Inhibition of Intestinal Motility by the Putative BKCa Channel Opener LDD175

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LDD175 (4-chloro-7-trifluoromethyl-10H-benzo[4,5]furo[3,2-b]indole-1-carboxylic acid) is a benzofuroindole compound characterized previously as a potent opener of the large conductance calcium activated (BKCa) channels. Activators of the BKCa channels are potential therapies for smooth muscle hyperactivity disorders. The present study investigates the influence of LDD175 on the mechanical activity of the ileum smooth muscle. LDD175 inhibited spontaneous contractions of the ileum in a concentration-dependent manner (pEC50=5.9 ± 0.1) (Emax=96 ± 1.0% at 100 µM, n=3). It also remarkably inhibited contractions due to acetylcholine (ACh) (pEC50=5.3 ± 0.1)(Emax=97.7 ± 2.3%, n=6) and electrical field stimulation (EFS) (pEC50=5.5 ± 0.1) (Emax=83.3 ± 6.0%, n=6). In strips precontracted by 20 mM KCl, LDD175 significantly reduced the contractions yielding a pEC50 of 6.1 ± 0.1 and Emax of 96.6 ± 0.9%, (n=6). In 60 mM KCl, a concentration-dependent inhibition was observed with respective pEC50 and Emax values of 4.1 ± 0.1 and 50.8 ± 5.0% (n=3). BKCa channel blockers iberiotoxin (IbTX) and tetraethylammonium chloride (TEA, 1 mM) attenuated the relaxative effect of LDD175 but not barium chloride (BaCl2), and glibenclamide (KIR and KATP channel blockers, respectively). These data demonstrate the antispasmodic activity of LDD175 attributable to the potentiation of the BKCa channels.

Key words: LDD175, Benzofuroindole, BKCa Channels, Ileum, Antispasmodic, IbTX

INTRODUCTION

Large conductance Ca2+-activated K+ (BKCa) channels are a family of potassium-selective ion channels abundantly expressed in both excitable and non-excitable cells (Becker et al., 1995; Chang et al., 1997; Vergara et al., 1998; Kaczorowski and Garcia, 1999). These channels, when activated by membrane depolarization and/or intracellular Ca2+ (Toro et al., 1998), facilitate K+ efflux as a mechanism for recovering (repolarization), maintaining (clamping), and/or enhancing (hyperpolarization) the resting potential of the cell (Lawson, 1996). BKCa channels exhibit large single channel conductance (100-300 pS) even at low probability of opening (Marty, 1981), a property which justifies their potent role in the control of the membrane potential (Calderone, 2002). In myocytes, BKCa channel mediated hyperpolarization is thought to inhibit the activity of the Ca2+ channels and subsequently reduce the contractile activity of the smooth muscles (Malysz et al., 2004). The role of BKCa channels in modulating the excitation-contraction coupling processes of the smooth muscles is indispensable (Winquist et al., 1989; Suarez-Kurtz et al., 1991). Indeed, BKCa channels are feasible targets and their activators are promising interventions for disease states characterized by an increased tonus of smooth muscles (Edwards and Weston, 1995).

Butera et al. (2001) reported that benzofuroindole
compounds relaxed smooth muscles of the bladder via activation of the BK<sub>ca</sub> channels. They found out that these compounds were highly bladder selective agents and thus, suggested of their potential application in urge urinary incontinence (UUI). Gormemis et al (2005) synthesized novel benzofuroindole derivatives which showed efficacy in upregulating the activity of cloned BK<sub>ca</sub> channels. Among them, compound 22 (4-chloro-7-trifluoromethyl-10H-benzo[4,5]furo[3,2-b]indole-1-carboxylic acid, or in this study LDD175) (Fig. 1) showed considerable potency.

The present study evaluated the functional effects of LDD175 in the contractility of the guinea pig ileum. Experiments were performed to explore the antispasmodic activity of LDD175 and the mechanism involved. Here we report the spasmolytic activity of LDD175 mediated possibly through activation of the BK<sub>ca</sub> channels.

**MATERIALS AND METHODS**

**Ileal preparations and tension measurement**

All experiments were performed in line with the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and the Animal Care and Use Guidelines of Sahmyook University, South Korea. Male guinea pigs (300-400 g) were sacrificed by decapitation and the terminal ileum was removed, flushed of luminal contents and placed in Kreb’s solution composed of (in mM): 118 NaCl, 4.75 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 11.1 glucose). Segments of the ileum (1-2 cm long) were suspended in 20 mL baths containing Kreb’s solution maintained at 37°C, aerated with a mixture of 95% oxygen and 5% carbon dioxide. One end of the tissues was secured to a stationary rod and the other to Grass isometric force transducer (model FT-03; Grass instruments, Quincy, MA, USA). Each preparation was allowed to equilibrate for 60 minutes (with 15 min-intermittent washing) under a preload tension of 0.5 g. Three preparations were obtained from each animal: n values are number of preparations derived from different animals.

**In vitro experiments**

The first series of experiments was conducted to evaluate the relaxant effect of LDD175 on the spontaneous contractions of the guinea pig ileum. After equilibration, LDD175 (0.1-100 µM) or its vehicle was added cumulatively in the organ bath with a 10-minute incubation period per concentration. Incubation period was based on preliminary experiments showing such time was sufficient for LDD175 to exert relaxant activity. In the next separate experiments, the K<sup>+</sup> channel opening activity of LDD175 was explored as previously described (Davies et al., 1996; Kobayashi et al., 2000; Malysz et al., 2004). After equilibration, the preparations were contracted with high K<sup>+</sup> (20 or 60 mM KCl) and the responses were allowed to reach a plateau (after 10 min). The tissues were washed and again contracted with high K<sup>+</sup>. When the contractions became stable (after 20 minutes), LDD175 was added cumulatively in increasing concentrations. In some experiments, the preparations were exposed to 20 mM KCl for 20 minutes and to 100 nM iberiotoxin (IbTX), 1 mM tetraethylammonium chloride (TEA), 10 µM barium chloride (BaCl<sub>2</sub>), 10 µM glibenclamide or an equivalent volume of vehicle for 10 minutes before the addition of LDD175. Any relaxant effect of the vehicle was deducted from the apparent relaxative effect of LDD175. Time control experiments were also performed in this protocol.

The effect of LDD175 on acetylcholine (ACh)-induced contraction was assessed in a method demonstrated by Borrelli et al. (2006) but with some modifications. At the start of the experiment, the tissues were contracted with 1 mM ACh to determine maximal contraction. After washout, the tissues were allowed to equilibrate for 1 h and then contracted again with ACh (1 µM). Three control contractions were obtained at 20 min interval between stimulations and after washout, the contractile responses were repeated in the presence of increasing (noncumulative) concentrations of LDD175 added at least 10 minutes before stimulation with ACh (i.e. after washout).

Finally, the effect of LDD175 on electrical field stimulation (EFS)-induced contractions was investigated. After contraction with 1 mM ACh and a 1-h equilibration, EFS (1 Hz, 40 V, 5 ms pulse width) was applied via parallel stainless steel electrodes included in stationary rods, placed around the intestine. EFS-evoked contractions were similar in magnitude with that of 1 µM ACh. After stabilization of contractions, LDD175 (0.1-100 µM) was added in sequential concentrations. Time control and vehicle-