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Icilin-induced wet-dog shakes in rats are dependent on NMDA receptor activation and nitric oxide production

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ABSTRACT

Icilin is a cold channel agonist that produces vigorous wet-dog shaking in rats. The shaking is accompanied by an increase in the level of extracellular glutamate in the brain. Hence, we hypothesized that icilin-induced wet-dog shakes are dependent on increased glutamatergic transmission and nitric oxide (NO) production. Rats injected with icilin (0.5, 1, 2.5, 5 mg/kg, i.p.) displayed a dose-related increase in wet-dog shakes. Pretreatment with LY 235959 (1, 2 mg/kg, i.p.), a NMDA receptor antagonist, or L-NAME (50 mg/kg, i.p.), a NO synthase (NOS) inhibitor, attenuated icilin-induced wet-dog shakes. The shaking was also reduced by intracerebroventricular L-NAME (1 mg/rat, i.c.v.) administration, indicating that the stimulant effect of icilin is dependent on central NO production. Pretreatment with 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX) (10, 20 mg/kg, i.p.), an AMPA receptor antagonist, or ceftriaxone (200 mg/kg, i.p. for 5 days), a beta-lactam antibiotic and glutamate transporter subtype 1 (GLT-1) activator, did not alter the incidence of icilin-induced shaking. The present data reveal that icilin produces behavioral stimulation by a mechanism requiring NMDA receptor activation and nitric oxide production and suggest that glutamate and NO signaling play important roles in cold channel pharmacology.

1. Introduction

Icilin activates two transient receptor potential (TRP) channels, TRPM8 and TRPA1, located in the peripheral nervous system (McKemy et al., 2002; Peier et al., 2002; Reid et al., 2002; Nealen et al., 2003; Story et al., 2003; Bandell et al., 2004; Liu et al., 2006). Upon application to the skin or tongue, icilin produces "mild, pleasant sensations of coolness, similar to menthol but discrete and non-irritating" and is 400–600 times more potent than menthol (Wei, 1981; Wei and Seid, 1983; Tse and Wei, 1986; Behrendt et al., 2004). As a result, icilin is a potential alternative to menthol in many over-the-counter medications, and has therapeutic promise as an analgesic, antipruritic and anti-arthritic agent (Biró et al., 2005). The signature overt effect of icilin in rats is the dramatic, stimulant behavior it precipitates following intraperitoneal administration. The behavioral syndrome consists of excessive body grooming, abdominal writhing, ptosis, forepaw tremor, jumping and vigorous wet-dog shakes (Wei, 1976; Cowan, 1981). In addition to its stimulant effects, icilin increases extracellular glutamate in the brain and causes a hyperthermia that is attenuated by NMDA receptor antagonism or nitric oxide synthase inhibition (Werkheiser et al., 2007; Ding et al., 2008). Since icilin produces hyperthermia, shaking and increased extracellular glutamate, and NMDA receptor blockade and NOS inhibition attenuate icilin-induced hyperthermia, we hypothesized that NMDA receptor blockade and NOS inhibition will also attenuate icilin-induced shaking. This hypothesis was tested using two pharmacological agents: (−)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate] (LY235959), a selective NMDA receptor antagonist; and N(G)-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nonselective NOS inhibitor. Additionally, we investigated the effects of an AMPA receptor antagonist, 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX), and a GLT-1 transporter activator, ceftriaxone, on the frequency of icilin-induced wet-dog shakes (Rothstein et al., 2005).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Ace Laboratories, Boyertown, PA), weighing 100–125 g, were housed in groups of three for five days prior

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to use, with food and water available ad libitum. The temperature in the room was 23 ± 1 °C and a standard light–dark cycle was maintained with a timer-regulated light period from 0700 to 1900 h. The studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Temple University. Each rat was used only once, for a single experiment, and then euthanized immediately.

2.2. Compounds

Icilin, a gift from Delmar Chemicals Ltd. (Montreal, Canada), was suspended in 1% Tween 80/distilled water. LY 235959, L-NAME and DNQX (disodium salt) were purchased from Tocris Laboratories (Ellisville, MO, USA) and dissolved in physiological saline. Ceftriaxone hydrochloride was purchased from Apotex Corporation (Weston, FL, USA) and dissolved in physiological saline. For systemic administration, compounds were administered in a volume of 1 ml/kg and injected intraperitoneally (i.p.). For central administration, L-NAME was administered intracerebroventricularly (i.c.v.) in a volume of 5 µl.

2.3. Experimental design

All experiments were performed during the light phase between noon and 5 p.m. Each rat was weighed and acclimated in a plexiglas observation box (22 cm long; 18 cm wide; 25 cm high) 90 min before administration of test compounds.

2.3.1. Systemic experiments

Rats were injected with LY 235959 (0.5, 1, 2 mg/kg, i.p.), L-NAME (50 mg/kg, i.p.) or saline 30 min prior to icilin (0.5, 1, 2.5, 5 mg/kg, i.p.). Each compound has been investigated previously in our laboratories, and doses and pretreatment times were selected on the basis of results from those studies (Rawls et al., 2006; Ding et al., 2008). For DNQX experiments, rats were pretreated with DNQX (10, 20 mg/kg, i.p.) or saline and injected with a fixed dose (2.5 mg/kg, i.p.) of icilin 30 min later. For ceftriaxone experiments, rats were injected with ceftriaxone (200 mg/kg, i.p.) or saline once daily for 5 days. On day 6, rats were injected with icilin (0.5, 1, 2.5, 5 mg/kg, i.p.). The ceftriaxone design was based on evidence that the said dosing schedule increases GLT-1 transporter activity and expression in the rat brain (Rothstein et al., 2005; Rawls et al., 2007). The incidence of wet-dog shakes was counted for 30 min after icilin administration in all experiments. Following experimentation, each rat was placed in the prone position on the Perspex lid of the observation chamber, and tested for sedation. If the rat moved freely off the lid and on to the bench top, it was considered "not behaviorally depressed." None of the rats in our experiment were observed to be behaviorally depressed following experimentation.

2.3.2. Central experiments

Rats were anesthetized with an i.p. injection of ketamine hydrochloride (150 mg/kg) and acepromazine maleate (0.2 mg/kg). A polyethylene guide cannula was implanted stereotaxically into the right lateral ventricle (Rawls et al., 2006). Coordinates for the lateral ventricle were 0.8 mm posterior from bregma, 1.6 mm lateral from the midline, and 3.5 mm from the skull. Dental acrylic cement was used to secure the cannula to the cranium. The route of L-NAME administration was intracerebroventricular (i.c.v.) and performed by inserting the needle tip of a 10-µl syringe into a polyethylene cannula. The tip of the needle extended 1 mm beyond the tip of the cannula. In behavioral experiments, L-NAME (1 mg/rat, i.c.v.) or an equivalent volume (5 µl, i.c.v.) of saline was injected 30 min before icilin (2.5 mg/kg), after which wet-dog shakes were counted for 30 min. Following i.c.v. experiments, injection sites were verified with an injection of 0.1% Evan's blue (4 µl). The central dose of L-NAME was based on prior results from our laboratory (Rawls et al., 2006).

2.4. Data analysis

Data are expressed as mean wet-dog shakes ± S.E.M. (nonlinear regression, GraphPad Prism). One-way ANOVA was used to evaluate cumulative group means. Bonferroni's *post-hoc* analysis was performed after significance was determined by ANOVA. For the experiment investigating the effects of centrally administered L-NAME on icilin-induced shaking, the two groups were compared using a Students *t*-test. Values of $P<0.05$ were considered statistically significant in all cases.

3. Results

3.1. Effect of a NMDA receptor antagonist on icilin-induced wet-dog shakes

The effect of LY 235959 (0.5, 1, 2 mg/kg, i.p.) pretreatment on icilin-induced wet-dog shakes is presented in Fig. 1. One-way ANOVA revealed a significant main effect [$F(15, 176) = 10.34$, $P<0.0001$]. When given by itself, icilin (0.5, 1, 2.5 and 5 mg/kg, i.p.) produced a dose-dependent increase in the incidence of wet-dog shakes over the 30-min observation interval. Onset of shaking occurred within 2 min of icilin administration. In all cases, icilin-induced abdominal writhing, which preceded the onset of shaking and excessive body grooming that was evident for the duration of the experiment. The administration of LY 235959 (0.5, 1, 2 mg/kg, i.p.) by itself did not elicit abdominal writhing, wet-dog shakes or excessive grooming. When LY 235959 (1 mg/kg, i.p.) was given in combination with 2.5 or 5 mg/kg of icilin, the incidence of wet-dog shaking was reduced by about 60% and 46%, respectively ($P<0.01$). A higher dose of LY 235959 (2 mg/kg, i.p.) also antagonized wet-dog shaking induced by 2.5 or 5 mg/kg of icilin by about 50% and 56%, respectively ($P<0.01$). The lowest dose of LY 235959, 0.5 mg/kg, did not affect the number of wet-dog shakes produced by any of the doses (0.5, 1, 2.5, 5 mg/kg, i.p.) of icilin ($P>0.05$).

3.2. Effect of a NOS inhibitor on icilin-induced wet-dog shakes

The effect of systemically injected L-NAME (50 mg/kg, i.p.) on icilin-induced wet-dog shakes is presented in Fig. 2A. One-way ANOVA revealed a significant main effect [$F(7, 88) = 28.03$, $P<0.0001$]. Administration of

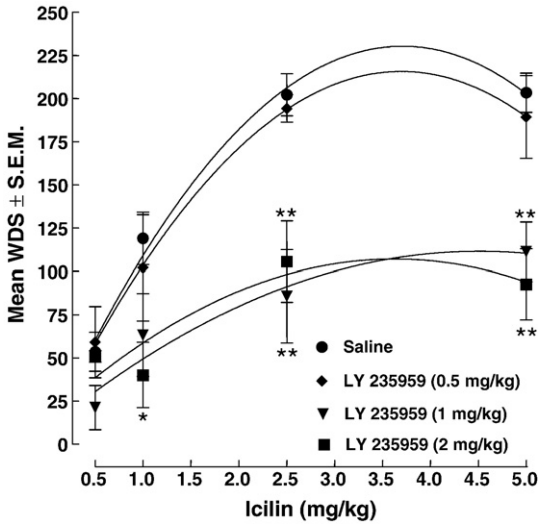

Fig. 1. Effect of LY 235959 on icilin-induced wet-dog shakes. Rats pretreated with LY 235959 (0.5, 1, 2 mg/kg, i.p.) or saline were injected 30 min later with icilin (0.5, 1, 2.5, 5 mg/kg, i.p.). Data from 12 rats per group are expressed as mean wet-dog shakes (WDS) ± S.E.M. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to rats pretreated with saline.