phospholipid (PL(-)) is required to generate high PIP2 sensitivity of Kir2 channel gating, but the PL(-) binding site and mechanism are yet to be elucidated. We used two electrode voltage clamp (TEVC) on channel activity by patch clamp measurements in PC12 cells expressing SK3 channels. Our objective is to determine if the TZD rosiglitazone (RGZ) is directly associated with the formation of kidney oedema. Recent studies have suggested the implication of the epithelial sodium channel expressed in the renal collecting duct for these adverse side effects. Indeed, the PPARγ stimulation by TZDs activate epithelial sodium channel probably by inducing the expression and the activation of SGK1 (Serum and Glucocorticoid-regulated Kinase 1). It is well known that the sodium and potassium transport are tightly linked at the kidney level. Our objective is to determine if the TZD rosiglitazone (RGZ) is directly implicated into the regulation of the potassium transport. To do so, we performed two electrode voltage clamp studies (TEVC) in Xenopus laevis oocytes expressing PPARγ receptor, wild type (wt) and mutant ROMK channels. We have shown that a 48h treatment with 10 μM RGZ produced a 2-fold increase of wt ROMK activity. This activation is blocked by GW9662, a PPARγ antagonist. We have also shown the implication of SGK1 into the regulation of ROMK activity by using an inhibitor of SGK1, SGK639 and by mutating the phosphorylation site of SGK1 on ROMK (ROMK-S44A). Finally, immuno-fluorescence experiments have shown the recruitment of ROMK at the cell’s membrane of the oocytes treated with RGZ. All the results suggest that RGZ increase the potassium current generated by ROMK by increasing the expression of SGK1 and promoting the recruitment of ROMK to the cell’s membrane.

3784-Pos Board B512
Enhancement of Current through TreK1 Two Pore Domain Channels by Flufenamic Acid
Ehab Al-Moubarak, Alistair Mathie.
Medway School of Pharmacy, University of Kent, Chatham, United Kingdom.
Two pore domain potassium (K2P) channels are responsible for background currents that regulate neuronal membrane potential and excitability. It has been shown that TREK1 K2P channels are implicated in pain and can be activated by flufenamic acid (FFA). The aim of this study was to investigate the mechanism of FFA on TREK1 channels. tsa201 cells were transiently transfected with wild type and mutated TREK1 channels. The whole cell patch clamp technique was used to obtain current recordings. Homology models for TREK1 channels were constructed using Modeller 9v8. FFA (100μM) activated TREK1 channels by 207% (n=7). From the mutational model of TREK1, it was predicted that the inner helix residues (A286 and G171) face the ion conductance pathway of TREK1 pore near its intracellular terminus. Mutating A286 and G171 to a bulky F residue, significantly reduced the TREK1 current (WT: 533 ± 87pA, (n=7); A286F: 54 ± 5pA (n=8); G171F: 112 ± 19pA (n=17)). However, FFA (100μM) recovered these reduced currents. TREK1 A286F current was enhanced by 823 ± 275% (n=9); and TREK1 G171F by 523 ± 181% (n=7). Consequently, it was hypothesized that FFA induces a counter-clockwise helical rotation that removes the bulky F side chain from the ion pathway. Accordingly, A287 may move to occupy the A286 pore position. Consistent with this hypothesis, the A287F mutation abolished the FFA effect on TREK1 (enhancement: 7 ± 8%, n=8). Moreover, although, increasing pH extr to 8.4 enhanced TREK1 current by 49 ± 6% (n=7), it did not substantially recover the reduced TREK1 A286F current (enhancement: 87 ± 13%, n=6). These results indicate that FFA may induce conformational changes at the TREK1 inner helix consistent with a counter clockwise helical rotation, which may contribute to TREK1 gating.

3785-Pos Board B513
Regulation of SK Channel Spatial Distribution by Tonic PKA
Kirthika Abiraman1, Anastasios Tzingounis2, George Lykotrafitis1.
1Department of Mechanical Engineering, University of Connecticut, Storrs, CT, USA, 2Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT, USA.
Small-conductance Ca2+-activated K+ (SK) channels mediate a ubiquitous expressed potassium conductance in the brain involved in synaptic plasticity and learning and memory. SK channel spatial distribution shows a polarized topographic expression being highly enriched in neuronal dendrites with limited expression on somatic membranes. However, the mechanism that controls this spatial distribution is unknown. To investigate the mechanisms that control the spatial distribution of SK channels at the single molecule level we combine single molecule force microscopy and toxin pharmacology.

Methods and results: AFM tip functionalized with apamin, a SK channel blocker, is used to detect the distribution of SK channels in living neurons by measuring the binding forces between apamin and SK channels. Employing the above technique, we test the effect of PKA on the clustering and dendritic localization of SK channels. Here, we show that SK2 channel nanoclustering is under the control of cAMP and PKA activity and demonstrate that SK2 channel polarized distribution in neurons is dictated by basal PKA activity.

Conclusion: Our work reveals a new level of regulation of SK2 channels by PKA and also demonstrates for the first time that SK2 spatial distribution is plastic.

3786-Pos Board B514
Evidence for the Interaction of Endophilin 3 with the SK3 Channels in PC12 Cells
Malika Janbein, Stephan Grissmer.
Ulm University, Ulm, Germany.
Small conductance calcium-activated (SK) channels play an important role by controlling the after-hyperpolarization of excitable cells. The level of expression and density of these channels is an essential factor for controlling different cellular functions. Several studies showed a co-localization of SK3 channels and Endophilin 3 in different tissues. Using the yeast two hybrid system and the GST pull down assay we demonstrated that Endophilin 3 interact with SK3 and Endophilin 3 in different tissues. We also showed that SK3 channel nanoclustering is under the control of cAMP and PKA activity and demonstrate that SK3 channel distribution in neurons is dictated by basal PKA activity.

Conclusion: Our work reveals a new level of regulation of SK3 channels by PKA and also demonstrates for the first time that SK3 spatial distribution is plastic.