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Differential effects of R-isovaline and the $GABA_B$ agonist, baclofen, in the guinea pig ileum

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ABSTRACT

R-isovaline is a non-proteinogenic amino acid which produces analgesia in a range of nociceptive assays. Mediation of this effect by metabotropic receptors for γ-aminobutyric acid (GABA) and glutamate, demonstrated by previous work, may depend on the type of tissue or receptor system. The objective of this study was to assess the activity of R-isovaline acting at $GABA_B$ and group II metabotropic glutamate receptors in guinea pig ileum, which is known to exhibit well-defined responses to $GABA_B$ agonists such as baclofen. The effects of bath-applied R-isovaline and RS-baclofen were examined on electrically evoked contractions of guinea pig ileum and during $GABA_B$ antagonism by CGP52432. In separate experiments, the group II metabotropic glutamate receptor agonist, LY354740 was applied to determine the functional presence of these receptors. R-isovaline (1–100 mM) decreased the amplitude of ileal muscle contractions and increased tension. RS-baclofen reduced contraction amplitude, but decreased tension. CGP52432 did not prevent the effects of R-isovaline on contraction amplitude, but antagonized effects of RS-baclofen on contraction amplitude. The group II metabotropic glutamate receptor agonist, LY354740, produced no detectable effects on evoked contractions. R-isovaline differed significantly from RS-baclofen in its actions in the guinea pig ileum, indicated in particular by the finding that CGP52432 blocked only the effects of RS-baclofen. The ileal tissue did not respond to a group II metabotropic glutamate receptor agonist, previously shown to co-mediate R-isovaline analgesia. These findings raise the possibility of a novel therapeutic target at unknown receptors for R-isovaline-like compounds in the guinea pig ileum.

1. Introduction

R-isovaline (2-Amino-2-methylbutanoic acid) is a non-proteinogenic amino acid with a chemical structure similar to the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Initial studies on biological systems showed that R-isovaline produces peripheral antinociception in rodent assays including the formalin foot assay and allodynia induced by prostaglandin E_2 or strychnine (Whitehead et al., 2012; MacLeod et al., 2010; Asseri et al., 2015). These studies on the analgesic effects were extended by our recent demonstration that isovaline co-administered with the hypnotic, propofol, produces general anesthesia (Whitehead et al., 2015). R-isovaline also prevents seizures in 4-aminopyridine and pilocarpine models (Yu et al., 2014, 2015; Shin et al., 2011). While the specific targets mediating the anesthetic and anticonvulsant effects are not known, $GABA_B$ receptors participate in the anti-allodynic effects of R-isovaline as indicated by their partial reversibility by the $GABA_B$ receptor antagonist, CGP35348. Both $GABA_{B1}$ and $GABA_{B2}$ subunits have been detected in keratinocytes and nerve endings, consistent with evidence for $GABA_B$ mediation of R-isovaline effects in the periphery (Whitehead et al., 2012; Corell et al., 2015).

Subsequent investigations have demonstrated an additional involvement of group II metabotropic glutamate receptors in R-isovaline analgesia in vivo. LY341495, a group II metabotropic glutamate receptor antagonist nearly abolished the antiallodynic effect of R-isovaline which was potentiated by LY487379, a positive allosteric group II metabotropic glutamate receptor modulator

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(Asseri et al., 2015). On the basis of these findings, we undertook the present experiments to shed further light on the receptor mechanisms of R-isovaline's effects in the guinea pig ileum which expresses functional $GABA_B$ and group II metabotropic glutamate receptors (Chen and Kirchgessner, 2002; Larzabal et al., 1999; Kirchgessner, 2001; Hyland and Cryan, 2010).

Originally developed by Paton and Zar (1968), this gut assay involves isolation of a small length of ileal tissue and applying transmural electric stimulation, increased luminal pressure, or receptor agonists to evoke muscle contraction. In the present experiments, we used transmural electric stimulation which elicits muscle contraction neurogenically, by activating the myenteric plexus. $GABA_B$ agonists attenuate spontaneous and stimulated contractions by actions that are dose-dependent and susceptible to blockade by $GABA_B$ antagonists (Kerr et al., 1990; Ong and Kerr, 1983; Giotti et al., 1983). In contrast, the effects of group II metabotropic glutamate receptor agonists have received less study. Chen and Kirchgessner (2002) showed that group II/III metabotropic glutamate receptor agonists inhibited N-type calcium channels in the myenteric plexus of guinea pig ileum. They also demonstrated immunoreactive staining of group II/III metabotropic glutamate receptors of neurons in the myenteric plexus of guinea pig ileum, implicating glutamatergic inputs. Hence, we hypothesized that R-isovaline would decrease contractions through the intrinsic activation of both $GABA_B$ and group II metabotropic glutamate receptors. We also sought to compare isovaline's effects to those of the prototypical $GABA_B$ agonist, baclofen, as well as the specific group II metabotropic glutamate receptor agonist, LY354740.

2. Materials and methods

2.1. Animals

Hartley guinea pigs of either sex weighing 300–400 g were housed at 21 °C and 55% relative humidity on a 12 h light dark-cycle with lights on at 07:00 AM. Food and water were available ad libitum. All procedures were carried out with approval from the Animal Care Committee at The University of British Columbia and complied with the Canadian Council on Animal Care. This study is reported according to ARRIVE guidelines (Kilkenny et al., 2010). A total of 28 tissues from 14 animals were used in these studies. These numbers were based on previous studies investigating the effects of baclofen in the guinea pig ileum (Kerr et al., 1990).

2.2. Experimental procedure

On the day of testing, animals were anesthetized with an intraperitoneal (i.p.) injection of pentobarbital (65 mg/kg). The abdomen of the animal was opened via a V-shaped incision and a separate incision was made into the chest cavity. The ileum was then located and a 20–30 cm segment was excised 2–3 cm distal to the ileocecal valve. The ileal segment was divided into 2–3 cm segments and quickly transferred into a 20 ml organ bath containing continuously aerated Tyrode's solution composed of (mM): NaCl 137, KCl 2.68, glucose 5.55, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $MgCl_2$ 1.05, and $CaCl_2$ 1.8 (pH 7.4). Ileal segments were mounted with 5–0 silk sutures threaded through one wall at each end of the segment with a preload tension of 1 g and allowed to equilibrate within the organ bath for one hour prior to testing. Tissues were primed for testing after the equilibration period via stimulation with parallel platinum electrodes delivering pulses (pulse width 10 ms, 0.1 Hz, sub-maximal voltage) generated by a Grass S9 stimulator (Natus Neurology Inc., Warwick, RI, USA) for 3 min followed by a 7-min recovery period, and repeated for 10 cycles. Contractions were measured isometrically with a Grass FT03 force transducer (Natus Neurology Inc., Warwick RI, USA), recorded with Powerlab/8sp (ADInstruments, Colorado Springs, CO, USA), and displayed using Chart 5 software (ADInstruments). Following priming, drugs were added 1 min after the start of electric stimulation and remained in the organ bath for 2 min before washing. In the antagonist studies, CGP52432 was added for a final bath concentration of 3 μM to the bath 5 min prior to the addition of RS-baclofen and R-isovaline. Effects of drugs on contractions were recorded as the measured change in tension in relation to the preload tension.

2.3. Statistical analysis

Muscle contraction traces were analyzed using Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA) by measuring peak amplitude of contraction force. Spike amplitudes with compounds present were compared to spike amplitudes obtained prior to compound administration and are expressed as a percentage of pre-compound amplitudes. Interpuls tension was determined by calculating the average cyclic minimum pre- and post-application and are expressed as a percentage change from pre-application values. In cases of normalization, data were normalized to pre-drug application values to account for differences in contractility and resting tension between tissues. Concentration-response curves were produced using a least squares fit of a log[in hibitor] vs response model according to the equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/\left(1 + 10^{[x - \log IC50]}\right)$$

Where Top and Bottom are plateaus of the curve and the IC_{50} referring to the concentration yielding a response halfway between Top and Bottom. Grubb's test was used for the detection of outliers which were excluded without replacement from analysis. A two-tailed, parametric *t* test was used to compare R-isovaline and RS-baclofen data. IC_{50}s for R-isovaline and RS-baclofen were taken to be different if there was no overlap of 95% confidence intervals (CIs). Data are presented as individual points with each point and summary bars presented as mean with 95% CI. A P value < 0.05 was considered to be statistically significant.

2.4. Drugs and chemicals

RS-Baclofen, LY354740, and CGP52432 were purchased from Tocris Bioscience (Ellisville, Mo, USA). Tetrodotoxin (TTX) was purchased from Alomone Labs (Jerusalem, Israel). R-isovaline was a generous gift from Nagase & Co. Ltd (Osaka, Japan). RS-baclofen and LY354740 was dissolved in equimolar sodium hydroxide solution, TTX was dissolved in water, and R-isovaline was dissolved in Tyrode's solution.

3. Results

3.1. Electric stimulation produces contractions mediated by neural tissue

Application of TTX (300 nM) abolished the electrically evoked ileal contractions (Fig. 1A). This blockade, which was reversible, demonstrated that the contractions resulted from action potential-dependent stimulation of neural tissue and not likely smooth muscle.