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INTRACELLULAR POSTSYNAPTIC CANNABINOID RECEPTORS LINK THYROTROPIN-RELEASING HORMONE RECEPTORS TO TRPC-LIKE CHANNELS IN THALAMIC PARAVENTRICULAR NUCLEUS NEURONS

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Abstract—In rat thalamic paraventricular nucleus of thalamus (PVT) neurons, activation of thyrotropin-releasing hormone (TRH) receptors enhances excitability via concurrent decrease in G protein-coupled inwardly-rectifying potassium (GIRK)-like and activation of transient receptor potential cation (TRPC)4/5-like cationic conductances. An exploration of intracellular signaling pathways revealed the TRH-induced current to be insensitive to phosphatidylinositol-specific phospholipase C (PI-PLC) inhibitors, but reduced by D609, an inhibitor of phosphatidylcholine-specific PLC (PC-PLC). A corresponding change in the *I–V* relationship implied suppression of the cationic component of the TRH-induced current. Diacylglycerol (DAG) is a product of the hydrolysis of PC. Studies focused on the isolated cationic component of the TRH-induced response revealed a reduction by RHC80267, an inhibitor of DAG lipase, the enzyme involved in the hydrolysis of DAG to the endocannabinoid 2-arachidonoylglycerol (2-AG). Further investigation revealed enhancement of the cationic component in the presence of either JZL184 or WWL70, inhibitors of enzymes involved in the hydrolysis of 2-AG. A decrease in the TRH-induced response was noted in the presence of rimonabant or SR144528, membrane permeable CB1 and CB2 receptor antagonists, respectively. A decrease in the TRH-induced current by intracellular, but not by bath application of the membrane impermeable peptide hemopressin, selective for CB1 receptors, suggests a postsynaptic intracellular localization of these receptors. The TRH-induced current was increased in the presence of arachidonyl-2′-chloroethylamide (ACEA) or JWH133, CB1 and CB2 receptor agonists, respectively. The PI3-kinase inhibitor LY294002, known to inhibit TRPC translocation, decreased the response to TRH. In addition, a TRH-induced enhancement of the low-threshold spike was prevented by both rimonabant, and SR144528. TRH had no influence on excitatory or inhibitory miniature postsynaptic currents, suggesting presynaptic CB receptors are not involved in this situation. Collectively, the data imply that activation of TRH receptors in these midline thalamic neurons engages novel signaling pathways that include postsynaptic intracellular CB1 and CB2 receptors in the activation of TRPC4/5-like channels.

Key words: midline thalamus, CB1 and CB2 receptors, diacylglycerol lipase, hemopressin.

INTRODUCTION

The tripeptide thyrotropin-releasing hormone (TRH) was initially discovered as a hypothalamic hormone whose receptors on adenohypophyseal thyrotrophs and lactotrophs regulate the release of the thyroid-stimulating hormone and prolactin, respectively (see Lechan and Fekete, 2006 for a review). Following its initial structural characterization, the subsequent detection of TRH and TRH receptors in various regions of the brain and spinal cord encouraged numerous studies that supported a role for this neuropeptide in a variety of central nervous system (CNS) functions ranging from arousal, thermogenesis, locomotion, analgesia, to mood and cognition (reviewed in Nillni and Sevarino, 1999). Additionally, such observations heightened interest in the potential of TRH and TRH analogs in neuroprotection and treatment of narcolepsy, certain forms of epilepsy, neuropsychiatric and mood disorders (reviewed in Gary et al., 2003; Yarbrough et al., 2007).

In the central nervous system (CNS), TRH has long been deemed to have a transmitter/modulatory role. Indeed, exogenous application of TRH has been shown to influence neuronal excitability through a postsynaptic action at receptors in the spinal cord (Nicoll, 1977; Fisher and Nistri, 1993; Kolaj et al., 1997), brainstem (Bayliss et al., 1992; Ishibashi et al., 2009; Parmentier et al., 2009), hypothalamus (González et al., 2009; Hara et al., 2009; Lyons et al., 2010; Zhang and van den Pol, 2012), hippocampus (Ebihara and Akaike, 1993; Deng

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Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; ABHD6, α/β-hydrolase domain 6; ACEA, arachidonyl-2'-chloroethylamide; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; Ba^{2+}, barium; Bis II, bisindolylmaleimide II; CNS, central nervous system; DAG, diacylglycerol; DAGL, diacylglycerol lipase; EGTA, ethylene glycol tetraacetic acid; FAAH, fatty acid amide hydrolase; GIRK, G protein-coupled inwardly-rectifying potassium; GPCR, G protein-coupled receptors; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LTS, low-threshold spike; MAGL, monoglycerol lipase; NCX, Na^{+}/Ca^{2+} exchanger; nRT, reticular nucleus; PC-PLC, phosphatidylcholine-specific PLC; PI3-kinase, phosphatidylinositol 3-kinase; PI-PLC, phosphatidylinositol-specific phospholipase C; PKC, protein kinase C; PVT, paraventricular nucleus of thalamus; TRH, thyrotropin-releasing hormone; TRPC, transient receptor potential cation; TTX, tetrodotoxin.

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et al., 2006) and thalamus (Broberger and McCormick, 2005; Zhang et al., 2013). Analyses of mechanisms involved in the TRH-induced excitatory responses have revealed a diversity that includes an increase in a nonselective cation conductance (e.g. Parmentier et al., 2009; Lyons et al., 2010; Zhang et al., 2013), involvement of the Na^+/Ca^{2+} exchanger (NCX; Parmentier et al., 2009; Zhang and van den Pol, 2012) and/or suppression of various potassium channels (e.g. Bayliss et al., 1992; Fisher and Nistri, 1993; Kolaj et al., 1997; Broberger and McCormick, 2005). Where TRH signaling has been assessed in neurons in more detail, it appears that TRH mediates its responses via receptors coupled with Gαq proteins (Deng et al., 2006; Ishibashi et al., 2009; Zhang et al., 2013), thus resembling the prototypic TRH receptor coupling in endocrine cells (Sun et al., 2003). Interestingly, in the CNS, mechanisms involved in downstream signaling can differ not only from endocrine cells but also between neuronal populations. For example, in locus coeruleus neurons, TRH promotes activation of phosphatidylinositol phospholipase C (PI-PLC) and subsequent generation of inositol 1,4,5-triphosphate (Ishibashi et al., 2009), thus resembling events in endocrine cells (Sun et al., 2003). In the lateral hypothalamus, TRH actions are attenuated by phospholipase C and NCX inhibitors and blockers of transient receptor potential cation (TRPC) channels (Zhang and van den Pol, 2012). By contrast, in the hippocampus, TRH activation of GABAergic interneurons does not appear to involve PLC and its downstream effectors (Deng et al., 2006).

In the thalamus, several reports have raised interest in the midline paraventricular nucleus (PVT) as a CNS site associated with arousal, vigilance and motivated behaviors (Van der Werf et al., 2002; Benarroch, 2008; Hsu and Price, 2009; Hsu et al., 2014; Colavito et al., 2015). Among thalamic regions, PVT uniquely displays TRH-like immunoreactive axons (Merchenthaler et al., 1988) likely arising from the hypothalamus, and a high density of TRH receptors (Heuer et al., 2000). In PVT, we recently reported (Zhang et al., 2013; Kolaj et al., 2014a) that exogenous application of TRH increases the excitability of a majority of neurons, inducing a G-protein-mediated inward current that involves a concurrent decrease in a G protein-coupled inwardly-rectifying potassium (GIRK)-like conductance and an increase in a TRPC-like conductance, possibly involving TRPC4/5 channels. Here, we explored further the nature of the signaling pathway. To our surprise, the TRPC-like component of membrane conductance contributing to the TRH-induced inward current was resistant to agents known to block the PI-PLC pathway, but was reduced with D-609, an inhibitor of phosphatidylcholine-specific PLC (PC-PLC), an enzyme that hydrolyzes phosphatidylcholine to diacylglycerol (DAG). When further investigation indicated that the TRH-induced TRPC-like current was reduced by suppressing DAG hydrolysis, we tested the hypothesis that a DAG metabolite, the endocannabinoid 2-arachidonoylglycerol (2-AG), was linked to the TRPC-like current. Our data suggest that this current is sensitive to pharmacological manipulation of 2-AG hydrolysis, also to intracellular blockade of CB1 receptors, and to a membrane permeable CB2 receptor blocker. These observations suggest a novel participation of intracellular cannabinoid receptors in this component of the TRH-induced response in these midline thalamic neurons.

EXPERIMENTAL PROCEDURES

In vitro tissue slice preparation

Experimental protocols were approved by the Ottawa Hospital Research Institute Animal Care and Use Committee, and utilized methods similar to an earlier investigation (see Zhang et al., 2013). Briefly, 21–35-day-old Wistar rats of either sex were housed in pairs in a temperature-controlled (22–24 °C) environment with a 12–12-h light/dark (LD) cycle, with food and water *ad libitum*. Animals were sacrificed by guillotine at ZT 2–6 h (during the subjective quiet-day period), the brain was quickly removed, immersed in oxygenated (95% O_2–5% CO_2) and cooled (< 4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 127, KCl 3.1, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 10 (osmolality 300–310 mOsm/kg; pH 7.3). Brain slices 350–400 microns in thickness were cut in the coronal plane with a Vibratome 3000 Plus Sectioning System (Vibratome, St Louis, MO, USA), incubated in gassed ACSF for > 1 h at room temperature, then transferred to a recording chamber and superfused (2–4 ml/min) with oxygenated ACSF at 32–34 °C.

Electrophysiological recordings and data analysis

In all experiments, the blind-patch technique was used for whole-cell current-clamp and voltage-clamp recordings using an Axopatch 200A or Axopatch 1D (Molecular Devices, Sunnyvale, CA, USA). Data were filtered at 2 kHz, continuously monitored and stored on a disk. A Digidata 1200B interface with Clampex software (pClamp 9; Molecular Devices) was used to generate current and voltage commands, and to store data. Recordings were obtained from PVT neurons using borosilicate thin-walled micropipettes (resistances of 3–7 MΩ) filled with (in mM): Kgluconate 130, KCl 10, $MgCl_2$ 2, HEPES 10, EGTA 1, Mg-ATP 2, Na-GTP 0.3 (pH adjusted with NaOH to 7.3, osmolality ~290 mOsm/kg). To record miniature IPSCs, Kgluconate was replaced with CsCl. All voltage-clamp experiments were done in the presence of tetrodotoxin (TTX, 1 μM). To evaluate the contribution of Na^+-dependent conductances we used low-Na^+ ACSF where NaCl was replaced with Tris–Cl (see Zhang et al., 2013). ACSF containing 1.3 mM barium (Ba^{2+}) was used to eliminate a contribution of GIRK channels to TRH-induced inward currents (Zhang et al., 2013). Series resistance was compensated for > 65%, and was regularly checked throughout the experiment by monitoring current responses to brief 5 mV hyperpolarizing steps. Membrane voltages were corrected for liquid junction potential (normal ACSF, −13 mV; low-Na^+ ACSF, −23 mV). Voltage-clamp analyses used a standard ramp protocol (step from −63 mV to −43 mV for 0.5 s and then ramped from −43 mV to −113 mV or −123 mV in 3 s followed by step back to −63 mV) from a holding potential of −63 mV. The net