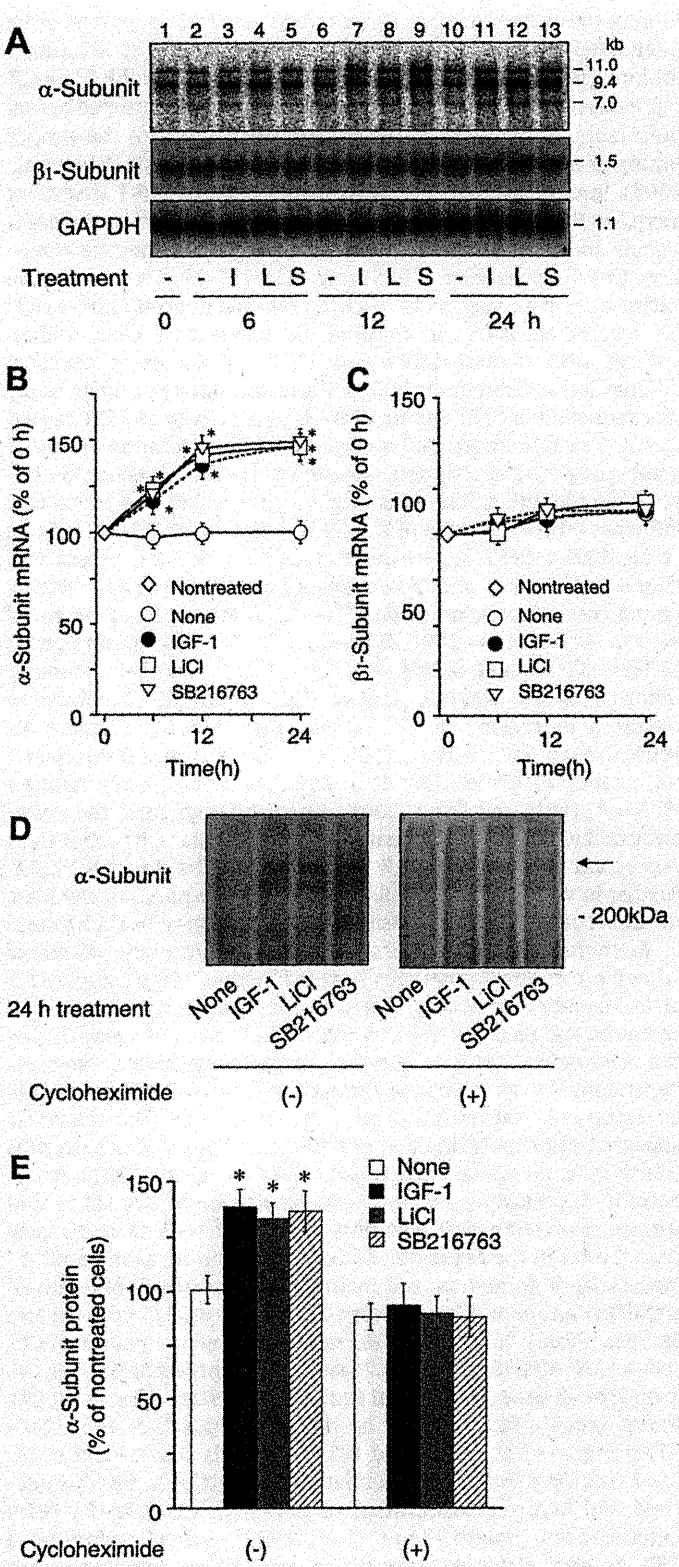

Fig. 7. Up-regulation of $Na_V1.7$ α-subunit (but not $β_1$-subunit) mRNA and $Na_V1.7$ α-subunit protein levels in cells treated with IGF-1, LiCl and SB216763. (A) Cells were treated without (–) or with 100 nM IGF-1 (I), 20 mM LiCl (L), 10 μM SB216763 (S) for up to 24 h; then poly $(A)^+$ RNA was extracted, electrophoresed, and transferred to membrane. The membrane was hybridized with each ^{32}P-labeled cDNA probe for $Na_V1.7$ α-subunit (top), Na^+ channel $β_1$-subunit (middle), or GAPDH (bottom) after removing the former probe. Levels of $Na_V1.7$ α-subunit mRNA (~9.4 kb), $β_1$-subunit mRNA and GAPDH mRNA were quantified by a bioimage analyzer. The relative level of (B) $Na_V1.7$ α-subunit/GAPDH mRNA or (C) $β_1$-subunit mRNA/GAPDH mRNA is shown. The relative level in untreated cells at 0 h is assigned a value of 100%. Mean ± S.E.M. ($n = 3$). $^*P < 0.05$, compared with untreated cells. (D) Cells were treated with or without 100 nM IGF-1, 20 mM LiCl, 10 μM SB216763 for 24 h in the absence or presence of 10 μg/ml cycloheximide. The whole-cell lysates were solubilized, size-fractionated by SDS-7.5% PAGE, and transferred to a membrane for the western blot analysis of $Na_V1.7$ α-subunit protein. (E) The relative level of $Na_V1.7$ α-subunit protein is shown. The relative level in untreated cells is assigned a value of 100%. Mean ± S.E.M. ($n = 3$). $^*P < 0.05$, compared with untreated cells.

accelerated transcription rate of $Na_V1.7$ α-subunit gene by 30% (Yanagita et al., 2009). IGF-1 and SB216763 also accelerated transcription rate of $Na_V1.7$ α-subunit gene by ~30% (Fig. 8A).

We then measured degradation rate of $Na_V1.7$ α-subunit mRNA by using actinomycin D. Cells were treated for the first 12 h with or without 100 nM IGF-1, 20 mM LiCl or 10 μM SB216763, then exposed to actinomycin D in the continuous absence or presence of either test compounds treatment, and subjected to northern blot analysis at the indicated times. As reported previously, LiCl did not alter half-life ($t_{1/2}$) of $Na_V1.7$ α-subunit mRNA (Yanagita et al., 2009). Neither IGF-1 nor SB216763 altered half-life ($t_{1/2}$) of $Na_V1.7$ α-subunit mRNA (Fig. 8B).

4. Discussion

In the present study, we found that chronic treatment of adrenal chromaffin cells with IGF-1 increased cell surface density of $Na_V1.7$ Na^+ channels via acceleration of Na^+ channel α-subunit gene transcription. In cells treated with IGF-1, veratridine-induced $^{22}Na^+$ influx and subsequent $^{45}Ca^{2+}$ influx and catecholamine secretion were augmented, whereas pharmacological properties of Na^+ channels characterized by neurotoxins (STX, veratridine, α- and β-scorpion venom, and PbTx-3) were similar to those of native Na^+ channels. These findings suggest that chronic IGF-1 treatment up-regulates cell surface expression of native Na^+ channels via acceleration of α-subunit gene transcription, enhancing Na^+ influx and subsequent Ca^{2+} influx and catecholamine secretion.

Intracellular signaling by the IGF-1 receptor is relatively well understood. The activated receptor mainly stimulates one or both of two cascades, (1) the Ras~MEK~ERK cascade or (2) the PI3K~Akt cascade. Activated Akt phosphorylates/inhibits GSK-3β, and phosphorylates/activates mTOR (Bondy and Cheng, 2004; LeRoith and Roberts, 2003; Nemoto et al., 2010b). In the present study, IGF-1-induced up-regulation of $Na_V1.7$ Na^+ channels was prevented by PI3K inhibitors (LY204002 or wortmannin), or Akt inhibitor (Akt inhibitor IV), but not by MEK inhibitors (PD98059 or U0126) or mTOR inhibitors (rapamycin). Potent GSK-3 inhibitors (LiCl, VPA SB216763 or SB415286) also up-regulated $Na_V1.7$ Na^+ channels, whereas simultaneous treatment of IGF-1 with GSK-3 inhibitors did not produce an additive increasing effect. In cultured bovine adrenal chromaffin cells, we have demonstrated that LiCl increased Ser^9-phosphorylation of GSK-3β and accumulation of β-catenin, a hallmark of GSK-3 inhibition (Yokoo et al., 2007; Nemoto et al., 2008). Inhibition of GSK-3β by LiCl or SB216763 decreased cell surface expression of insulin receptor (Yokoo et al., 2007) and IGF-1 receptor (Nemoto et al., 2010b) via regulating their mRNA level, as well as protein levels of IRS-1, IRS-2 and Akt via regulating proteasomal degradation and/or synthesis (Nemoto et al., 2006, 2008). In the present study, IGF-1 increased Ser^{437}-phosphorylation of Akt and Ser^9-phosphorylation of GSK-3β, and inhibited GSK-3β activity in cultured bovine adrenal chromaffin cells. Taken together these previous and present studies, chronic IGF-1 treatment up-regulates cell surface expression of $Na_V1.7$ Na^+ channels via PI3K~Akt~GSK-3β pathway.

The IGF-1 receptor is thought to mediate the majority of trophic effects produced by IGF-1 (Connor and Dragunow, 1998; Bondy and Cheng, 2004) such as neurogenesis and neuroprotection (Cui et al.,

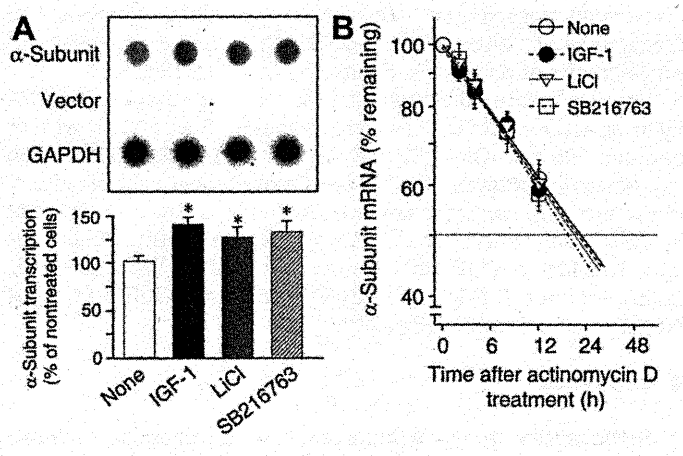

Fig. 8. Acceleration of transcription rate of $Na_V1.7$ α-subunit gene by IGF-1, LiCl and SB216763: no effect on $Na_V1.7$ α-subunit mRNA stability. (A) Cells were treated with or without 100 nM IGF-1, 20 mM LiCl, 10 μM SB216763 for 12 h; nuclei were isolated and used for in vitro nuclear run-on assay using [α-^{32}P]UTP. ^{32}P-Labeled transcripts were purified, and hybridized to 10 μg of pBII alone (Vector), or pBII containing $Na_V1.7$ α-subunit cDNA or GAPDH cDNA immobilized on membrane (upper panel). Data are typical of three independent experiments with similar results. Radioactivities in the upper panel were quantified by a bioimage analyzer, and transcription rates of $Na_V1.7$ α-subunit gene are shown in lower panel; a value of 100% represents the transcription rate obtained in cells not subjected to a 12 h incubation. (B) Cells were pretreated with or without 100 nM IGF-1, 20 mM LiCl, 10 μM SB216763 for 12 h and incubated with 10 μg/ml actinomycin D in the continuous presence or absence of 20 mM LiCl. At the indicated times, poly $(A)^+$ RNA was isolated and subjected to northern blot analysis. The level of $Na_V1.7$ α-subunit mRNA (~9.4 kb) was quantified by a bioimage analyzer. Mean ± S.E.M. ($n = 3$).

1998; Laurino et al., 2005; Brywe et al., 2005; Wang et al., 2010), whereas evidence has accumulated that IGF-1 receptor and insulin receptor resemble in the structure and features, and share the signaling cascade (Barbieri et al., 2003). In addition, the existence of hybrid heterodimers composed of one IGF-I and one insulin receptor monomer is well documented, and a physiological and pathological role for them has been shown (Belfiore et al., 2009; LeRoith and Roberts, 2003). In the present study, IGF-1 phosphorylated both IGF-1 receptor and insulin receptor. On the other hand, insulin phosphorylated insulin receptor alone, although very small content of tyrosine phosphorylation of IGF-1 receptor was observed only at high concentration (100 nM) of insulin. Thus, IGF-1 activates both IGF-1 receptor and insulin receptor, and stimulates subsequent PI3K~Akt~GSK-3β pathway, although we could not neglect the possibility the existence of IGF-1-sensitive and insulin-less sensitive hybrid heterodimers.

Long-term changes in neuronal synaptic function are correlated with, and in some cases have been shown to be dependent on, the induction of new programs of gene expression (Hyman and Nestler, 1996). In the present study, rise of [^{3}H]STX binding caused by IGF-1 was completely prevented by cycloheximide or actinomycin D, and rise of $Na_V1.7$ α-subunit protein level caused by IGF-1 or potent GSK-3 inhibitors (LiCl or SB216763) was also prevented by cycloheximide. IGF-1, LiCl or SB216763 increased steady-state level of $Na_V1.7$ α-subunit (but not β_1-subunit) mRNA, and accelerated $Na_V1.7$ α-subunit gene transcription without altering $Na_V1.7$ α-subunit mRNA stability. These results suggest that chronic treatment with IGF-1, LiCl, or SB216763 accelerates $Na_V1.7$ α-subunit gene transcription, thus leading to the increased steady-state level of $Na_V1.7$ α-subunit mRNA, and the increased cell surface expression of $Na_V1.7$. Recently, the promoter region of $Na_V1.7$ Na^+ channel gene (SCN9A) has been identified that it contains binding elements for following transcription factors: (i) Sp1, (ii) egr1, (iii) Brn3, and (iv) repressor element-1 silencing transcription factor (REST)/neuron-restrictive silencer factor (NRSF) (Diss et al., 2008). Among these transcription factors, REST has been reported to be modulated by IGF-1 (Di Toro et al., 2005; Bedini et al., 2008), lithium (Ishii et al., 2008), and VPA (Kim et al., 2007). REST represses multiple neuronal target genes in undifferentiated neural precursors of the central nervous system to control the proper timing of neural gene expression during neurogenesis (Ishii et al., 2008). In cultured SH-SY5Y neuroblastoma cells, IGF-1 treatment increases REST during the early stages of neural induction via MAPK signals and then declines REST with a progressive neurite extension: this disappearance of REST caused by IGF-1 is a key step of the extent of neurite outgrowth in differentiating neuroblastoma cells (Di Toro et al., 2005). In cultured rat neural stem cells, lithium reduces DNA binding activity of REST and increases neuronal differentiation (Ishii et al., 2008). These correlative findings imply that reduction of REST and/or DNA binding activity of REST caused by IGF-1 or lithium may be involved in the acceleration of $Na_V1.7$ α-subunit gene transcription, although the precise mechanism has yet to be identified. REST was originally identified as a factor that influences the expression of Nav1.2 Na^+ channels (SCN2A) (Chong et al., 1995): REST suppresses SCN2A gene in undifferentiated neural precursors, and down-regulation of REST/NRSF during neurogenesis causes induction of Nav1.2 to proper development of neuron (Ballas et al., 2001; Ishii et al., 2008). Among nine genes (SCN1A-SCN5A and SCN8A-SCN11A) of Na^+ channel α-subunit isoforms ($Na_V1.1$–$Na_V1.9$), putative REST binding sites are found in the promoter region of $Na_V1.2$ Na^+ channels (SCN2A) (Chong et al., 1995), $Na_V1.6$ Na^+ channels (SCN8A) (Valerie et al., 2005), $Na_V1.7$ Na^+ channels (SCN9A) (Diss et al., 2008), and $Na_V1.8$ Na^+ channels (SCN10A) (Puhl and Ikeda, 2008). These findings raise the possibility that IGF-1 or lithium treatment might regulate the cell surface expression of not only $Na_V1.7$, but also $Na_V1.2$, $Na_V1.6$, and $Na_V1.8$. Further in vivo and in vitro investigations are required to elucidate the effect of IGF-1 on the density of these isoforms of Na^+ channels.

Membrane responsiveness to both chemical and electrical stimuli is essential for nervous system function. Many components of excitability (e.g. voltage-gated ion channels, ligand-gated ion channels, and receptors for a variety of substances) develop during the embryonic period. Among these components, voltage-dependent Na^+ channels appear early in neuronal differentiation. In embryonic rat spinal cord cells, functional Na^+ channels appeared prior to $GABA_A$ and glutamate receptors: this pattern is common to all spinal cord regions (Walton et al., 1993). 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 cultured dorsal root ganglion neuron, PC12 cells and NG108-15 cells, $Na_V1.7$ was localized predominantly in the axon growth cone (Toledo-Aral et al., 1997; Kawaguchi et al., 2007). Nerve growth factor (NGF) up-regulates $Na_V1.7$ in PC12 cells (D'Arcangelo et al., 1993) and NG108-15 cells (Kawaguchi et al., 2007) during neuronal differentiation. Interestingly, tightly regulated and localized inactivation of GSK-3β is essential for NGF-induced axon growth (Zhou et al., 2004). Ser^9-phosphorylated GSK-3β accumulates at the top of the axon in hippocampal neurons (Jiang et al., 2005; Shi et al., 2004) or dorsal root ganglion neurons (Zhou et al., 2004). Inhibition of GSK-3β by GSK-3 inhibitors (lithium, SB216763, or SB415286) induces formation of multiple axons, whereas overexpression of constitutively active GSK-3β inhibits axon formation (Jiang et al., 2005; Yoshimura et al., 2006). Furthermore, PI3K~Akt~GSK-3β pathway is crucial for the neurotrophic (Cui et al., 1998; Laurino et al., 2005) and neuroprotective (Brywe et al., 2005; Wang et al., 2010) action of IGF-1. In cultured rat hippocampal neuronal cells, IGF-1 promotes membrane

expansion at the nerve growth cone via IRS/PI3K/Akt pathway (Laurino et al., 2005). In cultured mouse cerebellar granule cells, IGF-1 increases proliferation of the cells via Ser^9-phosphorylation/inhibition of GSK-3β, and this increasing effect of IGF-1 is antagonized by the over-expression of dominant active mutant of GSK-3β (Cui et al., 1998). IGF-1 protects human neuroblastoma cells against MPP^+-induced apoptosis via Akt/GSK-3β pathway (Wang et al., 2010), and also protects adult rat brain against hypoxia/ischemia-induced neuronal damage via Akt/GSK-3β pathway (Brywe et al., 2005). These findings imply that up-regulation of $Na_v1.7$ and axon growth induced by GSK-3β inhibition may contribute to the neurotrophic/neuroprotective action of IGF-1.

Recently, we found that Na^+ influx caused by veratridine increased Ser^9-phosphorylation/inhibition of GSK-3β and decreased Ser^{396}-phosphorylation of tau in a concentration-dependent manner (Kanai et al., 2009; Nemoto et al., 2010a). Increased Na^+ influx via up-regulated cell surface Na^+ channels enhances Na^+ influx-induced Ser^9-phosphorylation/inhibition of GSK-3β, and enhanced GSK-3β inhibition up-regulates cell surface Na^+ channels. A simple interpretation of these correlative findings is that the relationship between GSK-3β inhibition-induced up-regulation of Na^+ channels and Na^+ influx-induced inhibition of GSK-3β may act as a positive-feedback loop. This positive-feedback loop may be stimulated in response to GSK-3β inhibition induced by endogenous growth factors or exogenous therapeutic drugs or agents, such as IGF-1, insulin, lithium and VPA. IGF-1 might have the disease modifying benefits in the treatment of neuronal damage due to ischemia or neurodegenerative disease via modulating this positive-feedback loop (GSK-3β ⇄ Na^+ channel).

In conclusion, chronic treatment with IGF-1 up-regulates cell surface expression of native $Na_v1.7$ Na^+ channels via GSK-3 inhibition in cultured bovine adrenal chromaffin cells, thereby resulting in the enhancement of Na^+ influx, Ca^{2+} channel gating and catecholamine secretion. The present findings would provide insight into the neurotrophic and neuroprotective effects of IGF-1.

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References

Arsenijevic, Y., Weiss, S., 1998. Insulin-like growth factor-I is a differentiation factor for postmitotic CNS stem cell-derived neuronal precursors: distinct actions from those of brain-derived neurotrophic factor. J. Neurosci. 18, 2118–2128.

Ballas, N., Battaglioli, E., Atouf, F., Andres, M.E., Chenoweth, J., Anderson, M.E., Burger, C., Moniwa, M., Davie, J.R., Bowers, W.J., Federoff, H.J., Rose, D.W., Rosenfeld, M.G., Brehm, P., Mandel, G., 2001. Regulation of neuronal traits by a novel transcriptional complex. Neuron 31, 353–365.

Barbieri, M., Bonafè, M., Franceschi, C., Paolisso, G., 2003. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. Am. J. Physiol. Endocrinol. Metab. 285, E1064–E1071.

Bedini, A., Baiula, M., Spampinato, S., 2008. Transcriptional activation of human mu-opioid receptor gene by insulin-like growth factor-I in neuronal cells is modulated by the transcription factor REST. J. Neurochem. 105, 2166–2178.

Belfiore, A., Frasca, F., Pandini, G., Sciacca, L., Vigneri, R., 2009. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr. Rev. 30, 586–623.

Bondy, C.A., Cheng, C.M., 2004. Signaling by insulin-like growth factor 1 in brain. Eur. J. Pharmacol. 490, 25–31.

Brackenbury, W.J., Djamgoz, M.B., Isom, L.L., 2008. An emerging role for voltage-gated Na^+ channels in cellular migration: regulation of central nervous system development and potentiation of invasive cancers. Neuroscientist 14, 571–583.

Brywe, K.G., Mallard, C., Gustavsson, M., Hedtjärn, M., Leverin, A.L., Wang, X., Blomgren, K., Isgaard, J., Hagberg, H., 2005. IGF-I neuroprotection in the immature brain after hypoxia-ischemia, involvement of Akt and GSK3β? Eur. J. Neurosci. 21, 1489–1502.

Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26, 13–25.

Catterall, W.A., Goldin, A.L., Waxman, S.G., 2005. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol. Rev. 57, 397–409.

Cestèle, S., Catterall, W.A., 2000. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. Biochimie 82, 883–892.

Chong, J.A., Tapia-Ramírez, J., Kim, S., Toledo-Aral, J.J., Zheng, Y., Boutros, M.C., Altshuller, Y.M., Frohman, M.A., Kraner, S.D., Mandel, G., 1995. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. Cell 80, 949–957.

Coghlan, M.P., Culbert, A.A., Cross, D.A., Corcoran, S.L., Yates, J.W., Pearce, N.J., Rausch, O.L., Murphy, G.J., Carter, P.S., Roxbee, Cox.L., Mills, D., Brown, M.J., Haigh, D., Ward, R.W., Smith, D.G., Murray, K.J., Reith, A.D., Holder, J.C., 2000. Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. Chem. Biol. 7, 793–803.

Connor, B., Dragunow, M., 1998. The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res. Brain Res. Rev. 27, 1–39.

Cui, H., Meng, Y., Bulleit, R.F., 1998. Inhibition of glycogen synthase kinase 3β activity regulates proliferation of cultured cerebellar granule cells. Brain Res. Dev. Brain Res. 111, 177–188.

D'Arcangelo, G., Paradiso, K., Shepherd, D., Brehm, P., Halegoua, S., 1993. Neuronal growth factor regulation of two different sodium channel types through distinct signal transduction pathways. J. Cell Biol. 122, 915–921.

Diss, J.K., Calissano, M., Gascoyne, D., Djamgoz, M.B., Latchman, D.S., 2008. Identification and characterization of the promoter region of the Nav1.7 voltage-gated sodium channel gene (SCN9A). Mol. Cell Neurosci. 37, 537–547.

Di Toro, R., Baiula, M., Spampinato, S., 2005. Expression of the repressor element-1 silencing transcription factor (REST) is influenced by insulin-like growth factor-I in differentiating human neuroblastoma cells. Eur. J. Neurosci. 21, 46–58.

Gurvich, N., Klein, P.S., 2002. Lithium and valproic acid: parallels and contrasts in diverse signaling contexts. Pharmacol. Therapeut. 96, 45–66.

Hanson, M.G., Landmesser, L.T., 2004. Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. Neuron 43, 687–701.

Hua, J.Y., Smear, M.C., Baier, H., Smith, S.J., 2005. Regulation of axon growth in vivo by activity-based competition. Nature 434, 1022–1026.

Hyman, S.E., Nestler, E.J., 1996. Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am. J. Psychiatry 153, 151–162.

Ishii, T., Hashimoto, E., Ukai, W., Tateno, M., Yoshinaga, T., Saito, S., Sohma, H., Saito, T., 2008. Lithium-induced suppression of transcription repressor NRSF/REST: effects on the dysfunction of neuronal differentiation by ethanol. Eur. J. Pharmacol. 593, 36–43.

Isom, L.L., 2002. The role of sodium channels in cell adhesion. Front Biosci. 7, 12–23.

Jiang, H., Guo, W., Liang, X., Rao, Y., 2005. Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3β and its upstream regulators. Cell 120, 123–135.

Jope, R.S., 2003. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. Trends Pharmacol. Sci. 24, 441–443.

Jope, R.S., Johnson, G.V., 2004. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem. Sci. 29, 95–102.

Kajiwara, K., Yanagita, T., Nakashima, Y., Wada, A., Izumi, F., Yanagihara, N., 2002. Differential effects of short and prolonged exposure to carvedilol on voltage-dependent Na^+ channels in cultured bovine adrenal medullary cells. J. Pharmacol. Exp. Ther. 302, 212–218.

Kanai, T., Nemoto, T., Yanagita, T., Maruta, T., Satoh, S., Yoshikawa, N., Wada, A., 2009. Nav1.7 sodium channel-induced Ca^{2+} influx decreases tau phosphorylation via glycogen synthase kinase-3β in adrenal chromaffin cells. Neurochem. Int. 54, 497–505.

Kawaguchi, A., Asano, H., Matsushima, K., Wada, T., Yoshida, S., Ichida, S., 2007. Enhancement of sodium current in NG108-15 cells during neural differentiation is mainly due to an increase in NaV1.7 expression. Neurochem. Res. 32, 1469–1475.

Kim, S.J., Lee, B.H., Lee, Y.S., Kang, K.S., 2007. Defective cholesterol traffic and neuronal differentiation in neural stem cells of Niemann-Pick type C disease improved by valproic acid, a histone deacetylase inhibitor. Biochem. Biophys. Res. Commun. 360, 593–599.

Klugbauer, N., Lacinova, L., Flockerzi, V., Hofmann, F., 1995. Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells. EMBO J. 14, 1084–1090.

Laurino, L., Wang, X.X., de la Houssaye, B.A., Sosa, L., Dupraz, S., Cáceres, A., Pfenninger, K.H., Quiroga, S., 2005. PI3K activation by IGF-1 is essential for the regulation of membrane expansion at the nerve growth cone. J. Cell Sci. 118, 3653–3662.

LeRoith, D., Roberts Jr., C.T., 2003. The insulin-like growth factor system and cancer. Cancer Lett. 195, 127–137.

Maruta, T., Yanagita, T., Matsuo, K., Uezono, Y., Satoh, S., Nemoto, T., Yoshikawa, N., Kobayashi, H., Takasaki, M., Wada, A., 2008. Lysophosphatidic acid-LPA1

receptor-Rho-Rho kinase-induced up-regulation of Nav1.7 sodium channel mRNA and protein in adrenal chromaffin cells: enhancement of $^{22}Na^{+}$ influx, $^{45}Ca^{2+}$ influx and catecholamine secretion. J. Neurochem. 105, 401–412.

Nemoto, T., Kanai, T., Yanagita, T., Satoh, S., Maruta, T., Yoshikawa, N., Kobayashi, H., Wada, A., 2008. Regulation of Akt mRNA and protein levels by glycogen synthase kinase-3β in adrenal chromaffin cells: effects of LiCl and SB216763. Eur. J. Pharmacol. 586, 82–89.

Nemoto, T., Miyazaki, S., Kanai, T., Maruta, T., Satoh, S., Yoshikawa, N., Yanagita, T., Wada, A., 2010a. Nav1.7-Ca^{2+} influx-induced increased phosphorylations of extracellular signal-regulated kinase (ERK) and p38 attenuate tau phosphorylation via glycogen synthase kinase-3β: priming of Nav1.7 gating by ERK and p38. Eur. J. Pharmacol. 640, 20–28.

Nemoto, T., Satoh, S., Maruta, T., Kanai, T., Yoshikawa, N., Miyazaki, S., Yanagita, T., Wada, A., 2010b. Homologous posttranscriptional regulation of insulin-like growth factor-I receptor level via glycogen synthase kinase-3β and mammalian target of rapamycin in adrenal chromaffin cells: effect on tau phosphorylation. Neuropharmacology 58, 1097–1108.

Nemoto, T., Yokoo, H., Satoh, S., Yanagita, T., Sugano, T., Yoshikawa, N., Maruta, T., Kobayashi, H., Wada, A., 2006. Constitutive activity of glycogen synthase kinase-3β: positive regulation of steady-state levels of insulin receptor substrates-1 and -2 in adrenal chromaffin cells. Brain Res. 1110, 1–12.

Oh, Y., Waxman, S.G., 1994. The β_1-subunit mRNA of the rat brain Na^{+} channel is expressed in glial cells. Proc. Natl. Acad. Sci. U.S.A. 91, 9985–9989.

Patino, G.A., Isom, L.L., 2010. Electrophysiology and beyond: multiple roles of Na^{+} channel β subunits in development and disease. Neurosci. Lett. 486, 53–59.

Puhl 3rd, H.L., Ikeda, S.R., 2008. Identification of the sensory neuron specific regulatory region for the mouse gene encoding the voltage-gated sodium channel $Na_V1.8$. J. Neurochem. 106, 1209–1224.

Ryves, W.J., Harwood, A.J., 2001. Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. Biochem. Biophys. Res. Commun. 280, 720–725.

Shi, S.H., Cheng, T., Jan, L.Y., Jan, Y.N., 2004. APC and GSK-3β are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. Curr. Biol. 14, 2025–2032.

Shiraishi, S., Yanagita, T., Kobayashi, H., Uezono, Y., Yokoo, H., Minami, S., Takasaki, M., Wada, A., 2001. Up-regulation of cell surface sodium channels by cyclosporin A, FK506, and rapamycin in adrenal chromaffin cells. J. Pharmacol. Exp. Ther. 297, 657–665.

Takahashi-Yanaga, F., Taa, Y., Miwa, Y., Kubohara, Y., Watanabe, Y., Hirata, M., Morimoto, S., Sasaguri, T., 2003. Dictyostelium differentiation-inducing factor-3 activates glycogen synthase kinase-3β and degrades cyclin D1 in mammalian cells. J. Biol. Chem. 278, 9663–9670.

Toledo-Aral, J.J., Moss, B.L., He, Z.-J., Koszowski, A.G., Whisenand, T., Levinson, S.R., Wolf, J.J., Silos-Santiago, I., Halegoua, S., Mandel, G., 1997. Identification of PN1, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. Proc. Natl. Acad. Sci. U.S.A. 94, 1527–1532.

Torres-Aleman, I., 2010. Toward a comprehensive neurobiology of IGF-I. Dev. Neurobiol. 70, 384–396.

Trejo, J.L., Carro, E., Garcia-Galloway, E., Torres-Aleman, I., 2004. Role of insulin-like growth factor I signaling in neurodegenerative diseases. J. Mol. Med. 82, 156–162.

Urenjak, J., Obrenovitch, T.P., 1996. Pharmacological modulation of voltage-gated Na^{+} channels: a rational and effective strategy against ischemic brain damage. Pharmacol. Rev. 48, 21–67.

Valerie, L.D., Andrew, P.L., Miriam, H.M., 2005. Multiple transcripts of sodium channel SCN8A (NaV1.6) with alternative 5′- and 3′-untranslated regions and initial characterization of the SCN8A promoter. Genomics 85, 245–257.

Wada, A., Wanke, E., Gullo, F., Schiavon, E., 2008. Voltage-dependent $Na_V1.7$ sodium channels: multiple roles in adrenal chromaffin cells and peripheral nervous system. Acta Physiol. (Oxf.) 192, 221–231.

Walton, M.K., Schaffner, A.E., Barker, J.L., 1993. Sodium channels, $GABA_A$ receptors, and glutamate receptors develop sequentially on embryonic rat spinal cord cells. J. Neurosci. 13, 2068–2084.

Wang, L., Yang, H.J., Xia, Y.Y., Feng, Z.W., 2010. Insulin-like growth factor 1 protects human neuroblastoma cells SH-EP1 against MPP^{+}-induced apoptosis by AKT/GSK-3β/JNK signaling. Apoptosis 15, 1470–1479.

Waxman, S.G., Dib-Hajj, S., Cummins, T.R., Black, J.A., 2000. Sodium channels and their genes: dynamic expression in the normal nervous system, dysregulation in disease states. Brain Res. 886, 5–14.

Xia, Y., Fung, M.-L., O'Reilly, J.P., Haddad, G.G., 2000. Increased neuronal excitability after long-term O_2 deprivation is mediated mainly by sodium channels. Mol. Brain Res. 76, 211–219.

Xu, X., Shrager, P., 2005. Dependence of axon initial segment formation on Na^{+} channel expression. J. Neurosci. Res. 79, 428–441.

Yamamoto, R., Yanagita, T., Kobayashi, H., Yokoo, H., Wada, A., 1997. Up-regulation of sodium channel subunit mRNAs and their cell surface expression by antiepileptic valproic acid: activation of calcium channel and catecholamine secretion in adrenal chromaffin cells. J. Neurochem. 68, 1655–1662.

Yamamoto, R., Yanagita, T., Kobayashi, H., Yuhi, T., Yokoo, H., Wada, A., 1996. Up-regulation of functional voltage-dependent sodium channels by insulin in cultured bovine adrenal chromaffin cells. J. Neurochem. 67, 1401–1408.

Yanagita, T., Kobayashi, H., Yamamoto, R., Kataoka, H., Yokoo, H., Shiraishi, S., Minami, S., Koono, M., Wada, A., 2000. Protein kinase C-α and -ε down regulate cell surface sodium channels via differential mechanisms in adrenal chromaffin cells. J. Neurochem. 74, 1674–1684.

Yanagita, T., Kobayashi, H., Yamamoto, R., Takami, Y., Yokoo, H., Yuhi, T., Nakayama, T., Wada, A., 1999. Protein kinase C and the opposite regulation of sodium channel α- and β_1-subunit mRNA levels in adrenal chromaffin cells. J. Neurochem. 73, 1749–1757.

Yanagita, T., Kobayashi, H., Uezono, Y., Yokoo, H., Sugano, T., Saitoh, T., Minami, S., Shiraishi, S., Wada, A., 2003. Destabilization of $Na_V1.7$ sodium channel α-subunit mRNA by constitutive phosphorylation of extracellular signal-regulated kinase: negative regulation of steady-state level of cell surface functional sodium channels in adrenal chromaffin cells. Mol. Pharmacol. 63, 1125–1136.

Yanagita, T., Maruta, T., Nemoto, T., Uezono, Y., Matsuo, K., Satoh, S., Yoshikawa, N., Kanai, T., Kobayashi, H., Wada, A., 2009. Chronic lithium treatment up-regulates cell surface $Na_V1.7$ sodium channels via inhibition of glycogen synthase kinase-3 in adrenal chromaffin cells: enhancement of Na^{+} influx, Ca^{2+} influx and catecholamine secretion after lithium withdrawal. Neuropharmacology 57, 311–321.

Yanagita, T., Maruta, T., Uezono, Y., Satoh, S., Yoshikawa, N., Nemoto, T., Kobayashi, H., Wada, A., 2007. Lithium inhibits function of voltage-dependent sodium channels and catecholamine secretion independent of glycogen synthase kinase-3 in adrenal chromaffin cells. Neuropharmacology 53, 881–889.

Ying Wang, J., Peruzzi, F., Lassak, A., Del Valle, L., Radhakrishnan, S., Rappaport, J., Khalili, K., Amini, S., Reiss, K., 2003. Neuroprotective effects of IGF-I against TNFα-induced neuronal damage in HIV-associated dementia. Virology 305, 66–76.

Yokoo, H., Nemoto, T., Yanagita, T., Satoh, S., Yoshikawa, N., Maruta, T., Wada, A., 2007. Glycogen synthase kinase-3β: homologous regulation of cell surface insulin receptor level via controlling insulin receptor mRNA stability in adrenal chromaffin cells. J. Neurochem. 103, 1883–1896.

Yoshimura, T., Arimura, N., Kaibuchi, K., 2006. Signaling networks in neuronal polarization. J. Neurosci. 26, 10626–10630.

Yuhi, T., Wada, A., Kobayashi, H., Yamamoto, R., Yanagita, T., Niina, H., 1996. Up-regulation of functional voltage-dependent sodium channels by cyclic AMP-dependent protein kinase in adrenal medulla. Brain Res. 709, 37–43.

Zhou, F.Q., Zhou, J., Dedhar, S., Wu, Y.H., Snider, W.D., 2004. NGF-induced axon growth is mediated by localized inactivation of GSK-3β and functions of the microtubule plus end binding protein APC. Neuron 42, 897–912.

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Sevoflurane Inhibits the μ-Opioid Receptor Function Expressed in *Xenopus* Oocytes

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Key Words
μ-Opioid receptor · $G_{i/o}$-protein-coupled receptors · Sevoflurane · *Xenopus* oocyte

Abstract
Sevoflurane is widely used for anesthesia, and is commonly used together with opioids in clinical practice. However, the effects of sevoflurane on μ-opioid receptor (μOR) functions is still unclear. In this study, the effects of sevoflurane on μOR functions were analyzed by using *Xenopus* oocytes expressing a μOR fused to chimeric Gα protein G_{qi5} (μOR-G_{qi5}). Sevoflurane by itself did not elicit any currents in oocytes expressing μOR-G_{qi5}, whereas sevoflurane inhibited the [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO)-induced Cl^- currents at clinically used concentrations. Sevoflurane did not affect the Cl^- currents induced by AlF_4^-, which directly led to activation of G proteins. The inhibitory effects of sevoflurane on the DAMGO-induced currents were not observed in oocytes pretreated with the protein kinase C (PKC) inhibitor GF109203X. These findings suggest that sevoflurane would inhibit μOR function. Further, the mechanism of inhibition by sevoflurane would be mediated by PKC.

Introduction

Sevoflurane is widely used as an inhalation general anesthetic agent due to its low solubility in blood. Opioids are commonly used with sevoflurane at the same time. There have been some reports that have pointed out interactions between sevoflurane and opioid receptors (ORs) in animal experiments; the potency of sevoflurane was modulated by the endogenous μ-opioid system, but not by the κ- and δ-opioid systems in experiments using mice lacking the ORs [1]. In animals, morphine decreases the minimal alveolar concentration (MAC) of sevoflurane [2, 3]. However, it has been reported that morphine does not affect MAC for sevoflurane in humans. More recently, it has been reported that μOR-knockout mice have no different MAC of sevoflurane [4]. The interaction between sevoflurane and OR function is still controversial.

The ORs belong to the G-protein-coupled receptor family and three types of receptors, μ, δ and κ, have been identified by molecular cloning [5]. Within the three subtypes of these receptors, μORs are the major receptors that mediate the analgesic effects of opioids [5]. On the basis of second-messenger signaling, μOR couples to $G\alpha_{i/o}$ protein to cause inhibition of adenylate cyclase, in-

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hibition of voltage-dependent Ca^{2+} channels, and activation of G-protein-coupled inwardly rectifying K^+ channels [5]. There have been many reports that revealed direct effects of general anesthetics on G_q-coupled receptors [6–11]. As far as the functions of $G_{i/o}$-coupled receptors including μOR are concerned, much less is known about the direct effects of volatile anesthetics. Moreover, we recently reported that the volatile anesthetic halothane inhibited μOR function at clinical concentrations [12]. It would be interesting to study whether sevoflurane affects μOR functions.

The *Xenopus* oocyte expression system has widely been employed to study the functions of a number of G-protein-coupled receptors [13, 14]. In the case of G_q-coupled receptors, receptor stimulation results in activation of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes by G_q-mediated phospholipase C activation and subsequent formation of IP_3 and diacylglycerol [14]. The IP_3 formed causes release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels endogenously expressed in the oocytes [14]. However, in case of $G_{i/o}$-coupled receptors, analysis has been difficult, due to lack of appropriate analytical output in oocytes. We have established an assay method for $G_{i/o}$ PCRs by using a μOR fused to G_{qi5} (μOR-G_{qi5}) in *Xenopus* oocytes [12, 15].

We examined the effects of sevoflurane on the function of μOR using this assay system. Moreover, we investigated the mechanisms of the effects of sevoflurane on μOR.

Materials and Methods

Materials

Adult *Xenopus laevis* female frogs were purchased from Kato Kagaku (Tokyo, Japan). The Ultracomp *E. coli* Transformation Kit was from Invitrogen (San Diego, Calif., USA). Sevoflurane was purchased from Maruishi Pharmaceutical (Osaka, Japan). Purification of cDNAs was performed with a Qiagen purification kit (Qiagen, Chatworth, Calif., USA). Gentamicin, sodium pyruvate, [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and other chemicals were from Nacalai Tesque (Kyoto, Japan). The rat μOR was provided by Dr. N. Dascal (Tel Aviv University, Ramat Aviv, Israel). The chimeric $G\alpha_{qi5}$ was a kind gift from Dr. B.R. Conklin (UCSF, San Fransico, Calif., USA). Each of the cRNAs was prepared using a mCAP mRNA Capping Kit, and transcribed with a T7 RNA polymerase in vitro transcription kit (Stratagene, La Jolla, Calif., USA).

Preparation of Chimeric μOR-G_{qi5}

The tandem cDNAs of chimeric μOR-G_{qi5} were created by ligating the receptor cDNA sequences into the *Nhe*I site of G_{qi5} cDNAs. The sequences of all PCR products were confirmed by sequencing with ABI3100 (Applied BioSystems, Tokyo, Japan). All cDNAs for the synthesis of cRNAs were subcloned into the pGEMHJ vector, which provides the 5′- and 3′-untranslated regions of the *Xenopus* β-globin RNA [16], ensuring a high level of protein expression in the oocytes. Each of the cRNAs was synthesized using the mCAP mRNA Capping Kit, with the T7 RNA polymerase in vitro transcription kit (Ambion, Austin, Tex., USA) from the respective linearized cDNAs.

Recording and Data Analysis

Isolation and microinjection of *Xenopus* oocytes were performed as previously described [7, 9–12, 17–19]. *Xenopus* oocytes were injected with appropriate amounts of cRNAs (in 50 ng; μOR-G_{qi5}) and incubated with ND 96 medium composed of (in mmol/l); NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (pH 7.4, adjusted with NaOH), supplemented with 2.5 mmol/l sodium pyruvate and 50 μg/ml gentamicin for 3–7 days until recording. Oocytes were placed in a 100-μl recording chamber and perfused with modified Barth's saline (MBS) composed of (in mmol/l): NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 10, $MgSO_4$ 0.82, $Ca(NO_3)_2$ 0.33, and $CaCl_2$ 0.91, (pH 7.4 adjusted with NaOH) at a rate of 1.8 ml/min at room temperature. Recording and clamping electrodes (1–2 MΩ) were pulled from 1.2-mm outside-diameter capillary tubing and filled with 3 mol/l KCl. A recording electrode was imbedded in the animal's pole of oocytes, and once the resting membrane potential stabilized, a clamping electrode was inserted and the resting membrane potential was allowed to restabilize. A Warner OC 725-B oocyte clamp (Hampden, Conn., USA) was used to voltage clamp each oocyte at –70 mV. We analyzed the peak component of the transient inward currents induced by receptor agonists because this component is dependent on the concentrations of the receptor agonist applied, and is quite reproducible, as described by Minami et al. [10]. Sevoflurane was applied for 2 min before and during the application of DAMGO (1 μmol/l) to allow complete equilibration in the bath. The solutions of sevoflurane were freshly prepared immediately before use. We calculated the final concentration of sevoflurane in the recording chamber using a gas chromatography method, and accordingly the concentrations of sevoflurane represent the bath concentrations.

AlF_4^- was used as a direct activator of G proteins, and with this system we could bypass the signal to G proteins from activated receptors. Under a two-electrode voltage clamp, we injected 30 nl of solution containing NaF and $AlCl_3$ into the oocyte by using a pressure injector (PM2000B; MicroData Instruments, South Plainfield, N.J., USA). The concentrations of NaF and $AlCl_3$ used in this study were 20 mmol/l and 60 μmol/l, respectively.

To determine whether activation of protein kinase C (PKC) plays a role in anesthetic modulation of μOR-mediated events, oocytes were exposed to a PKC inhibitor, bisindolylmaleimide I (GF109203X; 200 nmol/l) [20], in MBS for 120 min. We compared the effects of anesthetics on DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes expressing μOR-G_{qi5} before and after the exposure to GF109203X.

Statistical Analysis

Results are expressed as percentages of control responses. The control responses were measured before and after each drug ap-

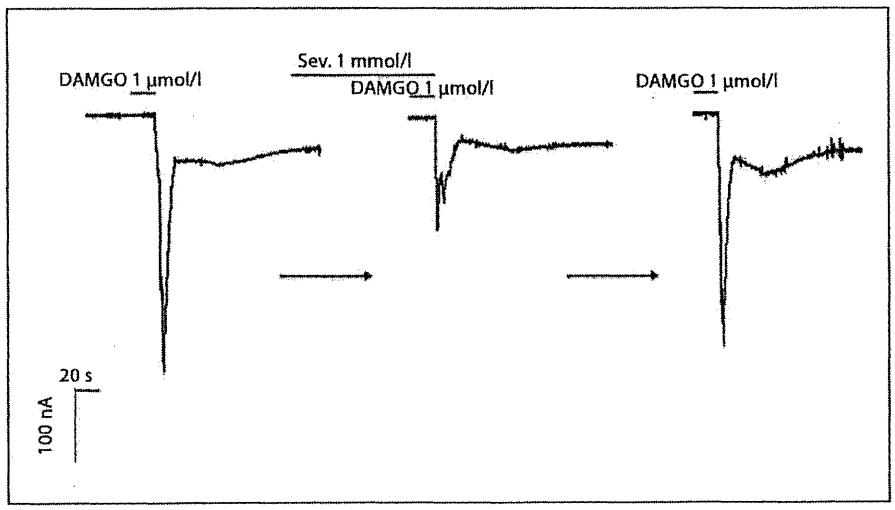


Fig. 1. Typical tracings of the effect of 1 mmol/l sevoflurane (Sev.) on the Cl^- current evoked by 1 μmol/l DAMGO in *Xenopus* oocytes expressing μOR-G_{qi5}.

plication, to take into account possible shifts in the control currents as recording proceeded. The 'n' values refer to the number of oocytes studied. Each experiment was carried out with oocytes from at least two different frogs. Statistical analyses were carried out by one-way ANOVA followed by Dunnett's correction and paired t test using GraphPad Prism 4 (GraphPad Softwear, Inc; La Jolla, Calif., USA). Values of $p < 0.05$ were considered to be significant.

Results

Pretreatment of sevoflurane by itself did not elicit any currents in oocytes expressing μOR-G_{qi5}, whereas the sevoflurane significantly inhibited DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents in a concentration-dependent manner (fig. 1); sevoflurane at 0.25, 0.5 and 1 mmol/l inhibited the DAMGO (1 μmol/l)-induced Cl^- currents to 84.7 ± 12.3, 50.6 ± 10.1 ($p < 0.01$), and 48.8 ± 8.2% ($p < 0.01$) of the control value, respectively (n = 8 each) (fig. 2).

AlF_4^- has been reported to bind to guanosine diphosphate on heterotrimeric G protein, and guanosine diphosphate-AlF_4^- complex promotes the dissociation of heterotrimeric G proteins into Gα and Gβγ subunits, which directly (without receptor stimulation) leads to the activation of G protein and subsequent G-protein-mediated pathways downstream [21]. The peak amplitude of AlF_4^--induced currents was 282 ± 121 nA (n = 6), and sevoflurane did not affect the AlF_4^--induced currents (418 ± 86.4 nA; n = 6) (fig. 3).

Treatment with a PKC inhibitor, GF109203X (200 nmol/l), which has a K_i value for inhibiting PKC activity of 20 nmol/l [20], produced enhancement of the initial Cl^- currents activated by DAMGO (1 μmol/l) (fig. 4a). After a 1-hour incubation of GF109203X, the response of DAMGO (1 μmol/l) increased to 2 times the initial currents (205 ± 27.6% of control), and this enhancement continued for 2 h (fig. 4b) (n = 6). Sevoflurane (1 mmol/l) inhibited DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents to 61.2 ± 15.8% of the control value (n = 6). However, the inhibitory effects of sevoflurane (1 mmol/l) on DAMGO (1 μmol/l)-induced currents were abolished after 2 h of pretreatment with GF109203X (115.8 ± 26.8% of control) (fig. 5).

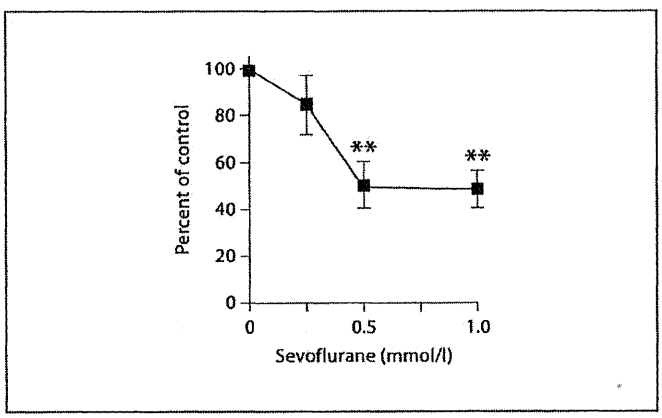


Fig. 2. Concentration-response curve for the inhibitory effects of sevoflurane on DAMGO (1 μmol/l)-induced Cl^- currents in oocytes expressing μOR-G_{qi5}. ** $p < 0.01$ vs. control. Values are expressed as means ± SEM.

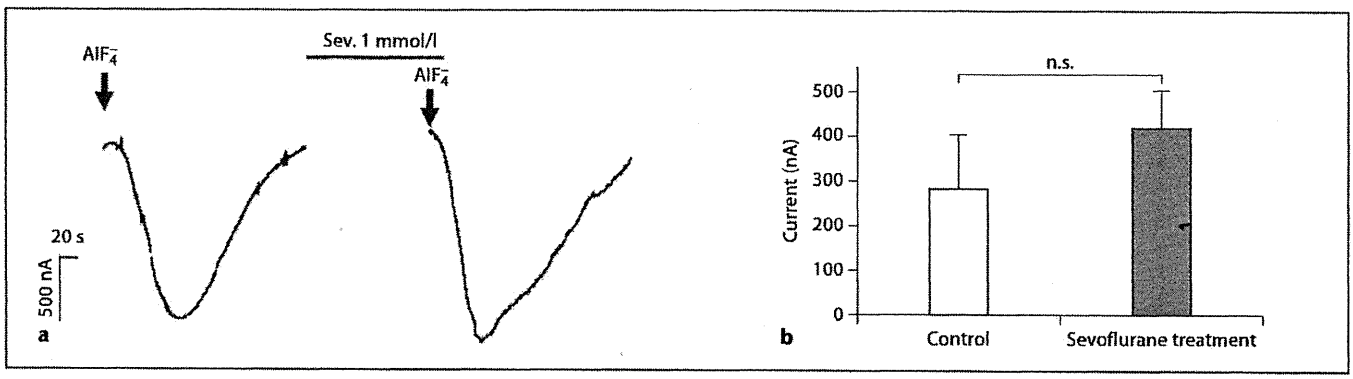


Fig. 3. Effects of sevoflurane (Sev.) on AlF_4^--induced currents in *Xenopus* oocytes. **a** Tracings were obtained from a single oocyte showing the effect of sevoflurane on AlF_4^--induced currents in oocytes expressing μOR-G_{qi5}. **b** Oocytes were injected with 30 nl test solution (20 mmol/l NaF and 60 μmol/l $AlCl_3$) in the presence (Sev. treatment) (n = 6) or absence (control) (n = 6) of 1 mmol/l sevoflurane. Data are expressed as means ± SEM of peak currents (nA).

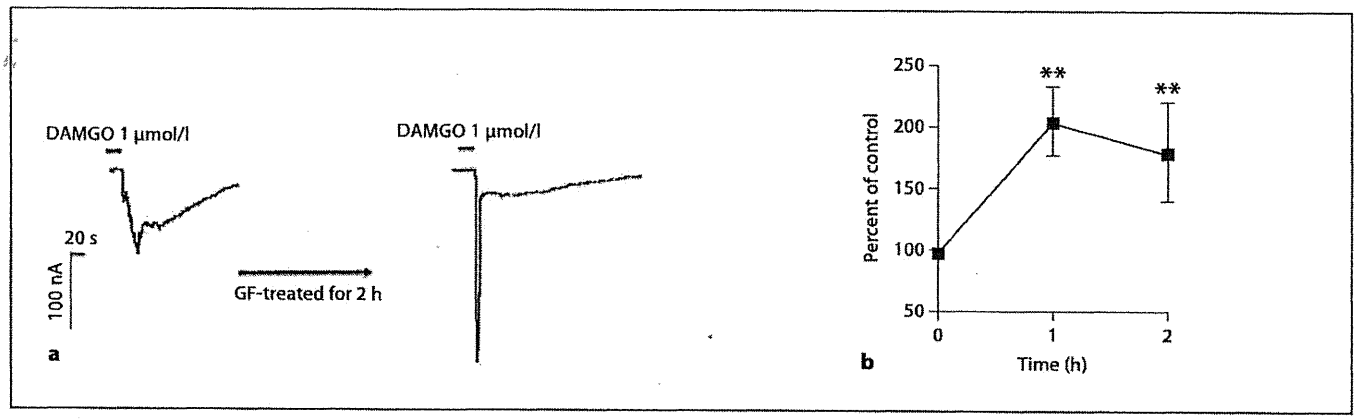
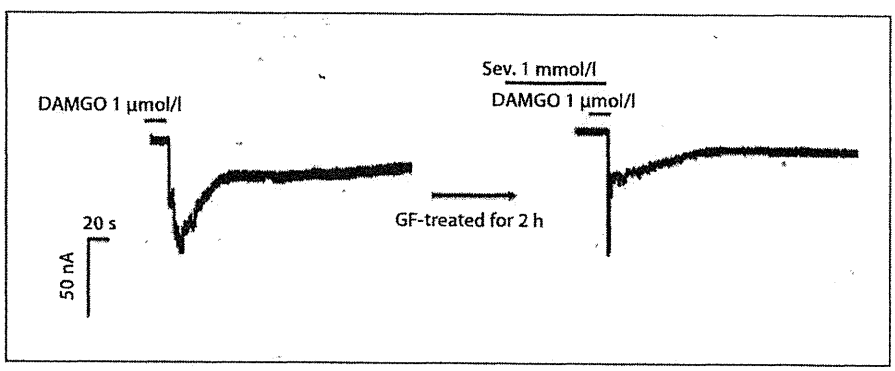


Fig. 4. Effects of bisindolylmaleimide I (GF109203X) on DAMGO-induced Cl^- current in oocytes expressing μOR-G_{qi5} receptor. **a** Tracings were obtained from a single oocyte showing the DAMGO (1 μmol/l)-induced currents in oocytes expressing μOR-G_{qi5} receptors before and after treatment with GF109203X (GF). Oocytes were incubated with 200 nmol/l GF for 2 h and were then stimulated by DAMGO. **b** Time course of effects of GF on DAMGO-induced Cl^- current in oocytes expressing μOR-G_{qi5} receptor. Oocytes were incubated with GF (200 nmol/l) for 120 min. DAMGO (1 μmol/l) was applied at 60 and 120 min during treatment of GF. Data represent means ± SEM of 6 oocytes. ** $p < 0.01$ vs. time at starting incubation with 200 nmol/l GF (0 h).

Fig. 5. Effects of bisindolylmaleimide I (GF109203X) on the inhibitory effects of sevoflurane (Sev.) on DAMGO (1 μmol/l)-induced currents. Tracings were obtained from a single oocyte showing the effect of sevoflurane on 1 μmol/l of DAMGO-induced currents in oocytes expressing μOR-G_{qi5} receptor before and after treatment with GF109203X (GF). Oocytes were incubated with 200 nmol/l GF for 2 h, and were then stimulated by DAMGO (1 μmol/l) in the presence of sevoflurane (1 mmol/l).



Discussion

We showed that sevoflurane had inhibitory effects on DAMGO-induced Cl^- currents in oocytes expressing μOR-G_{qi5}. In clinical situations, the free plasma concentration of sevoflurane was approximately 0.5 mmol/l [22, 23]. Sevoflurane suppressed DAMGO-induced Cl^- currents in oocytes expressing μOR-G_{qi5} at concentrations more than 0.5 mmol/l. Consistent with these reports, our present results suggest that anesthetic concentrations of sevoflurane would have inhibitory effects on μOR.

Our study raises the question of how sevoflurane inhibits μOR function. In our results, sevoflurane had little effect on AlF_4^--induced currents, suggesting that sevoflurane may not interfere with the signaling pathways downstream of activation of G proteins, such as phospholipase C activation, intracellular Ca^{2+} release, and Ca^{2+}-activated Cl^- channels. From these results, the action site of sevoflurane would be OR.

There is considerable evidence that PKC plays an important role in the regulation of OR function. A number of studies have reported that PKC is involved in morphine-induced tolerance in vivo [24–27]. In our present results, the PKC inhibitor GF109203X enhanced DAMGO-induced currents. These results suggested that PKC would inhibit the OR function. Moreover, the PKC inhibitor GF109203X abolished the inhibitory effects of sevoflurane on μOR function, suggesting that sevoflurane would inhibit μOR function by PKC-mediated pathways. In our study, unfortunately, we could not study how sevoflurane activates the PKC because of difficulties in measuring the activities of PKC in *Xenopus* oocyte preparation. However, there are several lines of evidence which reveal that sevoflurane activated PKC [28, 29]. To confirm this hypothesis, it could be required to investigate the region of μOR responsible for PKC action by using mutated μOR whose serine/threonine sites were point mutated.

In conclusion, we demonstrated that sevoflurane has significant inhibitory effects on the function of μOR at clinically relevant concentrations, and the inhibition might be mediated via PKC pathways. Although several investigations have reported the effects of opioids on sevoflurane anesthesia, the nature of the interaction between opioids and sevoflurane remains unclear. Our present results showed the inhibitory effects on μOR. To clarify the interaction between sevoflurane and opioid in the clinical situation, further study would be necessary.

References

1 Dahan A, Sarton E, Teppema L, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL: Anesthetic potency and influence of morphine and sevoflurane on respiration in mu-opioid receptor knockout mice. Anesthesiology 2001;94:824–832.

2 Katoh T, Uchiyama T, Ikeda K. Effect of fentanyl on awakening concentration of sevoflurane. Br J Anaesth 1994;73:322–325.

3 Katoh T, Ikeda K: The effects of fentanyl on sevoflurane requirements for loss of consciousness and skin incision. Anesthesiology 1998;88:18–24.

4 Koyama T, Mayahara T, Wakamatsu T, Sora I, Fukuda K: Deletion of mu-opioid receptor in mice does not affect the minimum alveolar concentration of volatile anaesthetics and nitrous oxide-induced analgesia. Br J Anaesth 2009;103:744–749.

5 Surratt CK, Adams WR: G protein-coupled receptor structural motifs: relevance to the opioid receptors. Curr Top Med Chem 2005; 5:315–324.

6 Durieux ME: Muscarinic signaling in the central nervous system: recent developments and anesthetic implications. Anesthesiology 1996;84:173–189.

7 Minami K, Uezono Y: Gq protein-coupled receptors as targets for anesthetics. Curr Pharm Des 2006;12:1931–1937.

8 Minami K, Uezono Y, Ueta Y: Pharmacological aspects of the effects of tramadol on G-protein coupled receptors. J Pharmacol Sci 2007;103:253–260.

9 Minami K, Gereau RW IV, Minami M, Heinemann SF, Harris RA: Effects of ethanol and anesthetics on type 1 and 5 metabotropic glutamate receptors expressed in *Xenopus* laevis oocytes. Mol Pharmacol 1998;53: 148–156.

10 Minami K, Minami M, Harris RA: Inhibition of 5-hydroxytryptamine type 2A receptor-induced currents by n-alcohols and anesthetics. J Pharmacol Exp Ther 1997;281: 1136–1143.

11 Minami K, Shiraishi M, Uezono Y, Ueno S, Shigematsu A: The inhibitory effects of anesthetics and ethanol on substance P receptors expressed in *Xenopus* oocytes. Anesth Analg 2002;94:79–83.

12 Minami K, Sudo Y, Shiraishi S, Seo M, Uezono Y: Analysis of the effects of anesthetics and ethanol on μ-opioid receptor. J Pharmacol Sci 2010;112:424–431.

13 Caulfield MP, Birdsall NJ: International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 1998;50:279–290.

14 Dascal N: The use of *Xenopus* oocytes for the study of ion channels. CRC Crit Rev Biochem 1987;22:317–387.

15 Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T, Kanaide M, Taniyama K, Sumikawa K, Uezono Y: mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. J Pharmacol Sci 2008;108:308–319.

16 Vorobiov D, Bera AK, Keren-Raifman T, Barzilai R, Dascal N: Coupling of the muscarinic m2 receptor to G protein-activated K(+) channels via Galpha(z) and a receptor-Galpha(z) fusion protein: fusion between the receptor and Galpha(z) eliminates catalytic (collision) coupling. J Biol Chem 2000;275: 4166–4170.

17 Minami K, Uezono Y, Sakurai T, Horishita T, Shiraishi M, Ueta Y: Effects of anesthetics on the function of orexin-1 receptors expressed in *Xenopus* oocytes. Pharmacology 2007;79: 236–242.

18 Minami K, Uezono Y, Shiraishi M, Okamoto T, Ogata J, Horishita T, Taniyama K, Shigematsu A: Analysis of the effects of halothane on Gi-coupled muscarinic M_2 receptor signaling in *Xenopus* oocytes using a chimeric G alpha protein. Pharmacology 2004;72: 205–212.
19 Minami K, Vanderah TW, Minami M, Harris RA: Inhibitory effects of anesthetics and ethanol on muscarinic receptors expressed in *Xenopus* oocytes. Eur J Pharmacol 1997; 339:237–244.
20 Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, Loriolle F, Duhamel L, Charon D, Kirilovsky J: The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J Biol Chem 1991;266:15771–15781.
21 Gilman AG: G proteins: transducers of receptor-generated signals. Annu Rev Biochem 1987;56:615–649.
22 Frink EJ Jr, Malan TP, Atlas M, Dominguez LM, DiNardo JA, Brown BR Jr: Clinical comparison of sevoflurane and isoflurane in healthy patients. Anesth Analg 1992;74:241–245.
23 Holaday DA, Smith FR: Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers. Anesthesiology 1981;54:100–106.
24 Smith FL, Gabra BH, Smith PA, Redwood MC, Dewey WL: Determination of the role of conventional, novel and atypical PKC isoforms in the expression of morphine tolerance in mice. Pain 2007;127:129–139.
25 Smith FL, Javed RR, Elzey MJ, Dewey WL: The expression of a high level of morphine antinociceptive tolerance in mice involves both PKC and PKA. Brain Res 2003;985:78–88.
26 Zeitz KP, Malmberg AB, Gilbert H, Basbaum AI: Reduced development of tolerance to the analgesic effects of morphine and clonidine in PKC gamma mutant mice. Pain 2001;94: 245–253.
27 Kelly E, Bailey CP, Henderson G: Agonist-selective mechanisms of GPCR desensitization. Br J Pharmacol 2008;153(suppl 1):S379–S388.
28 Hasegawa J, Takekoshi S, Nagata H, Osamura RY, Suzuki T: Sevoflurane stimulates MAP kinase signal transduction through the activation of PKC alpha and betaII in fetal rat cerebral cortex cultured neuron. Acta Histochem Cytochem 2006;39:163–172.
29 Bouwman RA, Musters RJ, van Beek-Harmsen BJ, de Lange JJ, Lamberts RR, Loer SA, Boer C: Sevoflurane-induced cardioprotection depends on PKC-alpha activation via production of reactive oxygen species. Br J Anaesth 2007;99:639–645.

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Short Communication

Possible Involvement of β-Endorphin in a Loss of the Coordinated Balance of μ-Opioid Receptors Trafficking Processes by Fentanyl

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KEY WORDS internalization/recycling pathway; opioids; receptor trafficking; fentanyl

BACKGROUND

It has been considered that opioid tolerance is, in part, the end result of a coordinated balance between processes that govern the desensitization, internalization, and resensitization of μ-opioid receptors (MOR) (Claing et al., 2002; Gainetdinov et al., 2004). However, a several line of evidence suggests that the trafficking properties of MORs driven by MOR agonists may depend on intrinsic characters of each agonist, and are still complicated. Previous biochemical studies on cultured enteric neurons have indicated that fentanyl induces either the functional desensitization or internalization of MORs (Minnis et al., 2003). In contrast, under the same condition, morphine does not promote the detectable internalization of MORs in cultured cells after prolonged or acute treatment in healthy animals, although it has been well-established that morphine causes the development of tolerance to its pharmacological actions (Minnis et al., 2003). However, recent studies have demonstrated that morphine activates MORs with promoting internalization of MORs via β-arrestin-2-dependent mechanisms in striatal neurons (Haberstock-Debic et al., 2005).

In the previous study, we demonstrated that repeated treatment with fentanyl, but not morphine, causes a rapid desensitization to its ability to block the hyperalgesia associated with the attenuation of MOR resensitization in mice with inflammatory pain (Imai et al., 2006). Based on this study, we hypothesized that released β-endorphin within the spinal cord under a chronic pain-like state may be implicated in the rapid development of tolerance to fentanyl, but not morphine and oxycodone. Namely, these findings raise the possibility that β-endorphin could attenuate the resensitization of MOR after the treatment with fentanyl, resulting in the high degree of tolerance to fentanyl-induced antihyperalgesic effects under long-lasting pain state. To further address this issue, this cell culture study was performed to investigate the effects of fentanyl on MOR internalization and resensitization in the presence or absence of β-endorphin.

MATERIALS AND METHODS

Baby hamster kidney (BHK) cells (Riken Cell Bank, Tsukuba, Japan) were grown in Dulbecco's

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modified eagle medium (DMEM: Invitrogen™) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Transient transfection was then performed with Effectene transfection reagent (Qiagen, Tokyo, Japan) in 0.2 μg of each cDNA according to the protocol provided by the manufacturer. Cells were used in confocal microscopy 16–24 h after transfection. cDNA for rat MOR was kindly provided by Dr. Dascal (Tel Aviv University). Venus, a brighter variant of yellow fluorescent protein (Nagai et al., 2002) was obtained from Dr. T. Nagai (Riken, Wako, Japan). Primers (5′-GGG GTA CCC CAT GGA CAG CAG CAC-3′) and (5′-GCG GCC GCG GGG CAA TGG AGC AGT-3′) were engineered to ligate the N-terminus of MOR by using standard molecular approaches with the polymerase chain reaction (PCR). Venus-fused MOR was created by ligating the MOR cDNA sequences into the *NotI* site of the corresponding Venus site. cDNA for transfection in BHK cells was subcloned into pcDNA3.1 (Invitrogen™ Life Technologies, CA). cDNA for rat β-arrestin 2 was generously provided by Dr. Y. Nagayama (Nagasaki University, Japan). For the analysis of the agonist-induced internalization of MORs, BHK cells that had been transfected with Venus-fused MORs and β-arrestin-2 were incubated in the absence or presence of 100 nM β-endorphin for 30 min at 37°C, and then treated with 10 μM morphine, 100 nM fentanyl or 10 μM oxycodone. To investigate the resensitization of MORs, the cells were incubated with 100 nM fentanyl or 10 μM oxycodone in the presence or absence of β-endorphin, and then apposed for 30 min, 90 min, 3 h, or 6 h at 37°C. The cells were subsequently fixed and examined by confocal microscopy as previously reported (Corbani et al., 2004). Venus was excited by a 488-nm laser was used to detect Venus fluorescence with a 505- to 530-nm band-pass filter, and images were obtained by placing the dish on the stage of an inverted Zeiss LSM510 META confocal microscope (Carl Zeiss, Jena, Germany). Data were stored on the hard disc with and analyzed with the Zeiss LSM software Zen 2009. For the quantitative analysis of agonist-induced internalization of MORs, BHK cells were fixed with 4% paraformaldehyde in PBS and stored at 4°C. The numbers of cells expressing Venus-fused MORs were counted. For counting cells whether Venus fluorescence was at the plasma membrane or in cytosol (internalization), we basically followed by Corbani et al. (2004). Localization of Venus-fused MORs in BHK cells was categorized as "mainly expressed at the plasma membrane," "not detected in plasma membrane but detected in cytosol," or "not detected" (whose localization was not belong to the former category), separated with a software Zen 2009 equipped with Zeiss LSM510 META confocal microscope, with reference to the control, not stimulated BHK cells. A total of 100 cells (counted mean 200–250 cells in sum of "the plasma membrane," "in the cytosol," plus "not detected") in six independent each dish. % Internalization was described as cytosol × 100/[plasma membrane + cytosol (total 100 cells)]. The drugs used in this study were fentanyl citrate (Hisamitsu Pharmaceutical, Tokyo, Japan), morphine hydrochloride

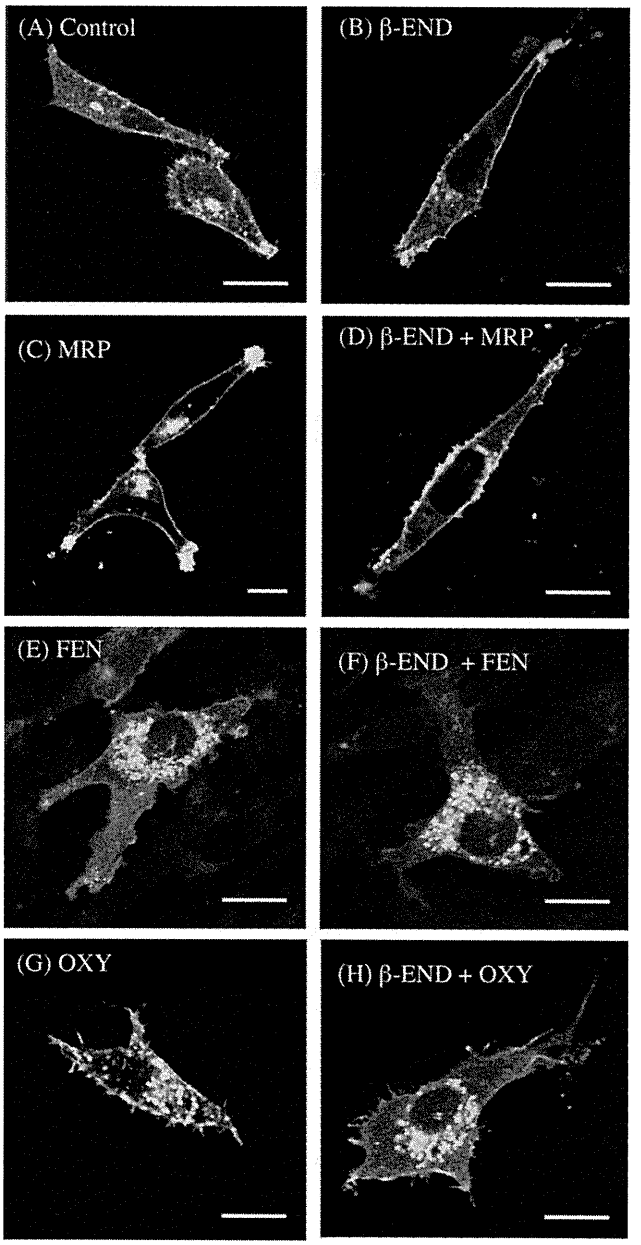

Fig. 1. Confocal imaging of agonist-induced internalization of MORs in BHK cells expressing Venus-fused MORs. The cells were incubated in the absence (**A**, **C**, **E**, and **G**) or presence (**B**, **D**, **F**, and **H**) of 100 nM β-endorphin (β-END) for 30 min at 37°C and then treated with 10 μM morphine (MRP; C, D), 100 nM fentanyl (FEN; E, F), or 10 μM oxycodone (OXY; G, H). The cells were subsequently fixed and examined by confocal microscopy. Yellow fluorescence from Venus indicates the localization of MORs in BHK cells. Scale bars, 10 μm.

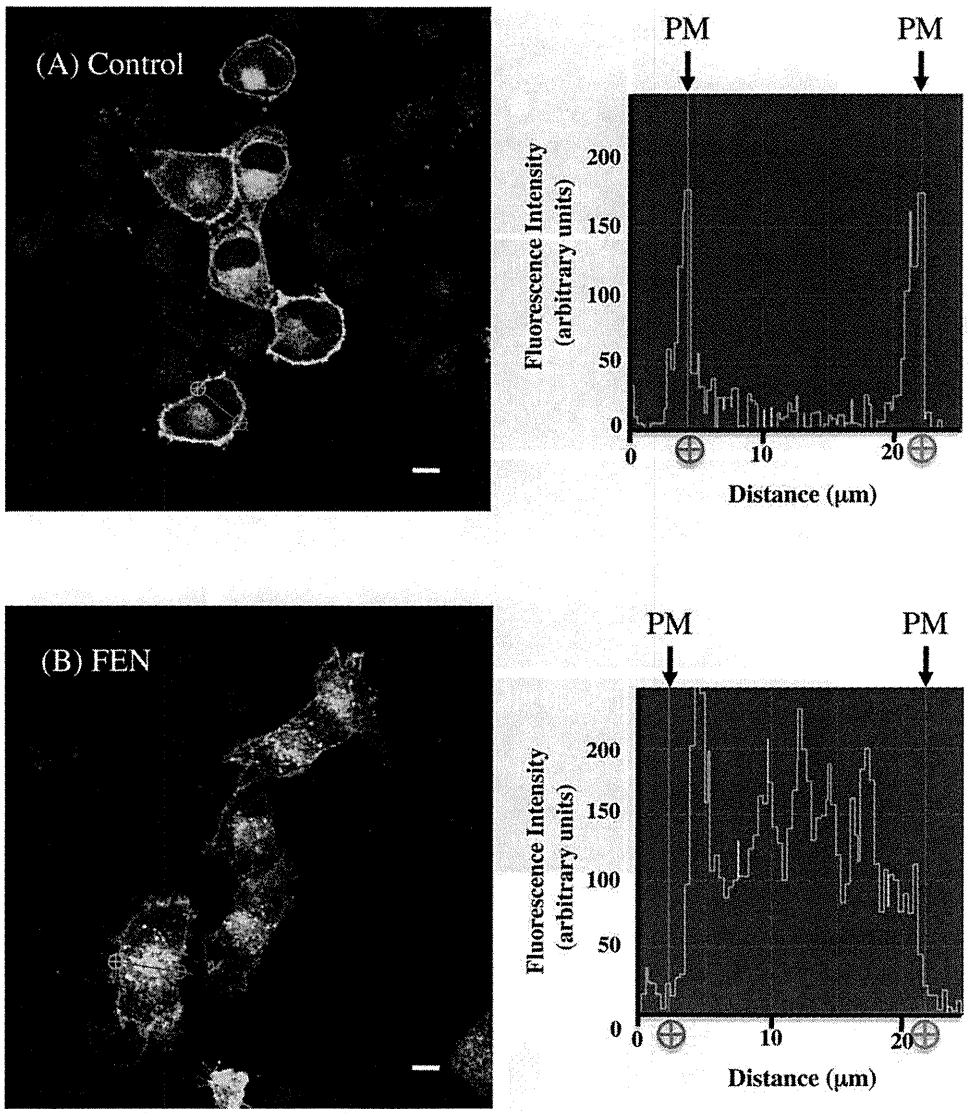

Fig. 2. Confocal imaging of agonist-induced internalization of MORs in BHK cells expressing Venus-fused MORs. Typical cells where most of MOR-Venus intensity was at the plasma membranes, [**A**, control cells (Control)] or in the cytosolic fraction [**B**, 100 nM fentanyl-stimurated for 30 min (FEN)]. PM; plasma membranes in BHK cells. Scale bars, 10 μm.

(Daiichi-Sankyo, Tokyo, Japan), oxycodone hydrochloride (a kind gift from Shionogi Pharmaceutical, Osaka, Japan), and β-endorphin (Sigma-Aldrich, St Louis, MO), which were dissolved in assay buffer.

RESULTS AND DISCUSSION

In this study, we assessed whether β-endorphin could affect the trafficking properties of MORs using immunocytochemical methods in BHK cells with confocal microscope. Confocal imaging of the BHK cells expressing Venus-fused MOR with β-arrestin-2 revealed that the yellow fluorescence was largely confined to the plasma membrane (Figs. 1A and 2A). In both the presence and absence of 100 nM β-endorphin, at which concentration there did not cause any internalization of MORs (Figs. 1B and 1C), cells expressing MORs treated with 10 μM morphine (Figs. 1C and 1D) showed little internalization of MORs, while the cells treated with 100 nM fentanyl (Figs. 1E, 1F, and 2B) and 10 μM oxycodone (Figs. 1G and 1H) showed robust internalization of the receptor. These findings were consistent with previous reports that fentanyl and etorphine caused partial internalization, while morphine failed to induce detectable MOR endocytosis (Koch et al., 2005). We next investigated the resensitization properties of MORs after the washing-out of agonists. In the absence of β-endorphin, internalized MOR returned to the plasma membrane from 90 min after the washing-out of fentanyl (Figs. 3B–3D). However, in the presence of β-endorphin, the internalized MOR induced by fentanyl

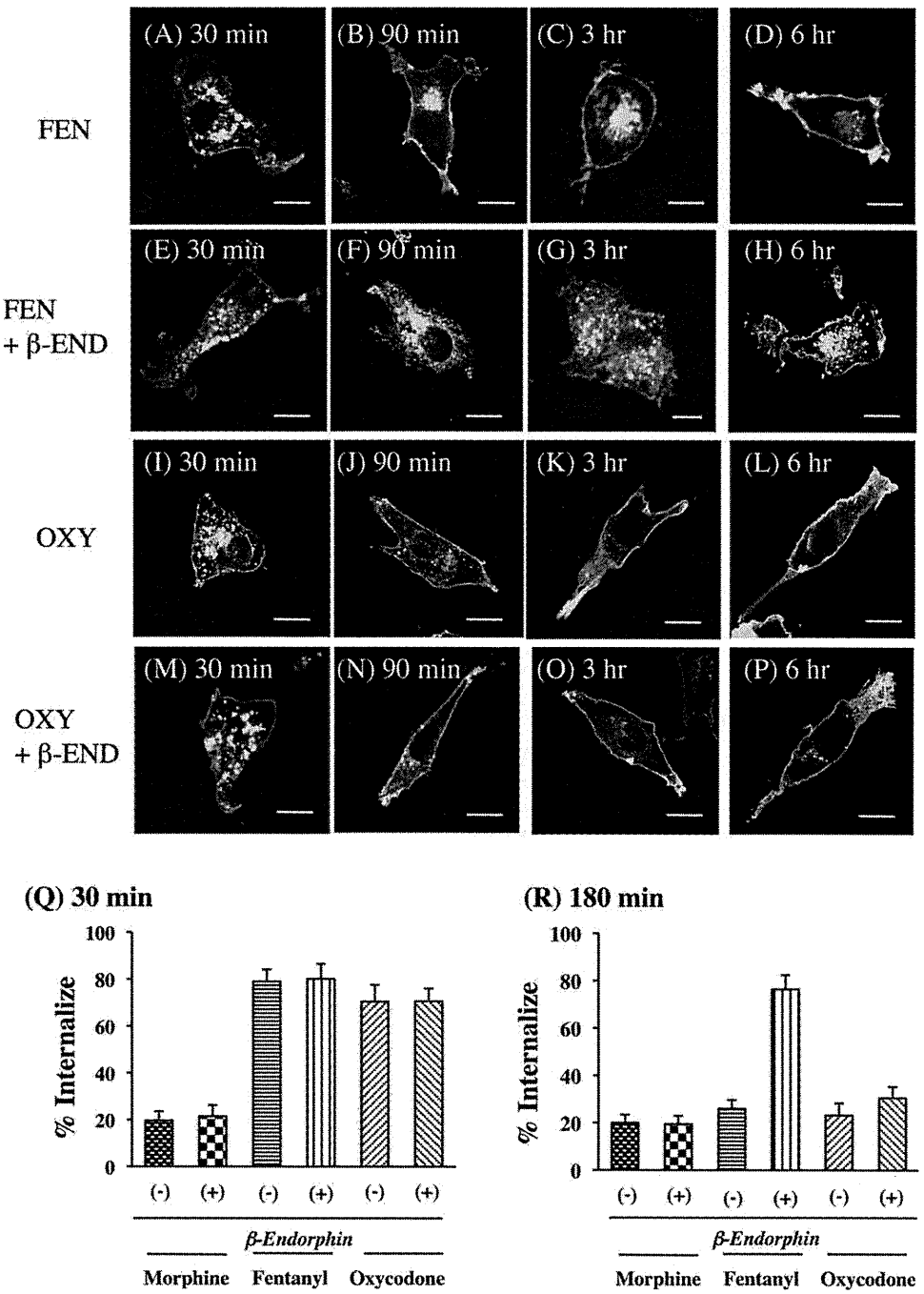

Fig. 3. Confocal imaging of resensitization of MORs in BHK cells expressing Venus-fused MORs. Cells were incubated with 100 nM fentanyl (**A–H**) or 10 μM oxycodone (**I–P**) in the absence (A-D and I-L) or presence (E-H and M-P) of β-endorphin, and then apposed for 30 min, 90 min, 3 h, or 6 h at 37°C. The cells were then fixed and counted by confocal microscopy. Yellow fluorescence from Venus indicates the cellular localization of MOR in BHK cells. Scale bars, 10 μm. Quantitative analysis of the % of the internalized cells expressing Venus-fused MORs treated with the drugs for 30 min (**Q**) or 180 min (**R**), respectively. The agonist concentrations represent the dose required to induce the maximal effect on receptor endocytosis for each drug. Each value represents the mean ± SEM of six separate experiments.

remained in the cytosolic fraction at 3–6 h after the washing-out of β-endorphin and fentanyl (Figs. 3F–3H). However, in both the presence and absence of β-endorphin, the internalized MOR induced by oxycodone returned to the plasma membrane after the washing-out of agonist in a time-dependent manner (Figs. 3I–3P). We performed quantitative analysis of the agonist-induced internalization of MORs after the washing-out of each agonist shown in Materials and Methods. At 30 min after the washing-out of agonists,

cells treated with fentanyl or oxycodone showed robust internalization of MORs (fentanyl: 79.0 ± 5.14%, β-endorphin fentanyl: 80.2 ± 3.7%, oxycodone: 70.5 ± 7.09%, β-endorphin oxycodone: 70.7 ± 5.35%), which was not seen in morphine-treated cells (morphine: 19.67 ± 3.93%, β-endorphin morphine: 21.5 ± 4.76%; Fig. 3Q). However, while there was no difference in the degree of oxycodone-induced MOR internalization between the presence and absence of β-endorphin 3 h after washing-out (oxycodone: 23.17 ± 5.12%, β-endorphin oxycodone: 30.5 ± 4.72%), in fentanyl-treated cells, β-endorphin caused the prolonged internalization of MORs and fluorescence was stayed in the cytosolic fraction (fentanyl: 27.67 ± 5.47%, β-endorphin fentanyl: 76.5 ± 6.02%; Fig. 3R).

It has been widely accepted that receptor desensitization, internalization and trafficking appear to play a key role in the development of opioid tolerance (Claing et al., 2002; Gainetdinov et al., 2004). The initial process in these events is the phosphorylation of intracellular domains of MOR. Phosphorylated MORs are mostly internalized via clathrin-coated pits into early endosomes and subsequently dephosphorylated by intracellular protein phosphatases. The dephosphorylated MORs might either be recycled to the plasma membrane or transported to lysosomes for degradation. A growing body (Smalheiser and Lugli) of evidence suggests that among diverse serine/threonine (Thr) residues of the intracellular domain of MOR, the phosphorylation of Ser 375 in the mouse MOR is essential for the internalization of MORs (Schulz et al., 2004). In a previous study, we found that repeated treatment with fentanyl, but not morphine, resulted in an increase in the levels of phosphorylated-MOR (Ser 375) associated with the enhanced inactivation of protein phosphatase 2A and a reduction in Rab4-dependent MOR resensitization in the spinal cord of mice that showed inflammatory pain (Imai et al., 2006). However, several lines of evidence indicate that, in response to pain stimulus, endogenous β-endorphin is released within some brain regions (Zubieta et al., 2001). We previously reported that β-endorphin released in the ventral tegmental area is a key factor in regulating the dysfunction of MOR to negatively modulate opioid reward under a neuropathic pain-like state (Niikura et al., 2008, 2010). Taken together, although further studies are still needed, these findings support the idea that inhibition of the resensitization system of MOR following chronic treatment with fentanyl in the presence of β-endorphin may be associated with antihyperalgesic tolerance to fentanyl under a chronic pain-like state.

In conclusion, we demonstrated here that unlikely morphine, either fentanyl or oxycodone induced a robust MOR internalization and, in turn, its resensitization. In the presence of β-endorphin, the internalized MOR induced by fentanyl, but not oxycodone, remained within the cytosolic fraction even after washing out. These findings strongly support that idea that fentanyl has different pharmacological profile form that of morphine or oxycodone.

REFERENCES

Claing A, Laporte SA, Caron MG, Lefkowitz RJ. 2002. Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog Neurobiol 66:61–79.

Corbani M, Gonindard C, Meunier JC. 2004. Ligand-regulated internalization of the opioid receptor-like 1: A confocal study. Endocrinology 145:2876–2885.

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. 2004. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 27:107–144.

Haberstock-Debic H, Kim KA, Yu YJ, von Zastrow M. 2005. Morphine promotes rapid, arrestin-dependent endocytosis of μ-opioid receptors in striatal neurons. J Neurosci 25:7847–7857.

Imai S, Narita M, Hashimoto S, Nakamura A, Miyoshi K, Nozaki H, Hareyama N, Takagi T, Suzuki M, Suzuki T. 2006. Differences in tolerance to anti-hyperalgesic effects between chronic treatment with morphine and fentanyl under a state of pain. Nihon Shinkei Seishin Yakurigaku Zasshi 26:183–192.

Koch T, Widera A, Bartzsch K, Schulz S, Brandenburg LO, Wundrack N, Beyer A, Grecksch G, Hollt V. 2005. Receptor endocytosis counteracts the development of opioid tolerance. Mol Pharmacol 67:280–287.

Minnis JG, Patierno S, Kohlmeier SE, Brecha NC, Tonini M, Sternini C. 2003. Ligand-induced mu opioid receptor endocytosis and recycling in enteric neurons. Neuroscience 119:33–42.

Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. 2002. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. Nat Biotechnol 20:87–90.

Niikura K, Narita M, Nakamura A, Okutsu D, Ozeki A, Kurahashi K, Kobayashi Y, Suzuki M, Suzuki T. 2008. Direct evidence for the involvement of endogenous β-endorphin in the suppression of the morphine-induced rewarding effect under a neuropathic pain-like state. Neurosci Lett 435:257–262.

Niikura K, Narita M, Butelman ER, Kreek MJ, Suzuki T. 2010. Neuropathic and chronic pain stimuli downregulate central μ-opioid and dopaminergic transmission. Trends Pharmacol Sci 31:299–305.

Schulz S, Mayer D, Pfeiffer M, Stumm R, Koch T, Hollt V. 2004. Morphine induces terminal micro-opioid receptor desensitization by sustained phosphorylation of serine-375. EMBO J 23:3282–3289.

Smalheiser NR, Lugli G. 2009. microRNA regulation of synaptic plasticity. Neuromolecular Med 11:133–140.

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. 2001. Regional μ opioid receptor regulation of sensory and affective dimensions of pain. Science 293:311–315.

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研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tomohisa Mori, Masahiro Shibasaki, Minako Abe, Yuya Udagawa, Tsutomu Suzuki.	Effects of dronabinol on morphine-induced dopamine-related behavioral effects in animals.	Synapse			acceptable
Tomohisa Mori, Yuka Sudo, Masahiro Shibasaki, Minoru Hojo, Hiroshi Yoneyama, Masato Harumiya, Noriyuki Nishida, Yasuhito Uezono, Tsutomu Suzuki.	Synergistic interactions between μ-opioid and cannabinoid CB_1 receptors: an in vivo and in vitro study.	J Pharmacol. Sci.			in preparation

分担研究者　山口重樹

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
細川豊史，井関雅子，奥田泰久，加藤実，佐伯茂，住谷昌彦，廣瀬宗孝，福井弥己郎，山口重樹	神経障害性疼痛薬物療法ガイドライン	日本ペインクリニック学会神経障害性疼痛薬物療法ガイドライン作成ワーキンググループ	神経障害性疼痛　薬物療法ガイドライン	真興交易	東京	2011	11-50
山口重樹，Donald R Taylor	癌患者への対応について述べよ	寺野彰，一杉正仁	カラーイラストで学ぶ集中講義：医事法学・法医学	メジカルビュー社	東京	2012	58-59
山口重樹，Donald R Taylor	望ましい緩和ケアにつて述べよ	寺野彰，一杉正仁	カラーイラストで学ぶ集中講義：医事法学・法医学	メジカルビュー社	東京	2012	60-61
山口重樹，Donald R Taylor	癌性疼痛とその治療について述べよ	寺野彰，一杉正仁	カラーイラストで学ぶ集中講義：医事法学・法医学	メジカルビュー社	東京	2012	62-64
山口重樹，Donald R Taylor	オピオイドをどのように使用すればよいですか？	宗圓聰，紺野愼一	運動器の痛みをとる・やわらげる	メジカルビュー社	東京	2012	112-116

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
山口重樹，北島敏光，Donald R Taylor	新しい疼痛コントロールの基本	レジデントノート	13(9)	1615-1621	2011
山口重樹，北島敏光，Donald R Taylor	新しいオピオイドを活用する，フェントス®テープ	緩和ケア	21(6)	578-581	2011

山口重樹，永尾勝，北島敏光，Donald R Taylor	痛みに対する緩和治療オピオイドの使い分け.	プロフェッショナルがんナーシング	1	320-323	2011
山口重樹，北島敏光，Donald R Taylor	高用量アセトアミノフェンの使い方，従来の約2倍量が投与可能に	日経メディカル	528	121-123	2011
山口重樹	最新のがん性疼痛マネジメントの知識.	ホスピスケア	22	17-42	2011
山口重樹，Donald R Taylor	オピオイドは患者を救うことができるのか？	ペインクリニック	32	629-630	2011
山口重樹，北島敏光，Donald R Taylor	プライマリケア医に必要な鎮痛薬の知識～アセトアミノフェンはプライマリケアに必須の鎮痛薬～	日本医事新報	4579	95-100	2012
矢吹省司，中村雅也，牛田享宏，山口重樹，西田圭一郎	運動器慢性疼痛の診療－現状をめぐる問題－	Locomotive Pain Froniter	1	5-13	2012
山口重樹	突出痛ごとに自己決定した用量のレスキュー・ドーズを使うことで，ADLを高く保ち，母親の介護を続けることが可能であった乳がん骨転移患者	がんの痛みをとる	11	6-7	2012

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神経障害性疼痛薬物療法ガイドライン

Guidelines for the Pharmacologic Management of Neuropathic Pain

一般社団法人日本ペインクリニック学会
神経障害性疼痛薬物療法ガイドライン
作成ワーキンググループ・編

The Committee for the Guidelines for the Pharmacologic Management of Neuropathic Pain of JSPC

真興交易㈱医書出版部

はじめに

神経障害性疼痛（神経障害痛と同義[脚注1]）は，“体性感覚系に対する損傷や疾患によって直接的に引き起こされる疼痛”と定義され[1]，疼痛疾患の中ではその重症度が高く，罹病期間が長い．さらに，著しい生活の質（quality of life：QOL）の低下をもたらす[2,3]．先進国の神経障害性疼痛の罹患率は1～7%と推定されており[2,4]，わが国では数百万人規模の神経障害性疼痛患者が存在すると推測される．

神経障害性疼痛にはさまざまな疾患が含まれている（表1）が，神経障害性疼痛患者の多くは特徴的な性質の痛みを訴え（表2），その病態には共通点があると考えられる．疼痛患者の診療の際に，①痛みの範囲が神経解剖学的に妥当であり，なおかつ体性感覚系の損傷や神経疾患を疑う症状を伴い，②神経系の損傷あるいは疾患を示唆するのに妥当な理学所見（客観的所見）や画像所見などがある時に，神経障害性疼痛と診断する（図1）[7]．

神経障害性疼痛の範疇に含まれる多様な疼痛疾患と，それに対する多様な治療法を一律に扱うことは困難であるが，神経障害性疼痛全般に対する初期診療としては薬物療法が挙げられる．これまでに，神経障害性疼痛を対象に多くの薬物についての知見が蓄積されてきているが，神経障害性疼痛は既存の治療薬に対する反応が不十分なことや，確立された神経障害性疼痛の薬物療法であってもその有効性を確実に予測することはできず，他薬を併用しなければならない

脚注1：「Neuropathic pain」の邦訳は，「神経障害痛，神経障害性疼痛」とされている（日本ペインクリニック学会用語集第3版）．2009年の用語委員会からの報告（長櫓　巧，寺井岳三，西江宏行，他：Neuropathic painの和訳：日本ペインクリニック学会・用語委員会報告．日本ペインクリニック学会誌2009；16：509-14）では，調査の最新時点では神経障害性疼痛という邦訳が最も使用頻度が高いことが明らかにされ，それに基づいて用語委員会がneuropathicの邦訳として“神経障害性”を提言している．さらに“末梢性神経障害性疼痛”が厚生労働省によって適応症として承認されている社会的背景を鑑み，本ガイドラインでは神経障害性疼痛という用語に統一することとした．