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Short communication

WIN 17317-3 blocks Ca^{2+}-activated K^+ channels and enhances motility of guinea-pig detrusor muscle

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Abstract

Patch-clamp experiments in both clonal GH3 cells and guinea-pig bladder myocytes reveal that 1-benzyl-7-chloro-4-(*n*-pentylimino)-1,4-dihydroquinoline hydrochloride (WIN 17317-3), a potent blocker of Kv1.3 channels and potential immunomodulator, reduces, in a reversible manner and at low micromolar concentrations, K^+ currents through Ca^{2+}-activated high conductance K^+ channels (BK channels). Blockade of BK channels is thought to account for the stimulatory effect of WIN 17317-3 on the contractility of guinea-pig bladder. This effect is not modified by tetrodotoxin (1 μM), but is abolished by nifedipine (0.1 μM). In conclusion, WIN 17317-3 lacks selectivity for the Kv1.3 channels, its postulated target for immunosuppression.

Keywords: WIN 17317-3; K^+ channel; Smooth muscle contractility

1. Introduction

A member of the voltage-dependent *Shaker* family of K^+ channels (Kv1), the Kv1.3 channel, is expressed at the plasma membrane of human lymphocytes and controls the membrane potential of both resting and activated T-cells (reviewed by Cahalan and Chandy, 1997; Garcia et al., 1998). Blockade of Kv1.3 channels by scorpion toxins, such as charybdotoxin and margatoxin, leads to membrane depolarization (Leonard et al., 1992), causing reduction in intracellular Ca^{2+} influx and cytosolic Ca^{2+} concentration upon activation of T-cell receptors. As a consequence, production of interleukin-2 and cellular proliferation are suppressed (Lin et al., 1991). The high affinity of scorpion toxins for Kv1.3 channels allowed the development of large-scale, high-capacity screening assays for modulators of this channel, which permitted the identification of several nonpeptidyl blockers (Hill et al., 1995; Michne et al., 1995; Slaughter et al., 1996; Felix et al., 1999). One of these, 1-benzyl-7-chloro-4-(*n*-pentylimino)-1,4-dihydroquinoline hydrochloride (WIN 17317-3), was initially described as a selective blocker of Kv1.3 ($IC_{50} = 0.3$ μM; Hill et al., 1995) and Kv1.4 ($IC_{50} = 0.3$ μM; Nguyen et al., 1996) channels. The blockade of Kv1.3 channels in T-lymphocytes by WIN 17317-3 was shown to decrease the production of interleukin-2 ($IC_{50} = 0.8–1$ μM; Hill et al., 1995), and inhibit clonal expansion ($IC_{50} \sim 5$ μM; Nguyen et al., 1996). Wanner et al. (1999), however, showed that WIN 17317-3 displays high-affinity binding ($Kd = 2.2 \pm 0.3$ nM) to type IIA Na^+ channels in rat brain tissue, and is a potent blocker ($K_i = 9$ nM) of these Na^+ channel currents in transfected CHO cells. We now present electrophysiological data indicating that WIN 17317-3 blocks Ca^{2+}-activated high conductance K^+ channels (BK channels) at low micromolar concentrations, and postulate that this blockade accounts for the observed stimulation of the contractility of guinea-pig detrusor muscle.

2. Materials and methods

2.1. Patch-clamp recordings of clonal GH3 cells and bladder detrusor myocytes

2.1.1. Cell culture

GH_3 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in

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HAM F-10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2.

2.1.2. Isolation of guinea-pig bladder myocytes.

Animals were kept following the precepts of humane care, in rooms with temperature control and light/dark cycle, and were asphyxiated by CO_2 inhalation. Myocytes from bladder detrusor muscle were isolated using the procedures and solutions described by Hu et al. (1997). Briefly, the detrusor muscle was dissected, cleaned of connective tissues and blood vessels and cut in small pieces, which were digested at 36 °C for two periods of 35 min each, in a saline solution containing (in mM): NaCl 135, KCl 5.4, $MgSO_4$ 2, glucose 5, HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[4-butanesulfonic acid]) 10 (pH = 7.4), and supplemented with (in mg/ml): collagenase-VIII 1, pronase-E 0.2, and bovine serum albumin 1. Single cells were obtained by gentle agitation through a Pasteur pipette and stored at 4 °C for 1 h before use in a saline solution, of the following composition (in mM): KCl 80, KH_2PO_4 30, $MgSO_4$ 5, Na_2ATP 5, creatine 5, taurine 20, pyruvic acid 5, DL-β-hydroxybutyric acid 5, and bovine serum albumin (1 mg/ml), pH = 7.4.

2.1.3. Patch-clamp recordings

BK channel currents were recorded from GH3 cells and bladder myocytes with the patch-clamp technique, in the whole-cell mode (Hamill et al., 1981). The cells were continuously superfused (0.3 ml/min) in the recording chamber with a saline solution of the following composition (in mM): KCl 145, $MgCl_2$ 2, $CoCl_2$ 2, glucose 5, and HEPES 10 (pH = 7.4). The intracellular (pipette) solution had the following composition (in mM): KCl 140, $CaCl_2$ 0.927, K_2EGTA 1, $MgCl_2$ 2, K_2ATP 2, HEPES 10 (pH = 7.4, *p*Ca = 6). The osmolarities of the extracellular and of the pipette solutions were calculated to be 327 and 318 mosmol/kgH_2O, respectively. The holding potential was set at 0 mV, the reversal potential for K^+ currents. Under these conditions, Ca^{2+} channel currents are suppressed by extracellular Co^{2+}, ATP-dependent K^+ currents are abolished by intracellular ATP, while both Na^+ and Kv1 channel currents are inactivated at the 0-mV holding potential. Test pulses of 200–400 ms, to +80 mV, were applied at 30-s intervals, to elicit BK channel currents. Preliminary experiments indicated that these currents are eliminated by iberiotoxin (10 nM), thus confirming that they are BK channel currents. These currents were recorded using a List EPC-7 amplifier (List-electronic, Darmstadt, Germany) digitized at 3 kHz (interface 1200, Axon Instruments, Foster City, CA, USA) and analyzed using the software pCLAMP 6.0.4 (Axon Instruments). Series resistances were in the range of 2–5 MΩ, and were compensated by 90–95%, whereas linear capacitance was fully compensated.

2.2. Isometric tension recordings

The urinary bladder was isolated from adult guinea-pigs and kept at 37 °C in a physiological saline solution containing (in mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.1, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 11, and HEPES 10 (pH = 7.3 after equilibration with 95% O_2 and 5% CO_2). Muscle segments (7–10-mm long) were mounted vertically between two metal stirrups, of which the lower was fixed and the upper was attached to a rigid wire connected to a force-displacement transducer (Grass FT-03; Grass Instruments, Quincy, MA, USA). A 1-g load was initially applied to the preparations, and the transducer

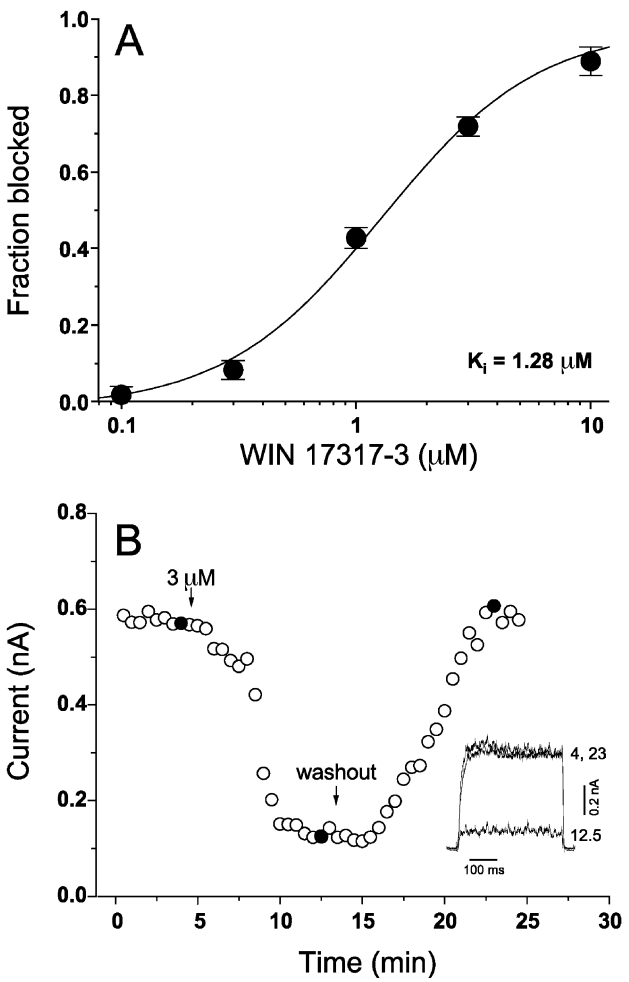

Fig. 1. Inhibition of BK channel currents by WIN 17317-3. BK channel currents were isolated as described in Section 2, and were recorded during 300-ms depolarizing test pulses, applied at 30-s intervals, from a holding potential of 0 to +80 mV. (A) GH_3 cells were exposed to increasing concentrations of WIN 17317-3 (abscissa) and the inhibition of BK channel currents is expressed as the fraction of current blocked by each concentration of WIN 17317-3 ($N = 4$ for each data point). (B) Blockade of the BK channel current in a guinea pig myocyte by 3 μM WIN 17317-3, and subsequent reversal upon washout of the drug from the medium. The inset shows the current recordings at the annotated times (min), which correspond to the solid circles in the plot.

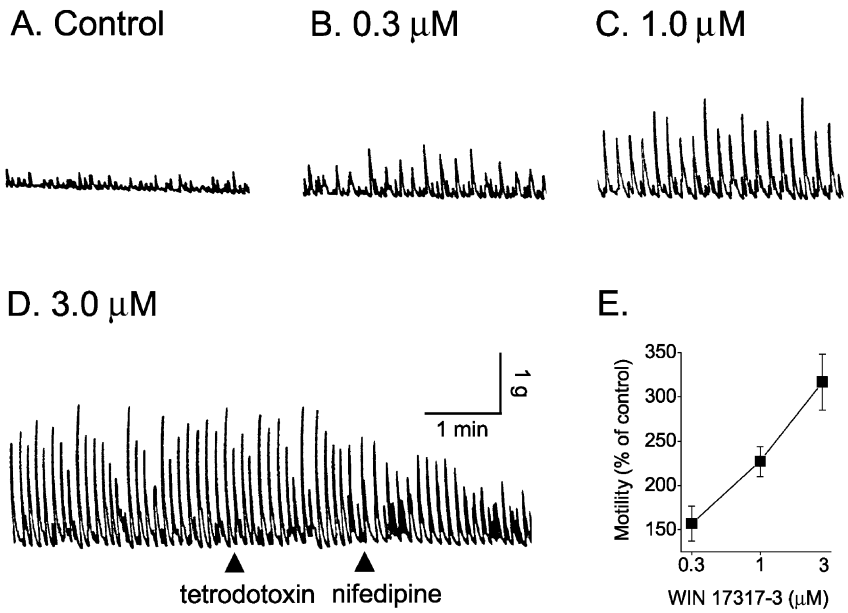

Fig. 2. Effects of WIN 17317-3 on the contractility of guinea-pig detrusor muscle. (A–D) Isometric tension recordings obtained from one segment of guinea-pig urinary bladder. The preparation was exposed, successively, for 20-min periods, to increasing concentrations of WIN 17317-3 (0.3–3 μM), and the recordings taken during the last 3 min of exposure to each concentration are shown. Tetrodotoxin (1 μM) and subsequently, nifedipine (1 μM), were added to the bathing medium, in the continuous presence of 3 μM WIN 17317-3. (B) Quantification of the effects of WIN 17317-3 on the motility of guinea-pig bladder. The mean amplitude of isometric tension recordings were measured and expressed (mean ± S.E.M., $N = 6$) relative to the control activity, which was taken as 1.0. The bladder motility in the presence of WIN 17317-3 (0.3–3 μM) was significantly different from the control ($P < 0.05$, paired *t*-test).

signals were amplified and recorded on a Grass polygraph (Model 7). The effects of WIN 17317-3 on the motility of the muscle segments were quantified by changes in the mean amplitude of the isometric tension recordings, averaged over 5 min. Data are expressed relative to the basal activity of each segment, recorded immediately before the first exposure to WIN 17317-3.

2.3. Drugs and chemicals

WIN 17317-3 was a gift from Dr. Gregory Kaczorowski, Merck Research Laboratories, Rahway, USA. Bovine serum albumin (fraction V), ATP, collagenase-VIII, pronase-E, taurine, creatine, HAM F-10 medium, HEPES, nifedipine, and tetrodotoxin were from Sigma (St. Louis, MO, USA). Fetal bovine serum and horse serum were obtained from GIBCO (Grand Island, NY, USA). All other reagents were grade A.

3. Results

3.1. Electrophysiological recording of BK channel currents

Clonal GH3 cells were used in a preliminary series of experiments to explore the effects of WIN 17317-3 on BK channels, and the results are shown in Fig. 1A. WIN 17317-3 caused a concentration-dependent inhibition of the BK channel currents with a K_i of 1.28 ± 0.15 μM ($N = 4$). Results obtained with bladder myocytes confirmed the inhibitory effect of WIN 17317-3 (> 0.3 μM) on BK channels (not shown). Fig. 1B shows one of four similar experiments and reveals the nearly complete blockade of BK channel currents by 3 μM WIN 17317-3 and the reversal of this effects by washout of the drug from the bathing medium.

3.2. Isometric tension recordings of guinea-pig detrusor muscle

WIN 17317-3 increases, in a concentration-dependent manner (0.3–3 μM), the myogenic activity of guinea-pig urinary bladder segments (Fig. 2). The increased contractility elicited by WIN 17317-3 was not affected by tetrodotoxin (1 μM) but was inhibited by nifedipine (0.1 μM).

4. Discussion

Two main pharmacological actions have been previously described for WIN 17317-3: one as a Kv1 channel inhibitor, with high selectivity for Kv1.3 and Kv1.4 (Hill

et al., 1995; Nguyen et al., 1996), and the other as a Na^+ channel blocker (Wanner et al., 1999). We now present direct electrophysiological data indicating that WIN 17317-3 blocks BK channels at low micromolar concentrations and propose that this mechanism underlies the ability of WIN 17317-3 to enhance smooth muscle contractility.

We have previously reported that peptidyl (charybdotoxin, iberiotoxin) and nonpeptidyl (paxilline, paspalitren) blockers of BK channels increase the motility of various guinea-pig and rat smooth muscles; these effects being insensitive to tetrodotoxin, but abolished by nifedipine (Suarez-Kurtz et al., 1991, DeFarias et al., 1996). In contrast, correolide, a potent nonpeptidyl blocker of Kv1 channels, fails to increase the contractility of guinea-pig detrusor muscle (Vianna-Jorge et al., 2000), whereas margatoxin, a selective peptidyl blocker of Kv1.1, Kv1.2 and Kv1.3 channels, only affects the spontaneous motility of detrusor muscle when applied at concentrations several orders of magnitude higher than its Kd for these Kv1 channels (Suarez-Kurtz et al., 1999). Taken together, these observations are consistent with our proposal that WIN 17317-3 enhances the contractility of guinea-pig detrusor muscle, by virtue of its inhibitory effect on BK channel currents, rather than on Kv1.3 channel currents. However, Hill et al. (1995) proposed that WIN 17317-3 is a poor inhibitor of BK channels, in view of its low potency ($IC_{50} = 14$ μM) for inhibition of ^{125}I-charybdotoxin binding to bovine aortic smooth muscle, as compared to its potency as a Kv1.3 blocker ($IC_{50} = 0.3$ μM). The discrepancy between our results regarding the potency of WIN 17317-3 as a blocker of BK channel currents in GH3 cells and in bladder myocytes and those reported by Hill et al. (1995) regarding its ability to displace ^{125}I-charybdotoxin bound to BK channels, might indicate that WIN 17317-3 and charybdotoxin bind to BK channels at different sites.

The stimulatory effects of WIN 17317-3 on smooth muscle contractility revealed in the present study are relevant to the development of WIN 17317-3 as an immunomodulator, because they were detected at concentrations (> 0.3 μM) not far from the drug's IC_{50} for its main target, namely, Kv1.3 channels (0.2–0.4 μM; Hill et al., 1995; Nguyen et al., 1996). Enhancement of the contractility of urinary smooth muscles is a potential source of undesirable side effects, which might hinder the development of WIN 17317-3 for clinical use. In addition, WIN 17317-3 is also a potent Na^+ channel blocker (Wanner et al., 1999), and displays weak affinity for L-type Ca^{2+} channels (Hill et al., 1995), which adds to the complexity of the pharmacodynamic spectrum of this drug.

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