functions (Lawrence and Jarrott 1996; Bradley et al. 1996). Recently, gene transfer using replication-deficient recombinant viral vectors to up- or down-regulate neurotransmitters has been used to examine the role of putative neurotransmitters in the NTS (Chen et al. 2006; Hirooka et al. 2003; Sakai et al. 2000). For example, injection of adenovirus vector (Ad) encoding endothelial nitric oxide synthase (eNOS) into the NTS to increase production of nitric oxide (NO) decreased heart rate and blood pressure (Hirooka et al. 2003; Sakai et al. 2000). Also, injection of Ad carrying the DNA sequence coding small hairpin RNA for angiotensin type 1 into the NTS caused a decrease in blood pressure with no effect on heart rate (Chen et al. 2006). Another class of viral vector, lentivirus, appears to be a very promising tool for gene transfer into the brain. Lentiviruses have been shown to transduce signals effectively in NTS cells (Coleman et al. 2003), and studies have shown that feline immunodeficiency virus (FIV), a type of lentivirus, may be an effective tool for transduction in gene therapy (Toupet et al. 2008). Although both Ad and FIV have been considered promising vectors for gene transfer, they exhibit unique characteristics. While Ad transfects both neuronal and glial cells types (Davidson et al. 1993), FIV has been found to preferentially transfect neurons in some areas of the brain (Alisky et al. 2003; Sinnayah et al. 2002). What type of cell a vector transfects may be important in dissecting how a neurotransmitter system works when that vector is used to up-regulate or down-regulate a neurotransmitter in a specific area of the brain. FIV's capacity to transduce signals in neurons exclusively would be a powerful tool in delineating the role of certain neurotransmitters such as neuronal nitric oxide synthase (nNOS), which in the NTS is only found in neurons (Lin et al. 2007; Lin and Talman 2005) while endogenous eNOS is found in other cells in the same region (Lin et al. 2007). In the brain stem, lentivirus has been shown to preferentially transfect neurons in the hypoglossal and dorsal vagal motor nuclei (Duale et al. 2005). The purpose of the current study was to test the hypothesis that FIV transduces signals in neurons in preference to glia in the rat NTS. We utilized immunofluorescent labels of neurons and glia to differentiate cell types and examined whether the expression of nNOS is altered after injection of FIV. Furthermore, we also studied if nNOS, which is expressed in NTS neurons (Lin et al. 2007; Lin and Talman 2005), and not glia, is expressed in FIV-transfected NTS neurons. Finally, we examined the nodose ganglion (NG) for evidence of transfection after injection of FIV vector into the NTS in an effort to determine whether there is uptake and retrograde transport of the vector from NTS to the NG, the site of sensory afferent neurons that send arterial baroreceptor, chemoreceptor, and cardiopulmonary afferent axonal projections to the NTS (Dampney 1994).

Methods

Viral Vectors

A replication-deficient FIV vector encoding nuclear-targeted LacZ gene (FIVLacZ) driven by cytomegalovirus (CMV) promoter was generated and titered by the University of Iowa the Gene Transfer Vector Core as described in detail previously (Brooks et al. 2002; Johnston et al. 1999). The vector was generated by inserting CMV-LacZ into the pVET_LFIV backbone and produced through a triple transfection of a shuttle, a backbone, and an envelope vector (Brooks et al. 2002). The titer of FIV vectors used in the current study was 4×10^9 pfu/ml.

Animals and Injections

All procedures conformed to standards established in the *Guide for Care and Use of Laboratory Animals* (National Academy Press, Washington, DC 1996). The Institutional Animal Care and Use Committees of the University of Iowa and Department of Veterans Affairs Medical Center, Iowa City reviewed and approved all protocols. Both institutions are AAALAC accredited. All efforts were made to minimize the number of animals used and to avoid their experiencing pain or distress.

Adult male Sprague–Dawley rats (275–340 g) were anesthetized with halothane (5% induction followed by 1.5–2.0% maintenance) delivered in 100% O₂ (2 L/min) by a nasal mask. The dorsal surface of the brain stem was exposed, as previously described (Talman 1989), and a glass micropipette filled with viral vectors was stereotactically placed (0.4 mm rostral to the calamus scriptorius, 0.5 mm from the midline, and 0.5 mm below the surface of the brain stem) unilaterally into the dorsolateral and medial subnuclear regions of the NTS at the level of the area postrema. Injections (individual increments of 25–50 nl to a combined total of 200 nl) were made over 15 min, and the pipette was left in place for 15 additional minutes to limit efflux of injectate from the pipette track. After recovery from anesthesia, the animal was returned to the animal care facility and then euthanized 1 ($n = 2$), 2 ($n = 2$), 4 ($n = 3$), and 15 ($n = 3$) days after the injection. Procedures for euthanasia and perfusion of the rats were the same as described in our earlier publications (Lin et al. 2007; Lin and Talman 2005, 2006). The brain stem was then removed, postfixed in 4% paraformaldehyde for 2 h and then cryoprotected for 2 days in 30% sucrose in phosphate buffered saline (PBS) at 4°C. Frozen 20- μ m coronal sections were cut with a cryostat and processed for double-label immunofluorescent staining for β -galactosidase (β Gal, the protein encoded by LacZ gene) and either glial marker glial fibrillary acidic protein (GFAP) or neuronal marker neuronal nuclear antigen (NeuN), or in some cases for β Gal and nNOS. The ipsilateral nodose ganglion was removed after the rat was perfused as described earlier (Lin et al. 1998). It was then postfixed for 1 h, transferred to 30% sucrose overnight, cut, mounted to slides, and processed for double-label immunofluorescent staining for β Gal and protein gene product 9.5 (PGP 9.5), which is present in all neurons of the NG (unpublished observation). As a positive control for retrograde transport (Sinnayah et al. 2002; Vasquez et al. 2001), adenovirus encoding LacZ gene (AdLacZ) of similar titer was injected unilaterally into the NTS of other rats ($n = 3$). The AdLacZ vectors were generated and titered by the University of Iowa Gene Transfer Vector Core. The AdLacZ injected rats were euthanized 15 days after the injection, and the NGs removed and processed as described previously for FIV-LacZ injection.

Immunofluorescent Staining

Procedures similar to those in our previous publications (Lin et al. 2007; Lin and Talman 2005, 2006) were used for immunofluorescent staining. For double-label immunofluorescent staining for β Gal and NeuN, brain stem sections were washed with PBS and then blocked with 10% donkey normal serum (Jackson ImmunoResearch Labs, USA) in PBS at 25°C for 1 h. Sections were then incubated in a mixture of rabbit anti- β Gal antibody (1:200, Chemicon, USA) and mouse anti-NeuN (1:50, Chemicon) in 10% donkey normal serum for 24 h in a humid chamber at 25°C. The sensitivity and specificity of anti- β Gal and anti-NeuN antibodies have been shown and the antibodies used in many studies (McHugh et al. 2007; Lin et al. 2007; Iwata et al. 2004; Wells et al. 1998). After being washed with PBS, sections were incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG (1:200, Jackson ImmunoResearch Labs) and rhodamine red X (RRX)-conjugated donkey anti-mouse IgG (1:200, Jackson ImmunoResearch Labs) in PBS for 20–24 h at 4°C. Similar procedures were used for double-label immunofluorescent staining of β Gal and GFAP, except that mouse anti-GFAP antibody (1:100, Sigma, USA) replaced NeuN antibody. The sensitivity and specificity of anti-GFAP antibody have been shown and the antibody used in many studies

(Lin et al. 2007; Debus et al. 1983). Procedures for double-label immunofluorescent staining of β Gal and nNOS were similar to those described previously except that sheep anti-nNOS antibody (1:1000, K205, a generous gift from Dr. P. C. Emson) replaced NeuN antibody and RRX-conjugated anti-sheep IgG (1:200, Jackson ImmunoResearch Labs) replaced anti-mouse antibody. The sensitivity and specificity of K205 sheep anti-nNOS antibody have been reported, and the antibody has been used in a number of publications (Lin and Talman 2006; Lin et al. 2004; Simonian and Herbison 1996; Herbison et al. 1996). Secondary antibodies for GFAP and nNOS were generated in donkey (Jackson ImmunoResearch Labs).

Stained sections were washed, air-dried, and mounted with Prolong Gold Antifade Reagents (Invitrogen-Molecular Probes, USA). Negative controls consisted of tissue processed (a) in the absence of primary antibodies or (b) after addition of only one of the primary antibodies. Similar procedures were used for double-label immunofluorescent staining of β Gal and PGP9.5 of NG except that Guinea pig anti-PGP9.5 (1:100, Chemicon) replaced NeuN antibody, and RRX-conjugated donkey anti-Guinea pig IgG (1:200, ImmunoResearch Labs) replaced anti-mouse antibody. Stained NTS sections and NG sections were examined with a Zeiss confocal microscope as described in the following section.

Confocal Laser Scanning Microscopy

Procedures similar to those in our previous publications were used (Lin et al. 2007; Lin and Talman 2006). Labeled sections were analyzed with a Zeiss LSM 510 confocal laser scanning microscope. In brief, we scanned specimens sequentially by two channels to separate labels. For optimal visualization of the relationship between RRX- and FITC-labeled elements, images from two channels were assigned the pseudocolor red (for RRX) and green (for FITC), and then were superimposed. Confocal images were obtained and processed with software provided with the Zeiss LSM 510. Adobe Photoshop image editing software (Adobe Photoshop CS2) was used to examine whether a structure had single labeling or double labeling by switching between channels on the monitor. Another image editing program, Microsoft PowerPoint (2003), was used to create montages. Contrast and brightness of images were the only variables we adjusted digitally.

Cell Counts

We counted stained cells in images of 3–4 selected sections from each rat. We avoided analysis of sequential sections so that sections used for counting were at least 20 μ m (the thickness of the sections) apart. Therefore, we minimized the possibility that the same stained neurons would be counted twice. Stained cells in each section were counted three times, first using the green channel for the number of β Gal positive cells, then the red channel for NeuN or GFAP positive neurons, and finally the combined channel for double-stained neurons. The percentage of single- to double-labeled cells in each section was calculated after each section had been counted. Percentages of single- to double-labeled cells in NTS subnuclei were expressed as mean $\pm$ SD.

Results

FIVLacZ in NTS

In control experiments, no fluorescent staining of either RRX or FITC was apparent when the respective primary antibody had been omitted (figure not shown). Confocal analysis of immunofluorescent staining for β Gal revealed localized and site-specific transfection in the NTS after FIVLacZ (Fig. 1b) had been microinjected to the NTS. FIVLacZ-transfected cells, exhibiting β Gal-immunoreactivity (IR), in the NTS appeared to be distributed evenly in the NTS. In contrast, the contralateral side of the NTS showed very few cells that were positive for β Gal-IR. The number of transfected cells in the NTS increased from the first through the

fifteenth day after injection. We observed 13 ± 3 cells per section in the injected NTS (at the site of injection) at 1 day after the injection, 37 ± 4 cells at 2 days, 233 ± 26 cells at 4 days, and 346 ± 38 cells at 15 days after the injection.

FIVLacZ Expressing Cell Types in NTS

Double-label immunofluorescent staining showed that NTS cells were positive for β Gal-IR after FIVLacZ injection, and often they were also positive for NeuN-IR (Fig. 2a–c) but negative for GFAP-IR (Fig. 2d–f). Of the NTS cells that were positive for β Gal-IR, $91.7 \pm 3.9\%$ also contained NeuN-IR while only $2.1 \pm 3.8\%$ also contained GFAP-IR. This analysis did not include rats that were euthanized 1 and 2 days after the injection because of the limited number of transfected cells seen at those two times. However, as was found at 4 and 15 days, the majority of transfected cells in the NTS of rats euthanized 1 and 2 days after the injection of FIVLacZ were positive for NeuN-IR.

FIVLacZ and nNOS in NTS

We observed that the staining pattern and intensity of nNOS-IR in the injected side of the NTS was not different from that of the contralateral side after the injection of FIVLacZ (Fig. 1e, f) at any of the time points. In addition, when confocal images for β Gal-IR and nNOS-IR were merged, we noted that some NTS cells that were positive for β Gal-IR were also positive for nNOS-IR (Fig. 2g, h). For example, in the medial subnucleus $22.1 \pm 7.1\%$ β Gal-IR cells were nNOS-IR positive. NTS cells that were labeled for β Gal-IR alone and nNOS-IR alone were also observed.

FIVLacZ and AdLacZ in NG

We did not observe any cells or fibers positive for β Gal-IR in the NG at any time point after FIVLacZ had been injected into the NTS (Fig. 3a). In contrast, consistent with findings in other areas of the brain (Sinnayah et al. 2002; Vasquez et al. 2001), many cells and fibers stained for β Gal were observed in the ipsilateral NG (Fig. 3a) after injection of AdLacZ into the NTS (Fig. 3b). Most of these β Gal-IR positive cells and fibers were also labeled for PGP9.5-IR (Fig. 3d)

Discussion

Our study demonstrates that FIV vectors transfect signals in NTS cells and that the majority of transfected NTS cells are neurons as indicated by their expressing NeuN-IR. In addition, our study shows that FIV vectors are not retrogradely transported from NTS to NG neurons.

Our finding that FIV vectors preferentially transfect neurons in the NTS is consistent with results of earlier studies with FIV or other lentiviral vectors in the hypoglossal and dorsal vagal motor nuclei in the brain stem (Duale et al. 2005) and in other brain areas (Sinnayah et al. 2002; Naldini et al. 1996). The reason that FIV demonstrates such tropism is not well understood; however, some conjecture that the presence of receptors for the virus in neurons may play a role (Vigna and Naldini 2000). Though the mechanism of its cytoselectivity remains to be elucidated, this selectivity makes FIV, in contrast to Ad, which transfects mainly glia (Allen et al. 2006), a suitable virus for gene transfection in NTS neurons. That property of the virus would likely yield more interpretable results when studies are aimed at peptides that typically reside in neurons. For example, when seeking to study the role of nNOS within the NTS where the enzyme is found exclusively in neurons (Lin et al. 2007; Lin and Talman 2005), FIV would target the site of the endogenous enzyme.

After FIV was injected into the NTS, we did not see signs of FIV transfection in the NG; therefore, we found no evidence that it underwent retrograde transport from the NTS. The observation that FIV is not transported in axons is consistent with reports in other brain areas

(Alisky et al. 2003; Sinnayah et al. 2002). The restricted transfection of FIV to the site of injection may be useful when gene transfer confined to a well-defined area is desired. On the other hand, after AdLacZ was injected into the NTS, we observed its expression in NG neurons and fibers. This property of Ad, uptake by nerve terminals at the primary injection site and retrograde transport to afferent neurons, has been shown in other reports (Sinnayah et al. 2002; Vasquez et al. 2001). Therefore, Ad may be a vector of choice when seeking to deliver gene to specific neurons that are not easily approachable. For example, a subgroup of neurons in the subfornical organ can be targeted by retrograde transport of AdLacZ from the supraoptic nucleus (Sinnayah et al. 2002). In the NTS, Ad may be useful as a retrograde tracer, by itself or combined with other methods, to study areas that project to this nucleus; but, as others have emphasized, with cautious interpretation of results given the local effects of that vector on glia at its site of introduction (Allen et al. 2006). In contrast to Ad, FIV would be the vector of choice when targeting neurons at the site where the vector had been introduced. In the latter case, resulting physiological changes could be more reliably attributed to effects on those local neurons.

There are other unique properties of FIV as a vector in transgene expression of non-dividing cells. One such characteristic is that transgene expression using FIV vector lasts for a longer period of time. Our preliminary data (not shown) indicated that expression of FIV lasted for at least 5 weeks, and one published study has demonstrated that transfection with lentivirus lasts for at least 3 months (Naldini et al. 1996). In addition, FIV vectors do not produce cytotoxicity and inflammation, which are common with the Ad vector (Witlox et al. 2007; Lowenstein and Castro 2003; Amalfitano and Parks 2002). Furthermore, our data suggest that transfection through use of FIV does not alter the expression of neuronal enzymes that have not been targeted by the vector. Specifically, we show that neurons that were transfected by FIVLacZ were able to express nNOS. Thus, it appears that FIV vector can be used as a powerful gene-transfer tool to target specific modulating systems, such as the nitroxidergic and glutamatergic system (Talman and Lin 2006; Talman 1997), that are implicated in NTS control of physiological functions (Lawrence and Jarrott 1996).

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References

- Alisky JM, Hughes SM, Davidson BL. Transduction of neurons lining the cerebral external capsules in mice with feline immunodeficiency virus based vectors. *Neurosci Lett* 2003;351:120–124. [PubMed: 14583396]
- Allen AM, Dosanjh JK, Erac M, Dassanayake S, Hannan RD, Thomas WG. Expression of constitutively active angiotensin receptors in the rostral ventrolateral medulla increases blood pressure. *Hypertension* 2006;47:1054–1061. [PubMed: 16618838]
- Amalfitano A, Parks RJ. Separating fact from fiction: assessing the potential of modified adenovirus vectors for use in human gene therapy. *Curr Gene Ther* 2002;2:111–133. [PubMed: 12109210]
- Bradley RM, King MS, Wang L, Shu X. Neurotransmitter and neuromodulator activity in the gustatory zone of the nucleus tractus solitarius. *Chem Senses* 1996;21:377–385. [PubMed: 8670717]
- Brooks AI, Stein CS, Hughes SM, Heth J, McCray PM Jr, Sauter SL, Johnston JC, Cory-Slechta DA, Federoff HJ, Davidson BL. Functional correction of established central nervous system deficits in an animal model of lysosomal storage disease with feline immunodeficiency virus-based vectors. *Proc Natl Acad Sci USA* 2002;99:6216–6221. [PubMed: 11959904]
- Chen Y, Chen H, Hoffmann A, Cool DR, Diz DI, Chappell MC, Chen AF, Morris M. Adenovirus-mediated small-interference RNA for in vivo silencing of angiotensin AT1a receptors in mouse brain. *Hypertension* 2006;47:230–237. [PubMed: 16380517]

- Coleman JE, Huentelman MJ, Kasparov S, Metcalfe BL, Paton JF, Katovich MJ, Semple-Rowland SL, Raizada MK. Efficient large-scale production and concentration of HIV-1-based lentiviral vectors for use in vivo. *Physiol Genomics* 2003;12:221–228. [PubMed: 12488511]
- Dampney RAL. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994;74:323–364. [PubMed: 8171117]
- Davidson BL, Allen ED, Kozarsky KF, Wilson JM, Roessler BJ. A model system for in vivo gene transfer into the central nervous system using an adenoviral vector. *Nat Genet* 1993;3:219–223. [PubMed: 8387378]
- Debus E, Weber K, Osborn M. Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each of the neurofilament triplet polypeptides. *Differentiation* 1983;25:193–203. [PubMed: 6198232]
- Duale H, Kasparov S, Paton JF, Teschemacher AG. Differences in transductional tropism of adenoviral and lentiviral vectors in the rat brainstem. *Exp Physiol* 2005;90:71–78. [PubMed: 15542614]
- Herbison AE, Simonian SX, Norris PJ, Emson PC. Relationship of neuronal nitric oxide synthase immunoreactivity to GnRH neurons in the ovariectomized and intact female rat. *J Endocrinol* 1996;8:73–82.
- Hirooka Y, Sakai K, Kishi T, Ito K, Shimokawa H, Takeshita A. Enhanced depressor response to endothelial nitric oxide synthase gene transfer into the nucleus tractus solitarii of spontaneously hypertensive rats. *Hypertens Res* 2003;26:325–331. [PubMed: 12733701]
- Iwata N, Mizukami H, Shirotani K, Takaki Y, Muramatsu S, Lu B, Gerard NP, Gerard C, Ozawa K, Saido TC. Presynaptic localization of neprilysin contributes to efficient clearance of amyloid-beta peptide in mouse brain. *J Neurosci* 2004;24:991–998. [PubMed: 14749444]
- Johnston JC, Gasmi M, Lim LE, Elder JH, Yee JK, Jolly DJ, Campbell KP, Davidson BL, Sauter SL. Minimum requirements for efficient transduction of dividing and nondividing cells by feline immunodeficiency virus vectors. *J Virol* 1999;73:4991–5000. [PubMed: 10233961]
- Kalia M, Mesulam M-M. Brain stem projections of sensory and motor components of the vagus complex in the cat: I. the cervical vagus and nodose ganglion. *J Comp Neurol* 1980;193:435–465. [PubMed: 7440777]
- Kalia M, Sullivan JM. Brainstem projections of sensory and motor components of the vagus nerve in the rat. *J Comp Neurol* 1982;211:248–264. [PubMed: 7174893]
- Lawrence AJ, Jarrott B. Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius. *Prog Neurobiol* 1996;48:21–53. [PubMed: 8830347]
- Lin LH, Talman WT. Nitroxidergic neurons in rat nucleus tractus solitarii express vesicular glutamate transporter 3. *J Chem Neuroanat* 2005;29:179–191. [PubMed: 15820620]
- Lin LH, Talman WT. Vesicular glutamate transporters and neuronal nitric oxide synthase colocalize in aortic depressor afferent neurons. *J Chem Neuroanat* 2006;32:54–64. [PubMed: 16735103]
- Lin LH, Cassell MD, Sandra A, Talman WT. Direct evidence for nitric oxide synthase in vagal afferents to the nucleus tractus solitarii. *Neuroscience* 1998;84:549–558. [PubMed: 9539225]
- Lin LH, Edwards RH, Fremereau RT, Fujiyama F, Kaneda K, Talman WT. Localization of vesicular glutamate transporters colocalizes with and neuronal nitric oxide synthase in rat nucleus tractus solitarii. *Neuroscience* 2004;123:247–255. [PubMed: 14667459]
- Lin LH, Taktakishvili O, Talman WT. Identification and localization of cell types that express endothelial and neuronal nitric oxide synthase in the rat nucleus tractus solitarii. *Brain Res* 2007;1171:42–51. [PubMed: 17761150]
- Lowenstein PR, Castro MG. Inflammation and adaptive immune responses to adenoviral vectors injected into the brain: peculiarities, mechanisms, and consequences. *Gene Ther* 2003;10:946–954. [PubMed: 12756415]
- McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 2007;317:94–99. [PubMed: 17556551]
- Naldini L, Blomer U, Gage FH, Trono D, Verma IM. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci USA* 1996;93:11382–11388. [PubMed: 8876144]

- Sakai K, Hirooka Y, Matsuo I, Eshima K, Shigematsu H, Shimokawa H, Takeshita A. Overexpression of eNOS in NTS causes hypotension and bradycardia in vivo. *Hypertension* 2000;36:1023–1028. [PubMed: 11116119]
- Simonian SX, Herbison AE. Localization of neuronal nitric oxide synthase-immunoreactivity within subpopulations of noradrenergic A1 and A2 neurons in the rats. *Brain Res* 1996;732:247–252. [PubMed: 8891292]
- Sinnayah P, Lindley TE, Staber PD, Cassell MD, Davidson BL, Davisson RL. Selective gene transfer to key cardiovascular regions of the brain: comparison of two viral vector systems. *Hypertension* 2002;39:603–608. [PubMed: 11882616]
- Talman WT. Kynurenic acid microinjected into the nucleus tractus solitarius of rat blocks the arterial baroreflex but not responses to glutamate. *Neurosci Lett* 1989;102:247–252. [PubMed: 2682388]
- Talman WT. Glutamatergic transmission in the nucleus tractus solitarii: from server to peripherals in the cardiovascular information superhighway. *Braz J Med Biol Res* 1997;30:1–7. [PubMed: 9222396]
- Talman, WT.; Lin, LH. Glutamatergic and nitroxidergic neurotransmission in the nucleus tractus Solitarii. In: Misu, Y.; Goshima, Y., editors. *Neurobiology of DOPA as a neurotransmitter*. CRC Press; Boca Raton: 2006. p. 203-216.
- Toupet K, Compan V, Crozet C, Mourton-Gilles C, Mestre-Frances N, Ibos F, Corbeau P, Verdier JM, Perrier V. Effective gene therapy in a mouse model of prion diseases. *PLoS ONE* 2008;3:e2773. [PubMed: 18648643]
- Vasquez EC, Beltz TG, Haskell RE, Johnson RF, Meyrelles SS, Davidson BL, Johnson AK. Adenovirus-mediated gene delivery to cells of the magnocellular hypothalamo-neurohypophyseal system. *Exp Neurol* 2001;167:260–271. [PubMed: 11161614]
- Vigna E, Naldini L. Lentiviral vectors: excellent tools for experimental gene transfer and promising candidates for gene therapy. *J Gene Med* 2000;2:308–316. [PubMed: 11045424]
- Wells DJ, Maule J, McMahon J, Mitchell R, Damien E, Poole A, Wells KE. Evaluation of plasmid DNA for in vivo gene therapy: factors affecting the number of transfected fibers. *J Pharm Sci* 1998;87:763–768. [PubMed: 9607956]
- Witlox MA, Lamfers ML, Wuisman PI, Curiel DT, Siegal GP. Evolving gene therapy approaches for osteosarcoma using viral vectors: review. *Bone* 2007;40:797–812. [PubMed: 17189720]

Abbreviations

Ad	Adenovirus vector
AdLacZ	Adenovirus vector with LacZ gene
β Gal	β galactosidase
Ce	Central subnucleus
CMV	promotor Cytomegalovirus promotor
DMV	Dorsal motor nucleus of vagus
FITC	Fluorescein-5-isothiocyanate
FIV	Feline immunodeficiency virus vector
FIVLacZ	FIV vector with LacZ gene
Gr	Gracilus nucleus
GFAP	Glial fibrillary acidic protein
LacZ	Gene encodes β galactosidase
NeuN	Neuronal nuclear antigen
NG	Nodose ganglion
Nnos	Neuronal nitric oxide synthase

PGP9.5	Protein gene product 9.5
NTS	Nucleus tractus solitarii
PBS	Phosphate buffered saline
RRX	Rhodamine red X
Tr	Tractus solitarius

Fig. 1.

Confocal images (*in gray scale*) of the rat NTS after FIVLacZ had been injected unilaterally into this nucleus, which then was subjected to immunofluorescent staining for β Gal (**a, b**) and nNOS (**e, f**). Numerous cells positive for β Gal-IR are observed in the injected side (**b**). A few transfected cells in the adjacent areas, such as the dorsal motor nucleus of vagus (DMV), area postrema (AP), and gracilis nucleus (Gr) were also noted. The contralateral NTS (**a**) shows almost no transfected cells. The schematic sketches of **a** and **b** are shown in **c** and **d**, respectively. **e** and **f** show confocal images of nNOS-IR of the same section as **a** and **b**, respectively. Note that the staining pattern and distribution of nNOS-IR in the NTS and in

adjacent areas in the injected side (**f**) are similar to that of the contralateral side (**e**).

Abbreviation: Tr, tractus solitarius. *Scale bar* = 100 μ m

Fig. 2.

Pseudocolored confocal images of rat NTS after FIVLacZ (a–f) had been injected into this nucleus, which was then subjected to double-label immunofluorescent staining for β Gal (green) and NeuN (red), or for β Gal (green) and GFAP (red), or for β Gal (green) and nNOS (red). **c** is a merged confocal image of **a** (FIVLacZ transfected, green) and **b** (NeuN-IR, red). White arrows in **a–c** indicate representative cells that are stained for both β Gal-IR and NeuN-IR and thus appear yellow in the merged image. Note that the majority of FIVLacZ transfected NTS cells also contained NeuN-IR. **f** is the merged confocal image of **d** (FIVLacZ transfected, green) and **e** (GFAP-IR, red). Blue arrows in **d** and **f** indicate cells that are positive for β Gal-IR alone. Empty white arrows in **e** and **f** indicate cells or processes that are positive for GFAP-IR alone. The majority of FIVLacZ-transfected NTS cells do not contain GFAP-IR. **i** is a merged confocal image of **g** (FIVLacZ transfected, green) and **h** (nNOS-IR, red). White

arrows in **g–i** indicate cells that are stained for both β Gal-IR and nNOS-IR. *Scale bar* = 20 μ m

Fig. 3. Confocal images (*in gray scale*) of the ipsilateral NG with immunofluorescent staining for β Gal and PGP9.5 after FIVLacZ or AdLacZ had been injected unilaterally into the NTS. Neither cells nor fibers labeled for β Gal-IR are seen in the ipsilateral NG after FIVLacZ had been injected to the NTS, as shown in **a**. **c** is a confocal image of PGP9.5-IR of the same section to show the structure of the NG section in **a**. **b** is a confocal image of NG showing cells (indicated by *arrows*) and fibers expressing β Gal-IR after injection of AdLacZ into the NTS. **d** is confocal image of PGP9.5 showing some NG cells (*arrows*) that are double labeled for β Gal-IR and PGP9.5-IR. Scale bar = 100 μ m