

C103

Control of spike frequency adaptation in medial entorhinal cortex layer II stellate cells

F. Khawaja, A. Alonso and C.W. Bourque

Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, QC, Canada

Spike-frequency adaptation (SFA) controls neuronal excitability in cortical neurons and is attributed to Ca^{2+} -dependent K^+ (K_{Ca}) currents which underlie most of the medium and slow afterhyperpolarizations (mAHP and sAHP) [1].

The entorhinal cortex (EC), part of the medial temporal lobe, serves as a 'cross-road' between the cortex and the hippocampus and is implicated in memory and learning [2]. Recent studies of cortical cells show that a reduction in SFA and post-burst AHP currents correlates with enhanced learning capability [3]. Also, medial EC (MEC) layer II stellate cells (SCs) provide one of the most prominent cortical inputs to the hippocampus [4]. Therefore, we investigated how SFA and the mAHP and sAHP are modulated in these cells.

Recordings were obtained under the whole-cell patch clamp configuration in a rat *in vitro* slice preparation. The internal solution contained (mM): 120 KMeSO_4 , 10 Hepes, 0.2 EGTA, 20 KCl, 2 MgCl_2 , 7 diTrisPhCr, 4 Na_2ATP and .3 TrisGTP. The external solution contained 126 NaCl, 2.5 KCl, 2 CaCl_2 , 2 MgCl_2 , 25 NaHCO_3 and 10 glucose. Kynurenic acid (2 mM) and picrotoxin (100 μM) were used to block synaptic transmission. For statistics, we used paired Student's t tests. Linopirdine (20 μM) and ZD-7288 (30 μM) were used to block M and H currents, respectively. Ca^{2+} -dependent (Ca^{2+} -dep.) currents contributing to the mAHP and sAHP were induced by applying a 100 ms step depolarization to +20 mV from a holding potential of -50 mV. The Ca^{2+} -dependent currents ($n = 9$; Table 1, Expt 1) consisted of a medium (Ca^{2+} -dep. mAHP) and a slow component (Ca^{2+} -dep. sAHP) in SCs. Apamin, a blocker of SK channels, mediates the Ca^{2+} -dependent K^+ component of the mAHP in cortical neurons [1]. However, we found that whereas the mAHP of MEC layer II non-SCs could be inhibited by 300 nM apamin ($p = 0.0194$; $n = 3$), that of MEC Layer II SCs was not affected (300 nM; $n = 9$; $p = 0.6982$). Stimulation of cAMP production has been shown to suppress sAHP in cortical neurons, without affecting the mAHP [1]. Application of forskolin (50 μM) and Ro20-1724 (200 μM), which stimulates cAMP production, suppressed both the mAHP ($p = 0.0028$) and the sAHP ($p = 0.0059$; $n = 8$) in SCs. To invoke repetitive firing, we applied 6 s 150 pA pulses. Application of forskolin (25 μM) decreased the SFA index ($p = 0.0012$) and post-burst AHP area ($p = 0.0070$; $n = 4$). We conclude that the Ca^{2+} -dep. mAHP is not mediated by SK channels in SCs. Also, the Ca^{2+} -dep. mAHP, sAHP and SFA are modulated by the cAMP pathway in SCs. Table 1. Results

Experiment	1		2		3		4		5	
	Control	+ $\text{Cd}^{2+}/\text{Co}^{2+}$	(SCs) Control	+ Apamin	(non-SCs) control	+ Apamin	Control	+ Forskolin & Ro20-1724	Control	+ Forskolin
mAHP amplitude	166.4 ± 20.69	70.47 ± 13.31	229.5 ± 38.43	226.7 ± 37.17	165.7 ± 33.71	36.67 ± 5.369	184.8 ± 23.11	62.06 ± 13.46	—	—
sAHP amplitude	36.67 ± 15.18	0.536 ± 0.539	16.39 ± 6.400	14.80 ± 5.920	—	—	32.71 ± 6.436	8.821 ± 1.287	—	—
Spike freq. adap. index	—	—	—	—	—	—	—	—	39.82 ± 2.003	23.08 ± 1.428
Post-burst AHP area	—	—	—	—	—	—	—	—	54.61 ± 5.190	28.60 ± 2.098

All values are given as means ± S.E.M.

Stocker M *et al.* (2004). *Toxicon* **43**, 933-949.

Squire LR *et al.* (2004). *Annu Rev Neurosci* **27**, 279-306.

Saar D & Barkai E (2003). *Eur J Neurosci* **17**, 2727-2734.

Insausti R *et al.* (1997). *Hippocampus* **7**, 146-183.

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

C104

The involvement of Kv7/M-current in regulating CA1 pyramidal cell dendritic excitability

M. Shah and D.A. Brown

Pharmacology, University College London, London, UK

The M-current is a non-inactivating potassium current that plays a critical role in regulating neuronal excitability. Neuronal M-channels are composed of KCNQ2-5 (Kv 7.2-7.5) subunits (Wang *et al.* 1998; Shah *et al.* 2002; Hadley *et al.* 2003). Recent evidence indicates that KCNQ subunits are expressed in hippocampal CA1 dendrites (Roche *et al.* 2002; Shah *et al.* 2002) but their functional role therein is unknown. We have used pharmacological tools to assess if functional M-currents are present in CA1 pyramidal cell dendrites and how they affect dendritic excitability.

Whole-cell current-clamp recordings were obtained from the somata and dendrites of visually identified CA1 pyramidal neurones in hippocampal slices obtained from 5-7 week old Sprague-Dawley rats (see Shah *et al.* 2004). Bath application of the KCNQ channel opener retigabine (10 μM) induced a significant hyperpolarization (3.0 ± 0.3 mV, $n=9$, $p<0.05$; mean ± s.e.m) of the somatic resting membrane potential (RMP) and a substantial decrease in the somatic input resistance (RN) when measured positive to -70 mV. Hence, greater current was required to evoke action potentials. Conversely, the KCNQ channel blocker, linopirdine (10 μM) significantly depolarized somatic RMP (8.75 ± 1.9 mV, $n=6$, $p < 0.05$), enhanced RN and substantially reduced the current required to induce action potentials. In contrast, retigabine had little effect on dendritic RMP (1.4 ± 0.6 mV, $n=8$) or RN. Despite this, greater dendritic current injection was required to record action potentials in the dendrite in the presence of retigabine. Linopirdine did depolarize the dendritic RMP (4.3 ± 0.4 mV, $n=3$) but did not affect dendritic RN. Notwithstanding, more action potentials were recorded in the dendrite in the presence of linopirdine.

To test for effects of M-current on subthreshold activity, effects of KCNQ channel modulators were assessed on single and repetitive somatic and dendritic simulated excitatory postsynaptic potentials (αEPSPs). Linopirdine significantly increased the amplitude and decay time constant of single somatic but not dendritic αEPSPs , by $41.8 \pm 8.1\%$ ($n=6$, $p < 0.05$) and $32.1 \pm 12\%$ ($n=6$, $p<0.05$) at -60 mV, respectively. It did not, however, modify the summation of a train of 5 αEPSPs evoked at 20 Hz or 40 Hz. Retigabine did not affect the amplitude of somatic or dendritic αEPSPs , but accelerated their decay and significantly reduced somatic αEPSP summation (% reduction = $47.2 \pm 3.6\%$, $n=8$, $p < 0.05$, at -60 mV).

From these observations it is concluded that M-channels are predominantly expressed in the soma/axon region (see Devaux *et*

al. 2004), but that alterations of somatic M-current can, nonetheless, affect dendritic excitability.

Devaux et al. (2004). J Neurosci 24, 1236-1244.

Hadley et al. (2003). J Neurosci 23, 5012-5019.

Shah et al. (2002). J Physiol 544, 29-37.

Shah et al. (2004). Neuron 44, 495-508.

Work was supported by the Wellcome Trust and European Union.

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

C105

Reductions of hyperpolarization-activated inward currents of human neocortical neurones correlate with severity of epilepsy

S. Wierschke¹, T. Lehmann², C. Dehnicke³, R. Nitsch¹ and R.A. Deisz¹

¹Centre for Anatomy, Institute of Cell Biology and Neurobiology, Charité Universitätsmedizin Berlin, Berlin, Germany, ²Neurosurgery, Charité Universitätsmedizin Berlin, Berlin, Germany and ³Epileptology, Epilepsy Centre Berlin-Brandenburg, Berlin, Germany

Hyperpolarization-activated inward currents (I_H) have diverse functions in central neurones, including participation in oscillatory behaviour, stabilization of resting membrane potential and attenuation of temporal summation of EPSPs. The reduced magnitude of I_H in a rodent model of epilepsy (Strauss et al. 2004) and the augmentation of I_H by anticonvulsants (Poolos et al. 2002) further indicates that a reduced I_H may contribute to hyperexcitability in epileptic disorders.

We further evaluated properties of I_H in human neocortical slices obtained from epilepsy surgery tissue (Kole et al. 1999). All patients (except one tumour case) suffered from pharmacoresistant epilepsy, i.e. had received various antiepileptic drugs. The methods were similar to those described previously (Deisz, 1999; Strauss et al. 2004). Hyperpolarising command potentials (2s, from -60 to -140 mV) evoked increasing inward currents, which were reduced by ZD7288 (n=6). The current densities, estimated from the currents at -140 mV and the membrane capacitance, averaged -2.6 ± 1.7 pA/pF (mean \pm s.d.; n=236) in neurones from human cortex and -6.9 ± 3.5 pA/pF (n=66) in rat cortical neurones ($P < 0.001$; t test). The time constant of fast activation averaged 43.5 ± 34.2 ms (n=264) in human neurones and 17.0 ± 10.3 ms (n=69) in rat neurones ($P < 0.001$). Application of 1 mM Ba^{2+} reduced instantaneous (Kir-type) currents of human neurones and increased I_H (control: -322 ± 292 pA; Ba^{2+} : -437 ± 382 pA; n=65; $P < 0.001$). The time constant of the fast activation (in Ba^{2+} , at -140 mV) averaged 68.8 ± 38.7 ms in human (n=65) and 50.7 ± 15.9 ms in rat neurones (n=27; $P = 0.0213$). The voltage of half-maximal activation ($V_{1/2}$) of isolated I_H averaged -101.1 ± 8.7 mV in human (n=8) and -92.5 ± 4.7 mV in rat neurones (n=8; $P = 0.029$). The magnitude of I_H in human neurones was unaffected by application of lamotrigine (100 μ M) alone (n=22; $P = 0.67$) or in the presence of 1 mM Ba^{2+} (n=46; $P = 0.76$). In 41 tissues we estimated a burden of disease from the patients' seizure

frequencies (annual number of grand mal (GM) \times years of epilepsy). We averaged the I_H densities of the neurones in control conditions from each tissue (2-11 neurones, average 4.8) and divided the tissues into two groups (below or above 15 GM). The tissues from patients with few GM (including the tumour case) had significantly higher average I_H densities (-3.2 ± 1.4 pA/pF; n=21) compared to the group with many GM (-2.2 ± 0.8 pA/pF; n=20; $P = 0.013$). The group with few GM (average total count of GM 1.6) had the same average age (38.1 ± 14.8 years) as the group with many GM (average total count 352 GM, average age: 38.1 ± 15.1 years, $P = 0.98$).

Our data indicate that specimen from patients with many seizures contain neurones with significantly lower average I_H densities. The difference in current magnitude, kinetics and $V_{1/2}$ values between rat and human epileptogenic cortex, may indicate a reduced contribution of HCN1 subunits in epileptogenic neocortices.

Deisz RA (1999). Neuropharmacology 38, 1755-1766.

Kole MHP et al. (1999). Soc Neuroscience 25, 844.

Poolos NP et al. (2002). Nature Neurosci 5, 767-773.

Strauss U et al. (2004). Eur J Neurosci 19, 3048-3058.

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

C106

The KCNQ2 (Kv7.2) gene is required for functional M-channels in embryonic mouse superior cervical ganglion (SCG) neurones

G.M. Passmore¹, J. Robbins², F. Abogadie¹ and D.A. Brown¹

¹Pharmacology, University College, London, UK and

²Pharmacology, King's College, London, UK

M-channels in rat (Wang et al. 1998; Hadley et al. 2003) and mouse (Selyanko et al. 1999) SCG neurones are most likely composed primarily of Kv7.2 and 7.3 subunits of the Kv7 potassium channel family. However, since all Kv7 subunits can generate 'M-like' currents (Selyanko et al. 2000), and since rat SCG neurones express three Kv7 subunits (Kv7.2, 7.3 and 7.5: Hadley et al. 2003), we have used Kv7.2 gene-deleted mice (Watanabe et al. 2000) to further assess the contribution of this particular subunit to M-channels in SCG neurones.

Homozygote Kv7.2-null mice do not survive after birth, so we used neurones from SCGs isolated from E17 embryos and cultured for 1-2 days in vitro. M-currents (I_M) were measured in neurones from wild-type (+/+), heterozygous (+/-) and homozygous (-/-) gene-deleted embryos using amphotericin-perforated patch pipettes (see Hadley et al. 2003).

Double gene-deletion totally eliminated M-current expression. Thus, sustained outward currents recorded at -20 mV holding potential were significantly ($P < 0.05$, ANOVA) reduced from 12.6 ± 2.1 pA pF⁻¹ (n=18) in +/+ cells to 4.7 ± 1.6 pA pF⁻¹ (n=17) in -/- cells, and no deactivation I_M -tails could be detected in -/- cells on stepping to -50 mV. Further, the M-channel enhancer retigabine (10 μ M) increased outward currents in +/+ cells, but not in -/- cells.

Single gene-deletion partly reduced I_M . Retigabine produced significantly ($P < 0.05$, ANOVA) less increase in the standing outward current at -20 mV in +/- cells and the deactivation tail-current at -50 mV was reduced by ~60% (4.5 ± 1.1 pA pF⁻¹ (n=18) in +/+ cells; 1.8 ± 0.2 pA pF⁻¹ (n=33) in +/- cells). Further, this residual current was less sensitive to tetraethylammonium (TEA) than that in +/+ cells (tail-current reduction by 10 mM TEA being $91.6 \pm 3.5\%$ (n=9) in +/+ cells and $56.2 \pm 8.3\%$ (n=7) in +/- cells). This suggests some substitution of TEA-sensitive Kv7.2 subunits by TEA-insensitive subunits such as Kv7.5 in +/- mouse embryos (see Hadley et al. 2003). These results accord with an obligatory contribution of Kv7.2 subunits to embryonic M-channel formation. Cells from gene-deleted mice were also hyper-excitable, so reduced I_M might explain the seizures that occur in human neonates with Kv7.2 mutations.

Hadley JK et al. (2003). J Neurosci 23, 5012-5019.

Selyanko AA et al. (1999). J Neurosci 19, 7742-7756.

Selyanko AA et al. (2000). J Physiol 522, 349-355.

Wang H-S et al. (1998). Science 282, 1890-1893.

Watanabe H et al. (2000). J Neurochem 75, 28-33.

Supported by the UK MRC. We thank Dr Hitoshi Sasai (Japan Tobacco Inc., Yokohama, Japan) for supplying KCNQ2 gene-deleted mice.

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

SA54

Alternative splicing of calcium-activated potassium channels

M.J. Shipston

University of Edinburgh, Edinburgh, UK

Alternative pre mRNA splicing and gene duplication represent distinct evolutionary mechanisms to generate functional diversity of pore-forming α -subunits of potassium channels. Large conductance calcium- and voltage activated potassium (BK) channels are encoded by a single gene that undergoes extensive alternative pre mRNA splicing resulting in BK channels with dramatically different phenotypes. Splicing can modify calcium sensitivity and channel kinetics, regulate cell surface expression and switch channel regulation by reversible protein phosphorylation and other intracellular signalling cascades. Importantly the splicing decision itself is also dynamically regulated, with distinct patterns of splice variants expressed in different cell types, at different stages in development of the nervous system and in response to a physiological challenge. The functional consequence of alternative splicing on BK channel properties, regulation and function in the nervous system will be explored.

Supported by The Wellcome Trust

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

SA55

Physiology of SK channels

J.P. Adelman and J.G. Maylie

Vollum Institute, OHSU, Portland, OR, USA

Small conductance Ca-activated K channels (SK channels) are important determinants of neuronal excitability. SK channels are gated solely by intracellular Ca ions and are activated by increased levels of cytosolic Ca such as occur during an action potential. SK channel activity exerts a repolarizing effect that continues past the action potential spike and in many neurons contributes to a prolonged afterhyperpolarization. Structurally, SK channels are tetramers of the α , pore-forming subunits, together with four calmodulins (CaM). CaM binds to the intracellular C-terminus of the channel subunits. Ca binding to the N-lobe E-F hands of CaM induces channel gating. Recent studies reveal that the SK channel complex contains several additional, constitutively associated regulatory proteins. Moreover, the molecular composition of the SK channel complex may vary in distinct subcellular compartments, suggesting that the same expressed α subunit may serve a variety of roles depending upon subcellular address and macromolecular identity. The distinct coupling of SK channels to different Ca sources in dendrites, where they affect dendritic integration, and in spines where they modulate NMDA receptor function and impact synaptic plasticity supports this concept.

Supported by NIH grants.

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

SA56

Roles beyond the resting potential for B6 mice with gene-targeted deletion of the Kv1.3 *Shaker* ion channel

D.A. Fadool¹, K. Biju¹, T. Kristal¹ and J.M. Overton²

¹Program in Neuroscience & Molecular Biophysics, Florida State University, Tallahassee, FL, USA and ²Program in Neuroscience, Exercise Science, & Nutrition, Florida State University, Tallahassee, FL, USA

Recent evidence has uncovered unexpected functions for a particular *Shaker* family member, Kv1.3, through gene-targeted deletion approaches. Using whole-cell electrophysiology, we have previously demonstrated that the Kv1.3 current component of mitral cells in the olfactory bulb is modulated by insulin receptor kinase and neurotrophin TrkB, two receptor tyrosine kinase (RTKs) pathways. Upon deletion of the Kv1.3 channel, the expression of the RTKs and their associated scaffolding proteins was perturbed as determined by SDS-PAGE and the mitral cells were insensitive to insulin and BDNF, the preferred ligands for these RTKs. The action potential frequency, 10 to 90% rise time, afterhyperpolarization, and duration was significantly changed in mitral cells of the Kv1.3-null animals. In light of these marked electrical and biochemical changes in neuronal signaling in the olfactory bulb, we were intrigued to explore the ability of the mice to smell. Contrary to our expectation, mice were not anosmic when tested for simple metrics of olfactory ability that did not require learning or memory. In fact, using odorant habituation paradigms, Kv1.3-null mice had the capacity to discriminate odorant molecules that differed by one to four carbon atoms and when challenged with a memory-based, two-choice paradigm, Kv1.3-null mice were found to have significantly different odorant thresholds; detecting odorants that were 10,000 to 100,000 x lower in concentration than those of wildtype animals.

Mice were also monitored in environmental metabolic chambers designed for continuous determination of oxygen consumption, locomotor activity, and ingestive behaviors. The body weight of Kv1.3-null mice was less than that of the wildtype mice even though total caloric and water intake was not different. To test whether deletion of the Kv1.3 gene could prevent weight gain, we bred Kv1.3-null animals with mice lacking the melocortin receptor gene (MC4R) that is found to be defective in human obesity. MC4R mice are hyperphagic and have a late-onset weight gain typical of type 2 diabetes. Progeny were initially solely monitored for weight gain (early developmental and adult late onset) for 1 yr. Mice with the double allelic combination (mmkk) were not significantly different in weight than that of the control wildtype (MMKK) while the MC4R-null mice (mmKK) gained significant weight at 2 months that continued to rise throughout adulthood, suggesting that the deletion of the Kv1.3 gene can prevent weight gain (ANOVA, snk, $n = 36$). Currently we are testing 3 of the allelic combinations (mmkk, MMKK, and mmKK) with a battery of mouse behavioural and cellular phenotypic screens. In our analysis we hope to characterize the degree to which obesity affects olfactory signaling and the role in which Kv1.3 ion channel mediates energy homeostasis. Due to the combined increased olfactory ability of the Kv1.3-null mice and altered electrical signaling of the mitral cell neurons, we explored the potential anatomical changes in the olfactory bulb of the null mice to find that the mean size of the

glomeruli were both significantly smaller and more numerous. We thus tested the hypothesis that axonal projections were altered in the Kv1.3-null mice by crossing them with two different lines of mice in which a single odorant receptor (called M72 or P2) is linked to the expression of lacZ-tagged tau protein. We conjectured that neurotrophins (a 7x increase by SDS-PAGE) may be differentially regulating axonal growth in the absence of the ion channel thereby allowing projections to more than one glomeruli. Visualization of the axonal projections from olfactory sensory neurons (OSNs) containing the P2 receptor protein did not reveal any obvious differences in the location and single glomerular projection between the wildtype and the Kv1.3-null animals. For OSNs containing the M72 receptor protein, however, axons typically projected to three glomeruli as opposed to a single glomerulus in the Kv1.3-null animals and the total number of OSNs expressing M72 receptor protein was significantly reduced. Although the pattern of OSN expression of M72 receptor protein in the olfactory epithelium was not altered, that of the P2 receptor protein was located in the most ventral region of the olfactory epithelium in the null-mice as opposed to its typically dorsal region of expression in wild-type mice.

These data suggest that the Kv1.3 channel plays a far more reaching role than that classically defined for a potassium channel - to shape excitability by influencing the membrane potential - and may have important regulatory roles for olfactory sensory processing, protein-protein interactions, olfactory coding and axonal targeting, and energy homeostasis.

Supported by National Institutes of Health (NIH DC03387).

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

SA57

Regulation of pre- and post-synaptic excitability at an auditory relay synapse

I.D. Forsythe, J. Johnston, A. Skrzypiec and S. Griffin

MRC Toxicology Unit, University of Leicester, Leicester, UK

It is difficult not to get excited about potassium channels, but that is exactly why they are there: – dominating the resting membrane potential, adjusting membrane time-constants, action potential thresholds, firing rates and of course, action potential repolarization and AHP generation. The number and diversity of their genes and spliced variants is exceeded only by the breadth and subtlety of their actions. And therein lies the physiological problem; most potassium channels have now been cloned and the recombinant channels characterised, but correlating the properties of these expressed channels with their native counterparts is complicated by heterogeneous subunit composition and ambiguous pharmacology. We have been studying neuronal excitability and synaptic transmission in the brainstem auditory pathway, focussing on the calyx of Held synapse onto the medial nucleus of the trapezoid body (MNTB). This glutamatergic synapse is a highly secure synapse, with over 2000 release sites generating EPSCs

of up to 20 nA, giving a high safety factor for postsynaptic action potential generation in the MNTB neurone, even at frequencies in excess of 200 Hz. If it were not for the exquisite tuning of the postsynaptic excitability by a range of potassium channels, the information contained in such a large synaptic input would be lost. Combined application of voltage-clamp, specific pharmacological antagonists and immunohistochemistry, have shown that Kv3 and Kv1 channels are located in specific neuronal compartments and have distinct functions. The high-voltage activated Kv3 channels enhance repolarization and minimise action potential duration, while the low-voltage activated Kv1 channels suppress excitability at voltages around threshold (Brew & Forsythe, 1995; Dodson et al. 2002). Both channel types are present in the presynaptic calyx and have analogous functions (Dodson et al. 2003). There is also support for activity-dependent regulation of Kv3 and Kv1 conductances (Song et al. 2005; Barnes-Davies et al. 2004).

In the presence of Kv1 and Kv3 antagonists (100 nM dendrotoxin-I and 3mM tetraethylammonium, respectively) half of the total outward current remains unblocked. Toxins and antagonists are available for most potassium channel subtypes, so we have taken a pharmacological, molecular and biophysical approach to characterise these other endogenous K⁺ currents. RT-PCR demonstrates that TASK, TWIK and TREK channels are expressed in the MNTB. Whole-cell patch recordings demonstrate that multiple twin-pore potassium channels (K2p, KCNK) dominate the resting membrane potential in the auditory brainstem and cortex. In addition, these channels make a major contribution to membrane conductance across the physiological range.

I will briefly describe the basic physiology of the auditory brainstem, review the potassium channel families known to regulate excitability in this region, and present our recent evidence for the role of the twin-pore channels in regulating membrane conductance at rest and on depolarisation.

Barnes-Davies et al. (2004). *Eur J Neurosci* 19, 325-333.

Brew & Forsythe (1995). *J Neuroscience* 15, 8011-8022.

Dodson et al. (2002). *J Neuroscience* 22, 6953-6961.

Dodson et al. (2003). *J Physiol* 550, 27-33.

Song et al. (2005). *Nat Neurosci* 8, 1335-1342.

Supported by the MRC.

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

SA58

Neuronal KCNQ channels: from human mutations to novel functional and pharmacological roles

M. Taglialatela¹, M. Soldovieri², F. Miceli², E. Panza², V. Barrese², F. Iannotti², F. Boscia², M. Martire³ and L. Annunziato²

¹Dept. of Health Science, University of Molise, Campobasso, Italy,

²Dept. Neuroscience, University of Naples Federico II, Naples, Italy

and ³Inst. Pharmacology, Catholic University of Rome, Rome, Italy

Membrane proteins of the potassium (K⁺) channel family play critical roles in controlling neurotransmitter release, neuronal

excitability, heart rate, insulin secretion, epithelial electrolyte transport, smooth muscle contraction, and cell volume. The KCNQ subfamily of voltage-gated K⁺ channels is formed by five members with diverse functional properties. In fact, KCNQ1 subunits underlie the slow repolarizing current (IKs) of the cardiac action potential; mutations in the KCNQ1 gene cause one form of the hereditary cardiac arrhythmia known as Long QT Syndrome. On the other hand, KCNQ2 and KCNQ3 subunits form heteromultimeric complexes whose functional properties recapitulate those of a neuronal-specific K⁺-selective current termed M-current (IKM); IKM plays a dominant role in controlling neuronal excitability and is characterized by a low activation threshold, slow activation kinetics and absence of inactivation. Mutations in KCNQ2 and KCNQ3 genes are responsible for a rare autosomal-dominant neonatal epilepsy known as Benign Familial Neonatal Convulsions (BFNC). KCNQ4 subunits are also expressed in the nervous system, being altered in some rare forms of autosomal dominant deafness. Finally, KCNQ5 subunits contribute to IKM heterogeneity, co-assembling with other KCNQ subfamily members.

The molecular mechanisms responsible for IKM dysfunction prompted by several BFNC mutations are heterogeneous; in fact, while some mutations affect the number of functional channels incorporated in the plasma membrane, possibly by reducing the cytoplasmic protein stability and causing an enhanced proteasomal degradation (1), others may cause permeation or gating defects in normally-assembled channels (2).

Beside their involvement in epilepsy, KCNQ2/3 channels appear as promising targets for pharmacological interventions directed against human hyperexcitability. Openers of neuronal KCNQ channels have been shown to be effective in a broad range of in vitro and in vivo seizure models, and are currently undergoing clinical testing. Some of these molecules also possess neuroprotective actions in in vitro and in vivo models of neurodegenerative diseases (3). On the other hand, IKM inhibitors may improve the symptoms of neurodegenerative conditions associated with neurotransmission

deficits, such as Alzheimer disease, and are currently undergoing investigation as cognition enhancers. These pharmacological tools have been instrumental in defining the role of presynaptic IKM in neurotransmitter release from central nerve endings (4).

In this presentation, we will attempt to describe the heterogeneous consequences on IKM function caused by several BFNC mutations identified by our group, also in the context of the recent progress in the definition of the structural elements involved in gating and permeation in mammalian voltage-gated K⁺ channels (5). Furthermore, we will explore the pharmacological implications of IKM modulation by drugs and neurotransmitters.

Soldovieri MV, Castaldo P, Iodice L, Miceli F, Barrese V, Bellini G, Miraglia Del Giudice E, Pascotto A, Bonatti S, Annunziato L & Tagliatela M (2006). *J Biol Chem* 281, 418-428.

Castaldo P, Miraglia Del Giudice E, Coppola G, Pascotto A, Annunziato L & Tagliatela M (2002). *J Neurosci* 22, RC199 (1-6).

Boscia F, Annunziato L & Tagliatela M (2006). *Neuropharmacology* 2006 (submitted).

Martire M, Castaldo P, D'amico M, Preziosi P, Annunziato L & Tagliatela M (2004). *J Neurosci* 24, 592-597.

Long SB, Campbell EB & Mackinnon R (2005). *Science* 309, 897-903.

Supported by: European Union Specific Targeted Research Project (STREP) PL 503038; Italian National Institute of Health (Ministero della Salute) grant RF/2002, RF/2003 and RC/2002; Italian Ministry of the University and Research (MIUR) FIRB RBNE01XMP4 and RBNE01E7YX_007; Telethon (Project GGP030209); Regione Campania Regional Law n.5 of 28/3/2002 (year 2003) and Centro Regionale di Competenza per il Trasferimento Tecnologico Industriale della Genomica Strutturale e Funzionale degli Organismi Superiori (GEAR).

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