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Kv7 (KCNQ) channel openers induce hypothermia in the mouse

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ABSTRACT

Kv7 channels, encoded by corresponding *kcnq* genes, are expressed both centrally and peripherally where they serve to dampen neuronal activity. While Kv7 channel openers have shown efficacy in neurological and neuropsychiatric disease models, the impact of Kv7 channel activation on physiological endpoint markers have not been addressed in detail. In this study we assessed the effect of a range of Kv7 channel openers with different affinity for neuronal Kv7.2-5 channel subunits on body temperature regulation in mice. Female NMRI mice were acutely exposed to vehicle (10% Tween-80, i.p.), retigabine (3–30 mg/kg, i.p., pan-Kv7 channel opener), (S)BMS-204352 (60–240 mg/kg, i.p., Kv7.4/5 channel-preferring opener), ICA-27243 (1–10 mg/kg, i.p., Kv7.2/3 channel-preferring opener), or S-(1) (10–60 mg/kg, i.p., Kv7.2/3 channel-preferring opener), and rectal body temperature was measured 15–120 min post-injection. Retigabine (>10 mg/kg), ICA-27243 (≥10 mg/kg), and S-(1) (≥30 mg/kg) dose-dependently lowered rectal body temperature with maximal doses of each Kv7 channel opener inducing a marked drop (>4 °C) in rectal temperature. The Kv7 channel openers showed differential temporal pharmacodynamics, which likely reflects their different pharmacokinetic profiles. Pretreatment with the pan-Kv7 channel blocker XE-991 (1.0 mg/kg, i.p.) completely reversed the hypothermic effect of the pan-Kv7 opener, retigabine (15 mg/kg), whereas ICA-27243-induced hypothermia (10 mg/kg) could only be partially prevented by XE-991. Because ICA-27743 and S-(1) are Kv7.2/3 channel subunit-preferring compounds, this suggests that the Kv7.2/3 channel isoform is the predominant substrate for Kv7 channel opener-evoked hypothermia. These data indicate the physiological relevance of Kv7 channel function on body temperature regulation which may potentially reside from central inhibitory Kv7 channel activity.

Kv7 channels, being the molecular correlate of the M-current, are voltage-dependent potassium channels composed of homo- and heteromeric complexes of five different Kv7 subunits (Kv7.1-5, encoded by the *kcnq*1-5 genes). Unlike Kv7.1, all Kv7.2-5 subunits are consistently found expressed in the CNS [11]. In contrast, all Kv7 channels are expressed in various peripheral tissues [6]. Kv7 channels represent attractive targets for the development of therapeutics for neuronal hyperexcitability disorders, because activation of Kv7 channel function leads to dampening of neuronal excitability. Accordingly, the principal Kv7 channel opener, retigabine, has recently been approved for adjunctive therapy in partial-onset seizures, thus lending strong support to the therapeutic applicability in human CNS diseases characterized by neuronal hyperfunction.

The generalized dampening effect on neuronal activity following activation of Kv7 channels has spurred extensive experimental Kv7 channel research in regard to neuropsychiatric disease states with hyperexcitability features, including anxiety, schizophrenia and mania [8–10,18,23]. In contrast, the impact of Kv7 channel activation on physiological endpoint markers has not been addressed in detail. Most consistently, retigabine is demonstrated to exhibit potent inhibitory effects on peripheral smooth muscular tissue contractility, indicating that physiological effects of Kv7 channel openers may have profound impact on basal physiological parameters, including vasoregulation [6]. However, Kv7 channel openers could potentially also exhibit physiological effects residing from regulation of other autonomous functions. Because Kv7 channels are expressed in the hypothalamus and thalamus [2,5,26], being key regions involved in thermoregulation, we speculated whether pharmacological stimulation of Kv7 channel function could potentially affect body temperature regulation. Hence, we assessed the effect of a range of Kv7 channel openers on rectal body temperature in mice. To date, no Kv7 channel subtype selective compounds exist, and to deduce whether a principal Kv7 channel isoform could be involved in Kv7 channel-associated thermoregulation, we compared the effect of retigabine, a pan Kv7.2-5 channel opener [6], with various subtype-preferring Kv7 openers, i.e. ICA-27243, S-(1) (both Kv7.2/3 preferring [27,29]), and (S)BMS-204352 (Kv7.4/5 preferring [13]), respectively.

Female NMRI mice (20–25 g; Harlan, the Netherlands) were used. Animals were allowed a minimum of 7 days acclimatisation to the laboratories before use. Mice were housed in groups

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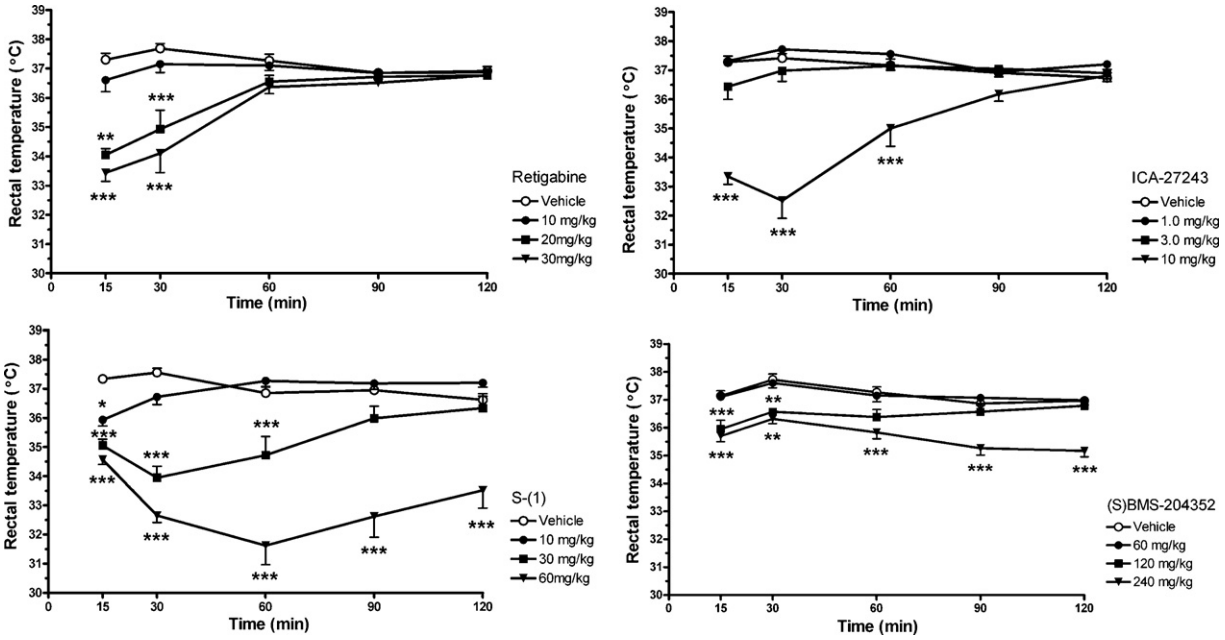

Fig. 1. Kv7 channel openers induce hypothermia in the mouse in a dose- and time-dependent (15–120 min) manner. Retigabine (10–30 mg/kg), ICA-27243 (1.0–10 mg/kg), S-(1) (10–60 mg/kg), (S)BMS-204352 (60–240 mg/kg) and vehicle as control. $^{**}p < 0.01$; $^{***}p < 0.01$ compared to vehicle control group.

of 8 in Macrolon III cages (20 cm × 40 cm × 18 cm) contained in Scantainers (Scanbur A/S, Ejby, Denmark) under a 12 h light/dark cycle (lights on: 06:00 h) with free access to food (standard laboratory pellets) and tap water. Experiments were performed between 9:00 h and 13:00 h in temperature and humidity-regulated rooms (22–24 °C; relative humidity: 60–70%). All procedures were carried out in accordance with internationally accepted principles for the care and use of laboratory animals and were approved by the Danish Committee for Animal Research.

Retigabine, ICA-27243, S-(1), (S)BMS-204352 and XE-991 were synthetized by NeuroSearch and dissolved in a 10% Tween 80 solution in saline and adjusted to pH 7.4. Flumazenil was from Sigma (St. Louis, MO). All compounds were administered via the intraperitoneal (i.p.) route in an injection volume of 10 ml/kg. All drug doses were calculated using free base weights.

Core body temperature was electronically measured in mice using a rectal probe (Type DM 852, Ellab, Copenhagen). Following lubrication with vaseline, the temperature probe was inserted in the rectum (approximately 2 cm) for approximately 5 s until a stable temperature was reached. For assessment of time–response effects, rectal temperature measurements were taken 15–30–60–90–120 min after drug dosing. After each measurement, the mouse was returned to its home cage. In antagonist interaction experiments, XE-991 (1.0 mg/kg) or flumazenil (10 mg/kg) was administered 15 min prior to retigabine (15 mg/kg) with rectal temperature measurement 15 min, thereafter. All experiments were conducted using 6 mice per group.

Statistical comparisons between groups were carried out by a one-way ANOVA with Tukey's post hoc test (XE-991 interaction study) or a repeated measurement two-way ANOVA followed by Bonferroni's multiple comparison test (dose–response studies). Data were considered statistically significant when $p < 0.05$. All data are presented as mean ± S.E.M.

Retigabine, ICA-27243, S-(1) and (S)BMS-204352 all caused a dose-dependent reduction in rectal temperature in female NMRI mice (Fig. 1). Retigabine (20–30 mg/kg, $p < 0.001$) induced hypothermia with the effect peaking (maximum hypothermia: 3.9 °C) within the first 15 min of drug administration. In contrast, ICA-27243, S-(1) and (S)BMS-204352 showed a more protracted hypothermic response. While ICA-27243 (10 mg/kg, $p < 0.001$) evoked a significant drop in rectal temperature at 15–60 min post-injection (maximum hypothermia, 30 min: 4.9 °C), the hypothermic effect of S-(1) (10–60 mg/kg, $p < 0.001$; maximum hypothermia, 60 min: 5.2 °C) and (S)BMS-204352 (120–240 mg/kg, $p < 0.001$; maximum hypothermia, 120 min: 1.8 °C) lasted throughout the monitoration period. No pro-convulsant activity was observed at any drug or dose administered (data not shown). When analyzing pooled data from all individual vehicle groups ($n = 24$) across the different time–response experiments, administration of vehicle *per se* induced a weak hyperthermic response (+0.33 °C) in female NMRI mice when comparing 15 min (37.3 ± 0.08 °C) and 30 min (37.6 ± 0.10 °C) post-injection times ($p < 0.05$, one-way ANOVA with Tukey's post hoc test). This is in accordance with previous reports on female mice being slightly less susceptible to stress-induced hyperthermia during rectal temperature probing as compared to male mice [17,25].

To address the principal relevance of Kv7 channel activation on the hypothermic response, the Kv7 channel blocker XE-991 (1.0 mg/kg) was administered prior to retigabine or ICA-27243 using an individual drug dose inducing a robust hypothermic response at 15 min post-dosing, equivalent to a rectal temperature drop of 2.9 ± 0.3 °C (retigabine, 15 mg/kg) and 4.0 ± 0.3 °C (ICA-27243, 10 mg/kg), respectively (Fig. 2A and B). While XE991 had no effect on rectal temperature *per se*, the Kv7 channel blocker fully prevented the hypothermic effect of retigabine ($p < 0.001$ vs. retigabine + vehicle; $p > 0.05$ vs. vehicle + vehicle, Fig. 2A). XE-991 also counteracted ICA-27243 induced hypothermia ($p < 0.01$ vs. retigabine + vehicle, Fig. 2B), however, with the reversal being incomplete ($p < 0.001$ vs. vehicle + vehicle). In addition, pre-treatment with the $GABA_A$ receptor antagonist flumazenil partially reversed retigabine-induced hypothermia (Fig. 2C).

All Kv7 channel openers showed a fast onset of hypothermic activity within 15 min post-administration. The hypothermic action was associated with stimulation of Kv7 channel function, examplified by the XE-991-sensitive modulation of retigabine and ICA-27243 induced hypothermia, respectively. While the