PS P82

Electrical properties of neurosecretory Dahlgren cells in the isolated spinal cord of the euryhaline flounder (*Platichthys flesus*)

M.J. Brierley, W. Lu, R.J. Balment, D. Riccardi and C.R. McCrohan

School of Biological Sciences, University of Manchester, Manchester, UK

The flounder is capable of full osmoregulatory adaptation to both freshwater (< 10 mosmol l^{-1}) and seawater (1000 mosmol l^{-1}) environments as part of its circannual migration. This involves major functional changes in osmoregulatory organs such as gut, gills, bladder and kidney. The caudal neurosecretory system (CNSS), located in the terminal segments of the spinal cord, plays an important role in this process. Magnocellular Dahlgren cells synthesise and secrete neuropeptide hormones, including urotensin I, urotensin II and possibly parathyroid hormone-related peptide, which are involved in this osmoregulatory adaptation. This study describes the electrophysiological properties underlying the repetitive firing activity of Dahlgren cells.

Isolated CNSS taken from humanely killed fish fully adapted to seawater conditions were mounted in a cooled (9–11 °C) interface recording chamber and continuously superfused (0.5 ml min^{-1}) with aerated flounder Ringer solution. Intracellular sharp microelectrode current clamp recordings were made in the presence of 1 μM tetrodotoxin (TTX). Other agonists and antagonists were superfused over the CNSS for at least 15 min before taking further recordings. Statistics are presented as means ± S.E.M. (n = number of neurons); P values were calculated using Student's two-tailed paired t test.

The rising phase of Dahlgren cell action potentials (*ca* 80 mV amplitude) consists of TTX-sensitive Na^+ (*ca* 50 mV) and nifedipine- (10 μM) sensitive Ca^{2+} (*ca* 30 mV) components. A sag potential (1–4 mV) was generated in response to hyperpolarising square wave current injection (−0.3 to −1.2 nA), which was followed by a depolarising after potential (DAP, 2–9 mV). Both of these responses were voltage dependent (Brierley *et al.* 2001) and may contribute to repetitive firing activity. The hyperpolarisation-activated sag potential was absent during perfusion with tetraethylammonium (TEA, 1 mM; $n = 3$) or Cs^+ (1 mM; $n = 3$) ions, indicating that it is mediated by a potassium conductance. Nifedipine (10 μM) significantly reduced DAP amplitude ($n = 3$, $V_M = -45$ mV, −1.2 nA pulses, 95.0 ± 5.0 % reduction, $P < 0.0001$) suggesting it was mediated by L-type Ca^{2+} channels. DAPs were not generated in nominally Ca^{2+} free Ringer solution, and were maintained when Ba^{2+} (1 mM) was substituted for Ca^{2+} ($n = 2$) as the charge carrying cation. Sag potentials persisted in the presence of nifedipine or Ba^{2+}, and in nominally Ca^{2+}-free Ringer solution. Since peptide secretion is likely to depend on firing pattern and rate in Dahlgren cells, modulation of sag potential and DAP may contribute to changes in secretory activity during seawater–freshwater adaptation.

Brierley MJ *et al.* (2001). *J Exp Biol* **204**, 2733–2739.

This work was this work was supported by a BBSRC research grant.

All procedures accord with current UK legislation

PS P83

Sodium extrusion in junctional region of rat muscle

R. Creese *, S.D. Head *† and P.W. Sampson ‡

† *Biomedical Division, Imperial College, London SW7 2AZ, *St Mary's Hospital Medical School, W2 1PG and ‡Shedingham Medical Centre NR26 8RT, UK*

The use of rat diaphragm muscle combined with tracer studies together with slicing methods gives the possibility of separating junctional (end-plate) from non-junctional exchange (Creese *et al.* 1977), and the response of the sodium efflux to the effects of exposure to depolarising drugs can be followed.

Rats were killed humanely by stunning and exsanguination, before rapid removal of the diaphragm muscle. Following equilibration in a solution with labelled sodium, and wash in inactive solution, the steady-state efflux in untreated diaphragm muscles gave a rapid fraction and a single exponential curve with a half-time of 5.8 min (30 min wash at 38 °C, rate constant: 0.119 min^{-1}, 95 % limits 0.109, 0.126 min^{-1}, regression from 46 muscles).

Brief exposure to depolarising drugs plus labelled sodium produces loading in the junctional region of rat diaphragm, as shown by the additional radioactivity in slices of muscle in the region of the endplates, above the level assigned to the ends of the muscle. Decamethonium (100 mmol l^{-1}, 30 s) plus labelled sodium, gave junctional sodium (mmol mg^{-1}), and the subsequent wash in inactive solution showed curvature in semilog plot and a power curve which could be linearised by a change to reciprocals, with efflux proportional to the square of the junctional sodium: the slope of the reciprocal plot was 0.306×10^{-3} min^{-1} (limits 0.301, 0.312, regression from 54 muscles), and the slope of the log–log plot was close to 2.0. In frog muscle, efflux of labelled sodium can give power curves which may be interpreted as indication of multiple carrier sites on the membrane transport complex, or in terms of a two-compartment model which would involve both the intra-fibre tubular system and the sarcoplasmic sodium (Keynes & Steinhardt, 1968).

In prolonged treatment with depolarising drugs, the resting potential is spontaneously restored (Creese & Mitchell, 1981). When exposure to decamethonium (100 mmol l^{-1}) was increased to 30 min followed by 30 s with labelled sodium plus decamethonium, the efflux from the junctional region reverted to the exponential form, with a half-time 3.0 min (from medians of the semilog plot, with rate constant −0.220 min^{-1}, limits: 0.21, 0.24, regression from 68 muscles).

With carbachol 100 mmol l^{-1} plus labelled sodium (30 s) the efflux of junctional sodium appeared to be completely prevented by ouabain (100 mmol l^{-1}, 32 muscles), with partial inhibition of non-junctional sodium.

Creese R & Mitchell LD (1981). *J Physiol* **313**, 173–186.

Creese R *et al.* (1977). *J Physiol* **272**, 295–316.

Keynes RD & Steinhardt RA (1968). *J Physiol* **198**, 581–599.

All procedures accord with current UK legislation

PS P84

ClC-2 does not represent the swelling-activated Cl^- channel in CAD cells

V.L. Harvey, C. Garner and R.L. McDonald

Department of Chemical and Biological Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK

Using the whole-cell configuration of the patch-clamp technique and video-imaging analysis, we have recently reported activation of the volume-regulated anion channel (VRAC) in response to hypotonic stress (Harvey *et al.* 2002, 2003). In the present study, we have further characterised the VRAC and investigated its molecular identity.

Whole-cell patch-clamp studies were performed as previously reported (Harvey *et al.* 2003). Data reported below were obtained by 100 ms step depolarisations from −80 to +100 mV from a holding potential of −60 mV, and are expressed as mean ± S.E.M. current densities (pA pF^{-1}). Significant differences were determined using Student's paired *t* test or ANOVA followed by the Tukey HSD test.

Cells were bathed in isotonic bath solution containing (mM): NaCl 110, $CaCl_2$ 1, $MgCl_2$ 1, Hepes 10, adjusted with NaOH (pH 7.3) and mannitol (300 mosmol $(kg\ H_2O)^{-1}$). They were dialysed with a pipette solution containing (mM): NaCl 50, EGTA 1, $MgCl_2$ 1, Hepes 10, adjusted with NaOH (pH 7.3) and mannitol (290 mosmol $(kg\ H_2O)^{-1}$). Under these conditions CAD cells displayed a maximum outward current of 8.4 ± 2.4 pA pF^{-1} and maximum inward current of −0.30 ± 0.44 pA pF^{-1} ($n = 16$). After a 5 min exposure to an iso-ionic hypotonic solution (230 mosmol $(kg\ H_2O)^{-1}$), the outward current increased to 48.2 ± 6.5 pA pF^{-1} and the inward current increased to −15.0 ± 3.0 pA pF^{-1} ($n = 16$, $P < 0.001$). The reversal potential (E_{rev}), −14.7 ± 1.4 mV ($n = 16$), was similar to the theoretical E_{rev} for a Cl^--selective conductance under these conditions. Some of the data above have previously been reported (Harvey *et al.* 2003). Bath perfusion with the Cl^- channel antagonist tamoxifen (10 μM) reduced the outward current (56.4 ± 15.6 pA pF^{-1}) to 22.1 ± 4.8 pA pF^{-1} ($n = 4$, $P < 0.05$), but had no significant effects on the inward current. This inhibition was reversed following washout with hypotonic solution (53.8 ± 26.3 pA pF^{-1}, $n = 4$). The molecular identity of the VRAC is controversial, with two members of the voltage-dependent Cl^- family, ClC-2 and ClC-3, proposed as the most likely candidates. The expression of members of the ClC family was examined in CAD cells and liver tissue, isolated from humanely killed rats, using RT-PCR. Using primers specific for ClC-1, -2, -3, -4 and -5 (ClC-2, ClC-3; Enz *et al.* 1999), transcripts for ClC-2, -3, -4 and -5 were identified in liver; but only ClC-3, -4 and -5 were identified in CAD cells.

In conclusion, CAD cells possess swelling-activated currents that display properties consistent with those of the VRAC reported in other cell types. Furthermore, the biophysical properties of the VRAC, and RT-PCR analysis suggest that ClC-2 does not represent the molecular candidate for the VRAC in CAD cells.

Enz R *et al.* (1999). *J Neurosci* **19**, 9841–9847.

Harvey V *et al.* (2002). *J Physiol* **554.P**, 72*P*.

Harvey V *et al.* (2003). *J Physiol* **548.P**, P4.

All procedures accord with current UK legislation

PS P85

The effects of genistein on the contractions of vas deferens isolated from hypothyroide rats

Z. Fatehi-Hassanabad, M. Fatehi, S. Parvardeh and M. Nassiri Asl

Department of Physiology and Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashad, Iran

Hypothyroidism is an endocrine disorder. It is well known that changes in thyroid hormone level have a profound influence on intestinal smooth muscle function. Recent studies have suggested that tyrosine kinase participates in the activation of the G-protein-mediated signal transduction pathway of smooth muscle contraction. However, the effect of hypothyroidism on other smooth muscles and involvement of tyrosine kinase in any related effects on contractile responses in this pathological condition remains uncertain. Thus, the purpose of this study was to investigate whether tyrosine kinase was involved in any possible changes in contractile responses to noradrenaline (NA) and nerve stimulation in the isolated rat vas deferens removed from hypothyroid animals.

In order to inhibit tyrosine kinases, we used genistein (a tyrosine kinases inhibitor). Control and hyothyroid rats were humanely killed and segments 2 cm long were removed from the prostatic portion of the vas deferens and mounted in a 50 ml organ bath. An isometric transducer recorded contractile responses to electrical field stimulation and NA. Data are expressed as means ± S.E.M. and analysed by one-way analysis of variance (ANOVA) and values of $P < 0.05$ were taken as significant.

Electrically evoked contraction in hypothyroid rats was reduced to 0.83 ± 0.04 g, compared to control of 1.7 ± 0.10 g. Maximum contractile response to NA (100 μM) was reduced to 0.38 ± 0.06 g *vs.* control, 1.09 ± 0.1 g. Contractile response to electrical stimulation was inhibited by genistein: 21.33 ± 3.67 % *vs.* control, 84.33 ± 2.18 %.

These results show that the contractile responses of vas deferens to electrical stimulation and maximum responses to NA were significantly decreased in hypothyroid rats. The inhibitory effects of genistein on contractile responses of the preparations from hypothyroid rats was less than that in control rats. This phenomenon may be due to a decrease in tyrosine kinase activation in hypothyroid rats, which decreases the Ca^{2+} sensitivity of actomyosin.

All procedures accord with current local and national guidelines

PS P86

Endogenous interleukin-1 plays a role in LTP induction

F.M. Ross, S.M. Allan, N.J. Rothwell and A. Verkhratsky

School of Biological Sciences, 1.124 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK

Exogenous administration of the pro-inflammatory cytokine interleukin-1 (IL-1) inhibits long-term potentiation (LTP) in several regions of the hippocampus, both *in vitro* and *in vivo* (O'Connor & Coogan, 1999). Recent evidence suggests a role for endogenous IL-1 in LTP induction and/or maintenance (Schneider *et al.* 1998; Yirmiya *et al.* 2003). The aim of this study was to investigate the role of endogenous IL-1 in LTP using IL-1 receptor I knock out (IL-1RI KO) mice.

Field excitatory postsynaptic potentials (fEPSP) were recorded from the CA1 region of hippocampal slices prepared from IL-1RI

KO mice or wild-type controls (C57 BL/6xSV129) (21–35 days old) humanely killed by cervical dislocation. Slices were incubated in normal aCSF at room temperature (21–24 °C) for at least 60 min in a holding chamber gassed with 95 % O_2–5 % CO_2. After this time individual slices were moved to a submerged chamber with the perfusing aCSF at 21–24, 29–30 or 34–36 °C. LTP was induced using a theta-burst stimulation (TBS) protocol (5 trains of 15 bursts each consisting of 4 pulses at 100 Hz. with an inter-burst interval of 200 ms with the trains given at 0.1 Hz.). Data are expressed as means ± S.E.M. analysed 55–60 min after TBS. Statistical analysis within slices between pre- and post-TBS fEPSP slope values was done using Student's paired *t* test. $P < 0.05$ was taken to be significant.

The magnitude of LTP at 21–24 °C did not differ between IL-1RI KO mice and wild-type (210 ± 30 % of control, $n = 6$, $P < 0.05$ and 189 ± 19 % of control, $n = 5$, $P < 0.01$, respectively). Similarly at 29–31 °C TBS resulted in statistically significant potentiation in hippocampal slices from wild-type mice and in slices from IL-1RI KO mice. Paired-pulse stimulation (inter-burst interval 40–800 ms) produced potentiation of fEPSP at all inter-burst intervals. There was no difference in the degree of potentiation obtained between wild-type and IL-1RI KO mice. TBS caused significant potentiation of fEPSP slope at 34–36 °C in slices from wild-type mice (203 ± 8 % of control, $n = 4$, $P < 0.05$) but not in slices from IL-1RI KO mice (134 ± 13 %, $n = 4$).

In conclusion, these results suggest that under certain circumstances endogenous IL-1, through the activation of IL-1RI, is involved in aspects of LTP and hence learning and memory.

O'Connor JJ & Coogan AN (1999). *Exp Physiol* **84**, 601–614.
Schneider H *et al.* (1998). *Proc Natl Acad Sci U S A* **95**, 7778–7783.
Yirmiya R *et al.* (2003). *Neurobiol Learn Mem* **78**, 379–389.

This work was supported by MRC.

All procedures accord with current UK legislation

PS P87

Molecular cloning and characterisation of the gene encoding corticotropin-releasing hormone in the European flounder, *Platichthys flesus*

W. Lu, S. Gumusgoz, M.J. Brierley, J. Warne, C.R. McCrohan, R.J. Balment and D. Riccardi

School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK

In mammals, corticotropin-releasing hormone (CRH) is the primary hypothalamic factor mediating stress-induced adrenocorticotropin (ACTH) secretion from the anterior pituitary. However, there are limited studies of this neuroendocrine peptide in non-mammalian vertebrates. In euryhaline fish, CRH may have an integrative role in the control of reproductive, osmoregulatory and nutritional systems. The caudal neurosecretory system (CNSS) is a neuroendocrine system distinct from brain and has been shown to be responsible for secretion of two major neuropeptides, urotensin I and II. Here we report cloning and sites of expression of CRH from the CNSS of the euryhaline flounder.

The CRH precursor cDNA was cloned by reverse transcriptase-polymerase chain reaction (RT-PCR) and screening of a CNSS cDNA library. The CNSSs were isolated from humanely killed fish. RNA was extracted using the guanidine thiocyanate method and utilized for both RT-PCR and library construction. RT-PCR was carried out using degenerate oligonucleotide primers based on the conserved amino acid sequence of CRH from a range of species. A CNSS cDNA library was constructed into the phage vector lTriplEx2 and screened at high stringency using a specific CRH probe obtained from degenerate RT-PCR. The CRH precursor consists of 168 amino acid residues and the carboxyl terminus represents the 41-amino-acid mature peptide, preceded by Arg–Arg and followed by Gly-Lys as putative cleavage sites. The deduced amino acid sequences of CRF and UI peptides from the same fish exhibit a sequence identity of 51.2 %. The genomic organisation, determined by PCR using specific primers, shows a single intron in the 5′-UTR region, and that the whole CRH coding sequence is contained in a single exon. Using conditions that gave no cross-hybridisation with other genes, Northern blot analysis of a range of flounder tissues confirmed that the CNSS is the major site of expression of CRH gene and also shows the possibility of multiple polyadenylation signals in the 3′ untranslated region. RT-PCR using specific primers indicated the presence of CRH transcripts in other tissues, such as brain, optic nerve, spinal cord, gill, head kidney, kidney, intestine and rectum. The primary structure of CRH shows a close similarity between flounder, tilapia and catfish, with more variation compared to orthologous vertebrate and other teleost CRHs. Whether these structural differences reflect the varied and contrasting functions of CRH remains to be investigated.

This work was supported by a grant from the BBSRC.

All procedures accord with current UK legislation

PS P88

Interactions between modulators of hTREK1 reveal divergent regulatory mechanisms for hypoxia and alkalosis

P. Miller*, C. Peers† and P.J. Kemp*

*Schools of *Biomedical Sciences and †Medicine, University of Leeds, Leeds LS2 9JT, UK*

The human tandem-P domain K^+ channel, hTREK1, is O_2 sensitive and hypoxia occludes its modulation by arachidonic acid (AA), membrane deformation (Miller *et al.* 2003*a*) or intracellular acidosis (Miller *et al.* 2003*b*). Although these findings question the neuroprotective role of hTREK1 during the acidosis/anoxia of central ischaemia, its role during physiological alkalosis has not been investigated. Here we report the effects of increased intracellular pH on the ability of hypoxia to inhibit, and AA to activate, this channel.

Experiments employed whole-cell or cell-attached patch clamp recordings from HEK293 cells stably transfected with hTREK1. Data reported are derived from currents recorded at a pipette potential of either +60 mV (whole-cell) or −130 mV (cell-attached) from a holding potential of –70 mV or 0 mV, respectively (see Miller *et al.* 2003*a* for details).

Following intracellular alkalinization with 20 mM trimethylamine (TMA), whole-cell current density was significantly reduced from 456 ± 50 to 223 ± 39 pA pF^{-1} ($n = 6$; mean ± S.E.M.). Further significant reduction was observed when TMA was co-applied with hypoxia such that current density was decreased to 110 ± 20 pA pF^{-1} ($P < 0.01$; Student's paired *t* test for each manoeuvre). These effects were mirrored by alkalinization with ammonium chloride in the absence and presence of hypoxia ($n = 6$). Furthermore, the order of application of TMA and hypoxia did not affect the ability of each to inhibit currents. Thus, hypoxia reduced current density from 435 ± 64 to 326 ± 44 pA pF^{-1} whilst co-application of TMA

reduced further the current density to 122 ± 23 pA pF^{-1} (n = 9; P < 0.02 for each manoeuvre). Although alkalosis was a potent inhibitor of hTREK1 current, it was without effect on AA action. Thus, following inhibition with TMA, 10 μM AA evoked a robust activation from 236 ± 35 to 567 ± 70 pA pF^{-1} (n = 9); this was completely occluded by hypoxia. In contrast, AA was without effect when hypoxia was pre-applied (in the presence of TMA; n = 11). The inability of TMA to block activation by AA was conserved in cell-attached recordings where TMA evoked mild but significant inhibition of currents from 21.3 ± 3.2 to 17.2 ± 3.2 pA and co-application of AA caused robust activation to 86.7 ± 31.5 pA (n = 7; P < 0.05); hypoxia completely reversed this effect.

In conclusion, although hypoxia occludes the activation of hTREK1 by either acidosis or arachidonic acid, it is without effect on the inhibitory effect of alkalosis, suggesting that regulation by oxygen/arachidonic acid and alkalosis are mechanistically distinct.

Miller P *et al.* (2003*a*). *J Physiol* **548**, 31–37.

Miller P *et al.* (2003*b*). *J Physiol* **551.P**, C23.

Funded by GlaxoSmithKline, British Heart Foundation and Wellcome Trust.

PS P89

Pharmacological induction of hyperactivity in embryonic chicks by the administration of 4-aminopyridine fails to accelerate joint formation but results in an increased capacity for skeletal muscle growth

J.C. Lewthwaite*, K.J. Lamb*, A. Somaiya*, J.-P. Lin†, D. Simon†, E. Kavanagh*, C.P.D. Wheeler-Jones* and A.A. Pitsillides*

**Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK, †Paediatric Neurology Guy's and St Thomas' Hospitals, London, UK and ‡Orthopaedic and Trauma Surgery, Imperial College School of Medicine, London, UK*

The temporal and spatial sequence of events involved in joint cavity formation during limb development is dependent upon embryonic movement. Many studies into chick embryo diarthrodial joint development have concluded that a lack of muscular movement results in the fusion of opposing joint elements and absence of articular cavities. This study has addressed the hypothesis that an enhancement of muscle contraction, with drugs such as 4-aminopyridine (4-AP), will accelerate embryonic chick joint cavitation.

Fertilised White Leghorn eggs were treated by methods conforming to UK legislation with sterile-filtered 4-AP at 2 mg ml^{-1} in Tyrode solution (TS) applied directly to the chorioallantoic on each day of treatment. Three groups of chicks received 4-AP, group 1 at stage 36–37, group 2 at stages 36–39 and group 3 at stages 36–43. Another three groups of chicks received TS, as a control, at the same times. One day after the final treatment, chicks were killed humanely.

Skeletal movement in control embryos consisted of variable periods of spontaneous muscular activity (approximately 15 seconds per minute studied, range: 10–40 s min^{-1}). Treatment with 4-AP at stage 36 induced an immediate (within 1 min) increase in the frequency of skeletal movement (greater than 35 s min^{-1}, range: 35–45 s min^{-1}) which was sustained for at least 24 h after this initial dose, and maintained by subsequent 4-AP treatments. This increased twitch frequency had no apparent effect on body weight, longitudinal growth or cartilage/bone formation rates in limb skeletal elements when compared to the stage-matched controls. Intriguingly, however, direct measurement of fibre number in hindlimb adductor muscles, showed that prolonged 4-AP treatment (days 10–18) produced significant increases in muscle fibre density compared to the TS control.

In contrast to the well-established cavitation-inhibitory influence of embryonic immobilisation, this study indicates that 4-AP-induced increases in frequency of skeletal movement fails to significantly modify the spatiotemporal pattern of embryonic joint cavity formation in the developing limb, and that such treatment also has little discernable effect on chick weight or skeletal element development and growth. In marked contrast, however, the imposition of defined 4-AP-induced embryonic hyper-mobility for prolonged periods during development stimulates increases in muscle fibre density, suggesting that such treatment may enhance the capacity of post-hatch growth in the chick.

All procedures accord with current UK legislation

PS P90

Spontaneous action currents in spinal cord neurones

K.T. Wann, E. Corson, J.S. Schweizer and U.V. Boolaky

Welsh School of Pharmacy, Redwood Building, King Edward VII Ave, Cardiff CF10 3XF, UK

Amyotrophic lateral sclerosis (ALS) is accompanied by muscle fasciculation and spasticity. Changes in membrane excitability of motor neurones may contribute to the disease progression of ALS (Bostock *et al.* 1995) and, given a possible role in precipitating neuronal degeneration in ALS, represent a target for therapeutic intervention. We have therefore investigated the 'excitability state' of motor neurones maintained in culture.

Using whole-cell and cell-attached patch-clamp methods we investigated spontaneous electrical activity in primary spinal cord cultures, isolated from humanely killed Balb-C mice (E13). The bath contained Locke solution (mM: 140 NaCl; 3 KCl; 2 $MgCl_2$; 2 $CaCl_2$; 10 Hepes; 10 glucose; buffered to pH 7.4 using NaOH). The recording pipette contained (mM): 140 KCl; 5 NaCl; 1 $MgCl_2$; 1 $CaCl_2$; 11 EGTA; Hepes 10; buffered to pH 7.2 with KOH.

Successful giga-ohm seals were formed in 68/116 cells (seal resistance 1–4 GΩ). Whole-cell measurements indicated that motor neurones in culture have healthy resting potentials (RMPs; mean ± S.D. −57 ± 11 mV; n = 19). In the cell-attached configuration, spontaneous biphasic waveforms, known as action currents (ACs), were observed at 0 mV pipette potential (RMP) in 40 % of cells (n = 148). We have recorded these ACs over pipette potentials −60 to +40 mV. Often inward single channel potassium currents followed the ACs (n = 7). The presence of ACs at RMP was not age dependent and interestingly activity was of two types. Cells fired continuously and/or in bursts. The frequency of firing was voltage dependent and the interspike interval clearly decreased with patch hyperpolarisation (n = 8). Furthermore, the intraburst interval decreased with patch hyperpolarisation (n = 6). The action currents were completely abolished in the presence of 10 μM TTX. Glutamate receptor antagonists (100 μM NBQX and D-AP5) reduced the overall number of cells firing to 28 % (n = 20).

The resting membrane potential values and spontaneous electrical activity we have observed here indicate that the neurones are fully functional within our culture system (Latham

et al. 2000). Spontaneous action currents, are indicative of freely changing membrane potential within the neurones. Our results suggest that a percentage of these cells may be 'pacemaker' cells which continue to fire without glutamatergic input. Such features of cultured motor neurones may provide reliable indices against which to test pharmacological intervention.

Bostock HM *et al.* (1995). *Brain* **118**, 217–225.
Latham PE *et al.* (2000). *J Neurphysiol* **83**, 828–835.

All procedures accord with current UK legislation

PS P91

Single ion channels in spinal cord neurones

U.V. Boolaky and K.T. Wann

Welsh School of Pharmacy, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK

In amyotrophic lateral sclerosis (ALS) hallmark symptoms include motor neurone hyperexcitability (Bostock *et al.* 1995; Zanette *et al.* 2002) raising the possibility that abnormal membrane conductance of motor neurones underlies the disease progression (Zanette *et al.* 2002). Furthermore, oxidative stress, a putative factor in ALS, causes irreversible depolarisation and enhanced calcium influx precipitating cell death (Herson & Ashford, 1997). Maintenance of the resting membrane potential (RMP) may prevent cell death and we describe here which channels in motor neurones may contribute to RMP.

Spinal cord cultures (islolated from E13 Balb-C mice, humanely killed) were grown as previously described (Boolaky *et al.* 2002). Motor neurones were identified by both morphology and immunohistochemistry. Whole-cell, excised inside-out and cell-attached recordings were made. The bath contained (mM): 140 NaCl; 3 KCl; 2 $MgCl_2$; 2 $CaCl_2$; 10 Hepes; 10 glucose; 1 μM TTX; buffered to pH 7.4 using NaOH. The pipette solution was (mM): 140 KCl; 5 NaCl; 1 $MgCl_2$; 1 $CaCl_2$; 11 EGTA; Hepes 10; buffered to pH 7.2 with KOH. In some experiments 10 mM TEA was added to the pipette.

In whole-cell measurements motor neurones have healthy RMPs (-57 ± 11 mV; mean ± S.D., $n = 19$). Single channels were present in 45% of the cells. The predominant channel at 0 mV pipette potential (PP) was of small conductance, the mean inward conductance being 46 ± 11 pS, the current reversing at a PP of -57 ± 21 mV ($n = 25$). At RMP the mean unitary current was -2.6 ± 0.6 pA ($n = 13$). The open probability (P_o) was voltage independent. An intermediate conductance channel (149 ± 9 pS) was present in 23% of cells. The channel current reversed at a PP of -67 ± 25 mV ($n = 10$) and the activity was voltage independent. A large TEA-sensitive channel with a mean conductance of 203 ± 28 pS ($n = 6$) was present in cell-attached patches and in excised inside-out patches (169 ± 34 pS, $n = 6$). The channel current showed inward rectification in asymmetrical K^+ (reverse gradient). This channel was both voltage and calcium dependent, the P_o increasing with depolarisation and increased 'intracellular' calcium.

Three channels which may contribute to resting conductance have been identified in motor neurones in culture. The large conductance channel is clearly the maxi-K potassium channel. The effects of glutamate excitotoxicity and oxidative stress on these channels is being investigated.

Boolaky UV *et al.* (2002). *Amyotroph Lateral Scler Other Motor Neuron Disord* **3** (S2), 80.
Bostock HM *et al.* (1995). *Brain* **118**, 217–225.
Herson PS & Ashford MLJ (1997). *J Physiol* **501**, 59–66.
Zanette G *et al.* (2002). *Clin Neurophy* **113**, 1688–1697.

All procedures accord with current UK legislation

PS P92

Profiling of Ion channel gene expression in mouse heart

J. Liu*, C. Pritchard†, R. Billeter‡, P. Underhill†, M. Lei* and D. Noble*

** Department of Physiology, University of Oxford, Oxford OX1 3PT, †MRC Mammalian Genetics Unit, Harwell, Oxon OX11 0RD and ‡BMS, University of Leeds, Leeds LS2 9JT, UK*

Electrophysiological properties vary in different regions of the heart, which is essential for effective cardiac function. Increasing evidence suggests that cardiac electrical heterogeneity is attributable to regional variability in distribution of ion channel and related genes. Dramatic progress in genomics has led to the discovery of a large number of ion channel genes; how these genes are expressed in specific regions of the heart and function in relation to its physiological function is largely unknown.

Hearts were excised from 20–25 g adult C57BL mice after humane killing by cervical dislocation. Total RNA was prepared from sinoatrial node (SAN), atrioventricular node (AVN), atrium and ventricles as described by Wittwer *et al.* (2002). Mouse Known Gene Array slides of 7.5 K (provided by the MRC HGMP Resource Centre) were used to identify the ion channel genes expressed in different regions of mouse heart. Amplification of cDNA by SMART was described by Vernon *et al.* (2000); labelling and hybridisation of cDNA were described by Smith *et al.* (2003). Reproducibility of signals is controlled by independent repeat hybridisations using different RNAs, colour swap and duplicate spots on the same array.

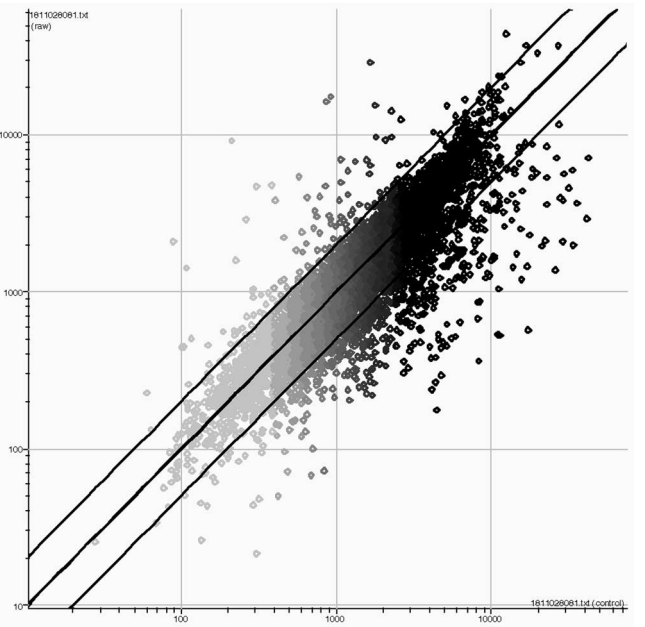

Figure 1. An example of scattered plot of comparison of gene expression between AVN (left top) and atrium (right bottom).

We have compared relative expression levels of ~500 channel and related genes in SAN, AVN, atrium and ventricle. Our

preliminary results showed that there are regional differences in channel gene expression in murine heart. Channel genes *Kcnj1*, *Kcnj8*, *Kcnj10*, *Kcna5*, and *Kcnh2*, *Scn8a* and *Scn1b* and *Hcn4* are highly expressed in nodal tissue (SAN/AVN), while channel genes *Kcnj16*, *Kcna4*, *Kcnb1* and *Gja1* (Cx43) are more highly expressed in muscle regions. In the profile of all genes, we found more differentially expressed genes between nodal and muscle regions, than between the two nodal regions. The gene expression profile between SAN and AVN is similar; this could be due to the functional and structural similarities between these two nodal regions. Further experiments using real time PCR and Western blot have been planned to validate these results.

Smith L *et al.* (2003). *Nucl Acids Res* **31**, e9.
Vernon SD *et al.* (2000). *J Mol Diagn* **2**, 124–127.
Wittwer M *et al.* (2002). *FASEB J* **16**, 884–886.

This work was supported by The Wellcome Trust.

All procedures accord with current UK legislation