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Icilin evokes a dose- and time-dependent increase in glutamate within the dorsal striatum of rats

Short Communication

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Summary. Icilin, the peripheral cold channel agonist, activates TRPM8 and TRPA1, localized on dorsal root ganglia and trigeminal neurons in rats. Icilin precipitates immediate wet-dog shakes in this species, which are antagonized by centrally acting mu and kappa opioid agonists, implicating the central nervous system in the behavioral response. We studied the effect icilin has on glutamate levels in the dorsal striatum, a brain region involved in movement. Icilin (0.25, 0.5 and 0.75 mg/kg, i.p.) elicited a dose- and time-dependent increase in glutamate within the striatum, indicative of icilin's neurochemical effect in rats.

Keywords: Icilin – TRPA1 – TRPM8 – Dorsal striatum – Glutamate

Introduction

Icilin is a ''super cold'' peripheral agonist at two transient receptor potential channels, TRPM8 and TRPA1, located on dorsal root ganglia and trigeminal neurons in rats (Wei and Seid, 1983; McKemy et al., 2002; Peier et al., 2002; Story et al., 2003). Icilin is 400–600 times more potent than menthol and may be an efficacious substitute for menthol in many over-the-counter medications. Icilin has potential as an analgesic, antiarthritic, antipruritic, and for treatment of hemorrhoids (Wei and Seid, 1983; Wei, 2005). Even with its therapeutic promise, the mechanism of icilin's activity in vivo, downstream of activation of TRP channels, remains unclear.

When given intraperitoneally (i.p.), icilin produces robust wet-dog shaking in rats that is antagonized by centrally acting mu and kappa opioid agonists, but not by ICI 204448, the peripherally directed kappa agonist (Werkheiser et al., 2004). We believe that icilin evokes changes in neurochemistry within the dorsal striatum, a region implicated in stereotypic and habitual movement (reviewed by Wolf et al., 2004). We used microdialysis to study glutamatergic changes in the rat dorsal striatum following submaximal behavioral doses of icilin to better understand icilin's effects in vivo.

Materials and methods

Animals and surgery

Male Sprague Dawley albino rats (150–175 g, Ace Laboratories, Boyertown, PA) were maintained according to Temple University Institutional Animal Care and Use Committee (IACUC) regulations. Rats were housed in groups of 4 at 23 ± 1 °C with food and water provided *ad libitum*. A standard light–dark cycle was maintained with a timer-regulated light period from 0700 to 1900 h.

The animals were anesthetized with ketamine + acepromazine (150 mg/kg + 2.5 mg/kg, i.p.) and implanted with a CMA/12 microdialysis cannula (CMA/Microdialysis, Chelmsford, MA) into the dorsal striatum using the following stereotaxic coordinates: AP 0.7, ML 3.0, DV 4.0 (Paxinos and Watson, 1998).

Microdialysis

Two days after surgery, the dummy probe was removed and replaced with a 2-mm CMA/12 microdialysis probe. Rats were acclimated in individual Plexiglas observation boxes and artificial CSF [NaCl (147 mM), $CaCl_2$ (1.2 mM), KCl (2.7 mM), $MgCl_2$ (0.85 mM)] was introduced into the probe at 2 µl/min. There was an initial 90-min washout period prior to baseline. Sample collections were taken in 15-min intervals for the duration of the experiment. Following four consecutive baseline collections (immediately after time 0), rats were given vehicle (1% Tween 80) or icilin (0.25, 0.5, 0.75 mg/kg, i.p.) at 1 mL/kg body weight. Samples were

collected for 120 min following icilin and stored at −80 °C for neurochemical analysis.

Histology

Rats were euthanized by inhalation of CO_2, brains were extracted, placed in 4% formaldehyde in phosphate buffered saline (pH = 7.0), and sections sliced and stained with 0.5% cresyl violet to determine probe placement and damage. Samples from rats with probe placement in the dorsal striatum were analyzed using HPLC.

Amino acid analysis

For glutamate derivitization, 5 µl of dialysate or amino acid standard was mixed with 5 µl sodium borate (8 mM, pH = 9.5), 5 µl KCN (12 mM) and later mixed with 4 µl naphthalene dicarboxylate acid, and derivitized for 5 min. Fifteen µl of the mixture was injected onto a 5-micron C-18 reverse phase column (150 × 4.6 mm) (Phenomenex Inc., Torrance, CA) with a LKB 2150 HPLC pump (Pharmacia Biotech, Upsala, Sweden) and eluted with 20 mM sodium citrate buffer (pH = 7.5) containing 50% methanol, using a linear gradient with 100% methanol. The flow rate was 0.60 ml/min. Glutamate was detected with a model HP 1050 diode array detector (Hewlett Packard Company, Atlanta, GA). Glutamate was identified by overlaying absorption spectra at 420 and 440 nm and quantifying at 420 nm using HP Chemstation software (Hewlett Packard Company) based on peak area by comparison with an external standard calibration curve ranging from 0.5 to 5 µM. The detection limit was 500 nM, based on signal-to-noise ratio.

Materials

Icilin (AG-3–5) was a gift from Delmar Chemicals Ltd. (Montreal, Canada) and suspended in 1% Tween 80. Amino acids were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO) and dissolved in double distilled water.

Statistical analysis

Baseline values were obtained prior to icilin or vehicle (1% Tween 80) administration. Each post-baseline sample was expressed as the percentage of respective baseline values. One way analysis of variance was applied to each time point and compared to vehicle-treated rats and analyzed using Newman–Keuls test (PharmTools Pro, The McCary Group, Emmaus, PA). Results were considered significant at $p < 0.05$.

To reflect overall changes in glutamate levels, AUC values were calculated from 15–120 min following icilin treatment, using KaleidaGraph (Synergy Software, Reading, PA) and expressed in histograms. One way analysis of variance was applied to each of the AUC values and analyzed using Newman–Keuls test (PharmTools Pro). Results were considered significant at $p < 0.05$.

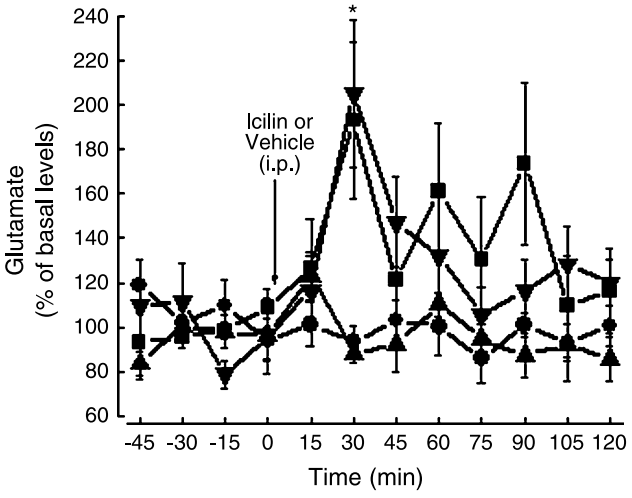

Fig. 1. Icilin [0.5 (▼) and 0.75 (■) mg/kg, i.p.] elevates basal glutamate levels within the striatum when compared with vehicle (●). Values are expressed as mean percent of baseline ± SEM of 6–10 rats. The highest increase in glutamate levels occurred at 30 min (*$p < 0.05$), which correlated with the incidence of shaking observed in vivo. Icilin, at 0.25 mg/kg (▲), did not significantly elevate glutamate levels within the striatum

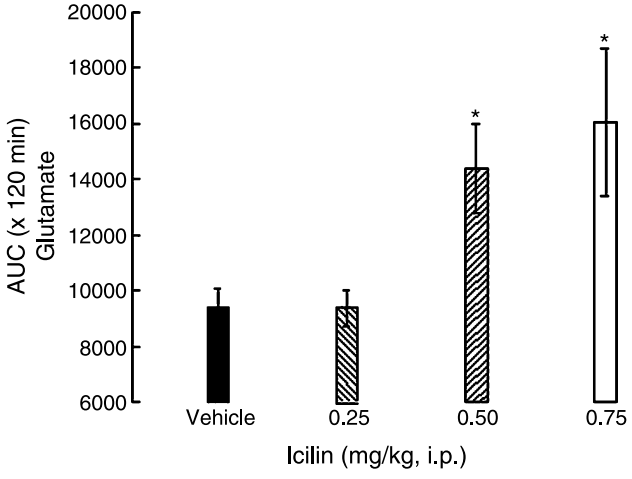

Fig. 2. AUC data show a dose-dependent increase in basal glutamate levels in rats treated with icilin (0.5, 0.75 mg/kg), compared to vehicle, within the dorsal striatum (*$p < 0.05$). Values are mean AUC ± SEM

Results and discussion

Submaximal behavioral doses of icilin (0.25, 0.5 and 0.75 mg/kg, i.p.) produced a dose- and time-dependent increase in basal glutamate levels within the striatum compared to control. At 30 min following icilin, there was an approximate 180% increase in basal glutamate levels in rats given 0.5 and 0.75 mg/kg icilin compared to vehicle-treated animals (Fig. 1). This increase in glutamate correlated with the incidence of wet-dog shaking observed in icilin-treated rats. Likewise, there was a dose-dependent increase in AUC values, significant at 0.5 and 0.75 mg/kg icilin (Fig. 2). With these preliminary experiments, we show for the first time the neurochemical effect that icilin exerts on the central nervous system of rats downstream of activation of TRPA1 and TRPM8 channels within the periphery.

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