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Participation of preoptic area TRPV4 ion channel in regulation of body temperature

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ABSTRACT

Transient receptor potential vanilloid 4 (TRPV4) ion channel is a non-selective cation channel and its role in cutaneous thermosensation is emerging. It is expressed in many areas of the brain including the preoptic area (POA)/anterior hypothalamus which is the key neural site for thermoregulation. The present study was conducted to find out the role of TRPV4 ion channel in the POA in thermoregulation. Rats preimplanted with guide cannulae with indwelling styli 2.0 mm above the POA received TRPV4 agonist/antagonist/isotonic saline injections bilaterally in the POA using an injector cannula in three separate groups of six rats each. Body temperature (Tb) was recorded telemetrically by preimplanted radio transmitter in the peritoneal cavity. The injection of TRPV4 agonist (GSK1016790A) in the POA decreased Tb while its antagonist (RN1734) increased Tb. Immunohistochemical localization showed presence of TRPV4 ion channel in the POA. The results of the present study suggest that TRPV4 ion channels in the POA may play an important role in thermoregulation.

1. Introduction

The role of preoptic area (POA) in thermoregulation comes from studies involving direct thermal stimulation of this neural structure. Magoun et al. (1938) observed that localized brain heating evokes panting in anesthetized cats. The POA warming produced cutaneous vasodilatation, sweating, panting and other heat loss behavioral responses (Adair, 1977; Boulant et al., 1980; Hellstorm and Hammel, 1967). Both electrolytic and chemical lesions of the POA produced hyperthermia in rats and cats (Gamble and Patton, 1953; Satinoff et al., 1982; Szymusiak et al., 1985). Intracerebral injections of various neurotransmitters in the POA also influenced Tb e.g. local application of norepinephrine at mPOA produced hypothermia (Datta et al., 1987; Mallick et al., 1988).

Recently, transient receptor potential (TRP) channels super family have received considerable attention. The TRPV subfamily TRPV (1 – 4) initially located in sensory nerve and skin is proposed to be involved in peripheral thermotransduction mechanism (Acs et al., 1996; Nedungadi et al., 2012; Peier et al., 2002). In vitro study shows TRPV (1 – 4) channels responding to a broad range of temperature from warm to hot (Caterina et al., 1997, 1999; Güler et al., 2002; Peier et al., 2002; Smith et al., 2002; Watanabe et al., 2002a, 2000b; Xu et al., 2002). TRPV (1 – 4)channels are expressed in number of brain areas including the POA (Acs et al., 1996; Caterina et al., 1997; Güler et al., 2002; Nedungadi et al., 2012). The role of POA TRPV1 channel in thermoregulation has been well studied. Peripheral or central administration of TRPV1 agonist, capsaicin induces hypothermia (Hori et al., 1984; Jancso-Gabor et al., 1970a, 1970b; Kumar et al., 2011).

TRPV4 channel, another member of subfamily TRPV may too have a role in thermoregulation, as they are activated by temperature ranging from 25 to 34 °C as studied in HEK 293 cell line (Güler et al., 2002; Watanabe et al., 2002a). Besides temperature, TRPV4 ion channels are activated by a variety of physical (cell swelling, mechanical stimuli, moderate heat) and chemical stimuli (Nilius et al., 2003, 2004; Watanabe et al., 2002a, 2002b). Initially the role of TRPV4 in osmotic regulation was observed in knockout mouse model of TRPV4 (Mizuno et al., 2003). TRPV4 ion channels are widely expressed in heart, endothelium, liver, kidney, urine bladder, keratinocytes, placenta, lung, trachea, salivary glands including brain (Strotmann et al., 2000; Liedtke et al., 2000; Wissenbach et al., 2000; Delany et al., 2001). Latest studies show the presence of TRPV4 channel in the POA (Güler et al., 2002). Role of TRPV4 ion channels in thermal selection behavior has also been shown in knockout mice (Lee et al., 2005). However, the role of TRPV4 ion channels of the POA in thermoregulation has not been addressed. The present study was undertaken to examine the effect of microinjection of TRPV4 ion channel agonist and its antagonist in the POA on Tb along with localization of TRPV4 channel in the POA.

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2. Materials & methods

2.1. Animals

Adult male Wistar rats (n = 24) weighing between 200 and 250 g (breed, reared and maintained in Central Animal Facility of All India Institute of Medical Sciences, New Delhi, India, 623/1AEAC/11) were used for the experiments. The animals were housed in separate polypropylene cages (40 × 28 × 15 cm), in an animal room with controlled room temperature (25 ± 1 °C) and having 14 h light period (light above 200 lx) and 10 h dark period (dark below 5 lx) schedule with light on at 6:00 h till 20:00 h. Food and water were provided ad libitum. All procedure were conducted in accordance with the rules of the Committee for the purpose of Control and Supervision for Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethics Committee, AIIMS, New Delhi, India. The experimental procedures were also in compliance with the Directive 2010/63/EU of the European parliament and of the Council of 22 September 2010.

2.2. Surgical procedure

Surgery was performed under aseptic conditions using pentobarbitone sodium anaesthesia (Aldrich Thomas Co, USA, 40 mg/kg BW, iP) to implant peritoneal transmitter and guide cannulae in the brain. For intracerebral microinjection bilateral guide cannulae made from stainless steel tube 24 G with indwelling styli were implanted aimed 2.0 mm above the POA, according to stereotaxic coordinate (AP 7.8, V 7.5, L ± 0.6) as per D Groot's atlas (De Groot, 1959). A radio transmitter TA10TAF40 (Data Science International, USA) was implanted in peritoneal cavity to record Tb. The rats were allowed seven days to recover from the surgical trauma.

2.3. Drugs and vehicle for microinjection

The drugs used in this study TRPV4 agonist GSK1016790A and TRPV4 antagonist, RN1734 was purchased from Sigma-Aldrich, Co. USA. They were dissolved in 0.9% pyrogen-free saline having 6% Tween 80 + 1% ethanol which was also used as the control/ vehicle (Jancso-Gabor et al., 1970). Anti-TRPV4 polyclonal antibody (produced in rabbit), was used as primary antibody while anti rabbit IgG- Atto 488 antibody (produced in goat) was used as secondary antibody in immunohistochemistry for localization of TRPV4 channel in the POA. All the antibodies were procured from Sigma-Aldrich Co. USA.

2.4. Recording procedure and experimental schedule

The study was conducted in four groups of 6 rats in each. Animals of Group 1 received TRPV4 agonist GSK1016790A, Group 2 received TRPV4 antagonist RN 1734, and Group 3 received vehicle while immunohistochemistry was performed in Group 4. The Tb was measured from 10.00 to 16.00 h and injection was given at 12.00 h. Three control recordings of Tb were taken on alternate days. Microinjection studies were done on 4th alternative day after control studies. Injections (0.4 μg/0.2 μl) were given bilaterally into the POA at a rate of 0.1 micro litre /min through a 32 G injector cannula inserted through the guide cannulae. The injector cannula was 2 mm longer than the guide cannulae. Tb was recorded telemetrically (DSI, USA) at 15 s interval. Temperature data of 15 min epochs were averaged for statistical analysis.

2.5. Histology and perfusion

At the end of the experiments the rats of Group 1, 2 and 3 were anaesthetized with pentobarbitone sodium (50–60 mg/kg B.W, I.P) and 2% ferric chloride (0.4 μg/0.2 μl) was injected in the POA through the injector cannula to mark the injection sites. The rats were perfused transcardially first with 100 ml of isotonic saline (0.9%) and then with 10% formaldehyde with 0.3% potassium permanganate to fix the brain tissue as described earlier (Bagga et al., 1981). Brains were removed from the skull. 10 μm thick paraffin sections of the brains were made and stained with hematoxylin-eosin, for histological examination of the injection sites which appeared blue on histological sections as ferric chloride reacted with potassium permanganate and formed Prussian blue (Fe_4 [Fe (CN) $_6$]$_3$·xH_2O).

2.6. Immunohistochemistry

The immunohistochemistry was performed to localize the TRPV4 ion channel in the POA as described earlier (Tóth et al., 2005). The rats of Group 4 were perfused with formalin transcardially. Brains were removed and placed in 10% formalin for 24 h and transferred into 15% and 30% sucrose solution consequently for 48 h. Brains were placed in the cryomedia with OCT (−20 °C) for 15 min and 16 μm coronal cryosection were taken. The sections were placed on poly-L-Lysine coated glass slides (Sigma Aldrich, USA) and stored at 4 °C. Before processing slides, the sections were rehydrated three times each for 20 min by phosphate buffer saline (PBS) and then preincubated for 2 h at room temperature with 10% bovine serum albumin (Sigma Aldrich, USA) to prevent binding of unspecific antibody to receptors. The sections were incubated for 24 h at 4 °C with rabbit polyclonal antibody that recognizes N-Terminus of TRPV4 (Sigma Chemical Co. USA), diluted in 1:100 in PBS). After three round of washing with PBSTX (Phosphate Buffer Saline TritonX) for 5 min each, the sections were again incubated for 2 h with secondary antibody, anti- rabbit IgG-Atto 488. After 2 h fluoroshield media (Sigma Aldrich, USA) was added and covered with cover slips. The brain sections were captured on fluorescent and confocal microscopes (Leica microsystem, Germany) at 10X and 20X magnifications respectively.

2.7. Statistical analysis

The pre and post injection data, after giving TRPV4 agonist, antagonist and vehicle microinjection in Groups 1, 2 and 3, were compared with repeated measure ANOVA test using Graph Pad Prism 6 software. The 2 h pre injection data (Mean ± S.D) of Tb was compared with every 15 min (Mean ± S.D) post injection data of body temperature for 6 h.

3. Results

Effect of microinjection of TRPV4 channel agonist, antagonist and vehicle in the POA on Tb in freely moving rats and localization of thermo TRPV4 channels in the POA are described in the results.

3.1. Effect of TRPV4 agonist microinjection in the POA on body temperature

In Group1, effect of TRPV4 agonist (GSK1016790A, 0.4 μg/0.2 μl) microinjection in the POA in 6 rats on Tb is shown in Fig. 1A.

The Tb during 2 h pre injection period was 37.47 ± 0.2 °C (Mean ± SD). After the microinjection of TRPV4 agonist, GSK1016790A, there was significant fall in the Tb during first one hour of injection. The temperature started falling 15 min after the injection and the effect lasted for more than one hour. The maximum fall in Tb was about 0.9 °C and it was observed around 75 min of injection.

3.2. Effect of TRPV4 antagonist microinjection in the POA on body temperature

The effect of TRPV4 antagonist RN 1734 microinjection in the POA

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