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PC14

Lentiviral and adenoviral vectors for targeting raphe serotonergic neurones based on a transcriptional amplification strategy

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Serotonin (5HT), one of the principal neuromodulators in the mammalian brain, is implicated in a variety of disorders such as pain, depression and schizophrenia. Many aspects of serotonergic transmission remain unknown, necessitating the development of new research tools. Previously, we successfully used viral vectors for cell-specific gene expression in the brain in order to selectively study or modulate the function of targeted neurones (Duale et al. 2007; Wang et al. 2006; Chiti & Teschemacher, 2007). Here we present novel lenti- and adenoviral vectors suitable for selective gene expression in raphe 5HT neurones. For targeting we used partial sequences (length 3.6kb, 2kb, and 1kb) of the natural promoter of rat tryptophan hydroxylase 2 (TPH2), the rate limiting enzyme in 5HT synthesis, obtained by PCR from rat brain genomic DNA. Lentiviral vectors for expression of EGFP were prepared using standard protocols (Liu et al. 2008) and stereotaxically microinjected into the rat raphe nuclei (under a mixture of ketamine (60 mg/kg) and medetomidine (250 μg/kg) i.m. anaesthesia). Specificity was then determined by immunofluorescence using anti-GFP and anti-TPH2 antibodies. The 3.6kb and 2kb promoter sequences conferred specific expression (co-localisation >95%), while the specificity of the 1kb promoter was only ~78%. However, native promoters were weak, and expression could only be detected using anti-GFP antibodies. To overcome this limitation, we employed a previously established transcriptional amplification strategy which involves cell-specific co-expression of a potent chimeric transactivator (Liu et al. 2008; Liu et al. 2006). This strategy increased the potency of 3.6kb and 2kb TPH2 promoters, leading to visible EGFP expression, while maintaining 5HT neurone specificity at 99% (n= 700 cells). Adenoviral vectors based on the 3.6kb construct were generated which caused visible EGFP expression in 5HT neurones in organotypic brainstem slice cultures. Moreover, it was possible to visually identify EGFP-positive axons with multiple small varicosities. Using previously established methods (Chiti & Teschemacher, 2007), we made the first microamperometric recordings of quantal 5HT release, and the first patch clamp recordings from EGFP-expressing 5HT neurones of the rat raphe. We believe that these viral vectors have great potential for in vivo and in vitro studies into the function of central 5HT neurones.

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PC15

The presence of mechanosensitive channels in the rainbow trout (*Oncorhynchus mykiss*) heart

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Mechanosensitivity describes the response to mechanical stimulation in cells, this may occur through the activation of mechanosensitive channels (MSCs). MSCs have been studied in microorganisms and shown to act as mechanoelectrical transducers of the forces exerted upon the cell membrane of the microorganism and so allow the cell to respond to mechanical stimuli (Martinac and Kloda, 2003). More recently, studies have found the degree of mechanosensitivity of mammalian cardiac tissue may contribute to electrical instability and arrhythmic disposition of the heart after myocardial infarction (Kamkin et al, 2005).

In this study we looked at the physiological effect of stretch upon the ventricular monophasic action potential (MAP) of the isolated whole *O.mykiss* heart (n=8). A range of filling pressures (1kPa – 4kPa) were used to stretch the heart and in some experiments outflow from the heart was inhibited to induce a large stretch of the myocardium. MAPs were recorded from the surface of the spontaneously beating heart.

An increase in filling pressure from 1kPa to 4 kPa shortened the MAP at 25% repolarisation (one way repeated measures ANOVA, P=0.017). Clamping outflow from the heart shortened this further (P=0.001). No significant differences were found in the MAP at 50% repolarisation time with either changes in filling pressure or clamping outflow. Although no significant difference in repolarisation time at 90% was seen with increased filling pressure, significant elongation (P<0.05) was seen when outflow was clamped. These experiments show that the MAP of *O. mykiss* is altered, due to stretch, in a similar manner to that which has been described in mammals (see, White, 2006) and may indicate the presence of non-selective cation MSCs in the *O.mykiss* ventricle. To investigate this further, we have cloned the trout variants of both TRPC1, which has been reported to be a store operated $Ca2^+$ MSC, and TREK-1 which has been identified as a twin pore K^+ MSC. We will use this information to look at the evolution of these channels and to determine their presence in different tissues from *O. mykiss*.

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PC16

The role of reverse-mode NCX in the contractility of the rainbow trout heart

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The Ca2+ that initiates contraction in the rainbow trout heart arrives at the myofilaments primarily through L-type Ca2+ channels in the sarcolemmal membrane (Vornanen et al., 2000). However, at specific times during the action potential, Ca2+ can also be brought across the sarcolemma by the Na+-Ca2+ exchanger (NCX) operating in reverse-mode (revNCX)(Hove-Madsen et al., 2000). The relative importance of this route of Ca2+ influx was assessed in single isolated cardiac myocytes and isolated muscle preparations using the specific inhibitor of revNCX, KB-R7943.

Rainbow trout (range 75 g to 600 g body weight) were killed humanely (UK Home Office Schedule 1 method). The heart was removed and prepared for either dissection of trabecular bundles or for enzymatic digestion. We evaluated the role of KB-R7943 on contractility by assessing isometric force production of isolated atrial and ventricular muscle preparations, and by assessing the percentage shortening of freshly isolated atrial and ventricular myocytes responding to field stimulation. The contribution of revNCX to the Ca2+-transient was assessed using whole-cell voltage-clamp and Fura-2 fluorescence measurements.

In ventricular myocytes, KB-R7943 significantly reduce cell shortening (by 40% from control, n=7, Mann-Whitney Rank Sum, P<0.05) at a concentration of 0.3 μM. The decrease in shortening was greater (by 75% from control, n=6, Mann-Whitney Rank Sum, P<0.05) at a concentration of 5 μM. This reduction is probably due to a decrease in intracellular Ca2+ concentration as our preliminary data shows 5 μM KB-R7943 reduces the amplitude of the Ca2+-transient by approximately 50 %. The reduction in contractility in response to treatment with KB-R7943 was greater in atrial than ventricular cells such that 5 μM KB-7943 completely abolished contraction (n=3).

The relative importance of revNCX decreased as contraction frequency was increased (from 0.2 to 0.8 Hz) in both atrial and ventricular cells. We attribute this to a frequency-induced shortening of action potential duration which reduces opportunity for Ca2+ to enter the cell on revNCX (see Birkedal & Shiels, 2007). In isolated muscle preparations, application of 5 μM KB-R7943 had no effect on peak isometric tension in either atrial (n=7, Mann-Whitney Rank Sum, P<0.05) or ventricular (n=6, Mann-Whitney Rank Sum, P<0.05) muscle over the entire range of contractile frequencies examined (0.2 – 2.0 Hz). This dose may be too low for trabecular muscle preparations.

We conclude that revNCX is an important Ca2+ influx pathway in trout myocytes and is relative importance is affected by the frequency of contraction.

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PC17

Caveolar remodelling in rabbit left ventricular myocytes after cell isolation

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Introduction: Caveolae (CAV) are centres of cardiac signal transduction, often studied in isolated cardiomyocytes (M). Little is known about CAV preservation in isolated M, so we monitored this for up to 8h after cell isolation.

Methods: Left ventricular M were isolated from New Zealand (NZ) white rabbit (<1kg) after Schedule 1 killing (conforming to UK Home Office regulations). After excision, the heart was swiftly connected to a Langendorff system, perfused with physiological saline, then cardioplegically arrested (high K+), followed by enzymatic digestion (L-type collagenase; Sigma-Aldrich). Isolated M were stored in saline with BSA and trypsin inhibitor at 22°C (pH 7.4) until fixation with Karnovsky's formaldehyde-glutaraldehyde mix at 0h, 3h or 8h after isolation. M were resin embedded, sectioned longitudinally (80nm), and imaged by transmission electron microscopy. CAV in direct contact or 'within reach' of the sarcolemma (CAVs; centre <50nm from sarcolemma) were distinguished from internal ones (CAVi). For each M, CAVs and CAVi were quantified within a 1μm sub-sarcolemmal space of 9-12 sarcomeres (6 M at each time point). Kruskal-Wallis and Ranksum tests were used (p<0.05).

Results: The density of CAVi and CAVs decreases with time after isolation (Fig.1). The proportion of CAVs increases from 66% at 0h (70% at 3h) to 80% at 8h. At 0h, no CAVs were in a 'semi-open state', i.e. with wide connection between CAV lumen and