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Research Report

Icilin-evoked behavioral stimulation is attenuated by alpha$_2$-adrenoceptor activation

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ABSTRACT

Icilin is a transient receptor potential cation channel subfamily M (TRPM8) agonist that produces behavioral activation in rats and mice. Its hallmark overt pharmacological effect is wet-dog shakes (WDS) in rats. The vigorous shaking associated with icilin is dependent on NMDA receptor activation and nitric oxide production, but little else is known about the biological systems that modulate the behavioral phenomenon. The present study investigated the hypothesis that alpha$_2$-adrenoceptor activation inhibits icilin-induced WDS. Rats injected with icilin (0.5, 1, 2.5, 5 mg/kg, i.p.) displayed dose-related WDS that were inhibited by pretreatment with a fixed dose of clonidine (0.15 mg/kg, s.c.). Shaking behavior caused by a fixed dose (2.5 mg/kg) of icilin was also inhibited in a dose-related manner by clonidine pretreatment (0.03–0.15 mg/kg, s.c.) and reduced by clonidine posttreatment (0.15 mg/kg, s.c.). Pretreatment with a peripherally restricted alpha$_2$-adrenoceptor agonist, ST91 (0.075, 0.15 mg/kg), also decreased the incidence of shaking elicited by 2.5 mg/kg of icilin. Pretreatment with yohimbine (2 mg/kg, i.p.) enhanced the shaking induced by a low dose of icilin (0.5 mg/kg). The imidazoline site agonists, agmatine (150 mg/kg, i.p.) and 2-BFI (7 mg/kg, i.p.), did not affect icilin-evoked shaking. These results suggest that alpha$_2$-adrenoceptor activation inhibits shaking induced by icilin and that increases in peripheral, as well as central, alpha$_2$-adrenoceptor signaling oppose the behavioral stimulant effect of icilin.

1. Introduction

Icilin (AG-3-5) activates transient receptor potential cation channel subfamily M member 8 (TRPM8) (McKemy et al., 2002; Peier et al., 2002; Reid et al., 2002; Nealen et al., 2003; Story et al., 2003; Bandell et al., 2004; Biró et al., 2005; Liu et al., 2006). TRPM8 channels are densely expressed in sensory neurons within the dorsal root ganglion, and their activation by icilin and other

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cooling compounds (e.g., menthol, WS-12, and CPS-369) produces sodium and calcium ion influx that results in cellular depolarization and the feeling of cold (Nealen et al., 2003; Babes et al., 2004). Despite the potential of TRPM8 ligands as analgesic, antipruritic and antiarthritic agents, the endogenous and exogenous substances that modulate their pharmacological effects *in vivo* are largely unknown. The trademark overt pharmacological effect of icilin in rats is vigorous wet-dog shakes (WDS) (Wei, 1976, 1981; Werkheiser et al., 2006, 2007, 2009), and this shaking behavior provides a sensitive, reproducible, and quantifiable endpoint to investigate TRPM8 pharmacology *in vivo* and to identify, and exclude, receptor systems that shape the pharmacological response to TRPM8 channel activation.

Our studies have traditionally focused on the identification of downstream signaling events that underlie icilin-induced behavioral activation. For example, we have demonstrated that icilin-induced WDS are dependent on NMDA receptor activation and nitric oxide production but not on AMPA receptor or glutamate transporter subtype 1 (GLT-1) activation (Werkheiser et al., 2009). We have also shown that mu and kappa opioid receptor agonists reduce icilin-induced shaking (Werkheiser et al., 2006). The present study tested the overall hypothesis that alpha$_2$-adrenoceptor activation opposes icilin-induced behavioral activation. The hypothesis was based in part on the tenet that increased sympathetic activity contributes to shaking movements in rats and that suppression of sympathetic discharge by an alpha2-adrenoceptor agonist (clonidine) would reduce shaking caused specifically by icilin (Wei and Seid, 1983). The hypothesis was also based on a recent finding that alpha2-adrenoceptors and TRMP8 channels are co-expressed on peripherally located sensory neurons and that alpha$_2$-adrenoceptor activation inhibits TRPM8 channel signaling (Bavencoffe et al., 2010).We specifically investigated the effect of: 1) clonidine on the development and maintenance of icilin-induced shaking; 2) N-(2,6-diethylphenyl)-4,5-dihydro-1H-imidazol-2-amine hydrochloride (ST-91), a peripherally restricted alpha$_2$-adrenoceptor agonist, on the development of icilin-induced shaking; and 3) yohimbine, an alpha$_2$-adrenoceptor antagonist, on icilin-induced shaking. We also studied the effects of imidazoline site agonists on icilin-induced shaking behavior.

2. Results

2.1. *Clonidine pretreatment reduces icilin-induced shaking*

Effects of graded doses of clonidine (0.03, 0.6, 0.15 mg/kg) on WDS produced by a fixed dose of icilin (2.5 mg/kg) are presented in Fig. 1. One-way ANOVA revealed a significant drug effect [F (3, 31)=5.351, P=0.0049] for the experiment. Injection of icilin (2.5 mg/kg) by itself produced 148±32.5 WDS over the 30-min observation interval. Dunnett's post-hoc analysis indicated that the total number of WDS produced by icilin was significantly inhibited by pretreatment with either 0.06 mg/kg (P<0.05) or 0.15 mg/kg (P<0.01) clonidine. Specifically, 0.06 mg/kg and 0.15 mg/kg of clonidine inhibited the incidence of shaking by approximately 41% and 77%, respectively. The onset of shaking, and excessive grooming,

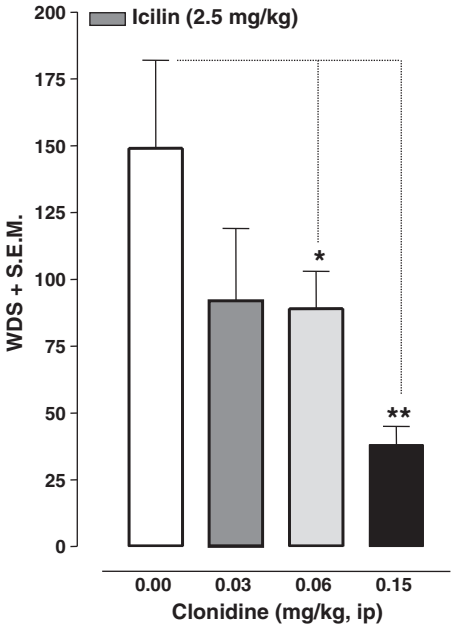

Fig. 1 – Clonidine pretreatment reduces WDS induced by a fixed dose of icilin. Rats pretreated with clonidine (0.03, 0.06, 0.15 mg/kg) or saline were injected 15 min later with icilin (2.5 mg/kg). Data from 6 to 8 rats per group are expressed as mean WDS+S.E.M. *P<0.5 or **P<0.01 compared to rats pretreated with saline.

began within 2 min of icilin injection. The behaviors persisted for the duration of the 30-min observation period, and were preceded, in all cases, by abdominal writhing. Although clonidine pretreatment (0.06, 0.15 mg/kg) did significantly inhibit the total extent of shaking induced by icilin, it did not significantly delay the onset of shaking. The administration of clonidine (0.03, 0.06, 0.15 mg/kg) by itself did not elicit abdominal writhing, wet-dog shaking, excessive grooming, or sedation.

Separate experiments examined the effect of a fixed dose of clonidine (0.06 mg/kg) on WDS elicited by progressively increasing doses of icilin (0.5, 1, 2.5, 5 mg/kg) (Fig. 2). Two-way ANOVA (pretreatment, icilin dose) indicated significant effects of pretreatment [F (1, 48)=61.37, P<0.0001] and dose [F (3, 48)=16.92, P<0.0001]. Icilin by itself produced dose-related shaking (0.5 mg/kg, 58±16.1; 1 mg/kg, 144±19.8; 2.5 mg/kg, 189±27.0; and 5 mg/kg, 190±18.2). Post-hoc analysis indicated that WDS associated with each icilin dose (0.5, 1, 2.5, 5 mg/kg) was significantly inhibited by pretreatment with clonidine (0.15 mg/kg). Specifically, clonidine pretreatment (0.15 mg/kg) produced the following effects on icilin-induced shaking (icilin dose, % inhibition, significance): (0.5 mg/kg, 75%, P<0.01); (1 mg/kg, 78%, P<0.01); (1 mg/kg, 60%, P<0.01); and (2.5 mg/kg, 52%, P<0.05).

2.2. *Clonidine posttreatment attenuates icilin-induced shaking*

Results from the experiment investigating the effect of clonidine posttreatment (0.6 mg/kg) on WDS produced by icilin (2.5 mg/kg) are presented as time-course data in Fig. 3.