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LOSS OF INHIBITION BY BRAIN NATRIURETIC PEPTIDE OVER P2X3 RECEPTORS CONTRIBUTES TO ENHANCED SPIKE FIRING OF TRIGEMINAL GANGLION NEURONS IN A MOUSE MODEL OF FAMILIAL HEMIPLEGIC MIGRAINE TYPE-1

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Abstract—Purinergic P2X3 receptors (P2X3Rs) play an important role in pain pathologies, including migraine. In trigeminal neurons, P2X3Rs are constitutively downregulated by endogenous brain natriuretic peptide (BNP). In a mouse knock-in (KI) model of familial hemiplegic migraine type-1 with upregulated calcium $Ca_V2.1$ channel function, trigeminal neurons exhibit hyperexcitability with gain-of-function of P2X3Rs and their deficient BNP-mediated inhibition. We studied whether the absent BNP-induced control over P2X3Rs activity in KI cultures may be functionally expressed in altered firing activity of KI trigeminal neurons. Patch-clamp experiments investigated the excitability of wild-type and KI trigeminal neurons induced by either current or agonists for P2X3Rs or transient receptor potential vanilloid-1 (TRPV1) receptors. Consistent with the constitutive inhibition of P2X3Rs by BNP, sustained pharmacological block of BNP receptors selectively enhanced P2X3R-mediated excitability of wild-type neurons without affecting firing evoked by the other protocols. This effect included increased number of action potentials, lower spike threshold and shift of the firing pattern distribution toward higher spiking activity. Thus, inactivation of BNP signaling transformed the wild-type excitability phenotype into the one typical for KI. BNP receptor block did not influence excitability of KI neurons in accordance with the lack of BNP-induced P2X3R modulation. Our study suggests that, in wild-type trigeminal neurons, negative control over P2X3Rs by the BNP pathway is translated into tonic suppression of P2X3Rs-mediated excitability. Lack of this inhibition in KI cultures results in a hyperexcitability phenotype and might contribute to facilitated trigeminal pain transduction relevant for migraine.

Key words: purinergic receptor, nociception, sensory neurons, TRPV1, vanilloid receptor.

INTRODUCTION

The trigeminal ganglion (TG) is a part of the trigeminovascular system that plays an integral role in regulating pain transduction in primary headache conditions, such as migraine (Noseda and Burstein, 2013). Trigeminal sensory neurons express ATP-gated P2X3 receptors (P2X3Rs) and capsaicin-sensitive transient receptor potential vanilloid-1 (TRPV1) receptors (Vulchanova et al., 1997; Julius and Basbaum, 2001; North, 2003), which mediate nociceptive responses and are thought to contribute to migraine pathophysiology (Yan and Dussor, 2014). Using a knock-in (KI) mouse model, expressing voltage-gated $Ca_V2.1$ channels with the R192Q missense mutation in its α_{1A} subunit that was shown to lead to familial hemiplegic migraine type-1 (FHM1; Ophoff et al., 1996; Ferrari et al., 2015), we have observed a selective upregulation of P2X3Rs function in TG neurons (Nair et al., 2010). This phenomenon is manifested as increased neuronal excitability in response to P2X3Rs activation (Hullugundi et al., 2014). Such results may help to explain the migraine-like pain behavior exhibited by R192Q KI mice (Chanda et al., 2013).

While numerous endogenous modulators upregulate P2X3Rs, to date only brain natriuretic peptide (BNP) and its natriuretic peptide receptor type-A (NPR-A) have been reported to induce constitutive downregulation of P2X3-mediated responses in wild-type (WT) TG neurons (Vilotti et al., 2013; Marchenkova et al., 2015). Indeed, inactivation of BNP signaling with selective NPR-A antagonist anantin or siBNP enhances P2X3Rs-mediated ion currents under voltage clamp conditions, thereby unmasking a background inhibition of P2X3R activity (Vilotti et al., 2013; Marchenkova et al., 2015). Most interestingly, in R192Q KI mice, the BNP-dependent P2X3R modulation appears to be disabled and might contribute to the gain-of-function of R192Q KI trigeminal neurons (Marchenkova et al., 2016). Because

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Abbreviations: α,β-meATP, α,β-methylene adenosine 5-triphosphate; AP, action potential; BDNF, brain-derived neurotrophic factor; BNP, brain natriuretic peptide; CGRP, calcitonin gene-related peptide; FA, fast-adaptive; FHM1, familial hemiplegic migraine type-1; KI, knock-in; MF, multiple-firing; NS, non-spiking; P2X3R, P2X3 receptor; SS, single-spike; TG, trigeminal ganglion; TRPV1, transient receptor potential vanilloid-1; WT, wild type.

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these functional data were obtained by recording membrane currents from neurons, they did not shed light on the neurophysiological correlates of these phenomena as trigeminal sensory neurons encode their responses as a series of action potentials (APs) (Sunada et al., 1990; Chudler et al., 1991; Coste et al., 2008).

The aim of the present study was to find out if BNP/NPR-A-mediated constitutive inhibition of P2X3Rs might actually influence the firing properties of WT neurons. In addition, we wished to explore if BNP acts selectively on P2X3R-mediated activation or on other forms of neuronal depolarization (i.e. current injection- or capsaicin-mediated depolarization). In particular, since the AP threshold is more negative in KI neurons, we investigated whether downregulation of BNP-mediated inhibition might change not only AP patterns but also the readiness to generate APs by shifting the spike threshold to more negative values. All these objectives required a current clamp study.

Thus, in order to uncover any constitutive effect of endogenous BNP, we blocked NPR-A receptors with its selective antagonist anantin (Weber et al., 1991; Yu et al., 2006; Abdelalim and Tooyama, 2011; Vilotti et al., 2013) in analogy with the previously reported protocol (Vilotti et al., 2013). Firing of trigeminal sensory neurons from WT or R192Q KI mice was investigated in response to current pulses as well as brief application of P2X3 or TRPV1 receptor agonists α,β-methylene adenosine 5-triphosphate (α,β-meATP) and capsaicin, respectively (Hullugundi et al., 2014).

Our data suggest that the negative inhibition of P2X3R activity by the BNP/NPR-A pathway results in a decreased P2X3R-mediated excitability of trigeminal neurons in WT cultures. In KI cultures, however, lack of efficient P2X3Rs downregulation contributes to the neuronal hyperexcitability phenotype.

EXPERIMENTAL PROCEDURES

Mouse trigeminal ganglion cultures

Experiments were performed on cultured TG neurons from FHM1 R192Q KI and WT mouse littermates. The colony of KI mice was bred and maintained locally, in accordance with the Italian Animal Welfare Act, after an initial transfer from Leiden University Medical Centre (van den Maagdenberg et al., 2004). All experimental protocols were approved by the SISSA ethics committee and are in accordance with EU guidelines (2010/63/EU) and Italian legislation (D.L. 4/3/2014, No. 26). Every effort was made to minimize the number of animals used for the experiments and their suffering. The genotyping was performed by PCR, as previously reported (Nair et al., 2010) after the mice were sacrificed for culture preparation: hence, at the time of electrophysiological recording the genotype of the tested neurons from each dish was not determined, thus ensuring blind analysis conditions. Primary cultures of P12-P14 mouse TG were prepared as described previously (Simonetti et al., 2006; Hullugundi et al., 2014) and used 24 h after plating. In brief, trigeminal ganglia were isolated from mice killed by cervical dislocation under general anesthesia induced by slowly raising the level of CO_2. Ganglia were cut and dissociated for 12 min at 37 °C in an enzyme mixture containing 0.25 mg/mL trypsin, 1 mg/mL collagenase, and 0.2 mg/mL DNase (Sigma, Milan, Italy) in F-12 medium (Invitrogen Corp, San Giuliano Milanese, Italy). Cells were plated on poly-L-lysine-coated petri dishes in F12 medium with 10% fetal calf serum.

Electrophysiology

After 24 h in culture, trigeminal neurons were superfused continuously (2–3 mL/min) with physiological solution containing (in mM): 152 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES (pH adjusted to 7.4 with NaOH), as previously described (Nair et al., 2010; Hullugundi et al., 2013). Cells were patch-clamped in the whole-cell configuration, using glass pipettes (4–5 MΩ resistance) filled with the following solution (in mM): 125 K-gluconate, 5 KCl, 2 $MgCl_2$, 2 Mg_2ATP_3, 10 HEPES, and 10 EGTA (pH adjusted to 7.2 with KOH). The K^+ equilibrium potential, calculated with the Nernst equation, was equal to −105 mV, and the liquid junction potential was 14.6 mV. Collected data were corrected accordingly.

Recordings were obtained from small- and medium-sized TG neurons (capacitance below 25 pF) under current-clamp conditions, using a Patch Clamp PC-501A amplifier (Warner Instrument Corporation). Experimental conditions were very similar to those of our previous study (Hullugundi et al., 2014), except for the membrane holding potential, as cells were held at −70 mV (after correction for the liquid junction potential). Electrophysiological responses were filtered at 5 kHz and acquired by means of a DigiData 1200 interface and pClamp 8.2 software (Molecular Devices). Input resistance was measured by applying hyperpolarizing pulses of −5 or −2 pA, while cell capacitance was estimated from the whole-cell capacitance facility.

Depolarizing current pulses lasting for 300 ms with 45 pA amplitude were used to stimulate neurons. As previously shown (Hullugundi et al., 2014), such stimulation is sufficient to elicit cell-specific firing activity from TG neurons of small and medium size. In accordance with our former studies, the P2X3R selective agonist α,β-meATP (Sigma, Milan, Italy) was applied for 2 s using a fast superfusion system (Rapid Solution Changer RSC-200; BioLogic Science Instruments, Claix, France) at a concentration of 10 μM to produce near-maximal P2X3R activation (Sokolova et al., 2006). Capsaicin (Sigma) was applied for 3 s at a concentration of 1 μM to elicit stable TRPV1 receptor-mediated responses (Simonetti et al., 2006; Nair et al., 2010; Hullugundi et al., 2014). The selective NPR-A antagonist anantin (500 nM) was applied to the cultures overnight (24 h) to block NPR-A receptor activity (Yu et al., 2006; Vilotti et al., 2013; Marchenkova et al., 2015); control dishes were incubated for the same period of time without anantin.

Data analysis

Data are expressed as mean ± standard error of the mean, with n indicating the number of analyzed cells.