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A novel form of capsaicin-modified amygdala LTD mediated by TRPM1

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ABSTRACT

Recently we have shown that capsaicin attenuates the strength of LTP in the lateral amygdala (LA) and demonstrated that this effect is mediated by the transient receptor potential (TRP) channel TRPV1. Here we further show that capsaicin, which is thought to act primarily through TRPV1, modifies long term depression (LTD) in the LA. Yet the application of various TRPV1 antagonists does not reverse this effect and it remains in TRPV1-deficient mice. In addition, voltage gated calcium channels, nitric oxide and CB1 receptors are not involved. Using pharmacology and *TRPM1*$^{-/-}$ mice, our electrophysiological data indicate that capsaicin-induced activation of TRPM1 channels contribute to the induction of LA-LTD. Whereas LA-LTD in general depends on the acitvation of NMDA receptors- and group II metabotropic glutamate receptors (mGluR), the modifying effect of capsaicin on LA-LTD via TRPM1 appears to be specifically mediated by group I mGluRs and in interaction with another member of the TRP family, TRPC5. Additionally, intact GABAergic transmission is required for the capsaicin-effect to take place. This is the first documentation that beside their function in the retina TRPM1 proteins are expressed in the brain and have a functional relevance in modifying synaptic plasticity.

1. Introduction

The LA serves as the first processing stage of sensory and cortical information to form emotional memory. The cellular basis of emotional learning and memory are long-term increases (LTP) or decreases (long-term synaptic depression, LTD) in synaptic efficacy between neurons. Several forms of LTP have been described in the LA both *in vitro* (Huang & Kandel, 1998; Humeau, Shaban, Bissiere, & Luthi, 2003; Weisskopf, Bauer, & LeDoux, 1999) *and in vivo* (Doyere, Schafe, Sigurdsson, & LeDoux, 2003). Depending on the induction protocols and the stimulated afferents the rise in intracellular calcium required to induce LA-LTP is mainly mediated by NMDA receptors, voltage-gated Ca^{2+} channels ((Bauer, Schafe, & LeDoux, 2002; Fourcaudot et al., 2009) or group I metabotropic GluRs (Lee, Lee, & Choi, 2002).

LTD in the LA has been shown to be dependent on activation of postsynaptic NMDARs (Drephal, Schubert, & Albrecht, 2006; Kaschel, Schubert, & Albrecht, 2004; Muller, Albrecht, & Gebhardt, 2009), group II mGluRs or L-type calcium channels (Kaschel et al., 2004; Lucas, Bortolotto, Collingridge, & Lodge, 2013; Muller et al., 2009; Tchekalarova & Albrecht, 2007).

A number of studies have indicated that members of the transient receptor potential (TRP) channel family, a family of non-selective cation channels, play a role in LTP or LTD in various brain regions including the hippocampus (Chavez, Chiu, & Castillo, 2010; Gibson, Edwards, Page, Van Hook, & Kauer, 2008; Li et al., 2008; Menigoz et al., 2015), entorhinal cortex (Banke, 2016), nucleus accumbens (Grueter, Brasnjo, & Malenka, 2010) and superior colliculus (Maione et al., 2009). In the LA, we have previously demonstrated the involvement of the highly calcium permeable TRP of vanilloid type 1 (TRPV1) channel in attenuating LTP (Zschenderlein, Gebhardt, von Bohlen und Halbach, Kulisch, & Albrecht, 2011). In contrast, LA-LTP seemed to be unaffected by the absence of TRPC5 (Riccio et al., 2009). Recently, TRPV1 has been implicated in fear and anxiety-like behavior, as reduced anxiety-like behavior, fear conditioning and stress sensitization have been demonstrated in mice where TRPV1 was genetically deleted or pharmacologically blocked (Aguiar et al., 2014; Marsch et al., 2007; Santos, Stern, & Bertoglio, 2008). In addition, we have shown that activation of TRPV1 receptors by capsaicin rescues LTP in slices derived from swim-stressed mice (Kulisch & Albrecht, 2012). Although in several brain regions TRPV1 also plays a role

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in LTD (Chavez et al., 2010; Gibson et al., 2008; Grueter, Rothwell, & Malenka, 2012; Grueter et al., 2010; Maione et al., 2009) there was no previous evidence for the involvement of TRPV1 in LTD in the LA. However, recently it has been shown that capsaicin is able to activate not only TRPV1 but also TRPM1, a member of the melastatin subfamily (Shen et al., 2009). The TRPM subfamily of ion channels comprises eight members divided into three main groups: TRPM1/3, TRPM4/5, and TRPM6/7 (Harteneck, 2005). TRPM1 was identified in 1998 as a protein down-regulated in highly metastatic melanoma cells (Duncan et al., 1998). It is known that TRPM1 is a putative tumor suppressor cloned from murine cells of the melanocyte lineage (Fang & Setaluri, 2000; Xu, Moebius, Gill, & Montell, 2001). A role in regulation of melanin content in melanocytes has been described (Oancea et al., 2009). In addition, TRPM1 is expressed in ON bipolar cells and is required for the depolarizing light response in retinal ON-bipolar cells (Morgans et al., 2009).

TRPM1 proteins are functional ion-conducting plasma membrane channels (Lambert et al., 2011) which seem to be slightly more permeable to Na^+ than to Ca^{2+} ($P_{Na}/P_{Ca} < 10$) (Oancea et al., 2009). The expression of the TRPM1 gene in the LA (Lein et al., 2007) and in the anterior and posterior amygdaloid complex has previously been demonstrated (Kasukawa et al., 2011). Although significant levels of mRNA expression for TRPM1 were also detected in other brain areas (Fonfria et al., 2006; Kasukawa et al., 2011; Kunert-Keil, Bisping, Kruger, & Brinkmeier, 2006; Oancea et al., 2009), a physiological function for TRPM1 in the brain outside of the retina has not been demonstrated thus far.

The aim of our present study is to further analyze the effect of capsaicin on changes in synaptic plasticity in the LA. We found an input-specific effect of capsaicin on LA-LTD induced by paired pulse low frequency stimulation (PP-LFS) of external capsule (EC) fibers in coronal and horizontal brain slices. The results obtained in horizontal slices by applying various TRPV1 antagonists and the use of knockout models showed that the capsaicin-modified EC-induced LTD is not mediated by TRPV1 receptors. Instead, it appears to be mediated by TRPM1 receptors, which for the first time suggests a function of TRPM1 in synaptic plasticity. Further, our results indicate that group I mGluRs and/or NMDARs are involved in mediating this effect, as well as an at this point unclear interaction between TRPM1 and members of the TRPC family. Whether the activation of TRPCs by mGluR1 is required to induce LTD in lateral amygdala neurons is unknown. We thus sought to elucidate the contribution of TRPCs in LA-LTD by blocking TRPC channels and monitoring changes in field potential amplitude. In contrast to LA-LTP our results suggest that TRPC channels are relevant in the induction of LTD in the amygdala.

2. Methods

2.1. Animals and housing

The experiments were performed with male C57BL/6J mice (8–16 weeks of age for field potential recordings and immunohistochemistry and 5–8 weeks of age for patch clamp experiments, respectively). Breeder pairs for B6,129SNos1^{tm1Plh} (nNOS$^{-/-}$) and TRPV1 knockout mice (B6.129S4-Trpv1^{tm1Jul}/J) were obtained from the Jackson Laboratory (Maine, USA). Heterozygous (+/-) mice were bred to produce NOS$^{-/-}$ mice. Heterozygous *Trpm1*$^{tm1Lex/tm1Lex}$, hereafter referred to as TRPM1$^{+/-}$ mice, were generated by Lexicon Genetics and acquired from the European Mouse Mutant Archive (www.emmanet.org). Homozygous mice NM_018752.2 (Trpm1) (genetic background: 129SvEvBrd) were obtained from the lab of Christian Harteneck (Tübingen, Germany). To preclude bias, the experimenters measuring LTD were blinded to the genotype of the mice analyzed. Four month old TRPM1$^{-/-}$ and eight month old TRPM1$^{+/-}$ mice were tested. The genotype of each mouse used in this study was confirmed by PCR. Animals were housed in standardized conditions with an artificial 12-h dark–light cycle, room temperature of 22 °C, and approximately 80% humidity. Mice had free access to food and water. All of the experimental protocols were approved by government authorities (Landesamt für Gesundheit und Soziales Berlin ID: T0344/05 and T0381/11) and performed according to the German Animal Welfare Act of May 25, 1998, and the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize suffering. As a minimum three animals and as a maximum 8 animals were used for each extracellular experiment. As a rule 4–5 mice were used for every drug treatment or different mice strains (n = number of slices). For patch clamp recordings excitatory postsynaptic currents from single LA neurons derived from 22 mice were included into the analysis.

2.2. Slice preparation and Electrophysiology

Mice were anesthetized either with ether or with 4% isoflurane (Kulisch, Eckers, & Albrecht, 2011), and decapitated. The brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) (in mM: 129 NaCl, 3 KCl, 1.6 $CaCl_2$, 1.8 $MgSO_4$, 1.25 NaH_2PO_4, 10 glucose, and 21 $NaHCO_3$). For extracellular recordings, hemisected horizontal slices (400 μm) were prepared using a vibroslicer (Campden Instruments, Silbey, UK) and placed in an interface chamber, where they were allowed to equilibrate for 120 min at 35 °C. The slices were superfused continuously with ACSF (1.2 mL/min). The pH was maintained at 7.4 (95% O_2 and 5% CO_2). For patch-clamp recordings horizontal slices (300 μm) were prepared using a Camden slicer 7000 smz (Camden Instruments, Silbey, UK) and stored at room temperature under submerged conditions at least one hour before being transferred into the recording chamber. For extracellular recordings, glass microelectrodes (Science Products, Hofheim, Germany) were filled with ACSF (tip resistance 3 MΩ) and placed in the LA to record field potentials. Patch pipettes (5–8 MΩ) were filled with internal solution composed of 135 mM K-gluconate, 6 mM KCl, 10 mM HEPES, 2 mM Na_2ATP, 2 mM $MgCl_2$, 0.2 EGTA, 0.3 Na-GTP, 5 phosphocreatine and were pulled from borosilicate glass capillaries (Science Product GmbH, Hofheim, Germany) using a Puller PC 10 (Narishige, Japan). Patch clamp recordings were obtained from neurons located in the LA using an Axopatch 200B amplifier (Axon Instruments Inc., Foster City, CA). Signals were filtered with 3 kHz, digitized at 10 kHz and acquired using the Clampex 9.0 software (Axon Instruments Inc., Foster City, CA). In horizontal slices bipolar stimulation electrodes were used to stimulate fibers within the LA (IN) or to stimulate fibers within the external capsule (EC). In coronal slices cortical or thalamic inputs were stimulated. An input/output response curve was constructed by varying the intensity of the single-pulse stimulation and averaging six responses per intensity. The stimulus intensity that evoked field potential amplitudes or EPSCs equal to 50% of the maximal response was then used for all subsequent stimulations. Once a stable baseline of responses had been obtained for at least 20 min in extracellular recordings, low frequency stimulation (LFS; 1 Hz, 15 min) or paired pulse LFS (PP-LFS; interstimulus interval: 40 ms) were applied to induce LTD (Muller et al., 2009). Subsequent responses to single stimuli were recorded for at least 60 min (in patch clamp recordings: 25 min), and their amplitude quantified as a percent change with respect to baseline.

2.3. Drug application

All drugs were bath-applied at the indicated concentrations starting at least 30 min before LFS. We alternated between control