C39

POTENTIATION OF THE VOLUME-REGULATED ANION CURRENT BY SULPHONYLUREAS IN RAT PANCREATIC β-CELLS.

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Stimulation of insulin release by sulphonylureas is thought to involve the inhbition of K_{ATP} channels. The therapeutic action of sulphonylureas, however, occurs at high glucose concentrations where K_{ATP} channels may already be closed. This study has investigated additional ionic actions of sulphonylureas on β-cells. Islets were isolated from rat pancreas (animals killed humanely by stunning and cervical dislocation), dispersed into single cells by exposure to a low [Ca^{2+}] buffer and cultured for 2-10 days. Electrical and ion channel activity were recorded using the patch-clamp technique. $^{86}Rb^+$ efflux (an index of net K^+ permeability) was measured with intact perifused islets. β-cell volume was measured in isolated cells using a video-imaging technique.

In the absence of glucose, tolbutamide (100 μM) caused a transient depolarisation of β-cell membrane potential. In the presence of 5 mM glucose, tolbutamide evoked a sustained period of electrical activity, whilst at 10 mM glucose the drug evoked a pronounced "silent" depolarisation. Tolbutamide inhibited $^{86Rb+}$ efflux in the absence of glucose, reflecting K_{ATP} channel inhibition. However, the drug caused a transient stimulation of Rb^+ efflux in the presence of 10 mM glucose, indicative of a K_{ATP} channel-independent mechanism. In conventional whole-cell recordings, tolbutamide had no significant effect on activity of the volume-regulated anion channel (VRAC) current. However in intact cells, tolbutamide increased the mean amplitude ($I_{vrac,ave}$) of the VRAC current in a glucose-dependent manner with a EC_{50} value of approximately 85 μM. For example, in the presence of 10 mM glucose, ($I_{vrac,ave}$) was –9.2 ± 0.28 pA and –16.4 ± 1.6 pA (mean ± SEM, both n=14; $P<0.01$ by paired t-test) in the absence and presence of 100 μM tolbutamide respectively. In single channel recordings, tolbutamide increased channel open probability from 0.11 ± 0.03 to 0.34 ± 0.08 (both n=8; $P<0.01$ by paired t-test) with no significant effect on current amplitude. Tolbutamide was found to increase β-cell volume by approximately 7% ($P<0.001$) in the presence, but not in the absence of glucose. Finally, tolbutamide-induced electrical activity in β-cells was suppressed by 4,4'-dithiocyanatostilbene-2,2'-disulphonic acid (DIDS; 100 μM and by 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB; 50 μM), two VRAC inhibitors.

It is concluded that tolbutamide can induce electrical activity by potentiating the VRAC current. This effect is probably not due to a direct effect of the drug on the channel, but could be secondary to an effect on β-cell glucose metabolism.

This work is supported by the Wellcome Trust. SLD is a MRC postgraduate student.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C40

PROTEIN KINASE C-δ MEDIATES THE ANTI-SECRETORYEFFECT OF 17β-OESTRADIOL THROUGH INHIBITION OF KCNQ1 CHANNELS.

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17β-oestradiol (E2) is responsible for the rapid activation of a number of signalling pathways in various cell types. In rat distal colonic crypts, it has been shown to activate PKCα and PKCδ and to increase intracellular calcium. This activation is associated with a number of events including inhibition of a basolateral potassium channel (Harvey 2001). Inhibition of this channel could be the key factor behind the gender-specific anti-secretory effect of E2 previously reported by our group. In this study, we clarify the interplay between oestrogen-activated signalling intermediates (PKC), a possible membrane target of estrogen (KCNQ1) and its impact of whole tissue physiology (anti-secretory effect).

Colonic epithelia were isolated from humanely killed Sprague-Dawley rats; mounted in an Ussing chamber and short circuit current (I_{SC}) were measured as an index of chloride secretion. Bacterial toxins (cholera toxin and heat stable E coli toxin) were used to stimulate transepithelial secretion. E2 at physiological concentrations (1-100 nM) decreased I_{SC} in a dose dependent manner (1 nM, 85±7%; 10 nM, 68±5%; 100 nM, 47±4%). This effect was dependent on PKCδ but not PKCα activity. Bicarbonate-dependent chloride secretion was unaffected by E2 (control 25±4, E2 23±5 μA cm^{-2}) suggesting no involvement of CFTR channels or anion exchangers. Therefore we examined the effect of E2 on other ion transporters involved in secretion. In apically permeabilized epithelia Na^+/K^+-ATPase activity was unaffected by E2 (control 32±5, E2 30±3 μA cm^{-2}). Next we examined the effect of E2 on basolateral potassium conductances. E2 inhibited a chromanol 293B sensitive conductance (control 57±6, E2 7±3 μA cm^{-2}), which indicated the involvement of KCNQ1 channels. Therefore, we investigated the interaction between PKCδ and the KCNQ1 channel protein. Exposure of isolated rat colonic crypts to E2 (10nM) for 15 minutes resulted in the activation of PKCδ only in the female and not in male rats. Additionally, KCNQ1 co-immunoprecipitated with PKCδ; this association was increased in the presence of E2, but only in crypt cells isolated from female rats. In vitro kinase assays using GST-tagged KCNQ1 intracellular termini as substrates revealed that PKCδ specifically phosphorylates the N terminus of KCNQ1. Taken together these data suggest the anti-secretory effect of E2 is mediated via rapid activation of PKCδ, which in turn inhibits a potassium channel (KCNQ1) crucial for the secretory processes (Kunzelmann 2002).

Harvey BJ et al. News Physiol Sci. 2001;16:174-7.

Kunzelmann K, Mall M. Physiol Rev. 2002;82(1):245-89.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C41

THE EFFECT OF THE REMOVAL OF EXTRACELLULAR SODIUM ON VOLTAGE-DEPENDENT K^+ CHANNEL CURRENTS IN RAT SMALL PULMONARY ARTERIAL MYOCYTES (PAMS)

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The effect of Na^+ removal was studied using a patch clamp technique in single PAMs at room temperature. Male Wistar rats (225-300 g) were humanely killed and PAMs were isolated from small pulmonary arteries (⟨400 μM external diameter) using collagenase and papain (Smirnov *et al.*, 2002). Voltage-dependent K^+ (Kv) current (I_{Kv}) was isolated using 1 μM paxilline and 10 μM glybenclamide (to eliminate BK_{Ca} and K_{ATP} respectively) in the external physiological saline solution (PSS) containing (mM): 130 NaCl, 5 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, 10 glucose, pH=7.2. Cells were dialysed with a pipette solution of composition (mM): 130 KCl, 0.5 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 10 EGTA, pH=7.2, unless mentioned otherwise. I_{Kv} was investigated using a 2 s voltage ramp applied every 20 s between -100 to 100 mV from a holding potential of -80 mV.

Removal of extracellular sodium (Na^+-free PSS, equimolar substitution with N-methyl-D-glucamine) augmented I_{Kv} from -60 to 10 mV causing a leftward shift in I_{Kv} activation. Changes in the I_{Kv} activation were described using the Boltzmann function and showed a significant decrease in the I_{Kv} half-activation potential (V_a) from 6.5±2.4 mV (PSS) to -16±3 mV (Na^+-free PSS) (n=19, p⟨0.0001, paired Students *t* test), yielding a mean relative shift in the I_{Kv} activation (ΔV_a) of -22.4±3.1 mV (n=19). Values are given as mean±s.e.m and statistically compared using unpaired *t* test, unless stated otherwise.

Incubation of PAMs with 100 μM amiloride, an inhibitor of the Na^+-Mg^{2+} exchanger, for 6 min caused a leftward shift (ΔV_a =-9.5±2.4 mV, n=9, p⟨0.015) mimicking that observed in the Na^+-free PSS. Subsequent removal of Na^+ in the presence of amiloride had only a small additional effect on the I_{Kv} activation (ΔV_a =-5.6±3.3 mV, n=8, p⟨0.006). Similarly, increased pipette $MgCl_2$ to 10 mM produced a leftward shift (V_a =-7.3±3.6 mV, n=8, p⟨0.004) and, though not significantly, diminished the effect of Na^+-free PSS (ΔV_a =-16.3±2.5 mV, n=8, p⟨0.24) on the I_{Kv} activation. Equimolar replacement of EGTA with EDTA, a more potent chelator of Mg^{2+}, in the pipette solution also attenuated the shift in Na^+-free PSS (ΔV_a=-13.7±2, n=12, p⟨0.05). However, the addition of the Ca^{2+} chelator BAPTA (10 mM) to the pipette solution did not affect the Na^+-dependent shift in I_{Kv} activation (ΔV_a=-18±1.9 mV, n=5, p⟩0.3).

Our data suggests that Kv channels in PAMs can be potently regulated by Na^+ dependent mechanism(s) in the physiological range of membrane potentials. Our results are consistent with the inhibition of the Na^+-Mg^{2+} extrusion mechanism by the removal of extracellular sodium, however, the involvement of other Na^+-dependent mechanisms such as Na^+-H^+ and Na^+-Ca^{2+} exchangers cannot yet be excluded.

Smirnov,S.V. *et al.* (2002).*J. Physiol.* **538**., 867-878

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C42

EXPRESSION OF MESSAGE FOR TRPC CHANNEL PROTEINS AND THEIR ALTERNATIVE SPLICE VARIANTS IN PRIMARY CULTURE HUMAN AIRWAY SMOOTH MUSCLE AND HUMAN SECONDARY CELL LINES.

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In a previous report to the Society we presented data concerning Ca^{2+} handling and influx mechanisms in human cultured airway smooth muscle (ASM), and proposed that the latter was likely to be via one or more transient receptor potential (TRP) channels (McVicker et al., 2001). TRP channels were first discovered in mutated *trp* photoreceptors of *Drosophila*, and the mammalian TRP homologues have recently been unified into three subfamilies (TRPC, TRPM, and TRPV). The TRP-canonical (TRPC) subfamily (formally short-TRPs or STRPs) is most closely related to *Drosophila* TRP proteins. Comparatively little information is available concerning the expression of TRPC in human ASM, therefore in this study we compared the expression of message for all known human TRPC channels and alternative splice variants between several human secondary cell lines (HeLa, HEK-293, A549, JURKAT and MOLT-4) and primary cultured human ASM. ASM cells were cultured from tissue derived from 6 patients undergoing surgery, and passages 4-6 were used for experiments. This study was approved by the Guy's and St Thomas' Hospitals' Research Ethics Committee and informed consent was obtained from donors.

RNA was isolated from the cells and reverse transcribed to cDNA. The cDNA was used for RT-PCR (24-40 cycles) with the specific primers designed against the different *trpc* genes. RT-PCR revealed that message for TRPC1 (including short alternative splice variant) and TRPC3 were expressed in all secondary cell lines and ASM, except TRPC3 in A549. Conversely, message for TRPC4α, β, ε, η and ζ were only expressed in HeLa, HEK-293, and ASM. TRPC5 was found mainly in ASM and JURKAT cells and at very low levels (requiring 40 cycles) in HeLa, HEK-293 and MOLT-4. Message for TRPC6 full, and the alternative splice variant $\delta_{316\text{-}431}$ were expressed in HeLa, ASM, and A549 cells alone. No message for TRPC4 γ, TRPC6$\delta_{377\text{-}431}$ and TRPC7 was detected in any of the human cell lines or ASM. The results give a general profile of *trpc* expression in the different human cell lines, which can now be used to determine their suitability for expression, over-expression or knock down studies for the role of specific TRPC proteins in Ca2+ influx pathways.

McVicker CG, Snetkov VA, Ward JPT (2001) J.Physiol. 536P: S047

YMS holds an MRC PhD studentship; the work was funded in part by the Wellcome Trust.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC25

EXPRESSION OF POTASSIUM CHANNEL SUBUNITS KV1.3 AND KV2.1 IN A CONDITIONALLY IMMORTALIZED COCHLEAR CELL LINE AND IN HAIR CELLS FROM THE NEONATAL MOUSE

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We used a conditionally immortalized cell line US/VOT-36 derived from the mouse embryonic cochlea to search for subunits of the embryonic and neonatal hair cell potassium (K^+) currents $I_{K,emb}$ and $I_{K,neo}$. It was derived from the otocyst at embryonic day 10 (E10, as described for a similar cell line, Lawoko-Kerali *et al.* 2004) and is likely to form a multipotent cell line capable of differentiation into hair cell-like cells. Whole-cell patch clamp recordings from cells grown under differentiating conditions showed endogenously expressed, voltage-activated K^+ currents. They shared characteristics of delayed-rectifier K^+ currents recorded from embryonic hair cells of humanely killed mice between E16 and the day of birth (P0), namely acquisition of a 4-AP sensitive partially inactivating component and a shift to a more hyperpolarized and steeper voltage dependence of activation (Marcotti *et al.* 2003; Helyer *et al.* in press). In both VOT-36 and basal outer hair cells (OHCs) at E18, 4-AP application removed the inactivating component. The remaining current resembled currents recorded earlier in differentiation in VOT-36 and in basal E16 OHCs. RT-PCR showed the presence of mRNA for Kv1.3 and Kv2.1 subunits in cell line cDNA and in whole organ of Corti cDNA prepared from P3 mice. Neither subunit has previously been shown to be expressed in cells of the mammalian organ of Corti. The changes in electrophysiological properties described are consistent with the expression of these subunits in VOT-36 and OHCs. Using immunohistochemistry, we showed Kv2.1 was expressed specifically on the basolateral membrane of inner hair cells (IHCs), with less expression in outer hair cells (OHCs). It was also expressed in hair cells of the utricular macula. Kv1.3 was present in IHC, OHCs and vestibular hair cells but no other cells of the sensory epithelium and was distributed diffusely throughout the cells. These results show that conditionally immortalized cell lines derived from the early embryo can express functional K^+ channels during conditional differentiation that may be candidates for subunits underlying the currents expressed in mammalian hair cells during development of the sensory epithelium. We speculate that Kv2.1 and Kv1.3 may be molecular correlates of components of $I_{K,emb}$ and $I_{K,neo}$.

Helyer *et al.* (2004). Audiol Neurootol in press.

Marcotti *et al.* (2003). J Physiol **548**, 383-400.

Lawoko-Kerali *et al.* (2004). Mech Dev **121**, 287-99.

This work was supported by the Colt Foundation & Defeating Deafness.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC26

IMMUNOHISTOCHEMICAL LOCALISATION OF THE VOLTAGE GATED POTASSIUM ION CHANNEL SUBUNIT KV3.3 IN THE RAT MEDULLA OBLONGATA AND SPINAL CORD

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Voltage gated potassium channel subunits of the Kv3 subfamily (Kv3.1-Kv3.4) play a vital role in action potential repolarisation in neurons (Rudy and McBain). We have previously reported on the differential distribution of Kv3.1b, Kv3.2 and Kv3.4 (Brooke et al., 2004) in the medulla oblongata and spinal cord. Since cellular and sub-cellular differences in the pattern of ion channel expression can aid in defining specific roles of channels, here we examine the distribution of the Kv3.3 subunit.

Rats (100-200g, n=5) were injected intraperitoneally with 0.1ml 1% Fluorogold (Fluorochrome Inc.) and 7 days later were humanely killed by Sagatal (100mg/kg i.p) and perfused transcardially with 4% paraformaldehyde/0.1-0.5% glutaraldehyde. Sections (50 m) of medulla and thoracic spinal cord were cut and incubated in anti-Kv3.3 antibody (Alomone; 1:1K), followed by a Cy3 conjugated secondary antibody and dual labelled with various antibodies to neurochemical markers, visualised using a biotinylated conjugated secondary antibody and Streptavidin Alexa488.

Kv3.3-immunoreactivity (Kv3.3-IR) was observed in the somata of discrete neuronal populations throughout the spinal cord and medulla. Particularly dense labelling was observed in motor nuclei, dorsal column nuclei (DCN), dorsal medullary nucleus, lateral reticular nucleus, spinal trigeminal nucleus (SpV). A few strongly labelled cells were present in the medial subnucleus of the nucleus tractus solitarius (NTS), raphe nuclei and throughout spinal laminae III-X. Kv3.3-IR neurones did not contain 5-HT or tyrosine hydroxylase, but some cells co-localised with the 200Kd neurofilament, NF200, a marker for heavily myelinated neurones. Kv3.3 predominantly co-localised with Kv3.1b, but some Kv3.1b-IR cells did not contain Kv3.3-IR. Autonomic preganglionic nuclei were predominantly devoid of labelling. Kv3.3-IR was identified in presynaptic terminals throughout the brainstem and spinal cord by co-localisation with SV2 and/or electron microscopic examination. These terminals were immunopositive for markers of excitatory (VGluT2) or inhibitory (GlyT2) terminals.

This study indicates that Kv3.3 subunits are found in neuronal somata and presynaptic terminals throughout the spinal cord and brainstem. The Kv3 subunits are differentially distributed, suggesting specific functions in some cell types.

Brooke, R.E., Atkinson, L., Batten, T.F.C., Deuchars, S.A. and Deuchars, J. (2004). Association of potassium channel Kv3.4 subunits with pre- and post-synaptic structures in brainstem and spinal cord. Neuroscience, 126(4): 1001-1010

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC27

A COMPARISON OF PHOSPHATE ABSORPTION BY THE MOUSE AND RAT SMALL INTESTINE: AN *IN VIVO* STUDY.

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Early studies performed mainly *in vitro* using rat small intestine (SI) demonstrated that the duodenum and jejunum are responsible for the bulk of intestinal phosphate (Pi) absorption (Walling, 1977). The protein responsible for this process has been identified as NaPi IIb, a sodium-dependent transporter expressed at the enterocyte brush border membrane (Murer et al., 2001). More recent studies have demonstrated that this protein is expressed in the ileum, as well as the proximal SI of mice (Personal communication). Based on these discrepancies, we aimed to examine the involvement of distinct SI regions on the absorption of Pi *in vivo* in rats and mice.

Male Sprague-Dawley rats (250 g) were anaesthetized with pentobarbitone sodium (50 mg/kg i.p.). Segments of duodenum, jejunum or ileum (7-10 cm long) were selected and flushed with warm 0.9% saline, followed by air. Uptake buffer (500µl) containing 100µM Pi labeled with ^{32}P was instilled into the lumen and the segment was tied off. Blood was collected via cardiac puncture after 10 minutes and Pi absorption calculated from the ^{32}P activity in plasma and expressed as a percentage of that in the uptake buffer. At the end of experiments animals were humanely killed. Results are given as a mean percentage of Pi absorbed into 1ml of plasma by 5cm of intestine ± SEM (n=6). The same procedure was performed using male C57 BL/6 mice with the following minor modifications. Segments of proximal SI (7-10cm) comprised the duodenum and jejunum, whilst a distinct region of ileum was selected. For mice 200µl of uptake buffer was used to avoid intestinal distention.

Pi transfer from lumen to blood by the rat SI was highest in the duodenum (0.089 ± 0.025%) followed by the jejunum (0.021 ± 0.005%), with relatively little absorption occurring in the ileum (0.0068 ± 0.001%). In contrast, in the mouse, SI uptake was significantly higher in the ileum than in the duodenum/jejunum segment (0.290 ± 0.036 vs. 0.143 ± 0.027%, P⟩0.01 using an unpaired t test).

The mouse small intestine has the ability to absorb phosphate in all segments examined, with the highest rate of absorption occurring in the ileum. In contrast the ileum of the rat has little ability to absorb this anion. These findings highlight a distinct difference in the handling of phosphate by the rat and mouse SI.

Murer, H. *et al* (2001). *Mol.Membr.Biol.* **18**, 3-11.

Walling, M. W. (1977). *Am J Physiol* **233**, E488-E494.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC28

EFFECTS OF THE KV3.4 PEPTIDE TOXIN BDS-II ON NEURONES WITHIN THE DORSAL VAGAL NUCLEUS OF RAT

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Kv3 subunits of voltage gated potassium channels are predominantly located within fast spiking neurones, facilitating brief action potentials (Rudy et al., 1999). Problems arise when investigating the functional properties of the Kv3 subunits in native tissue due to formation of heteromultimers. We have localised Kv3.4 immunoreactivity and an absence of immunoreactivity for the other Kv3 subunits within neurones of the dorsal vagal nucleus (DVN, Brooke et al., 2004). The aim of this study was to investigate a functional role for the Kv3.4 subunit within DVN neurones, utilising a peptide toxin, blood depressing substance, reported to selectively act on the Kv3.4 subunit (Diochot et al., 1998).

For electrophysiology, male Wistar rats (15-21days) were humanely killed by anaesthetising with sodium pentobarbitone (120mg kg-1, I.P.) followed by transcardial perfusion with sucrose aCSF and subsequent decapitation. 300 µm coronal slices of the medulla oblongata were prepared. Whole cell patch clamp recordings were made from neurones within the dorsal vagal nucleus (DVN, n=10) at room temperature. Application of the Kv3.4 blocker BDS-II (50nM) increased the action potential duration (5.39±0.16ms to 7.04±0.17ms, mean±SEM, P<0.05, Student's paired t test) and increased the afterhyperpolarisation amplitude (15.4±0.85mV to 18.6±0.63mV, P<0.05). This suggests that the Kv3.4 subunit contributes to the repolarisation phase of action potentials within the DVN.

To investigate the presynaptic effect of BDS a stimulating electrode was placed in the solitary tract to evoke synaptic events within DVN neurones. BDS-II increased the amplitude of EPSPs (5.6±0.21mV to 7.3±0.21mV, P<0.05) and IPSPs (5.2±0.4 to 6.3±0.32, P<0.05) and decreased the paired pulse ratio (EPSPs 0.93 to 0.78, IPSPs 0.82 to 0.73, P<0.05), indicative of a presynaptic effect (n=8). Furthermore BDS-II significantly increased the frequency of spontaneous PSPs (0.227±0.06Hz to 0.373±0.04Hz, P<0.05) but did not significantly change the amplitude (n=4). In the presence of TTX (1mM) BDS-II did not alter the frequency or amplitude of mini PSPs (n=4) suggesting that BDS-II increases neurotransmitter release in an action potential dependent manner.

These data show that neurones within the DVN are sensitive to BDS-II consistent with the presence of channels formed by the Kv3.4 subunit. In the DVN these Kv3.4 containing channels regulate action potential duration and transmitter release.

Rudy, B. et al. (1999). Ann.N.Y.Acad.Sci. 868,1-12

Brooke, R.E. et al. (2004). Neurosci 126, 1001-10

Diochot, S. et al. (1998). J Biol Chem 273, 6744-9

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC29

REAL-TIME RT-PCR ANALYSIS OF VOLTAGE-GATED SODIUM CHANNEL TRANSCRIPTS IN LEECH GANGLIA AFTER INJURY

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A family of four voltage-gated sodium channels have been partially cloned in the medicinal leech, *Hirudo medicinalis* and are named LeNa1-4 (Blackshaw *et al.*, 2003). We have devised an isoform specific quantitative real-time RT-PCR technique to study the expression of the four voltage-gated sodium channel transcripts in the leech central nervous system following experimental nerve injury. The levels of LeNa transcripts were measured in identified segmental ganglia, 1 day and 7 days after bilateral transection of their nerve roots and compared to the levels in control ganglia (n=6 for all groups). The levels of three housekeeping genes, elongation factor 1-alpha, cyclophilin-A and 18S rRNA were assayed in parallel and used to normalise LeNa expression. There was a statistically significant reduction in the level of non-normalised LeNa1 and LeNa3 in ganglia 1 and 7 days after injury relative to control ganglia (Dunnett's t test $P<0.05$). There was no significant change in the level of non-normalised LeNa2 or LeNa4. A similar pattern of results was obtained when LeNa expression was normalised to each of the housekeeping genes but there were some inconsistencies in the statistical significance of the data. In order to confirm the dynamic expression of voltage-gated sodium channel isoforms independently of housekeeping gene expression, we analysed the ratios of the LeNa isoforms to each other. There was a highly significant reduction in the ratio of LeNa1/LeNa4 (mean ratio calibrated to control group which is set as 1±SEM, control = 1±0.03, 7 days = 0.65±0.04) and LeNa1/LeNa2 (control = 1±0.04, 7 days = 0.59±0.02) at 7 days after injury relative to control (Dunnett's t test $P<0.001$).

There was also a significant reduction in the ratio of LeNa3/LeNa2 (control = 1±0.12, 7 days = 0.56±0.06) and LeNa3/LeNa4 (control = 1±0.14, 7 days = 0.61±0.06) in ganglia at 7 days after injury relative to control (Dunnett's t test $P<0.05$). There was no significant difference in the ratio of LeNa2/LeNa4 and LeNa1/LeNa3 after injury relative to control ganglia. The ratio data supports the conclusion that LeNa1 and LeNa3 are specifically down-regulated in ganglia 7 days after injury in comparison to LeNa2 and LeNa4 which are expressed at control levels. Current experiments are aimed at analysing the expression of LeNa transcripts in identified neuronal types after axotomy.

Blackshaw SE *et al.* (2003) *J. Neurobiol.* 55(3):355-71

Julie Malek was supported by the Wellcome Trust.

PC30

MUTATIONS OF THE CONSERVED GLUTAMATE RESIDUES E30 AND E182 ALTER IONIC SELECTIVITY AND PH SENSITIVITY IN THE TANDEM PORE K+ CHANNEL TASK-1

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The amino acid residues situated in the pore domain and constituting the selectivity filter are highly conserved amongst potassium channels. Studies of Shaker (Larsson & Elinder 2000) and KcsA (Doyle 1998) have also shown a region at the extracellular end of S5 to contain a glutamate residue that both appears highly conserved within the voltage-gated potassium channel family and is thought to play a crucial role in C-type inactivation. This glutamate is also present at the equivalent position in M1 and M3 in the tandem pore K^+ channel TASK-1 and is conserved throughout the family. We have mutated in turn both glutamate residues (E30 and E182) to cysteine in TASK-1 and observed the effects on ionic selectivity and pH sensitivity.

Channels were expressed in oocytes taken from Xenopus frogs that had been anaesthetised by immersion in 0.3% w/v MS222 and killed by destruction of the brain and spinal cord. Two-electrode voltage clamp was used to measure the pH sensitivity, and the shift in reversal potential when K^+ in the external medium was replaced by Rb^+ or Na^+.

Surprisingly, these mutated residues, located outside the pore region of the channel, exhibited altered selectivity compared to wild type channels. P_{Rb}/P_K was significantly increased from 0.77 ± 0.02 (n=6) in wild type to 0.88 ± 0.03 (n=6) in E30C and 0.93 ± 0.02 in E182C (n=5; mean ± s.e.m; $P<0.05$, using ANOVA). Unlike wild type, both mutants were noticeably Na^+-permeant, with an increase in P_{Na}/P_K from 0.02 ± 0.003 (n=6) to 0.20 ± 0.04 (n=6) and 0.45 ± 0.03 (n=5; $P<0.001$) for E30C and E182C respectively.

We found that like wild type channels (pK_a of 6.02 at +40mV), E30C exhibited complete pH dependence, albeit with a reduced affinity for protonation, indicated by a pK_a of 6.60 at +40mV. However, E182C showed a reduced pH dependence, and incomplete channel closure at physiological pH. Fitting the residual pH-sensitive current gave a pK_a of 7.60 at +40mV.

The change in pH dependence and selectivity exhibited with these non-pore mutations suggests that these residues may have a role in channel gating. The equivalent residue in KcsA and shaker is hypothesised to H-bond with local residues to stabilise the inactivation gate in its open conformation (Larsson & Elinder 2000). Our preliminary model of TASK-1 also indicates that E30 and E182 may form H-bonds with local residues T103 and Q209.

Doyle et al (1998). Science 280: 69-77

Larsson & Elinder (2000). Neuron, 27: 573-583.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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MUTATION OF THE ISOLEUCINE RESIDUE, I94 IN THE K^+ CHANNEL CONSENSUS SEQUENCE ALTERS THE IONIC SELECTIVITY AND PH-DEPENDENCE OF THE TANDEM PORE K^+ CHANNEL TASK-3

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The K^+ channel selectivity filter is found in the pore (P-) region of the channel and contains a consensus sequence of amino acids, TxxTxGYG. Studies in voltage gated (Taglialatela et al., 1993) and inward rectifier (Passmore 2003) channels have shown the residue preceding the GYG triplet of the signature sequence is important in determining the relative permeability of the channel to Rb^+. In TASK-3 the equivalent residue is an isoleucine (I) in both pore domains. We have previously reported that mutation of this residue in TASK-1 alters channel selectivity (Yuill et al 2004). We have substituted a serine residue for the isoleucine at position 94 in P1 of TASK-3 and expressed the channel in Chinese hamster ovary (CHO) cells. Whole cell patch clamp was used to measure pH sensitivity, and channel selectivity was measured from the shift in reversal potential when 70mM K^+ was replaced by Rb^+ or Na^+.

The I94S mutant had a significant effect on the selectivity of the channel. The permeability ratio for Rb^+/K^+ was increased from 0.69 ± 0.004 (n=3; mean ± s.e.m.) in wild type channels, to 1.08 ± 0.02 (n=3; P<0.01) in I94S. The effect on the permeability of I94S to Na+ was striking, as wild type channels are relatively Na+ impermeant with a P_{Na}/P_K of 0.01 ± 0.004 (n=3). In I94S the P_{Na}/P_K was significantly increased to 0.97 ± 0.007 (n=3; P<0.01).

The I94S mutant also displayed an altered pH dependence compared to wild type channels, which have a pK_a of 6.45 at –100mV (70mM $[K+]_O$). I94S reduced pH sensitivity, with a basal level of activity present between pH 5 and 6.5, indicating incomplete channel closure. The residual pH sensitivity gave a pK_a of 6.86 at 100mV (n=3).

These results show that I94 is crucial for selectivity in TASK-3. They also suggest that H98 is not the only residue involved pH sensing. Therefore, like Lopes et al (2001), we also believe that the response to acidification in these channels must involve a gating process.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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INCREASED IRON TRANSPORT ACROSS RAT DUODENUM DURING PROLONGED HYPOXIA IS ASSOCIATED WITH REDUCED HEPCIDIN GENE EXPRESSION

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Control of intestinal iron absorption is crucial for body iron homeostasis. We have shown that short-term (3 d) hypoxia promotes iron uptake across the duodenal brush border membrane (O'Riordan et al. 1997). This present study reports the effect of prolonged hypoxia on mucosal iron uptake as well as iron transfer to the blood and the expression of hepcidin, a proposed negative regulator of duodenal iron transport (Laftah et al. 2004).

Sprague-Dawley rats (90-100 g) were exposed to 10% oxygen or room air for 28-33 d. During this time they were pair-fed and weighed weekly. Before experimentation, animals were terminally anaesthetized (pentobarbitone sodium, 90 mg.kg, i.p) and blood and liver samples were taken to determine haemoglobin (Hb) level and gene expression of hepcidin respectively (Laftah et al. 2004). For mucosal uptake and autoradiographic studies, 2 cm everted duodenum was pre-incubated for 5 min in oxygenated, well-stirred Hepes buffer containing 10 mM glucose (O'Riordan et al. 1997). The tissue was then exposed for 5 min to this buffer with added 0.2 mM 59Fe2+:ascorbate (pH 6) and then washed free of surface bound iron and fixed. Tissues were weighed, gamma-counted and processed for autoradiography (O'Riordan et al. 1997). Parallel in vivo experiments on rats under terminal anaesthesia (pentobarbitone sodium, 90 mg.kg, i.p) measured the rate of appearance of 59Fe in blood for <30 min after instillation of buffer containing 0.2 mM 59Fe2+:ascorbate into closed 5 cm duodenal loops (O'Riordan et al. 1995). Rats were then humanely killed.

Hypoxia increased Hb concentration by 47.2% (means±s.e.m.: 12.7±0.4 (n=5) *vs* 18.7±0.6 (7) g/100 ml p<0.001, Students unpaired t-test), and reduced the expression of liver hepcidin mRNA by 60% (2.70±0.56 (3) *vs* 1.08±0.34 (4) arbitrary absorbance units, p<0.05). Hypoxia promoted both mucosal iron uptake (70.6±6.0 (6) *vs* 206.6±57.5 (7) pmoles/g tissue, p<0.05) and lumen to blood iron transfer (20 min: 0.60±0.10 (8) *vs* 1.14±0.20 (8), 30 min: 0.70±0.11 [8] *vs* 1.92±0.50 (8) pmoles/ml blood/g mucosa, both p<0.05). Hypoxia did not affect villus length but autoradiographic analysis showed that this condition increased enterocyte 59Fe content at all stages of cell maturation, particularly the mid-villus region, without affecting the overall developmental profile of villus iron uptake.