Neuropharmacology xxx (2014) 1–10

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Serotonin induces depolarization in lateral amygdala neurons by activation of TRPC-like current and inhibition of GIRK current depending on 5-HT_{2C} receptor

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ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form
11 March 2014
Accepted 12 March 2014

Keywords:
Lateral amygdala
Serotonin
TRPC
GIRK
PIP2
PI3-kinase

ABSTRACT

Regional differences are known in the serotonin-induced modulation of neuronal activity within the amygdala. This *in vitro* study in rats focuses on analyzing the ionic mechanism underlying serotonin-induced depolarization in the lateral amygdala. Serotonin depolarized membrane potential by 5 mV, which is underlain by a serotonin-induced inward current at rest with a characteristic reversal potential of −105 mV. From pharmacological experiments, the 5-HT_{2C} subtype was singled out as the receptor subtype involved. Under blockade of K^+ channels by Ba^{2+}, 5-HT induced an inward current with no reversal at the range between −50 and −130 mV, which was identified as a TRPC-like current. This current was blocked by the specific phosphatidylinositol 3-kinse (PI3-kinase) inhibitor LY294002, pointing to its dependence on PI3-kinase. The Ba^{2+}-sensitive component, obtained by subtraction, showed a strong outward rectification and the reversal potential of K^+, indicating that this component results from a serotonin-induced inhibition of G-protein coupled inwardly rectifying K^+ channel (GIRK) current. By wortmannin, an inhibitor of both PI3-kinase and PI4-kinase, a serotonin-induced phosphatidylinositol 4,5-bisphosphate (PIP_2) depletion was revealed to underlie GIRK inhibition. Thus, the serotonin-induced current turned out to be caused by a combined occurrence of GIRK inhibition and PI3-kinase-dependent TRPC-like current. With serotonergic modulation, all these mechanisms should be recruited in lateral amygdala principal neurons and likely contribute to generation of region-specific neuronal activity patterns within the amygdala, which may at least partly implement its required role in fear and anxiety.

1. Introduction

The basolateral amygdaloid complex, composed of the lateral amygdala (LA) and basolateral amygdala (BLA), plays pivotal roles in fear learning and its extinction (LeDoux, 2000). Both the LA and BLA receive prominent serotonergic innervations from the dorsal raphe nucleus (Sadikot and Parent, 1990; Ma et al., 1991). Serotonin (5-HT) release in the amygdala is increased in stress, anxiety or fear (Kawahara et al., 1993; Kirby et al., 1995; Yokoyama et al., 2005). In the acute phase of selective serotonin reuptake inhibitor (SSRI) administration, fear response is enhanced, depending on a 5-HT_{2C} receptor-mediated mechanism (Burghardt et al., 2004, 2007). Thus, the significance of serotonergic modulation in fear and anxiety is well established in the amygdala neuron circuitry.

It is reported that the LA and BLA play differential roles in affective behaviors (Paré et al., 2004; Anglada-Figueroa and Quirk, 2005; Herry et al., 2008). Consistently, serotonergic modulations of intrinsic properties appear to be different in these two substructures within the BLA complex. In the LA, 5-HT reduces afterhyperpolarization (Faber and Sah, 2002), thereby increasing the excitability of LA principal neurons. In contrast, 5-HT depolarizes interneurons and hyperpolarizes projection neurons in the BLA (Rainnie, 1999). We revealed that 5-HT induces slow afterdepolarization (sADP) in LA principal neurons (Yamamoto et al., 2012), but not in BLA neurons. 5-HT also induces membrane depolarization in LA, but not BLA, neurons (Rainnie, 1999; Yamamoto et al., 2012). The 5-HT-induced depolarization enhances the sADP in the LA only. Thus, 5-HT appears to modulate LA and BLA neurons in clearly different manners, even though the electrophysiological

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http://dx.doi.org/10.1016/j.neuropharm.2014.03.007
0028-3908/

properties of neurons in the LA and BLA are essentially similar. However, the detailed ionic and molecular mechanisms underlying the serotonergic differential modulations in the LA and BLA are not known to date, and thus worth elucidating to further understand the role played by serotonergic innervation to the amygdala in fear and anxiety.

Although the sADP that we found in the amygdala depends on both 5-HT and neuronal activity, the membrane depolarization is an activity-independent phenomenon induced by 5-HT receptor activation only, and hence is supposed to be based on simpler mechanisms. The present study thus focuses on studying the ionic mechanism of the 5-HT-induced membrane depolarization in LA principal neurons. We first attempted to identify which 5-HT receptor subtype is responsible, and then to clarify the properties of underlying ionic currents. The data demonstrated a combined modulation of TRPC-like and GIRK currents by 5-HT_{2C} receptor activation.

2. Materials and methods

All experiments were performed in accordance with the guiding principles of the Physiological Society of Japan and were approved by the Animal Care Committee of Kanazawa Medical University.

2.1. Slice preparations

Wistar rats (21–28 days old) were decapitated under ether anesthesia. The brains were dissected out, and slices containing the lateral amygdala and basolateral nucleus were sectioned coronally with a microslicer at 300 μm (Pro7; Dosaka, Kyoto, Japan) in ice-cold artificial cerebrospinal fluid (ACSF) composed (in mM) of 234 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$ and 20 glucose. Slices were kept at room temperature for ≥60 min before experiments in normal ACSF composed (in mM) of 124 NaCl, 3.0 KCl, 2.0 $CaCl_2$, 2.0 $MgSO_4$, 1.3 NaH_2PO_4, 26 $NaHCO_3$, and 20 glucose. Recordings were made from slices submerged in normal ACSF at 30 °C. In experiments in which cadmium and barium were added to the external solution, $MgSO_4$ was replaced with $MgCl_2$ and NaH_2PO_4 was omitted. All solutions were bubbled with a mixture of 95% O_2 and 5% CO_2.

2.2. Electrophysiological recordings

The slices were placed in a recording chamber on the stage of an upright microscope (BX51WI, Olympus, Tokyo, Japan) with a 60× water-immersion objective (LUMPlanFI/IR, Olympus,). Whole cell recordings were made from the soma of visually identified pyramidal like neurons located in the lateral or basolateral amygdala. Recordings were continued in cells that had resting membrane potential below −55 mV only. The series resistance was compensated 80%. The change in series resistance was monitored, and if its deviation was more than 15%, the recording was discarded. In current- and voltage-clamp recordings, glass pipette electrodes were filled with a solution containing (in mM): 130 K-gluconate, 10 KCl, 2 $MgCl_2$, 2 Na_2-ATP, 0.4 Na_2-GTP, 0.2 EGTA, 10 HEPES, 5 K_2-Phosphocreatine with pH adjusted to 7.2–7.3 with KOH. Liquid junction potentials of K-gluconate-based internal solution were about 10 mV which were not corrected.

In all voltage clamp experiments, tetrodotoxin (TTX; 0.5 μM) was added into the external solution. To obtain I–V relationship of 5-HT-induced current at hyperpolarized potential, membrane potential was clamped at −50 mV and voltage ramp from −50 mV to −130 mV for 320 ms were applied. To obtain I–V relationship of 5-HT-induced current at depolarized potential, membrane potential was clamped at −70 mV and voltage ramps from −70 mV to +20 mV for 2.25 s were applied. In both cases, three traces were averaged and the average trace obtained before drug application was subtracted from the average trace obtained after drug application. Recordings were obtained with a Multiclamp 700A amplifier (Molecular Devices, Sunnyvale, CA, USA) and digitized at 10 kHz (Digidata1322A and pClamp9, Molecular Devices).

2.3. Drugs used

Depending on the purpose of experiments, we bath-applied one or more of the following drugs: α-m-5-HT (5 μM; Sigma, St. Louis, MO, USA), 2-APB (100 μM; Sigma), Calphostine C (4 μM; Tocris, Bristol, UK), chelerythrine (10 μM; Tocris), flufenamic acid (FFA; 100 μM; Sigma), linopirdine (40 μM; Sigma), LY294002 (40 μM; Sigma), MDL11939 (0.5 μM; Sigma), SB204741 (5 μM; Tocris), SB206553 (5 μM; Tocris), SB242084 (5 μM; Tocris), serotonin (5-HT; 5 μM; Wako, Osaka, Japan), SKF96365 (100 μM; Tocris), TTX (0.5 μM; Wako), wortmannin (50 μM; Wako).

2.4. Data analysis

Data are expressed as means ± s.e.m. Paired or unpaired *t*-test, or one-way ANOVA followed by Tukey's test was used for statistics with the significance level set at $p < 0.05$.

3. Results

3.1. Serotonin depolarizes the resting membrane potential of LA principal neurons

We recorded from visually identified LA and BLA principal neurons. The electrophysiological characteristics of these neurons were consistent with previous reports (Washburn and Moises, 1992; Faber et al., 2001). In the current-clamp mode, bath-application of 5-HT (5 μM) induced the resting membrane potential depolarization in LA neurons within several minutes (Fig. 1A; from −63.6 ± 0.6 mV to −58.7 ± 0.9 mV, $n = 12$, $p < 0.001$ vs control) and it was recovered after washing out 5-HT (Fig. 1A; −62.3 ± 0.7 mV, $n = 12$, not significant vs control). In BLA neurons, by contrast, bath-application of 5-HT failed to induce membrane depolarization (Fig. 1B; from −64.3 ± 0.7 mV to −64.9 ± 0.8 mV, $n = 14$, not significant).

3.2. An inward current is generated by 5-HT_{2c} receptor activation

Then, in voltage-clamped LA neurons, we applied voltage ramps to clarify the property of 5-HT induced current that underlies the 5-HT-induced depolarization. We first assumed an involvement of potassium currents in 5-HT-induced depolarization. Therefore, we checked the properties of the current at hyperpolarized voltage range. The membrane potential was held at −50 mV. We recorded the current induced by voltage ramps (from −50 mV to −130 mV) both in the presence and absence of 5-HT. To obtain the I–V relationship of the net 5-HT-induced current, the current induced without 5-HT was subtracted from that with 5-HT (Fig. 2A,B). The I–V relationship of 5-HT-induced current showed a slight outward rectification with the reversal potential at around −105 mV (Fig. 2C; −37.0 ± 4.4 pA at −50 mV, 42.5 ± 7.6 pA at −130 mV, $n = 10$). Then, to clarify which 5-HT receptor subtype is responsible for this current, we used a 5-HT receptor agonist and its

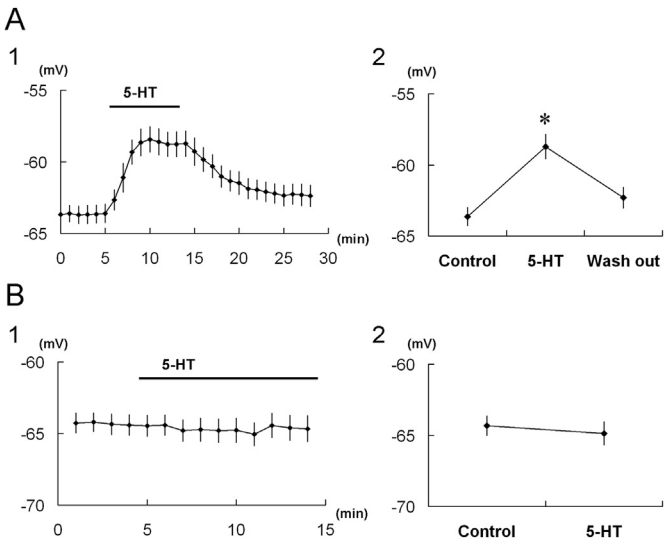

Fig. 1. 5-HT induced depolarization in LA neurons. ***A1***, The average time course of changes in resting membrane potential induced by 5-HT (5 μM) in LA neurons. Bath-application of 5-HT induced a depolarization. The resting membrane potential was restored after washout. ***A2***, Summary diagram of membrane potentials with or without 5-HT in LA neurons. 5-HT significantly depolarized the resting membrane potential. $^*p < 0.001$ vs control. ***B1***, The average time course of changes in resting membrane potential induced by 5-HT in BLA neurons. No obvious change of resting membrane potential was observed. ***B2***, Summary diagram indicating that the membrane potential remained unchanged after 5-HT application in BLA neurons.