Experimental Physiology – *Symposium Report*

Targeting specific neuronal populations using adeno- and lentiviral vectors: applications for imaging and studies of cell function

A. G. Teschemacher[1], S. Wang[2], T. Lonergan[2], H. Duale[2], H. Waki[2], J. F. R. Paton[2] and S. Kasparov[2]

[1]*Departments of Pharmacology and* [2]*Physiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK*

We employ viral vectors to address questions related to the function of specific types of neurones in the central control of blood pressure. Adenoviral vectors (AVVs) or lentiviral vectors (LVVs) can be used to visualize specifically living GABAergic or noradrenergic (NAergic) neurones or to interfere with intracellular signalling within these cell types. Here, we review recent *in vitro*, *in situ* and *in vivo* applications of these vectors in the rat brainstem as performed in our laboratories. In organotypic slice cultures prepared from defined cardiovascular brainstem areas, viral vectors were used to study the electrophysiological properties, intracellular signalling and gene expression in selected neuronal phenotypes. *In vivo*, vectors were microinjected into brainstem nuclei to inhibit specific aspects of cell signalling by expression of dominant negative proteins, for example. Outcomes for cardiovascular control were measured either acutely *in situ* or chronically *in vivo* with radio telemetry in freely moving rats. We showed that AVVs and LVVs have distinct properties that need to be considered prior to their application. For example, LVVs can be manufactured very quickly, have no immunogenicity and can be pseudotyped to display higher tropism for neurones than glia. However, comparatively lower production yields of LVVs may limit their use for some types of applications. In contrast, AVVs require a lengthy construction period, are easy to amplify to high yields at moderate cost but may trigger an immune response when used at high titres *in vivo*. These features make AVVs particularly suitable for *in vitro* applications. As the two vector types complement each other in several ways we generated a shuttle system that simplifies transfer of transgene cassettes between the backbones of AVVs and LVVs. Thus, AVVs and LVVs are powerful experimental tools that can be used in a variety of experimental designs *in vivo, in situ* and *in vitro*.

(Received 1 October 2004; accepted after revision 1 November 2004; first published online 12 November 2004)
Corresponding author A. G. Teschemacher: Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK. Email: Anja.Teschemacher@bristol.ac.uk

Viral vectors for cell-type specific transgenesis within the brainstem

Viral gene transfer in the central nervous system not only holds promise for the future in gene therapy of brain disorders but is also emerging as a powerful research tool for neuroscience. The function of every area of the brain depends on a concerted action of a variety of cellular phenotypes, including neurones with different neurochemical profiles, glial cells and local vasculature. To investigate the function of specific cell types within the brainstem, which are thought to play crucial roles in primary hypertension, we employed two types of viral vectors (Kasparov *et al.* 2004*a*). In this review, we describe a range of novel experimental approaches that we have developed to study both short-term modulation and long-term changes in intracellular signalling of GABAergic and NAergic neurones. This spectrum of techniques enables us to address a range of questions ranging from single-cell to whole animal cardiovascular system physiology.

Successful transgene expression in a certain type of cell depends on transductional and transcriptional factors. Transductional tropism of a vector determines the efficacy of delivery of a transgene into the cell type of interest. We use first-generation AVVs and

 DOI: 10.1113/expphysiol.2004.028191

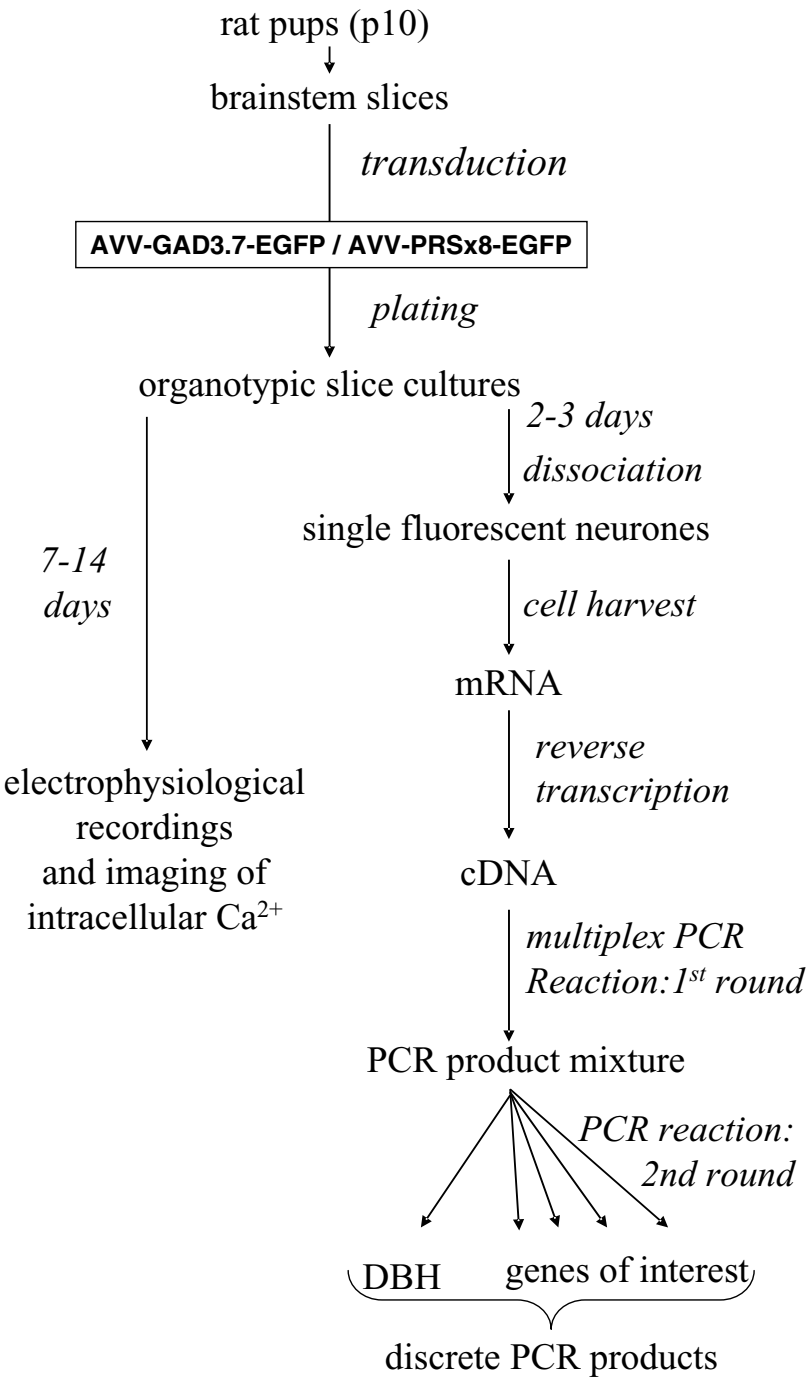


Figure 1. Application of viral vectors *in vitro* for studying single-cell physiology and gene expression
Transduction may be achieved either by 1 h of pre-incubation of slices in AVVs in dissection saline [Hanks' balanced salt solution (HBSS) without Ca^{2+} (Gibco 14175) with added glucose (total 25.6 mM), 10 mM $MgCl_2$, 1 mM Hepes, 1 mM kynurenic acid, 0.005% phenol red, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin] or by addition of AVVs to the wells at the time of plating [plating media: 50% Optimem-1 (Gibco 31985), 25% fetal bovine serum, 21.5% HBSS; 2.5% 1 M glucose, 1% penicillin and streptomycin 100 × (Sigma P-0781)]. With either transduction protocol an endpoint concentration of about 10^8 TU ml^{-1} is used. It should be noted that LVVs prove to be far less efficient under similar conditions and transduction benefits from incubation in plating media for 1 h at room temperature at 10^7 TU ml^{-1}. Owing to the comparatively low yield of LVVs preparations, increasing the endpoint concentration at the time of exposure is not a practical option. For tissue dissociation, brainstem areas containing EGFP-expressing NAergic neurones are punched from the slice cultures using a nuclease-free Pasteur pipette and expelled into 2 ml of pre-incubated (37°C for 30 min) digestion mix [dissection saline (see above) with 30 U ml^{-1} papain and 1.35 mg ml^{-1} L-cysteine (Sigma, cell culture grade)]. Following digestion at 37°C on an orbital shaker

vesicular stomatitis virus G (VSVG) glycoprotein-pseudotyped LVVs as previously described (Coleman *et al.* 2003; Graham & Prevec, 1995). Both vector types target neurones although AVVs transduce other cell types such as glia more readily than do LVVs (see Akli *et al.* 1993; Coleman *et al.* 2003; Davidson *et al.* 1993; Duale *et al.* 2005). In the rat brainstem, various neuronal populations, including NAergic neurones, are successfully transduced by AVVs as well as by LVVs (Duale *et al.* 2005). One prominent transductional difference between the two vector types is that AVVs will readily transduce axon terminals and can therefore be used for retrograde transduction when microinjected *in vivo* (Bilang-Bleuel *et al.* 1997; Kasparov *et al.* 2004*a*; Nishimura *et al.* 1998). In contrast, VSVG -coated LVVs transduce somata locally only in the area of microinjection (Mazarakis *et al.* 2001; Sinnayah *et al.* 2002).

On a practical note, although construction of a novel aclenoviral vector (AVV) takes several weeks to months, amplification of the vector is rapid thereafter and results in high yields of around 10^{11} transforming units (TU) within 2 weeks. In contrast, optimized production of lentiviral vector (LVV) requires not more than 1 week but yields only about 10^8 TU per preparation (Coleman *et al.* 2003). This renders AVVs more appropriate for high-throughput experimental series, in particular *in vitro* (see below), and LVVs more suitable for rapid small-scale investigations and *in vivo* brain micro-injections. Thus, AVVs and LVVs have complementary advantages for different experimental requirements. As the two vector types complement each other, and in order to maximize our flexibility of transductional and transcriptional targeting (see below), we generated a shuttle system that simplifies transfer of transgene cassettes between AVVs and LVVs backbones. This allows us to study the impact of transductional vector properties on transgene expression in specific cell types in the brain. Full details of this system are published elsewhere in this issue (see Duale *et al.* 2005).

Following transduction, expression of the transgene in the particular cell type of interest depends on the activity of the promoter used in the expression cassette. So-called 'constitutively active' promoters such as the human cytomegalovirus immediate early promoter (hCMV) operate in many cell types and are particulary active in glia (see Duale *et al.* 2005; Kügler *et al.* 2003; Stokes *et al.* 2003; Tsuchiya *et al.* 2002). However, in many neuronal types hCMV-driven expression is either absent or not sustainable (Glover *et al.* 2003; Kügler *et al.* 2003; Lonergan *et al.* 2004). This may be due to a number of response elements present in the hCMV promoter that render it a highly active promoter in many cell types but inactive in some where transcriptional enhancers such as the cAMP response element binding protein (CREB) pathway are down-regulated (Wilkinson & Akrigg, 1992).

for 50–60 min, the tissue is pelleted by gentle centrifugation. The digestion mix is removed and replaced by trypsin inhibitor (1 mg ml^{-1}; Sigma) in dissection saline. The tissue is then washed once with external solution (155 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose; pH 7.4) and gently triturated in 200 μl of fresh external solution with three fire-polished Pasteur pipettes of decreasing tip diameter. The dissociated cells are then plated onto two 35-mm culture dishes thinly coated with a 1 : 6 dilution of Matrigel (Becton Dickinson, USA) and containing 1 ml of external solution. Cells are allowed to settle for about 20 min at room temperature before harvesting. Healthy EGFP-positive cells appear with a well-defined smooth membrane surface under fluorescence microscopy and a halo under phase contrast microscopy. Many cells retain some dendritic arbor. For single-cell harvesting, patch pipettes of $\sim$2 μm tip diameter are pulled, filled with 9 μl nuclease-free water, fitted to a micromanipulator and lowered onto an adhered EGFP-positive neurone. Gentle suction is applied to attach the neurone firmly to the pipette tip. The pipette is then quickly raised and its contents expelled into a reaction tube containing RNAsinPlus (Promega) and reverse transcription buffer (Qiagen). Tubes are placed on dry ice until completion of cell harvesting. The samples are then thawed and vortexed briefly, followed by RNA denaturation at 60°C for 5 min, and cooling on ice. dNTPs (Qiagen), oligo(dT)15 primers (Promega), and Sensicript® reverse transcriptase (Qiagen) are added and reverse transcription is carried out in a reaction volume of 20 μl for 60 min at 37°C. The cDNA samples are then stored at −20°C until analysed by PCR. By using a multiplex protocol (Phillips & Lipski, 2000), co-expression of genes of interest in NAergic cells is determined. Briefly, a first round of PCR amplification (35 cycles) is carried out with cDNA template in the presence of 2 × PCR mastermix (Promega) and a mixture of external primer sets for various transcription products, including one for the NAergic marker dopamine-β-hydroxylase (DBH). The first-round reaction product is divided into 10 reactions and, in the second amplification round (45 cycles), PCR is performed separately for each transcript of interest using nested or seminested primer sets (see Fig. 3). Primers are designed to span introns to exclude contamination by genomic DNA. A positive control [cDNA from tissue punches expressing DBH (e.g. rostral ventrolateral medulla)] and a negative control (no cDNA transcription) are run with each reaction. DBH-negative cells are excluded from further analysis. Dissociation is carried out using sterile procedures. Cell harvest and reverse transcription take place under nuclease-free conditions: certified nuclease-free plasticware is used and all glassware is washed twice with 100% ethanol, rinsed with nuclease-free water and baked twice for 4 h at 160°C. Work surfaces and equipment (microscope stage, micromanipulator controls, pipette holder) are wiped with RNAse Zap (Ambion Inc, TX, USA) and nuclease-free water.

By transcriptional targeting, transgene expression can be restricted to specific cell types within the brain. Some promoters developed for neuronal transgene expression such as synapsin 1 and tubulin-α1 promoters show negligible activity in non-neuronal cell types. However, they do not discriminate between different types of neurones and are not suitable for selective targeting of any particular neuronal phenotype.

For targeting GABAergic brainstem neurones we use 3.7 kbp of the 5′ regulatory sequence of the mouse glutamic acid decarboxylase (GAD) gene, in addition to the first intron and exon of GAD (Katarova *et al.* 1998; Teschemacher *et al.* 2005). NAergic neurones are targeted using the artificial PRSx8 promoter (Hwang *et al.* 2001; Kasparov *et al.* 2004*a*; Lonergan *et al.* 2004; Teschemacher *et al.* 2005). As detailed below, these promoters can express fluorescent proteins to allow visualization of specific neurones within brain slices for single-cell experiments. Alternatively, proteins which chronically affect intracellular signalling can be expressed in order to determine cardiovascular changes at systems level *in situ* and *in vivo*.

Application of viral vectors *in vitro* for studying single-cell physiology and gene expression

For investigations of different neuronal phenotypes involved in blood pressure regulation at a single-cell level we use organotypic slice cultures of cardiovascular control centres of the rat brainstem. Cultures are prepared by the static method (Stoppini *et al.* 1991) following previously published protocols (Teschemacher *et al.* 2005). In culture, cells traumatized during preparation either regenerate or are degraded; glial cells proliferate to cover slice surfaces and support survival of healthy neurones for several weeks. Transduction occurs immediately prior to or at the time of plating by exposure to high vector titres (about 10^8 TU ml^{-1} endpoint dilution; see Fig. 1). As immune responses do not limit vector concentrations in tissue explants, AVVs are more suitable than LVVs for use *in vitro*.

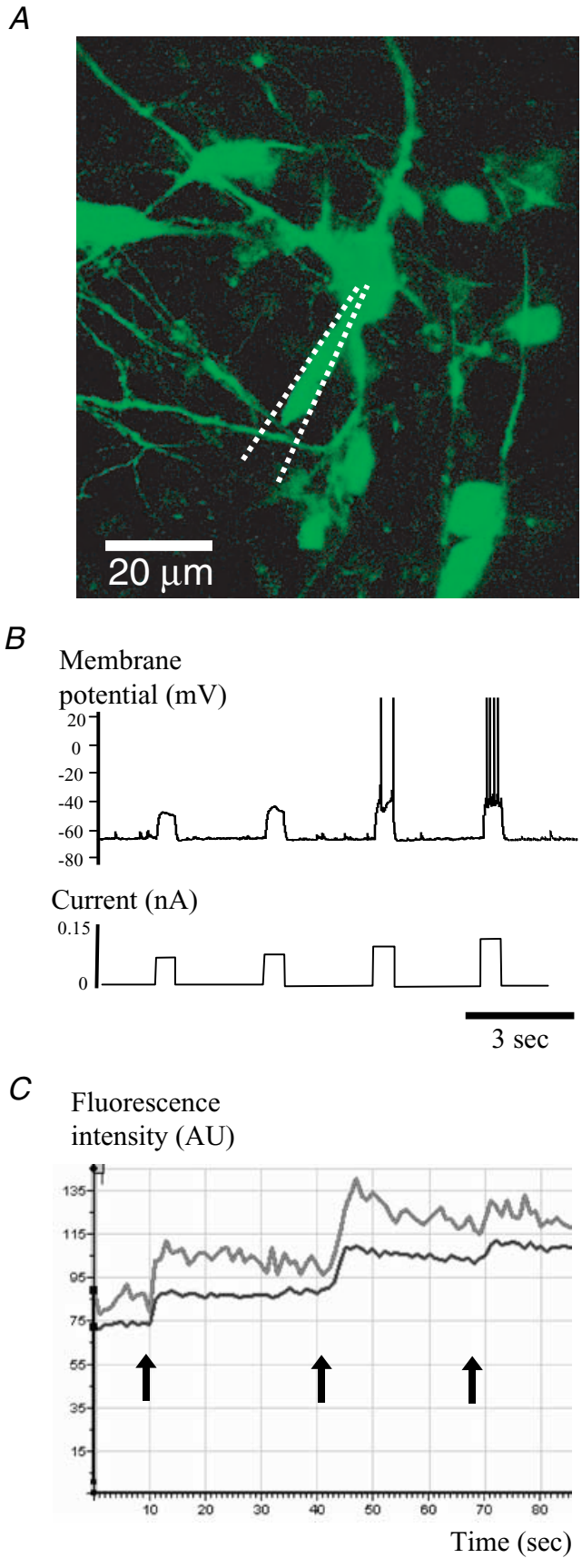
Depending on promoter strength and the time course of folding/maturation of the expressed protein, transgene expression plateaus within 2–3 days in culture and can be detected for several weeks afterwards.

Dynamic confocal imaging

Owing to the good optical properties of the organotypic cell culture membranes (Millipore) and the flattening of the tissue, which occurs over approximately 2 weeks in culture, very high-resolution confocal imaging of fluorescent neurones in slice cultures can be achieved (Kasparov *et al.* 2002; Teschemacher *et al.* 2005). For imaging, explants are excised together with the surrounding culture membrane and transferred to a glass-bottomed tissue chamber mounted on the microscope stage. The tissue is continuously superfused at 31 ± 1°C with artificial cerebrospinal fluid (Kasparov & Paton, 1999). All our imaging has been performed using an upright fixed-stage laser-scanning confocal microscope (Leica SP, Germany; see Kasparov & Paton, 2000; Teschemacher *et al.* 2005). The water immersion lenses used in this system have excellent optical characteristics – high numerical apertures and low optical distortions. For imaging in slice tissue, they are much more adequate than the long-working-distance air objectives used typically in scanners based on inverted microscopes. In addition, water immersion lenses also allow a clearer view of the upper surface of the slice, where fluorescent cells can be targeted by patch pipettes. Altogether, this set-up provides superior conditions for imaging such that ∼0.5-μm -wide objects, close to the theoretical limit of optical microscopy, may be visualized in three-dimensional (3-D) space (Kasparov *et al.* 2002) (see Fig. 2*A*). This technique is also well suited for imaging fluorescently tagged proteins and various signalling probes based on enhanced green fluorescent protein (EGFP) and its derivatives (Teschemacher *et al.* 2005).

Electrophysiological recordings from identified GABAergic neurones *in vitro*

The nucleus of the solitary tract (NTS) is an essential brainstem site that both generates cardiovascular activity and integrates cardiovascular afferent information (Paton *et al.* 2004; Paton & Kasparov, 2000; Spyer, 1994). GABA interneurones play an essential role in the NTS (Boscan *et al.* 2002; Durgam *et al.* 1999; Spyer, 1994; Vitela & Mifflin, 2001; Zhang & Mifflin, 1998) but had not previosly been possible to visualize them selectively in living tissue. We are now in a position to make electrophysiological recordings from identified NTS GABAergic interneurones. These are performed on the stage of the laser-scanning confocal microscope (see above). Slice cultures transduced with an AVVs containing the GAD3.7 promoter driving EGFP (AVV–GAD3.7–EGFP) are placed in the tissue chamber and an EGFP-expressing neurone is selected and patched. Patch pipettes are loaded with intracellular solution plus the Ca^{2+} indicator Rhod-2 (0.5 mM; tripotassium salt; Molecular Probes). The neurone to be patched is positioned centrally in the field of view using conventional epifluorescence microscopy. The patch pipette is prepositioned above the neurone close to the slice surface with a digitally controlled micromanipulator (Sutter Instruments, USA) under a 10 × objective. Confocal imaging with a 40 × objective is employed throughout the final stages of approach, seal formation and recording. The 488-nm wavelength



line of the argon laser is used to excite EGFP. The 633-nm HeNe laser line is used to obtain a differential interference contrast (DIC) image via a light detector placed beneath the recording chamber. DIC optics greatly facilitate visualization of the slice surface and the pipette tip. In order to confirm the identity of the target cell, EGFP and DIC channels are digitally overlaid on the computer screen prior to seal formation. The Rhod-2 dye is excited using a green HeNe line of 543 nm, typically at levels of < 0.2 mW, and Rhod-2-emitted light is sampled within the 560–640-nm band as defined by the spectral scanhead.

Once the whole-cell configuration has been established, the identity of the patched neurone can be confirmed by observing EGFP rising up into the patch pipette (Fig. 2*A*). Ca^{2+} imaging experiments commence after 20 min of whole-cell access to ensure equilibration of the indicator between the pipette and cytoplasm (Fig. 2*C*). The passive membrane properties of the recorded neurones can be evaluated by a series of hyperpolarizing and depolarizing currents delivered by the recording electrode (Fig. 2*B*). Depending on the experimental requirements, different scanning modes can be employed: a 2-D image can be taken every 1–10 s in *x*–*y*–*t* mode (the fastest commercially available scanners theoretically can take up to 20–50 images per second). When very fast events such as action potential-evoked Ca^{2+} transients need to be visualized, *x*–*t* line scans can be performed continuously at < 2.5 ms per scan. We can confirm that the same approach can be used to study selectively other targeted neuronal phenotypes (e.g. NAergic neurones) visualized by cell type-specific AVVs in slice culture.

Figure 2. Electrophysiological recordings from identified NTS GABAergic neurones
A, an angled view (10°) of the 3-D reconstruction of a confocal stack showing a patch pipette attached to a neurone transduced with AVV–GAD3.7–EGFP in an organotypic slice culture. Recordings are performed in whole-cell patch configuration using pipettes pulled to a resistance of 3–5 MΩ and filled with the following solution: 130 mM potassium gluconate, 10 mM Hepes, 5.5 mM EGTA, 4 mM NaCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM ATP, 0.5 mM GTP, 5 mM glucose; pH 7.3. For imaging of intracellular Ca^{2+}, the neurone is loaded by diffusion of 0.5 mM Rhod-2 from the pipette solution. Note that EGFP travels up into the pipette (contours of the pipette are indicated by white dotted lines), proving the identity of the recorded cell. Several other EGFP fluorescent neurones are visible in the background. Note that fine details such as small dendritic processes can be easily visualized. *B*, a trace of membrane potential of the EGFP-expressing GABAergic neurone shown in *A*. Depolarizations of the membrane were triggered by injections of depolarizing current via the recording pipette. Some spontaneous excitatory postsynaptic potentials are visible on the trace. *C*, intracellular Ca^{2+} concentration measurement in a GABAergic neurone. A neurone targeted using AVV–GAD3.7–EGFP was patched with a pipette containing the Ca^{2+} indicator Rhod-2. Step depolarizations caused by current injection via the recording electrode (black arrows) triggered Ca^{2+} elevations in the soma (lower trace) and in a proximal dendrite (upper trace) of that neurone.

Gene expression in single NAergic neurones

There is good evidence that one role for catecholamine-containing neurones within the NTS is in cardiovascular control (De Jong & Nijkamp, 1976; Kubo *et al.* 1987; Kubo & Misu, 1981). Reis *et al.* (1977) showed that pharmacological lesioning of catecholaminergic NTS neurones (the A2 cell group) produced a small increase in arterial pressure and caused instability. They suggested that A2 neurones facilitate baroreceptor activity in the NTS, consistent with a depressor response upon microinjection of noradrenaline in the NTS (De Jong & Nijkamp, 1976). In this regard, we have begun to study the role of these neurones in arterial pressure control in normo- and hypertensive animals. Furthermore, we have initiated gene-profiling studies of phenotypically identified A2 neurones.

Although high-throughput gene array analysis may provide information on the expression level of numerous genes in the whole brain or in an area of the brain, it cannot reveal the cellular source of the transcripts. In contrast, single-cell gene analysis helps to identify any disease-related genetic signatures within a known neuronal phenotype. To that end, cell-specific expression of fluorescent proteins allows single neurones of identified phenotype to be recovered individually. Their mRNA content can then be analysed using reverse transcription PCR. Slice cultures transduced with an AVVs containing the PRSx8 promoter driving EGFP (AVV–PRSx8–EGFP) are used after 3–5 days in culture when EGFP expression is maximal and before the increase in glial content interferes with the dissociation process. cDNA is produced by reverse transcription of the mRNA of single green fluorescent cells. In a multiplex PCR protocol (Phillips & Lipski, 2000), coexpression of genes of interest in NAergic cells is determined (see Fig. 3).

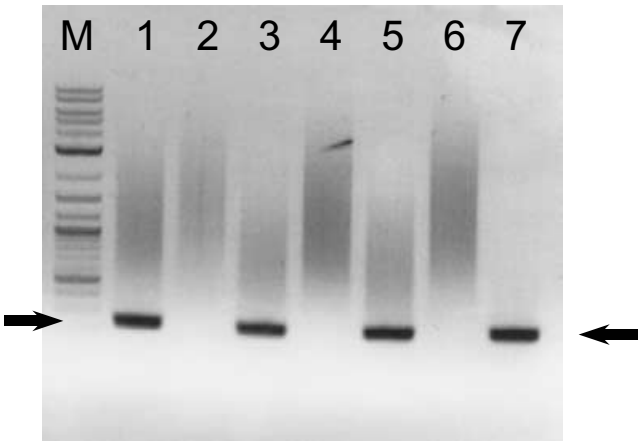


Figure 3. Gene expression in single NTS NAergic neurones *in vitro*
Single-cell reverse transcription PCR analysis of brainstem slice cultures transduced with AVV–PRSx8–EGFP. Neurones were dissociated and NAergic cells selected using a patch pipette (see Fig. 2 for details). Second-round PCR amplification products using DBH-specific seminested primers (modified from Phillips & Lipski, 2000) were run on an ethidium bromide-stained agarose (1%) gel. A band of the expected size was found in four of seven different single EGFP-positive cells (1–7) from a single harvesting session. Note that the lack of the expected PCR product can be due to the technical difficulties of cell sampling and does not necessarily mean that these cells were not NAergic. M: molecular marker. Arrows indicate the expected band size for the specific DBH amplification product.

Viral vector-mediated gene expression in discrete brainstem areas: application to whole animal systems physiology

Viral vectors have been used in our laboratories in two types of *in vivo* experiments:

(i) First, they can be injected into a particular brain nucleus of a rat and the outcome for blood pressure regulation and autonomic outflows studied chronically using radiotelemetry. Examples of this approach can be found in recent publications from our group (Waki *et al.* 2003*a*,*b*). In brief, radio transmitters are implanted under ketamine/medetomidine anaesthesia at least 7 days before any experimental protocol begins (Fig. 4). The tip of the catheter of the transmitter (TA11PA-C40; DSI) is inserted into the abdominal aorta caudal to the root of the left renal artery and held in place with tissue adhesive (Vetbond, 3M). The transmitter is sutured to the ventral wall of the abdominal cavity. Once the transmitter has been implanted blood pressure can be monitored by placing the animal cage on the receiver. For 7–10 days after the implantation the baseline cardiovascular parameters are evaluated. The animal is then re-anaesthetized and a viral vector is injected into the nucleus of interest (Fig. 4). For example, AVV–CMV–TeNOS, which expresses a dominant negative protein to the endothelial nitric oxide synthase, was used (Paton *et al.* 2001*a*; Waki *et al.* 2003*b*). The outcome of the viral gene transfer for mean arterial blood pressure, heart rate and activity of the sympathetic and parasympathetic autonomic outflows (derived from heart rate variability) is studied using an automated computer analysis package (Waki *et al.* 2004). The sites of injection can be marked by co-injection of a vector that encodes for a fluorescent protein for *post hoc* histological verification.

(ii) Second, animals may be pre-injected with a vector to express a dominant negative molecule or express another biologically active protein. After 5–7 days, when the expression reaches its maximum, they can be used in acute experiments. Examples of this approach can be found in Paton *et al.* (2001*a*) and Wong *et al.* (2002). In these studies pretransfected animals were used for generating *in situ* working heart–brainstem preparations (Paton, 1996). A variety of transgenes were expressed in order to establish the intracellular signalling pathway between angiotensin II

type 1 receptors and endothelial nitric oxide synthase in the NTS for baroreceptor reflex modulation.

Importantly, in the work cited above we used AVVs without cellular targeting specificity. More recently it has become evident, however, that for future analysis it is essential to refine this approach and to target our genetic manipulations to specific cellular phenotypes as characterized *in vitro* (see also Kasparov *et al.* 2004*a*,*b*) and *in situ* (Deuchars *et al.* 2000; Paton *et al.* 2001*b*,*c*).

Furthermore, LVVs have definite advantages for *in vivo* applications. As small vector quantities are sufficient for brain microinjections, the relatively low production yields with LVVs are not rate limiting. In addition, a lack of immune responses to LVVs is preferable to the inherent risk posed by high local AVVs titres (Duale *et al.* 2005).

Conclusion

We have briefly reviewed some of the applications for viral gene transfer in studies of central cardiovascular control that have evolved in our laboratory during the last few years. Our research has prompted us to refine our methods towards directing genetic manipulations to specific subsets of cells within the brainstem, rather than

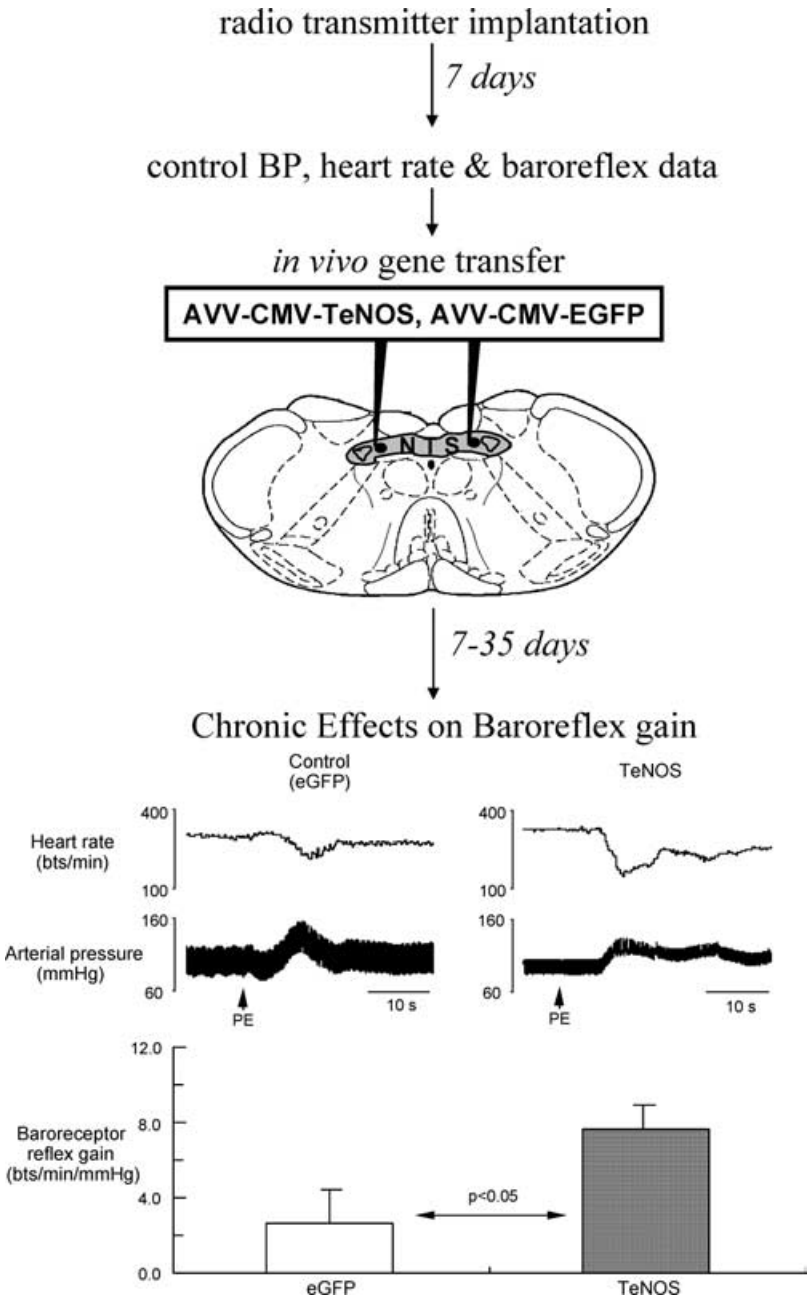


Figure 4. *In vivo* AVVs gene transfer into the NTS to express a dominant negative protein to block endogenous endothelial nitric oxide synthase (eNOS) activity
Rats are implanted with a radio transmitter for remote measurement of pulsatile arterial blood pressure (BP). Following recovery from surgery control data are obtained including cardiac baroreceptor reflex gain using either the spontaneous baroreceptor reflex method (see Waki *et al.* 2003*a*, *b*) or conventional intravenous injection of phenylephrine (PE). Animals are then re-anaesthetized and a bilateral microinjection of AVV is made into the caudal NTS. The experiment shown here used AVV–CMV–TeNOS. TeNOS is a truncated form of eNOS that acts as a dominant negative protein to block endogenous eNOS activity. The effect of viral transduction was to increase cardiac baroreceptor reflex gain, suggesting an essential role for eNOS in the NTS for governing the sensitivity of the cardiac baroreceptor reflex. Reproduced from Waki *et al.* (2003*b*), with permission.

applying them nondiscriminately. As described here, both AVVs and LVVs prove to be valuable tools for achieving neuronal phenotype-specific transgenesis, which can be used in a variety of experimental approaches *in vivo*, *in situ* and *in vitro*. As both types of vectors have unique properties, we have created a system to facilitate a one-step exchange of expression cassettes between the viral backbones (see Duale *et al.*, 2005, in this issue). We hope that the procedures described herein will advance our understanding of the physiology, intracellular signalling and gene expression profiles of specific subtypes of neurones in brainstem circuits controlling blood pressure in health and disease. Finally, we believe that only through a multidisciplinary, collaborative approach we can discover novel approaches for controlling arterial pressure.

References

Akli S, Caillaud C, Vigne E, Stratford-Perricaudet LD, Poenaru L, Perricaudet M, Kahn A & Peschanski MR (1993). Transfer of a foreign gene into the brain using adenovirus vectors. *Nature Genet* **3**, 224–228.

Bilang-Bleuel A, Revah F, Colin P, Locquet I, Robert J-J, Mallet J & Horellou P (1997). Intrastriatal injection of an adenoviral vector expressing glial-cell- line-derived neurotrophic factor prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc Natl Acad Sci USA* **94**, 8818–8823.

Boscan P, Kasparov S & Paton JF (2002). Somatic nociception activates NK1 receptors in the nucleus tractus solitarii to attenuate the baroreceptor cardiac reflex. *Eur J Neurosci* **16**, 907–920.

Coleman JE, Huentelman MJ, Kasparov S, Metcalfe BL, Paton JF, Katovich MJ, Semple-Rowland SL & Raizada MK (2003). Efficient large-scale production and concentration of HIV-1-based lentiviral vectors for use in vivo. *Physiol Genomics* **12**, 221–228.

Davidson BL, Allen ED, Kozarsky KF, Wilson JM & Roessler BJ (1993). A model system for in vivo gene transfer into the central nervous system using an adenoviral vector. *Nature Genet* **3**, 219–223.

De Jong W & Nijkamp FP (1976). Centrally induced hypotension and bradycardia after administration of alpha-methylnoradrenaline into the area of the nucleus tractus solitarii of the rat. *Br J Pharmacol* **58**, 593–598.

Deuchars J, Li Y-W, Kasparov S & Paton JFR (2000). Morphological and electrophysiological properties of neurones in the dorsal vagal complex of the rat activated by arterial baroreceptors. *J Comparative Neurol* **417**, 233–249.

Duale H, Kasparov S, Paton JF & Teschemacher AG (2005). Differences in transductional tropism of adenoviral and lentiviral vectors in the rat brainstem. *Exp Physiol* **90**, 71–78.

Durgam VR, Vitela M & Mifflin SW (1999). Enhanced gamma-aminobutyric acid-B receptor agonist responses and mRNA within the nucleus of the solitary tract in hypertension. *Hypertension* **33**, 530–536.

Glover CP, Bienemann AS, Hopton M, Harding TC, Kew JN & Uney JB (2003). Long-term transgene expression can be mediated in the brain by adenoviral vectors when powerful neuron-specific promoters are used. *J Gene Med* **5**, 554–559.

Graham FL & Prevec L (1995). Methods for construction of adenovirus vectors. *Mol Biotechnol* **3**, 207–220.

Hwang D-Y, Carlezon WA Jr, Isacson O & Kim K-S (2001). A high-efficiency synthetic promoter that drives transgene expression selectively in noradrenergic neurons. *Human Gene Ther* **12**, 1731–1740.

Kasparov S & Paton JFR (1999). Differential effects of angiotensin II in the nucleus tractus solitarius – II. Plausible neuronal mechanisms. *J Physiol* **521**, 227–238.

Kasparov S & Paton JFR (2000). Somatic gene transfer: implications for cardiovascular control. *Exp Physiol* **85**, 747–755.

Kasparov S, Teschemacher AG, Hwang DY, Kim KS, Lonergan T & Paton JF (2004*a*). Viral vectors as tools for studies of central cardiovascular control. *Prog Biophys Mol Biol* **84**, 251–277.

Kasparov S, Teschemacher A & Paton JFR (2002). Dynamic confocal imaging in acute brain slices and organotypic slice cultures using a spectral confocal microscope with single photon excitation. *Exp Physiol* **87**, 715–724.

Kasparov S, Teschemacher AG & Paton JFR (2004*b*). Application of viral gene transfer in studies of neurogenic hypertension. In *Cardiovascular Genomics*, ed. Raizada MK, Paton JFR, Katovich MJ & Kasparov S, pp. 243–264. Humana Press, Totowa.

Katarova Z, Mugnaini E, Sekerkova G, Mann JR, Aszodi A, Bosze Z, Greenspan R & Szabo G (1998). Regulation of cell-type specific expression of lacZ by the 5′-flanking region of mouse GAD67 gene in the central nervous system of transgenic mice. *Eur J Neurosci* **10**, 989–999.

Kubo T, Kihara M, Hata H & Misu Y (1987). Cardiovascular effects in rats of alpha 1 and alpha 2 adrenergic agents injected into the nucleus tractus solitarii. *Naunyn-Schmiedebergs Arch Pharmacol* **335**, 274–277.

Kubo T & Misu Y (1981). Pharmacological characterisation of the alpha-adrenoceptors responsible for a decrease of blood pressure in the nucleus tractus solitarii of the rat. *Naunyn-Schmiedebergs Arch Pharmacol* **317**, 120–125.

Kügler S, Kilic E & Bahr M (2003). Human synapsin 1 gene promoter confers highly neuron-specific long-term transgene expression from an adenoviral vector in the adult rat brain depending on the transduced area. *Gene Ther* **10**, 337–347.

Lonergan T, Teschemacher AG, Paton JF & Kasparov S (2004). Cellular phenotype specificity of viral vectors delivered into cardiovascular control centres in the brainstem. *J Physiol* **555P**, C27.

Mazarakis ND, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM, Kingsman AJ, Malley K & Mitrophanous KA (2001). Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum Mol Genet* **10**, 2109–2121.

Nishimura I, Uetsuki T, Dani SU, Ohsawa Y, Saito I, Okamura H, Uchiyama Y & Yoshikawa K (1998). Degeneration in vivo of rat hippocampal neurons by wild-type Alzheimer amyloid precursor protein overexpressed by adenovirus-mediated gene transfer. *J Neurosci* **18**, 2387–2398.

Paton JFR (1996). A working heart-brainstem preparation of the mouse. *J Neurosci Meth* **65**, 63–68.

Paton JFR, Deuchars J, Ahmad Z, Wong L-F, Murphy D & Kasparov S (2001*a*). Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat. *J Physiol* **531**, 445–458.

Paton JF, Deuchars J, Li Y & Kasparov S (2001*b*). Properties of solitary tract neurones responding to peripheral arterial chemoreceptors. *Neuroscience* **105**, 231–248.

Paton JFR & Kasparov S (2000). Sensory channel specific modulation in the nucleus of the solitary tract. *J Auton Nerv Syst* **80**, 117–129.

Paton JF, Li YW & Schwaber JS (2001*c*). Response properties of baroreceptive NTS neurons. *Ann NY Acad Sci* **940**, 157–168.

Paton JFR, Waki H, Raizada MK & Kasparov S (2004). Genes regulating cardiovascular function as revealed using viral gene transfer. In *Neural Mechanisms of Cardiovascular Regulation*, ed. Dunn NJ, Machado B & Pilowsky PM, pp. 399–409. Kluwer Academic Publishers, Boston.

Phillips JK & Lipski J (2000). Single-cell RT-PCR as a tool to study gene expression in central and peripheral autonomic neurones. *Auton Neurosci-Basic Clin* **86**, 1–12.

Reis DJ, Doba N, Snyder DW & Nathan MA (1977). Brain lesions and hypertension: chronic lability and elevation of arterial pressure produced by electrolytic lesions and 6-hydroxydopamine treatment of nucleus tractus solitarii (NTS) in rat and cat. *Prog Brain Res* **47**, 169–188.

Sinnayah P, Lindley TE, Staber PD, Cassell MD, Davidson BL & Davisson RL (2002). Selective gene transfer to key cardiovascular regions of the brain: comparison of two viral vector systems. *Hypertension* **39**, 603–608.

Spyer KM (1994). Central nervous mechanisms contributing to cardiovascular control. *J Physiol* **474**, 1–19.

Stokes CEL, Murphy D, Paton JFR & Kasparov S (2003). Dynamics of a transgene expression in acute rat brain slices transfected with adenoviral vectors. *Exp Physiol* **88**, 459–466.

Stoppini L, Buchs PA & Muller D (1991). A simple method for organotypic cultures of nervous tissue. *J Neurosci Meth* **37**, 173–182.

Teschemacher AG, Paton JF & Kasparov S (2005). Imaging living central neurones using viral gene transfer. *Advanced Drug Delivery Rev* **57**, 79–93.

Tsuchiya R, Yoshiki F, Kudo Y & Morita M (2002). Cell type-selective expression of green fluorescent protein and the calcium indicating protein, yellow cameleon, in rat cortical primary cultures. *Brain Res* **956**, 221–229.

Vitela M & Mifflin SW (2001). Gamma-aminobutyric acid (B) receptor-mediated responses in the nucleus tractus solitarius are altered in acute and chronic hypertension. *Hypertension* **37**, 619–622.

Waki H, Kasparov S, Katahira K, Shimizu T, Murphy D & Paton JF (2003*a*). Dynamic exercise attenuates spontaneous baroreceptor reflex sensitivity in conscious rats. *Exp Physiol* **88**, 517–526.

Waki H, Kasparov S, Wong L-F, Murphy D, Shimizu T & Paton JFR (2003*b*). Chronic inhibition of eNOS activity in NTS enhances baroreceptor reflex in conscious rats. *J Physiol* **546**, 233–242.

Waki H, Katahira K, Kasparov S, Murphy D & Paton JF (2004). Development of new software for the automation of analysis of cardiovascular autonomic function from chronic measurements of arterial pressure in conscious rats. *J. Physiol* **555**P, PC11.

Wilkinson GW & Akrigg A (1992). Constitutive and enhanced expression from the CMV major IE promoter in a defective adenovirus vector. *Nucleic Acids Res* **20**, 2233–2239.

Wong LF, Polson JW, Murphy D, Paton JF & Kasparov S (2002). Genetic and pharmacological dissection of pathways involved in the angiotensin II-mediated depression of baroreflex function. *FASEB J Online* **16**, 1595–1601.

Zhang J & Mifflin SW (1998). Receptor subtype specific effects of GABA agonists on neurons receiving aortic depressor nerve inputs within the nucleus of the solitary tract. *J Auton Nerv Syst* **73**, 170–181.

Acknowledgements

This work was supported by grants from the British Heart Foundation, Wellcome Trust, BBSRC and MRC. Our group has been collaborating with Professor D. Murphy's laboratory (URCN, University of Bristol) on AVVs gene delivery for the last few years. AVVs mentioned in this paper were derived from plasmids kindly supplied by Professor J. Uney (URCN, University of Bristol). Our LVVs have been derived from vectors developed in Professor M. Raizada's group by Dr M. Huentelman (Gainesville, FL, USA). We gratefully acknowledge the technical assistance of Dr N. Nikiforova and T. Saunders.