Neuroscience 156 (2008) 165–174

EFFECTS OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 ANTAGONIST A-425619 ON BODY TEMPERATURE AND THERMOREGULATION IN THE RAT

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Abstract—Transient receptor potential vanilloid 1 (TRPV1) receptor antagonists have gained much attention for their potential to treat inflammatory and neuropathic pain. However, systemic administration of TRPV1 antagonists induces a period of hyperthermia, a potential liability for small molecule development. Here we characterize the effects of the TRPV1 antagonist A-425619 on body temperature (T_b) in the rat when administered: (1) alone at different times of the circadian cycle, (2) as repeated hourly or daily treatment, (3) as pre-treatment to prevent capsaicin-induced hypothermia, (4) to capsaicin-desensitized animals, and (5) prior to a heat challenge. Changes in T_b were compared with compound exposure data, locomotor activity, and time course of efficacy in inflammatory pain models. Without affecting locomotor activity, oral administration of A-425619 induced a transient period of hyperthermia that was followed by a period of hypothermia, a profile unique among reported TRPV1 antagonists. Repeated hourly administration of A-425619 produced an increase in T_b similar to a single administration. A-425619 had no effect on T_b when administered to capsaicin-desensitized rats. The duration of A-425619-induced hyperthermia, but not hypothermia, was dependent on the time of the circadian cycle when administered. Pre-treatment with A-425619 attenuated capsaicin-induced hypothermia and did not potentiate T_b or alter thermoregulatory behavioral responses during a heat challenge. These results indicate that A-425619-induced hyperthermia is transient, circadian-dependent, not related to exposure levels, locomotor activity, or time course of analgesic action, and does not affect the ability to thermoregulate during a heat challenge.

Key words: hyperthermia, inflammation, capsaicin.

Originally described as the receptor for capsaicin and other vanilloid containing compounds on primary sensory neurons (Caterina et al., 1997), vanilloid receptor 1 (VR1) was later shown to be a member of the transient receptor potential family and renamed transient receptor potential channel, vanilloid subfamily member 1 (transient receptor potential vanilloid 1, TRPV1). TRPV1 is a non-selective cation channel expressed primarily on polymodal sensory neurons (C- and Aδ-fibers). TRPV1 has high Ca^{2+} permeability, approximately 10-fold greater than Na^{2+}, and activation can lead to sustained depolarization that can produce sensory neuron hyperexcitability. This TRPV1-mediated hyperexcitability is thought to play a crucial role in injury-induced increases in neuronal sensitivity in both inflammatory and neuropathic conditions (Nagy et al., 2004; Numazaki and Tominaga, 2004; Immke and Gavva, 2006; Levine and Alessandri-Haber, 2007; Szallasi et al., 2007).

Opening of TRPV1 is facilitated by low pH, heat (>43 °C), and several pro-inflammatory mediators, including: bradykinin, prostaglandin E_2, ATP, anandamide (AEA), and nerve growth factor. TRPV1$^{-/-}$ mice have normal responses to non-noxious and noxious mechanical stimuli, but have diminished responses to noxious heat and inflammation-induced thermal hyperalgesia. The role of TRPV1 in mediating inflammatory responses is well established and antagonists such as capsazepine inhibit hyperalgesic responses to capsaicin and reduce the effects of TRPV1 potentiating agents such as AEA (Smart et al., 2000; Smart and Jerman, 2000; Roberts et al., 2002; Dinis et al., 2004; Nagy et al., 2004). The involvement of TRPV1 in inflammation and a putative role in neuropathic pain has led to the development of TRPV1 antagonists as potential therapies for inflammatory and neuropathic conditions.

TRPV1 is also expressed in the preoptic/anterior hypothalamus (PO/AH; Acs et al., 1996; Szabo et al., 2002), an area involved in thermoregulation, and TRPV1 has been proposed to have a thermoregulatory role in mammals (for review: Caterina, 2007). Supporting this, systemic injection of capsaicin produces hypothermia in several species (Jancso-Gabor et al., 1970a), which is followed by a period of hyperthermia (Szikszay et al., 1982). Capsaicin-induced hypothermia is not seen in TRPV1$^{-/-}$ mice (Caterina et al., 2000) and can be prevented by TRPV1 antagonists such as capsazepine (Dogan et al., 2004; Swanson et al., 2005; Gavva et al., 2007a; Steiner et al., 2007), suggesting a direct involvement of TRPV1. TRPV1$^{-/-}$ mice also have an attenuated fever response to lipopolysaccharide injection (Iida et al., 2005). The specificity of a TRPV1-mediated mechanism is further supported by studies in capsaicin-desensitized rodents. S.c. capsaicin injection at high doses (e.g. 50 mg/kg) results in loss of TRPV1 expressing nerve fibers and TRPV1 immunoreactivity (Fitzgerald, 1983; Russell and Burchiel, 1984; Jancso et al., 1985; Holzer, 1991; Khan et al., 2004) and these capsaicin-desensitized rats have attenuated body temperature (T_b) responses to subsequent capsaicin injections (Jancso-Gabor et al., 1970a; Szikszay et al., 1982).

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Abbreviations: AEA, anandamide; CFA, complete Freud's adjuvant; MPN, medial preoptic nucleus; PO/AH, preoptic/anterior hypothalamus; PWT, paw withdrawal threshold; SCN, suprachiasmatic nucleus; T_b, body temperature; TRPV1, transient receptor potential vanilloid 1.

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doi:10.1016/j.neuroscience.2008.06.069

In addition to the hypothermic effects of TRPV1 activation, other lines of evidence suggest that TRPV1 may constitutively regulate T_b (Gavva et al., 2007b). For instance, capsaicin-desensitized mice and rats lose their ability to regulate T_b and cannot withstand a heat challenge (Jancso-Gabor et al., 1970a,b; Szelenyi et al., 2004). Additionally, TRPV1 antagonists induce hyperthermia in a number of different species, including: mice, rats, dogs, and monkeys (Swanson et al., 2005; Gavva et al., 2007b; Steiner et al., 2007). Therefore, constitutive TRPV1 activity may be an intrinsic, tissue specific property that is attenuated by antagonist administration. Alternatively, endogenous sensitizing factors for TRPV1, such as AEA (van der Stelt et al., 2005) or prostaglandins (Moriyama et al., 2005) may allow tonic channel activity at temperatures lower than 43 °C. The hyperthermic effects induced by TRPV1 antagonists pose safety concerns for the development of therapeutic antagonists (Gavva et al., 2008).

The present work characterizes the TRPV1 antagonist, A-425619 (Abbott Laboratories; Honore et al., 2005) in rat models of thermoregulation and demonstrates: (1) when administered alone, A-425619 induces a period of hyperthermia that is followed by a dose dependent hypothermic period—a profile unique among reported TRPV1 antagonists, (2) the duration of hyperthermia is dependent on the circadian cycle, whereas the magnitude and duration of the hypothermic period is circadian-independent, (3) A-425619 effects on T_b are dependent upon TRPV1 expression, (4) A-425619-induced changes in T_b are not due to changes in locomotor activity, (5) A-425619 dose-dependently attenuates capsaicin-induced hypothermia, (6) A-425619 does not affect the ability of rats to thermoregulate during a heat challenge, (7) A-425619 effects on T_b are temporally distinct from effects on inflammatory pain, and (8) A-425619 effects on T_b and against capsaicin-induced mechanical sensitivity do not attenuate with repeated daily dosing.

EXPERIMENTAL PROCEDURES

Experimental animals, capsaicin and complete Freud's adjuvant (CFA) injections

Adult male Sprague–Dawley rats, 190–210 g, were obtained from Harlan (Indianapolis, IN, USA) and housed with a 12-h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee and in accordance with IASP guidelines. Efforts were taken to minimize the number of animals used and the extent of their suffering. All behavioral experiments were conducted in a randomized, blinded fashion. For s.c. capsaicin injection, capsaicin (Sigma-Aldrich, St. Louis, MO, USA) was prepared by first dissolving in 70 °C Tween-80 and then diluting in saline to give final working concentrations in 7% Tween/93% saline. To induce capsaicin desensitization, 30 mg/kg/day of capsaicin was injected for three consecutive days. For intraplantar injection of CFA (Sigma), animals were lightly anesthetized with 2% isoflurane and 50 μl of 100% CFA was injected into the plantar surface of the left hind paw.

A-425619 preparation

A-425619 was synthesized as previously described (Gomtsyan et al., 2005) and was prepared in 10% PEG 400, 0.225% methyl cellulose with probe sonication and given orally for all experiments.

T_b assays

Core T_b and home cage activity data were monitored in unanesthetized, singly housed, freely moving rats using i.p. radio transmitters (model TA10TA-F40; Data Sciences International, St. Paul, MN, USA). For capsaicin-induced hypothermia experiments, capsaicin was administered at the time of peak core T_b, 1 h after onset of the dark cycle and A-425619 was administered 1 h before capsaicin injection. All experiments began 2 weeks after transmitter implantation. Following A-425619 and/or capsaicin administration, the maximum decrease in T_b during the hypothermic period (lowest absolute T_b), the overall hypothermic effect (evaluated as area under the curve), and maximum increase in T_b during the hyperthermic period were determined. Data were collected at 50 Hz in 60 s bins and analyzed with Dataquest Advanced Research Technology Analysis software v4.0 (Data Sciences International), and presented as a 30 min moving average (mean±S.E.M.).

The heat challenge assay was performed by heating animals in their home cage with a radiant heat source for 1 h. Ambient temperatures rose from 21 °C to a maximum temperature of 32 °C at 30 min, which was maintained for 30 min, a total heat challenge of 60 min. Subjects were randomly assigned to the following groups: 1) heat challenge only, 2) vehicle+heat challenge, 3) A-425619 (35 mg/kg)+heat challenge, 4) A-425619 (100 mg/kg)+heat challenge, or 5) capsaicin desensitized+heat challenge. Heat challenge was begun 0.5 h after vehicle or A-425619 administration.

Normal T_b of the rat displays a circadian rhythm with higher temperatures occurring during the dark cycle. To determine the effects of A-425619 on T_b at different times of the circadian cycle, we administered A-425619 (35 mg/kg) so that the peak of hyperthermia, which occurs 30 min post-administration, coincided with the beginning, middle, and end of the dark cycle.

Behavioral testing

Injection of CFA produces hypersensitivity to mechanical stimuli as evident by a decrease in mechanical paw withdrawal thresholds (PWT). To assess the effectiveness of A-425619 against CFA-induced mechanical sensitivity, the Randall Selitto test using the Ugo Basile Analgesy-Meter (Ugo Basile, Varese, Italy) was used to determine PWTs following intraplantar injection of CFA. Subjects were acclimated to testing procedures for at least two trials on three separate days before recording baseline PWTs. CFA-induced changes in mechanical sensitivity were determined 3 days after intraplantar CFA injection.

CFA-induced changes in thermal sensitivity were determined using the Hargreaves and hotplate assays 3 days after intraplantar CFA injection. For the Hargreaves assay, rats were placed on a glass plate over a light box (San Diego Instruments; San Diego, CA, USA) and a radiant heat stimulus was applied by focusing a beam of light on the glabrous surface of the paw. The light beam was turned off automatically by a photocell when the paw was withdrawn from the glass surface. Paw withdrawal latency was defined as the time from when the light source was turned on to the time the paw was withdrawn. For the hotplate assay, rats were placed on a Hotplate Analgesia Meter (Columbus Instruments; Columbus, OH, USA) heated to 58 °C. The mean of two trials was averaged with at least 5 min between trials. The latency to withdrawal the injected paw was determined with a cutoff time of 10 s was used to avoid tissue damage.

To evaluate the effects of A-425619 on spontaneous locomotor activity, rats were pre-treated with A-425619 1 h prior to placement into a novel environment (Plexiglas activity chambers 45×45×23 cm). Exploratory locomotor activity during the first 30 min was collected using the Photobeam Activity System (PAS)