Transcriptional up-regulation of cell surface Na$_V$1.7 sodium channels by insulin-like growth factor-1 via inhibition of glycogen synthase kinase-3β in adrenal chromaffin cells: enhancement of ^{22}Na$^+$ influx, ^{45}Ca^{2+} influx and catecholamine secretion

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ABSTRACT

Insulin-like growth factor-1 (IGF-1) plays important roles in the regulation of neuronal development. The electrical activity of Na$^+$ channels is crucial for the regulation of synaptic formation and maintenance/repair of neuronal circuits. Here, we examined the effects of chronic IGF-1 treatment on cell surface expression and function of Na$^+$ channels. In cultured bovine adrenal chromaffin cells expressing Na$_V$1.7 isoform of voltage-dependent Na$^+$ channels, chronic IGF-1 treatment increased cell surface [^{3}H]saxitoxin binding by 31%, without altering the Kd value. In cells treated with IGF-1, veratridine-induced ^{22}Na$^+$ influx, and subsequent ^{45}Ca^{2+} influx and catecholamine secretion were augmented by 35%, 33%, 31%, respectively. Pharmacological properties of Na$^+$ channels characterized by neurotoxins were similar between nontreated and IGF-1-treated cells. IGF-1-induced up-regulation of [^{3}H]saxitoxin binding was prevented by phosphatydil inositol-3 kinase inhibitors (LY204002 or wortmannin), or Akt inhibitor (Akt inhibitor IV). Glycogen synthase kinase-3 (GSK-3) inhibitors (LiCl, valproic acid, SB216763 or SB415286) also increased cell surface [^{3}H]saxitoxin binding by ∼33%, whereas simultaneous treatment of IGF-1 with GSK-3 inhibitors did not produce additive increasing effect on [^{3}H]saxitoxin binding. IGF-1 (100 nM) increased Ser^{437}-phosphorylated Akt and Ser^9-phosphorylated GSK-3β, and inhibited GSK-3β activity. Treatment with IGF-1, LiCl or SB216763 increased protein level of Na$^+$ channel α-subunit; it was prevented by cycloheximide. Either treatment increased α-subunit mRNA level by ∼48% and accelerated α-subunit gene transcription by ∼30% without altering α-subunit mRNA stability. Thus, inhibition of GSK-3β caused by IGF-1 up-regulates cell surface expression of functional Na$^+$ channels via acceleration of α-subunit gene transcription.

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1. Introduction

Insulin-like growth factor-1 (IGF-1) and its receptors are widely distributed throughout the nervous system during development, and IGF-1 has an important role in normal brain development, promoting neuronal growth, cellular proliferation and differentiation (Arsenijevic and Weiss, 1998; Connor and Dragunow, 1998; Torres-Aleman, 2010). In addition, IGF-1 has neuroprotective effects against hypoxia/ischemia-induced neuronal injury, neurodegenerative disease, and infectious neuronal disorders (Torres-Aleman, 2010; Trejo et al., 2004; Ying Wang et al., 2003).

Most of the physiological effects of IGF-1 are mediated by the IGF-1 receptor which, after ligand binding-induced autophosphorylation, associates with specific adaptor proteins and activates different second messengers. The activated receptor typically stimulates one or both of two cascades, (1) the Ras∼mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)∼ERK cascade, via the adaptor protein SHC, or (2) the phosphatydil inositol-3 kinase (PI3K)∼Akt cascade, via insulin receptor substrate (IRS) (Bondy and Cheng, 2004; LeRoith

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and Roberts, 2003). Activated Akt phosphorylates/inhibits glycogen synthase kinase-3 (GSK-3), and phosphorylates/activates mammalian target of rapamycin (mTOR) (Nemoto et al., 2010b). GSK-3 is one of the key molecules of neurotrophic and neuroprotective effects of IGF-1, insulin, lithium and valproic acid (VPA) (Gurvich and Klein, 2002; Jope, 2003). GSK-3, a serine/threonine protein kinase, is constitutively active in nonstimulated cells, causing phosphorylation and inactivation/degradation of signaling molecules (e.g., glycogen synthase), transcription factors (e.g., β-catenin), translation initiation factor eIF2B, and structural proteins (e.g., tau) (Jope and Johnson, 2004). GSK-3 consists of α and β isoforms and its activity is inhibited by therapeutic concentration of lithium or VPA (Ryves and Harwood, 2001; Gurvich and Klein, 2002).

It has become increasingly evident that coordinately regulated cell surface expression and electrical activity of Na$^+$ channels play crucial roles in the regulation of axon competition, axon pathfindings, synaptic formation, and maintenance and repair of neuronal circuits (Hanson and Landmesser, 2004; Hua et al., 2005; Xu and Shrager, 2005). In contrast, dysregulated expression and activity of Na$^+$ channels are associated with hypoxia/ischemia-induced cell injury (Urenjak and Obrenovitch, 1996), seizure (Xia et al., 2000), fatal cardiac arrhythmia (Catterall, 2000) and intolerable pain (Waxman et al., 2000). To understand the molecular basis for these physiological and pathological events, it is essential to explore the mechanisms whereby the expression and function of cell surface Na$^+$ channels are regulated.

Na$^+$ channel consists of the principal α-subunit, with or without β$_1$- to β$_4$-subunit (Isom, 2002; Catterall et al., 2005). The nine α-subunits (Na$_V$1.1-Na$_V$1.9) arise from nine different genes (SCN1A-SCN5A and SCN8A-SCN11A) (Catterall et al., 2005; Klugbauer et al., 1995; Toledo-Aral et al., 1997). Different α-subunits have distinct electrophysiological and pharmacological properties. The β-subunits are type 1 transmembrane proteins containing a single transmembrane segment, and interact with extracellular (e.g. neurofascin) and intracellular proteins (e.g. ankyrin), regulating gating kinetics and cell surface targeting of Na$^+$ channel (Catterall et al., 2005; Isom, 2002).

In adrenal chromaffin cells (embryologically derived from the neural crest), α-subunit isoform of Na$^+$ channels is Na$_V$1.7 [the tetrodotoxin (TTX)/saxitoxin (STX)-sensitive human neuroendocrine type Na$^+$ channel α-subunit (hNE-Na)] (Klugbauer et al., 1995; Catterall et al., 2005; Wada et al., 2008). In cultured bovine adrenal chromaffin cells, cell surface expression of Na$_V$1.7 is up- and down-regulated by extra- and intra-cellular signalings. Activation of protein kinase C (PKC)-ε (Yanagita et al., 1999, 2000) and ERK (Yanagita et al., 2003) destabilized Na$_V$1.7 mRNA, and activation of PKC-α (Yanagita et al., 1999, 2000) promoted internalization of Na$_V$1.7. Protein kinase A (Yuhi et al., 1996) promoted cell surface externalization of Na$_V$1.7. In addition, several bioactive signaling molecules and therapeutic drugs which would be expected as neuroprotective drugs [Lithium (Yanagita et al., 2009), lysophosphatidic acid (Maruta et al., 2008), insulin (Yamamoto et al., 1996), VPA (Yamamoto et al., 1997), cyclosporin A, FK506 (Shiraishi et al., 2001), and carvedilol (Kajiwara et al., 2002)] up-regulated cell surface Na$^+$ channels via multiple mechanisms. In the present study, we found that chronic IGF-1 treatment accelerated Na$_V$1.7 α-subunit gene transcription and up-regulated cell surface expression of Na$_V$1.7 via inhibition of GSK-3β, thereby resulting in the enhancement of Na$^+$ influx, Ca^{2+} channel gating and catecholamine secretion.

2. Materials and methods

2.1. Materials

Eagle's minimum essential medium was from Nissui Seiyaku (Tokyo, Japan). IGF-1 was from PeproTech (London UK). Calf serum and TRIzol reagent were from Invitrogen

Corp. (Carlsbad, CA USA). Actinomycin D, cytosine arabinoside, lithium chloride (LiCl), TTX, α-scorpion venom (Leiurus quinquestriatus quinquestriatus), β-scorpion venom (Centruroides sculpturatus), and ouabain were from Sigma (St. Louis, MO, USA). Ptychodiscus brevis toxin-3 (PbTx-3) was from Latoxan, Westbury, (N.Y., USA). Phenylmethylsulfonyl fluoride, leupeptin, aprotinin, sodium orthovanadate, sodium fluoride, Nonidet P-40, Tween-20, and sodium deoxycholate were from Nacalai Tesque (Kyoto, Japan). SB216763 and SB415286 were from Tocris Cookson Ltd. (Avonmouth UK). PD98059, wortmannin, LY294002, Akt inhibitor IV and rapamycin were from Calbiochem (San Diego, CA, USA). Oligotex-dT30 ⟨Super⟩ and mini Quick Spin RNA Columns were from Roche Diagnostics (Tokyo, Japan). BcaBEST labeling kit, and Non-interfering protein assay kit were from Takara (Kyoto, Japan). Rabbit polyclonal antibody against Na$_V$1.7 Na$^+$ channel α-subunit was from Alomone Labs Ltd. (Jerusalem, Israel). Mouse monoclonal antibody against Akt, and rabbit polyclonal antibodies against IGF-1 receptor β-subunit and insulin receptor β-subunit were from Santa Cruz Biotechnology (Santa Cruz. CA, USA). Mouse monoclonal antibodies against GSK-3β and phosphotyrosine (PY20) were from BD biosciences (San Jose, CA, USA). Rabbit polyclonal antibodies against phospho-GSK-3β (Ser[9]) and phospho-Akt (Ser[473]) were from Cell Signaling Technology (Beverly, MA, USA). RQ1 RNase-Free DNase, proteinase K, and U0126 were from Promega (Madison, WI, USA). [³H]STX (20-40 Ci/mmol), [α-³²P]dCTP (>3000 Ci/mmol), [α-³²P]UTP (800 Ci/mmol), [γ-³²P]ATP (∼3000 Ci/mmol), Hybond-N$^+$, Hybond-P, ECL Plus Western Blotting Detection Reagents, and Rapid-hyb buffer were from Amersham Biosciences (Buckinghamshire, UK). cDNA for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was from Clontech (Palo Alto, CA, USA). Can Get Signal was from Toyobo (Osaka, Japan). Phospho-glycogen syntase peptide-2 (GSK-3 substrate peptide) was from Upstate Biotechnology, Inc. (Lake Placid, NY, USA). Plasmid Bluescript II (pBII) was from Stratagene (La Jolla, CA, USA). Plasmids containing hNE-Na (Na$_V$1.7) cDNA (Klugbauer et al., 1995), and rat brain Na$^+$ channel β$_1$-subunit cDNA (Oh and Waxman, 1994) were generously donated by Drs. F. Hofmann (Technischen Universität München), and Y. Oh (University of Alabama), respectively.

2.2. Primary culture of adrenal chromaffin cells and drug treatment

Isolated bovine adrenal chromaffin cells were cultured (4 × l0^6/dish, Falcon; 35 mm in diameter) under 5% CO$_2$/95% air in a CO$_2$ incubator in Eagle's minimum essential medium containing 10% calf serum, and 3 μM cytosine arabinoside to suppress the proliferation of nonchromaffin cells (Yamamoto et al., 1996). Three days after plating, the cells were exposed to test compounds for up to 72 h. Test compounds were dissolved in distilled H$_2$O or dimethyl sulfoxide (DMSO), the final concentration of DMSO in the test medium being 0.25%. Treatment of chromaffin cells with 0.25% DMSO for 72 h did not alter [³H]STX binding, compared with nontreated cells. When chromaffin cells were purified by differential plating (Yamamoto et al., 1996), relative abundance of α- and β$_1$-subunit mRNAs/GAPDH mRNA was similar between conventional and purified adrenal chromaffin cells.

2.3. [³H]STX binding

Cells were washed with ice-cold Krebs–Ringer phosphate (KRP) buffer (mM) (154 NaCl, 5.6 KCl, 1.1 MgSO$_4$, 2.2 CaCl$_2$, 0.85 NaH$_2$PO$_4$, 2.15 Na$_2$HPO$_4$, 5 glucose, and 0.5% bovine serum albumin, pH 7.4), and incubated with 1–25 nM [³H]STX in 1 ml KRP buffer at 4 °C for 15 min in the absence (total binding) and presence (nonspecific binding) of 1 μM TTX (Yamamoto et al., 1996; Yanagita et al., 1999, 2000; Yuhi et al., 1996). The cells were washed, and solubilized in 10% Triton X-100, and counted for radioactivity. Specific binding was calculated as the total binding minus nonspecific binding.

2.4. ^{22}Na$^+$ influx, ^{45}Ca^{2+} influx, and catecholamine secretion

^{22}Na$^+$ influx was measured by incubating cells with 2 μCi ^{22}NaCl in culture medium or KRP buffer at 37 °C for 5 min without or with veratridine, α-scorpion venom, β-scorpion venom, PbTx-3, and ouabain, and for 1 min without or with nicotine or high K$^+$ solution. To measure ^{45}Ca^{2+} influx and catecholamine secretion, cells were incubated with 2 μCi ^{45}CaCl$_2$ for 5 min with or without 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 assay by HPLC, and the cells were washed, solubilized and counted for radioactivity (Yamamoto et al., 1996).

2.5. Western blot analysis of Akt, phosporylated Akt, GSK-3β, phosporylated GSK-3β, and Na$_V$1.7 Na$^+$ channel α-subunit

Cells were washed with ice-cold Ca^{2+}-free phosphate-buffered saline (PBS), and solubilized at 95 °C for 3 min in 500 μl of 2× SDS electrophoresis sample buffer. Total quantity of cellular proteins was measured by Non-interfering protein assay kit. The same amount of protein (20 μg/each lane) was separated by sodium dodecyl sulfate (SDS) −7.5% or −12% polyacrylamide gel electrophoresis (PAGE), and transferred onto a polyvinylidene difluoride membrane. The membrane was preincubated at room temperature 1% BSA in Tween-Tris-buffered saline [10 mM Tris–HCl (pH 7.4), 150 mM NaCl and 0.1% Tween-20], then reacted overnight at 4 °C with antibodies against Akt, Ser[473]-phosphorylated Akt, GSK-3β, Ser[9]-phosphorylated GSK-3β, and