Available online at www.sciencedirect.com
ScienceDirect
Neuropharmacology 53 (2007) 881–889
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Lithium inhibits function of voltage-dependent sodium channels and catecholamine secretion independent of glycogen synthase kinase-3 in adrenal chromaffin cells

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Received 7 May 2007; received in revised form 31 July 2007; accepted 20 August 2007

Abstract

Lithium has been proven to be effective in the therapy of bipolar disorder, but its mechanism of pharmacological action is not clearly defined. We examined the effects of lithium on voltage-dependent Na^+ channels, nicotinic acetylcholine receptors, and voltage-dependent Ca^{2+} channels, as well as catecholamine secretion in cultured bovine adrenal chromaffin cells. Lithium chloride (LiCl) reduced veratridine-induced $^{22}Na^+$ influx in a concentration-dependent manner, even in the presence of ouabain, an inhibitor of Na^+, K^+-ATPase. Glycogen synthase kinase-3 (GSK-3) inhibitors (SB216763, SB415286 or the GSK-3 inhibitor IX) did not affect veratridine-induced $^{22}Na^+$ influx, as well as inhibitory effect of LiCl on veratridine-induced $^{22}Na^+$ influx. Enhancement of veratridine (site 2 toxin)-induced $^{22}Na^+$ influx caused by α-scorpion venom (site 3 toxin), β-scorpion venom (site 4 toxin), or *Ptychodiscus brevis* toxin-3 (site 5 toxin), still occurred in the presence of LiCl in the same manner as in the control cells. LiCl also reduced veratridine-induced $^{45}Ca^{2+}$ influx and catecholamine secretion. In contrast, LiCl (≦30 mM) had no effect on nicotine-induced $^{22}Na^+$ influx, $^{45}Ca^{2+}$ influx and catecholamine secretion, as well as on high K^+-induced $^{45}Ca^{2+}$ influx and catecholamine secretion. Chronic treatment with LiCl at 100 mM (but not at ≦30 mM) significantly reduced cell viability in a time-dependent manner. These results suggest that lithium selectively inhibits Na^+ influx thorough Na^+ channels and subsequent Ca^{2+} influx and catecholamine secretion, independent of GSK-3 inhibition.

Keywords: Lithium; Na^+ channel; Ca^{2+} channel; Catecholamine secretion; Bipolar disorder; Adrenal chromaffin cells

1. Introduction

Lithium is the most commonly used drug for the treatment of bipolar disorder (BPD). Clinical applications suggested for lithium are initial treatment of new manic episodes and long-term stabilization of mood in BPD (Price and Heninger, 1994). Recent studies indicated that lithium also has neuroprotective effects against various noxious insults [e.g. ischemia (Nonaka and Chuang, 1998; Ren et al., 2003; Xu et al., 2003), glutamate excitotoxicity (Hashimoto et al., 2002) and β-amyloid neurotoxicity (Alvarez et al., 1999)]. Despite long-standing clinical use of lithium and intensive research, no consensus has been reached concerning the therapeutic mechanism of lithium (Shaldubina et al., 2001; Gurvich and Klein, 2002). The antiepileptic drug valproic acid (VPA) is also effective in acute mania of BPD (Gurvich and Klein, 2002) as well as lithium. Therefore, identification of common target molecule(s) or signaling pathways which are regulated by both lithium and VPA may provide insights into the mechanism of these drugs in BPD (Gurvich and Klein, 2002; Harwood and Agam, 2003). Based on this idea, numerous overlapping actions of both drugs have been reported so far [e.g. inhibition of glycogen synthase kinase-3 (GSK-3), depletion of inositol,

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doi:10.1016/j.neuropharm.2007.08.018

and enhancement of AP-1 DNA binding activity] (see reviews, Gurvich and Klein, 2002; Harwood and Agam, 2003; Wada et al., 2005).

Besides their common actions, VPA has been reported to inhibit voltage-dependent neuronal Na^+ channels (VanDongen et al., 1986; Van den Berg et al., 1993). In addition, randomized clinical trials have shown that other Na^+ channel inhibiting antiepileptic drugs (e.g. lamotrigine, riluzole, phenytoin and carbamazepine) are also effective for treating manic phase of BPD (Rogawski and Löscher, 2004). These findings raised a simple question of whether inhibition of Na^+ channels also was caused by lithium. However, little is known whether lithium directly interfere the function of Na^+ channels.

Voltage-dependent Na^+ channels consist of the principal α-subunit (~260 kDa), which may be associated with a noncovalently-attached β_1-subunit (~36 kDa) and a disulfide-linked β_2-subunit (~33 kDa) in some tissues and species (Catterall, 2000). Nine mammalian α-subunits ($Na_V1.1$–$Na_V1.9$) and four β-subunits (β_1–β_4) have been cloned. Dysregulated activity and expression of Na^+ channels are associated with epilepsy (Xia et al., 2000), fatal cardiac arrhythmia (Catterall, 2000), intolerable pain (Waxman et al., 1999), and hypoxia/ischemia-induced cell injury (Urenjak and Obrenovitch, 1996).

In cultured bovine adrenal chromaffin cells (embryologically derived from the neural crest), α-subunit isoform of Na^+ channels is $Na_V1.7$ [the tetrodotoxin/saxitoxin-sensitive human neuroendocrine type Na^+ channel α-subunit (hNE-Na)] (Klugbauer et al., 1995; Yanagita et al., 2003; Wada et al., 2004). We previously showed that both veratridine-induced Na^+ influx via Na^+ channels and nicotine-induced Na^+ influx via the nicotinic receptors increase Ca^{2+} influx via voltage-dependent Ca^{2+} channels, a prerequisite for exocytotic secretion of catecholamines, whereas high K^+ directly gates the Ca^{2+} channels without increasing Na^+ influx (Yamamoto et al., 1996). In the present study, we found that lithium selectively inhibited Na^+ influx through Na^+ channels and subsequent Ca^{2+} influx and catecholamine secretion independent of GSK-3 inhibition in adrenal chromaffin cells.

2. Methods

2.1. Materials

Eagle's minimum essential medium was from Nissui Seiyaku (Tokyo, Japan). Newborn calf serum was from Invitrogen Corp. (Carlsbad, CA, USA). Lithium chloride (LiCl), veratridine, α-scorpion venom (*Leiurus quinquestriatus quinquestriatus*), β-scorpion venom (*Centruroides sculpturatus*), ouabain, and cytosine arabinoside were from Sigma (St. Louis, MO, USA). *Ptychodiscus brevis* toxin-3 (PbTx-3) was from Latoxan, Westbury, (NY, USA). $^{22}NaCl$ (6–17 Ci/mmol) and $^{45}CaCl_2$ (0.5–2 Ci/mmol) were from GE Healthcare Biosciences (Piscataway, NJ, USA). Cell counting Kit-8 was from Dojindo (Kumamoto, Japan).

2.2. Primary culture of adrenal chromaffin cells

Isolated bovine adrenal chromaffin cells were cultured (4×10^6/dish, 35 mm in diameter; BD Biosciences, San Jose, CA, USA) under 5% CO_2/95% air in a CO_2 incubator in Eagle's minimum essential medium containing 10% calf serum (Yanagita et al., 2000, 2003), and 3 μM cytosine arabinoside to suppress the proliferation of nonchromaffin cells (Yamamoto et al., 1996; Yanagita et al., 2000, 2003).

2.3. $^{22}Na^+$ influx

$^{22}Na^+$ influx was measured by incubating cells with 2 μCi $^{22}NaCl$ in 1 ml KRP buffer at 37 °C for 5 min in the absence or presence of veratridine, α-scorpion venom, β-scorpion venom, PbTx-3, and ouabain, or for 1 min in the absence or presence of nicotine. The cells were washed 3 times with 2 ml ice-cold KRP buffer, solubilized in 10% Triton X-100, and radioactivity was counted by a liquid scintillation counter (Wada et al., 1985, 1992; Yamamoto et al., 1996; Yanagita et al., 2003).

2.4. $^{45}Ca^{2+}$ influx and catecholamine secretion

To measure $^{45}Ca^{2+}$ influx and catecholamine secretion, cells were incubated with 2 μCi $^{45}CaCl_2$ for 5 min without or with veratridine in KRP buffer, or for 1 min without or with nicotine or high K^+ solution. Incubation medium was saved into a test tube for catecholamine (epinephrine plus norepinephrine) assay by high performance liquid chromatography (Yamamoto et al., 1996). The cells were washed, solubilized in 10% Triton X-100, and radioactivity was counted by a liquid scintillation counter (Wada et al., 1985; Yamamoto et al., 1996).

2.5. Cell viability assay

Cell viability was evaluated with the cell counting kit-8, in which 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) is used as a substrate. WST-8 is reduced by intracellular dehydrogenases to generate WST-8-formazan, which has a maximal absorbance at 450 nM (Ishiyama et al., 1997). The cells (1×10^3/well) in 96-well plates (BD Biosciences) were incubated without or with LiCl for up to 24 h. Then, 10 μL of the kit reagent was added to each well, and the cells were incubated for an additional 2 h. The absorbance was measured at a test wavelength at 450 nm and a reference wavelength of 600 nm by using a microplate reader (SpectraMax 190, Molecular Devices). Cell viability was evaluated as the ratio of the absorbance of the sample to that of LiCl-nontreated cells at each time.

2.6. Statistical methods

$^{22}Na^+$ influx, $^{45}Ca^{2+}$ influx, catecholamine secretion and cell viability assay were measured in triplicate, and all experiments were repeated at least three times. Values are mean ± SEM. Significance ($p < 0.05$) was determined by one-way ANOVA with post-hoc mean comparison by Newman–Keuls multiple range test.

3. Results

3.1. LiCl: concentration-dependent inhibition of veratridine-induced $^{22}Na^+$ influx, $^{45}Ca^{2+}$ influx, and catecholamine secretion

Veratridine (100 μM), an activator of voltage-dependent Na^+ channels, caused the $^{22}Na^+$ influx (220.1 ± 13.0 nmol/4 × 10^6 cells) (Fig. 1A). LiCl reduced veratridine-induced $^{22}Na^+$ influx in a concentration-dependent manner ($IC_{50} = 23.4 \pm 2.1$ mM). The basal of $^{22}Na^+$ influx was not changed by LiCl at any concentration used, and a significant reduction by LiCl was observed at 1 mM.

As shown in Fig. 1B and C, veratridine produced $^{45}Ca^{2+}$ influx (3.52 ± 0.18 nmol/4 × 10^6 cells) and catecholamine secretion (2.40 ± 0.06 μg/4 × 10^6 cells), respectively. LiCl also