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REGULATION OF THE HUMAN SHORT TRANSIENT RECEPTOR POTENTIAL CHANNEL 3 (hTRP3) BY THE SERUM- AND GLUCOCORTICOID-INDUCED KINASE 1 (SGK1)

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Received: 4 July 2016; **Accepted:** 9 August 2016

ABSTRACT

The transient receptor potential channels (TRP channels) are widely expressed in a large number of various human and animal cell types. Most of the TRP channels are permeable for Ca^{2+} and some also for Mg^{2+}. TRP channels are divided into three main groups based on their structure: short, long and osm-like TRPs. The short TRP subfamily of cation channels contains mammalian TRPs, TRPL, and the *Drosophila* TRP. The mammalian TRP superfamily of cation channels contains four subfamilies (TRP1; TRP4, 5; TRP2 and TRP3, 6, 7) based on sufficiently similar sequence and function. The human short transient receptor potential channel 3 (hTRP3) is expressed at the highest levels in brain, and at much lower levels in small intestine, colon, testis, prostate, ovary, placenta and lung. Cytosolic Ca^{2+} ($[Ca^{2+}]_i$) plays a crucial role in various cellular functions of virtually all cell types and is thus under tight hormonal control. However, cellular mechanisms governing the regulation of human TRP3 abundance in the cell membrane are poorly understood. Surface abundance of the epithelial Na^+ channel is regulated by the Serum- and Glucocorticoid-Induced Kinase 1 (SGK1). The present study has been performed to explore whether human TRP3 is regulated by SGK1 and the related kinases SGK2, and SGK3. To this end, cRNA encoding human TRP3 (hTRP3) has been injected with or without cRNA encoding wild type SGK1, SGK2, and SGK3 into *Xenopus* Oocytes. In the presence of Cl^-, hTRP3 mediated Ca^{2+} entry leads to secondary activation of Ca^{2+}-sensitive Cl^- channels ($I_{Cl(Ca)}$). Coexpression of hTRP3 with SGK1 stimulates ($I_{Cl(Ca)}$) but not by SGK2, and SGK3. The observations suggest that SGK1 regulate hTRP3 and are thus likely to participate in the regulation of calcium homeostasis.

Keywords: *human TRP3, SGK isoforms, calcium homeostasis*

INTRODUCTION

Calcium ions (Ca^{2+}) are essential for many physiological processes, including fertilization, muscle contraction, hormone secretion, immune responses, brain functions, cell growth and last but not least, apoptosis(Orreniu*set al.,* 2003; Zhang *et al.*, 2015). The signal transduction capacity of Ca^{2+} depends on the 10,000-fold gradient across the plasma membrane with 2.5 mM extracellular and resting intracellular Ca^{2+} ion concentration of approximately 100 nM (Clapham, 1995). Increased cytosolic free Ca^{2+}levels are interposed by either Ca^{2+} inflow across the plasma membrane by using different Ca^{2+} channels (Barritt*et al.,* 2008) orrelease of Ca^{2+} from the internal Ca^{2+}stores such as the endoplasmic Reticulum (ER) (Parekh and Penner, 1997; Verkhratsky and Petersen, 2002).

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In mammals, Ca^{2+} concentration gradient between intracellular and extracellular fluids is tightly regulated by a complex homeostatic control mechanism involving fluxes of Ca^{2+} between theinterstitialfluid and the intestine, kidney, and bone (Mundy and Guise, 1999).The regulation of these fluxes islikely to come from more careful controlof three important hormones: thyrocalcitonin, parathyroid hormone (PTH), and Calcitriol (Boden and Kaplan, 1990). Several important cellular functions are dependent on the maintenance of the extracellular Ca^{2+} concentration within a very narrow range (Brown and MacLeod, 2001). Importantly, disturbances of this tightly controlled homeostatic system causesupsets inthe body's calcium metabolism that have expected effects, which can be attributed to effects on these cellular functions (Peacock, 2010).

Activation of the body's cells by hormones, neurotransmitters, and agents that stimulate the activity of the enzyme phospholipase C (PLC) leads to release of Ca^{2+} from distinct intracellular Ca^{2+} stores (Fan and Byron, 2000) and is followed by passive influx of Ca^{2+} from the extracellular space via a group of channels that have differently been

referred to as Ca^{2+} release activated Ca^{2+} channels (CRAC), store operated Ca^{2+} channels (SOC), and receptor operated Ca^{2+} channels (ROC) (Hoth and Penner, 1992; Zweifach and Lewis, 1995).

These store-operated channels serve refilling of the Ca^{2+} store by providing a Ca^{2+} entry pathway and may in addition control cell membrane potential and homeostasis of monovalent cations (Parekh and Penner, 1997). Because Ca^{2+} entering the cell replenishes the intracellular Ca^{2+} stores that act likes capacitors, it has also been called "CapacitativeCa^{2+} Entry, (CCE)" or "Store-Operated Ca^{2+} Entry, (SOCE)" (Putney, 1990).Transient Receptor Potential (TRP) ion channels or TRPs are thought to mediate CCE or SOCE (Montell, 1997).

TRPsare the largest group of non-selective and polymodalcation channels which pass Ca^{2+} (and other cations too) into the cell down its electrochemical gradient in response to different stimuli, thereby increasing the intracellular Ca^{2+} concentration and causing cell depolarization (Kumar *et al.*, 2014). TRP channels were first described in the fruit fly*Drosophila melanogaster*as a phototransduction*trp*mutant associated with a defect in light-induced calcium entry and a modified response to light (Minke, 1977; Hardie and Minke, 1992). Subsequently members of the TRP family have been identified in vertebrates, and other invertebrates, and in lower eukaryotes such as yeast and fungi. However, so far TRPs or their exact homologs have not been detected in plants (Kumar *et al.*, 2014).

TRPs are classified on the basis of their sequence homology and by the presence of specific signature domains and motifs such as the TRP-domain, TRP-box motifs, ankyrin repeats, etc. (Clapham, 2003). Based on amino acid sequence, homology with other TRP channels, and the presence of specific structural features, the *Drosophila* TRPshave been divided into seven subfamilies, namely, TRPC (Canoical or Classical), TRPV (Vanilloid-like), TRPM (Melastatin-like), TRPA (Ankyrin), TRPP (Polycystin), TRPML (Mucolipin) and TRPN (No Mechanoreceptor Potential C, NOMPC) (Nilius and Owsianik, 2011). However, the mammalian TRPs have been divided into six subfamilies (the TRPN family does not appear in mammals) (Hartenec*et al.*, 2000; Montell, 2005).

In mammals, TRP channels are ubiquitously expressed in almost all cell types and tissues, albeit at different levels (Kumar *et al.*, 2014). Most of the TRPs are selectively activated by specific ligands and are polymodal in nature (Baez-Nieto, *et al.*, 2011). TRP channels are regulated by multiple stimuli, both physical and chemical and a few members of TRPs are thermosensitive. The complex polymodalregulation of TRP channels by intracellular as well as extracellular components such as, pH, interacting proteins, etc. and the multiple routs of regulation by phosphorylation-dephosphorylation, suggest that these channels integrate multiple signaling events at the plasma membrane (Kumar *et al.*, 2014).

Seven mammalian TRPC channels have been isolated, which are further subdivided by sequence and function into four groups: TRPC1, TRPC2, TRPC3/6/7, and TRPC4/5 (Vazquez, *et al.*, 2004; Liao *et al.*, 2014), although it has been suggested that TRPC1 is dissimilar enough from TRPC4 and TRPC5 that it should comprise its own fourth group (Montell, 2005). All TRPC homologs studied were widely expressed within the central nervous system (CNS) and expression in peripheralnervous system (PNS)was often observed. In spite of this, each type of channel exhibited a different distribution profile (Riccio*et al.*, 2002).

Human TRP3is highly expressed in CNS and smooth and cardiac muscle cells, and likely to play a role in both excitable as well as non-excitable cells, being potentially involved in a wide spectrum of Ca^{2+}signalling mechanisms (Li *et al.*, 1999). TRP3 shows multiple potential sites for regulatory phosphorylation in both amino and carboxy termini located in the cytoplasm (Eder *et al.*, 2007). Interactions between different protein-interacting domains in TRP3 channels and a variety of regulatory proteins have been identified that are apparently essential for correct targeting or activation of TRP3 channels (Sinkins*et al.*, 2004).

The Serum- and glucocorticoid-induced protein kinase, SGK (denoted as SGK1), was identified as a novel serine/threonine protein kinase under transcriptional control by serum and glucocorticoids (Webster *et al.*, 1993). The SGK1 gene encodes a 50 kDa protein that is a member of the "AGC" family of serine/threonine protein kinases that includes protein kinases A (PKA),G (PKG), andC (PKC)(Bruhn *et al.*, 2010). SGK1 has two other protein homologues (SGK2 and SGK3), and there are four SGK1 isoforms that are products of alternate translation initiation and can localize to a variety of cellular compartments (Arteaga*et al.*, 2007).

SGK1 is a ubiquitously expressed serine–threonine kinase, highly expressed in the nervous system, playing an important physiological role in CNS in which it regulates different ion channels (Wesch*et al.*, 2010). SGK1 has previously been shown to regulate a wide variety of carriers and ion channels (Lang *et al.*, 2006), including the epithelial Ca^{2+} channels TRPV5 (Embark *et al.*, 2004) and TRPV6 (Sopjani*et al.*, 2010).Furthermore, SGK1 participates in the regulation of renal tubular Na^{+} reabsorption, salt appetite, and thus blood pressure by regulation of renal epithelial Na^{+} channel (ENaC) (Chen *et al.*,

1999).

SGK1 transcription is stimulated by increase in cytosolic Ca^{2+} activity $[Ca^{2+}]_i$ (Lang and Stournaras, 2013). SGK1 has been shown to be critically important for the Ca^{2+} entry into mast cells after activation of the IgE receptor (Sobiesiak*et al.*, 2009), an effect mediated by regulation of Ca^{2+} channel 1 (ORAI Calcium Release-Activated Calcium Modulator 1(Orai1)/ Stromal interaction molecule 1 (STIM1)) (Eylenstein*et al.*, 2011). Recent observations revealed a powerful effect of the SGK1 on Orai1 abundance, SOCE, activation and function of platelets (Borst*et al.*, 2012).

Interestingly, SGK contains a proline rich region (PXXP motif, where x denotes any amino acid) in its amino terminal regulatory domainand another protein interaction motif called PDZ domain in its carboxy terminal (O'Keeffe *et al.*, 2013).SGK1 includes three such PXXP motifs which could be involved in protein-protein interactions withproteins containing tryptophan-rich WW motif and thereby potentially modulate its activity (Zhou and Snyder, 2005). The name PDZ is derived from the first three proteins in which these domains were identified: PSD-95 (a 95 kDa protein involved in signaling at the post-synaptic density), DLG (the Drosophila melanogaster Discs Large protein) and ZO-1 (the zonulaoccludens 1 protein involved in maintenance of epithelial polarity) (Harris and Lim, 2001).

The aim of the current study was to investigate whether hTRP3 is regulated by SGK1 and/or the closely related isoforms SGK2, and SGK3. To this end, cRNA encoding wild-type hTRP3 has been injected into *Xenopus* oocytes with or without additional injection of cRNA encoding wild-type SGK1, SGK2, and SGK3. The experiments described here were performed by measuring ionic currents from *Xenopus* oocytes stably expressing hTRP3 using the two-electrode voltage-clamp technique.

MATERIALS AND METHODS

Molecular biology

Plasmid DNA of the human wild-type TRP3 (Zhu *et al.*, 1996; Zhu *et al.*, 1998), of human wild-type SGK1 (Waldegger*et al.*, 1997), human wild-typeSGK2 (Kobayashi *et al.*, 1999) and human wild-type SGK3 (Kobayashi *et al.*, 1999) were linearized with *Not*I(Source: *Nocardiaotitidis* bacteria) and transcribed *in vitro* with T7 RNA polymerase in the presence of the cap analog $m^7G(5')ppp(5')G$ at a concentrationof1mM.TemplatecDNAwasremovedby digestionwithRNase-freeDNaseI.Thecomplementary RNA (cRNA) was purified by phenol/chloroform extraction followed by precipitation with 0.5 volumes 7.5 M ammonium acetate and 2.5 volumes of ethanol to remove unincorporated nucleotides. The integrity of the transcripts was checked by denaturing agarose gel electrophoresis. The complete constructs were sequenced to prove the correct nucleotide exchange and to exclude any additional mutations.

Expression in Xenopus laevis oocytes

StagesV and VI oocytes were harvested from female wild type *Xenopuslaevis* (Knysna, Rep. South Africa) using previously described procedures (Wagner *et al.*, 2000). Briefly, frogs were anaesthetized by immersion in 0.1% 3-aminobenzoic acid ethyl ester in water. Small pieces of ovary were removed and the incision sutured. Frogs were placed in shallow water until full recovery of reflexes, and subsequently released into the tank. Oocytes were injected with 20ng/25nlcRNA of hTRP3 using a microinjection device (Bachofer, Reutlingen, Germany).In a second step, the oocytes were injected with 25nl water, as a control, or with 7.5ng/25nl water cRNA ofwild type kinases (SGK1, SGK2, and SGK3). All experiments were performed at room temperature (20–24 °C)2-3 days after injection of the respective cRNAs.

Voltage-clamp analysis

In two-electrode voltage-clamp experiments, currents were recorded during a 5-s linear voltage ramp from -150 mV to +50 mV. The intermediate holding potential between the voltage ramps was -50 mV. Data were filtered at 10 Hz and recorded with MacLab digital to analog converter and software for data acquisition and analysis (ADInstruments, Castle Hill, Australia). The bath solution contained 96 mMNaCl, 2 mMKCl, 1 mM $MgCl_2$, 1mM $BaCl_2$,10 µM methoxyverapamil, 5 mM HEPES, pH 7.4 with or without 10 mM $CaCl_2$. Oocytes were kept in modified Barth´s solution containing 88 mMNaCl, 1 mMKCl, 2.4 mM $NaHCO_3$, 0.8 mM $MgSO_4$, 0.3 mM$Ca(NO_3)_2$, 0.4 mM $CaCl_2$ and 5 mM HEPES, pH 7.4 supplemented with 25 µg/ml gentamycin. The final solutions were titrated to the pH indicated using HCl or NaOH. The flow rate of the superfusion was 20 ml/min and a complete exchange of the bath solution was reached within about 10 s. For determination of Ca^{2+} currents (I_{Ca}), the experiments were performed with Cl^- depleted oocytes (bathed for 24 hours in Cl- free medium) in the absence of extracellular Cl^- in the bath and KCl (3 M) filled agar bridges were used as reference electrodes to minimize liquid junction potentials. In the presence of Cl^- and absence of Cl^- channel inhibitors, the addition of 10 mM $CaCl_2$ induced an inward current ($I_{Cl(Ca)}$) which was created by entry of Ca^{2+} and subsequent activation of Ca^{2+} sensitive Cl^- channels (Hoenderop*et al.*, 1999b; Machaca and Hartzell, 1999). The peak inward current was taken as a measure for hTRP3 activity. $I_{Cl(Ca)}$ activity is synchronously triggered by the intracellular calcium concentration close to the membrane determined by hTRP3. Thus, $I_{Cl(Ca)}$ activity mirrors activation and inactivation kinetics of hTRP3(Miledi and Parker, 1984). Expression and currents may vary from batch to batch. Thus, care was taken to make comparisons

always within batches.

Statistical analysis

Data are expressed as mean ±s.e.m., where n is the number of oocytes investigated. All experiments were repeated with at least 3 batches of oocytes; in all repetitions qualitatively similar data were obtained. All data were tested for significance using paired or unpaired Student's*t*-test, and only results with $P<$ 0.05 were considered as statistically significant. The statistical software Origin 8.1 (OriginLab Corp., Northampton, MA) was used to perform all statistical analyses.

RESULTS

Activation of Ca^{2+} sensitive Cl^- channelsin hTRP3-expressing oocytes by cell membrane hyperpolarization.

The current was observed with *Xenopus* oocytes at 2 days after the injection of hTRP3 cRNA. In hTRP3-expressing oocytes, Ca^{2+} entry through hTRP3 triggered a hyperpolarization-activated inward current by activation of Ca^{2+} sensitive Cl^- channels ($I_{Cl(Ca)}$) (Fig. 1B). Addition of 10 mM $CaCl_2$ significantly increased this current (Fig. 1B).

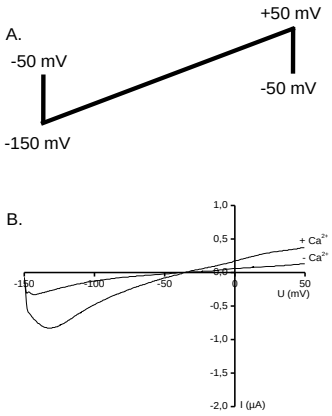

Fig. 1.hTRP3 mediated calcium currents indirectly activate an endogenous chloride conductance ($I_{Cl(Ca)}$). A: The cartoon at the top shows the applied two-electrode voltage-clamp protocol. B: Representive original voltage-clamp recording to Ca^{2+} induced Cl^- current ($I_{Cl(Ca)}$) from hTRP3-expressing oocytes elicited by linear voltage ramp from -150 mV to +50 mV delivered at 5 sec intervals from a holding potential of -50 mV. Currents were recorded in the presence and absence of 10 mM $CaCl_2$.

Stimulation of Ca^{2+} induced current by* SGK1 *in Xenopus oocytes

In the presence of Cl^- the Ca^{2+} entry through hTRP3 stimulated Ca^{2+} sensitive Cl^- channels leading to the appearance of a large Cl^- current ($I_{Cl(Ca)}$). As shown in Fig. 2, 10 mMCa^{2+}triggered a rapidly activating, slowly and partially inactivating inward current in hTRP3 expressing oocytes with additional expression of SGK1.

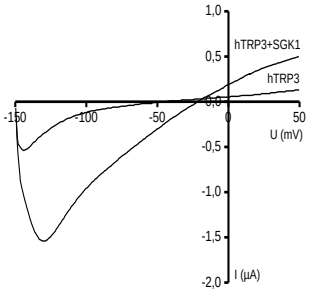

Fig. 2. Stimulation of the Ca^{2+} induced Cl^- current ($I_{Cl(Ca)}$) in *Xenopus* oocytes by the combined expression of hTRP3 and SGK1. Current-voltage (*I/V*) relationships of mean peak currents from *Xenopus* oocytes before and after application of 10 mM $CaCl_2$.

Effect of SGK2 and SGK3 on hTRP3 mediated currents in Xenopus oocytes

Further studies have been performed to determine the influence of SGK2 and SGK3 on hTRP3 mediated currents. The peak inward current was taken as a measure for hTRP3 activity. As reported earlier, the entry of Ca^{2+} triggers Ca^{2+} sensitive Cl^- channels (Miledi and Parker, 1984). As shown in figure 3, coexpression of hTRP3 together with SGK1 led to a significant increase of the current induced by addition of 10 mM $CaCl_2$. In contrast, the current was not increased by coexpression of hTRP3 with either SGK2 or SGK3.

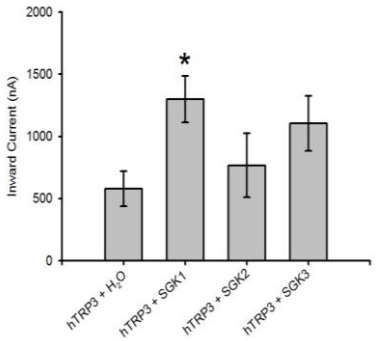

Fig. 3. Stimulation of the Ca^{2+} induced Cl^- current ($I_{Cl(Ca)}$) in *Xenopus* oocytes by the combined expression of hTRP3, SGK1 but not with SGK2 or SGK3. Bar graphs showing the mean peak inward current of hTRP3-expressing oocytes after application of 10 mM $CaCl_2$. Arithmetic means ± SEM. * indicates significant difference, (*$p < 0.05$).

DISCUSSION

The primary objective of the current study was to explore the impacts of serum- and glucocorticoid-induced kinases SGK1, SGK2, and SGK3 on hTRP3 Ca^{2+} channel activity.This investigation reveals a powerful effect of the serum- and glucocorticoid-induced kinase SGK1 on SOCE and discloses a completely novel signaling mechanism in the regulation of hTRP3 Ca^{2+} channel activity. This activity has obvious significantinduction by using SGK1, but not by SGK2 or SGK3.The effect of SGK1 on hTRP3 channel activity requires an intact catalytic subunit, wild-type SGK1(Kobayashi and Cohen, 1999;Boehmer*et al.,* 2003).

Importantly, the present investigation reveals that expression of hTRP3Ca^{2+} channels induces a Ca^{2+} entry allowing theintracellular accumulation ofCa^{2+} and generating a Ca^{2+}inward current. In the presence of Cl^-, Ca^{2+} influx through hTRP3 Ca^{2+} channels generates further Cl^- inward currents (Hoenderop et al, 1999a) by activation of endogenousCa^{2+}activated Cl^- channels in *Xenopus* oocytes (Callamaras and Parker, 2000).

SGK1 expression is upregulated by glucocorticoids (Webster *et al.,* 1993), aldosterone (Cowling and Birnboim, 2000), cell shrinkage (Waldegger*et al.,* 1997) and by a wide range of other factors (Lang and Cohen, 2001). Notably, SGK1 is under transcriptional control of Ca^{2+}ions inside the cells (Brickley*et al.,* 2013) and is thus likely to participate in the regulation of calcium homeostasis by regulation of differentcation channels including hTRP3 channels.

SGK1 has previously been shown to compromise Ca^{2+} influx into cells by regulating different Ca^{2+}-permeable TRP channels such as TRPV5 (Palmada*et al.,* 2005) and TRPV6 (Bohmer*et al.,* 2007). Moreover, SGK1 contributes to regulation ofthe epithelial Na^+/H^+ exchanger NHE3 (Yun *et al.,* 2002a) and a wide variety of K^+ channels (Laufer*et al.,* 2009; Lang *et al.,* 2009) such as the voltage gated K^+ channel Kv1.3 (Gamper*et al.,* 2002) and the renal epithelial K^+ channel ROMK1 (Yun *et al.,* 2002b).

Activation of $K^+$and Ca^{2+} channels is expected to hyperpolarize the cell membrane and thus enhance the driving force for Ca^{2+} entry through several Ca^{2+} channels. SGK1 expression (Klingel*et al.,* 2000; Taruno*et al.,* 2008) and activity (Imai *et al.,* 2003) are stimulated by increased cytosolic Ca^{2+} activity. Thus, at least in theory, SGK1 could serve as an amplifier of Ca^{2+} entry leading to induction of Ca^{2+} channel Orai1/STIM1 (Eylenstein*et al.,* 2011) and hTRP3 channels activity (Fig. 4).

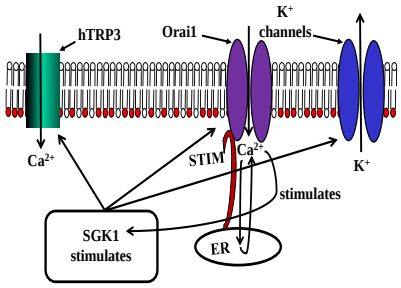

Fig. 4. Scheme illustrating the SGK1-sensitive regulation of hTRP3 channel activity. SGK1 participates in the regulation of transport proteins in plasma membrane, such as K^+ and Orai1/STIM1 channels, which have been shown to be SGK1 targets in different cell types.

Further experimental studies are required to confirm a potentially regulatory effect of SGK1 on hTRP3 channel activity. Theseexperiments will be needed to provide detailed information about the molecular mechanism behind SGK1-dependent regulation of hTRP3 channel activity.

CONCLUSIONS

In conclusion, the present observations identify hTRP3as a target of a regulatory mechanism involving the serine/threonine kinase. The SGK1 is a novel transcriptional and powerful regulator of hTRP3 which is at least partially effective through activation of Ca^{2+} entry as well as channel activity. Thus, SGK1-dependent hTRP3 regulations can influence SOCE and activation-dependent Ca^{2+} entry as well as Ca^{2+}-dependent mechanisms in brain, heart, and smooth muscle cells.

ACKNOWLEDGEMENTS

The authorgratefully acknowledges all members and professors of the Department of Animal Physiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt and of Physiology Institute, Faculty of Medicine, EberhardKarls University, Tuebingen, Germany, where the present work was performed, for their constant encouragement and kind help.

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تنظيم القناة المحتملة القصيرة ٣ للمستقبلات العابرة في الإنسان بواسطة إنزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد-١

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يتم التعبير على نطاق واسع عن القنوات المحتملة للمستقبلات العابرة (قنواتTRP) في عدد كبير من مختلف أنواع الخلايا البشرية والحيوانية. معظم قنوات TRP هي منفذه للكالسيوم والبعض أيضا للمغنسيوم. وتنقسم قنوات الترب إلى ثلاث مجموعات TRPرئيسية على أساس بنيتهاوتركيبيها: قصيرة، طويلة و TRPs مثل-OSM. فصيلة TRPالقصيرة من القنوات الموجبة التى تحتوي على TRPs الثدييات، TRPL، وTRPذبابة الفاكهة. فصيلة TRP القصيرة فى الثدييات من القنوات الموجبة التى تحتوي على أربعة تحت العوائل (TRP-١، TRP-٤، ٥، TRP-٢ وTRP-٣، ٦، ٧، ٨) على أساس تماثل كاف فى التسلسل والوظيفة. يصل التعبير عن القناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان إلى أعلى المستويات في الدماغ، و أدنى المستويات في الأمعاء الدقيقة والقولون، الخصية والبروستاتا والمبيض، المشيمة والرئة. يلعب الكالسيوم الخلوي دوراً حاسماً ومهماً في مختلف الوظائف الخلوية لجميع أنواع الخلايا تقريبا وبالتالي فهو تحت السيطرة الهرمونية الدقيقة. ومع ذلك، فأن الآليات الخلوية التي تتحكم فى تنظيم وفرة القناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان في غشاء الخلية غير مفهومة حتى الان. أن تنظيم وفرة قناة الصوديوم الطلائية على سطح الخلية يتم عن طريق انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد-١. وقد تم تنفيذ هذه الدراسة لاستكشاف ما إذا كان هناك تنظيم للقناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان عن طريق انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد-١والإنزيمات المتعلقة به مثل انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٢، و انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٣. وتحقيقاً لهذه الغاية، تم حقن الحامض النووي للقناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان مع أو بدون الحامض النووي البري لكل من انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد-١والإنزيمات المتعلقة به انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٢، و انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٣ فى بويضات ضفادعالزينوبس. في ظل وجود الكلوريد، فان دخول الكالسيوم عن طريق قناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان يؤدي إلى تفعيل وتنشيط قناة الكلوريد الحساسة للكالسيوم. تعبير القناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان مع انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد-١ يودي الى تحفيز قناة الكلوريد الحساسة الى الكالسيوم ولكن ليس مع انزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٢، وانزيم الكيناز المحدث بالمصل والجلوكوكورتيكويد- ٣. وتشير الملاحظات الى أن انزيم الكيناز المحدثبالمصل والجلوكوكورتيكويد-١ينظم القناة المحتملة القصيرة ٣ للمستقبلات العابرة في الانسان، وبالتالي فهو قد يشارك في تنظيم توازن الكالسيوم.