ELSEVIER

Neuroscience Letters 357 (2004) 159–162

Neuroscience Letters

www.elsevier.com/locate/neulet

Group II metabotropic glutamate receptors inhibit cAMP-dependent protein kinase-mediated enhancement of tetrodotoxin-resistant sodium currents in mouse dorsal root ganglion neurons

Dongni Yang[a], Robert W. Gereau IV[a,b,*]

[a]*Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030, USA*
[b]*Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA*

Received 16 October 2003; received in revised form 17 November 2003; accepted 24 November 2003

Abstract

Tetrodotoxin (TTX)-resistant sodium currents are important in nociception and nociceptive sensitization, which is partially due to their cAMP/protein kinase A (PKA)-mediated enhancement. Here we studied the effects of group II mGluR activation on TTX-resistant sodium currents in cultured mouse dorsal root ganglion (DRG) neurons. Activation of adenylyl cyclase with forskolin caused an increase in the amplitude of TTX-R currents and a leftward shift of the activation curve. When neurons were treated with ammonium pyrrolidinedithiocarbamate (APDC), a selective group II mGluR agonist, both the forskolin-induced increase in current amplitude and the shift of activation curve were blocked. LY341495, a group II mGluR antagonist, prevented these inhibitory effects of APDC. Our results suggest that group II mGluRs can negatively regulate TTX-R sodium currents in mouse DRG neurons.
© 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: mGluR; Forskolin; mGlu2; Pain; Nociception; Inflammation; Primary sensory neuron

Tetrodotoxin (TTX)-resistant sodium channels are expressed in primary nociceptive sensory neurons and are insensitive to tetrodotoxin at concentrations in the micromolar range [5]. These channels play a major role in generating the action potentials that signal pain and are thought to play an important role in inflammation-induced pain sensitization [8]. Reducing TTX-resistant currents by either knocking out $Na_{v1.8}$, the gene encoding the majority of these currents, or injecting anti-sense $Na_{v1.8}$ RNA can significantly decrease pain sensation or pain sensitization in various models [1,12–14,20]. Many inflammatory mediators, including prostaglandin E_2 (PGE_2), serotonin, adenosine, nitric oxide and endothelin-1 can enhance TTX-R sodium currents [11,21]. The cAMP/protein kinase A (PKA) pathway is known to play an important role in the modulation of TTX-resistant currents [3,6,10]. Considering the importance of TTX-resistant sodium currents in nociceptive transmission and inflammatory pain, agents that negatively regulate these currents could potentially reduce pain transmission or hypersensitivity.

Activation of mu-opioid receptors blocks PGE_2-induced enhancement of TTX-R, which is likely to contribute to the anti-nociceptive effects of opioids [9]. The group II mGluRs (mGlu2/3) are also candidate negative modulators of TTX-R. Systemic or intrathecal application of group II mGluR agonists can produce anti-nociceptive effects [7,16,17]. We recently reported that activation of peripheral group II metabotropic glutamate receptors decreases inflammation-induced thermal hyperalgesia and mechanical allodynia [18,19]. These receptors are known to couple to G_i, and in mouse DRG neurons mGlu2/3 negatively regulate cAMP/PKA-mediated sensitization of TRPV1 receptors by inhibiting adenylyl cyclase [18]. In this study, we examined whether group II mGluRs could also act as negative modulators of TTX-R currents in dorsal root ganglia (DRG) neurons.

Dorsal root ganglia isolated from 4–6-week-old male CD1 mice were enzymatically treated and mechanically triturated as described previously [18]. Dissociated cells were plated on

* Corresponding author. Present address: Washington University Pain Center, Department of Anesthesiology, Washington University School of Medicine, 660 S. Euclid Ave, Campus Box 8054, St. Louis, MO 63110. Tel.: +1-314-362-8312; fax: +1-314-362-8571.

E-mail address: gereaur@morpheus.wustl.edu (R.W. Gereau).

0304-3940/03/$ - see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2003.11.074

12-mm glass coverslips coated with poly-D-lysine and collagen (Sigma) and grown in medium containing Neurobasal medium, 10% fetal bovine serum, 100 U/ml penicillin/streptomycin and 2 mM Glutamax (all from Invitrogen) at 37 °C in humidified air with 5% CO_2.

Ammonium pyrrolidinedithiocarbamate (APDC) and LY341495 were purchased from Tocris Cookson (Ballwin, MO) and dissolved in NaOH of the same concentration or 1.2 times the concentration, respectively. Forskolin (Sigma) was dissolved in dimethyl sulfoxide (DMSO) with final concentration of DMSO in the external solution $< 0.1\%$. In all experiments where forskolin is used, 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor (5 μM, Sigma), was added.

DRG cells cultured for 12–24 h were used in whole-cell voltage-clamp experiments. Electrodes were pulled using a Narishige PC-10 puller to final resistance of 1–4 MΩ and fire-polished using Narishige MF-830. The internal solution contained (mM): CsCl 100, NaCl 10, $CaCl_2$ 0.1, EGTA 5, HEPES 5, TEA 30, $MgCl_2$ 4, Na-ATP 3, Na-GTP 0.5; pH 7.35. The external solution contained (mM): CsCl 10, NaCl 100, $CaCl_2$ 0.1, $MgCl_2$ 2, HEPES 10, TEA 30, $CdCl_2$ 0.1, glucose 10, $CoCl_2$ 2, 4-AP 1; pH 7.35. TTX (250 nM, Alomone Labs, Jerusalem, Israel) was added to the external solution to isolate TTX-resistant currents. All experiments were conducted at room temperature. Membrane currents were amplified using an Axonpatch-200B, low-pass filtered at 5 kHz and then digitized using Digidata 1322A and collected using pCLAMP8 software (all from Axon Instruments, Foster City, CA). Series resistance (3–8 MΩ) and capacitance currents were compensated. Conductance was calculated using the equation: $G = I/(V - V_m)$, where V is the reversal potential calculated from $[Na^+]_{internal}$ and $[Na^+]_{external}$. Activation and steady-state inactivation curves were fitted using a Boltzmann equation: $G = G_{max}/(1 + \exp((V_{0.5} - V_m)/k))$. Inactivation time constants were obtained by exponential fits of the falling-phase of currents. Data are expressed as mean ± S.E.M. Drug effects on TTX-R sodium currents were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons. The voltage of half-maximum activation, $V_{0.5}$, was obtained from each individual cell and shifts in $V_{0.5}$ were analyzed using paired Student's t-tests. Error probabilities of $P < 0.05$ were considered statistically significant. All statistical analysis was done using Graphpad Prism and Origin software.

Previous studies have shown that TTX-resistant sodium currents are enhanced by PKA activation [6,10]. In our DRG neuron cultures, we recorded sodium currents that persisted in the presence of 250 nM TTX (Fig. 1a). These currents activated around -20 mV and reached maximum conductance at about 10 mV. The average $V_{0.5}$ of TTX-R was -10.0 ± 0.1 mV. The steady-state inactivation started at around -50 mV and had $V_{0.5}$ at -23.6 ± 0.1 mV (Fig. 1).

Consistent with previous reports [6,10], the adenylyl cyclase activator forskolin dose-dependently increased

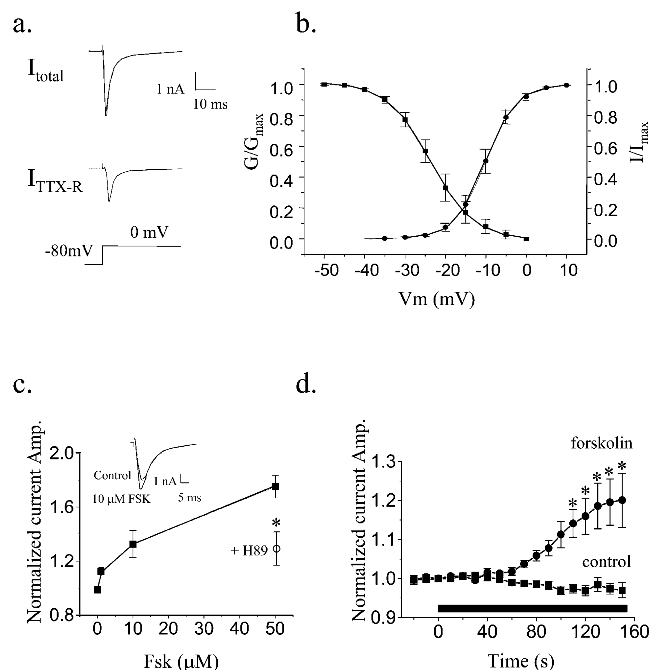

Fig. 1. TTX-resistant sodium currents in mouse DRG neurons are sensitized by adenylyl cyclase activation. (a) Representative traces of Na^+ currents. Neurons were clamped at -80 mV and a 50 ms voltage step to 0 mV was used to activate Na^+ currents. In the presence of 250 nM TTX, I_{TTX-R} was isolated. (b) Steady-state inactivation curve and activation curve of I_{TTX-R}. (c) Forskolin (2 min) dose-dependently increased the amplitude of TTX-R sodium currents. H89 (10 μM, open circle) significantly reduced the effect of 50 μM forskolin. $n = 5–11$. Inset: representative traces showing the effect of 10 μM forskolin. (d) Forskolin (1 μM, $n = 8$) increased the amplitude of TTX-resistant sodium currents. Bar represents forskolin perfusion. Cells were held at -80 mV, a 50 ms step to 0 mV was used to activate TTX-R. *$P < 0.05$ (two-way ANOVA).

TTX-resistant sodium currents (Fig. 1c), and the PKA inhibitor H89 (10 μM) significantly reduced the effect of 50 μM forskolin. High concentrations of forskolin have been reported to have non-specific effects on ion channels [2,4]. To minimize non-specific effects, 1 μM forskolin was used to study the effect of group II mGluR activation. In forskolin-treated neurons, G_{max} increased by 3 ± 1 μSiemens ($n = 7$, $P < 0.05$, paired t-test). Forskolin (1 μM) also increased the amplitude of TTX-resistant sodium currents activated by depolarization from -80 to 0 mV (Fig. 2a,b). The increase of G_{max} and current amplitude was accompanied by a leftward shift of the activation curve. The average change of $V_{0.5}$ is 4.7 ± 0.7mV ($n = 7$) (Fig. 2c). No change in the slope factor of the activation curve, steady-state inactivation, $T_{0.5}$ activation or Tau-inactivation was observed as compared to control neurons.

We previously showed that peripheral activation of group II mGluRs could inhibit PGE_2- or carrageenan-induced inflammatory pain [18,19]. One possible mechanism by which group II mGluRs could inhibit inflammatory pain is by blocking the cAMP/PKA-mediated enhancement of TTX-resistant sodium currents. We therefore studied the effect of activating group II mGluRs on TTX-R sodium

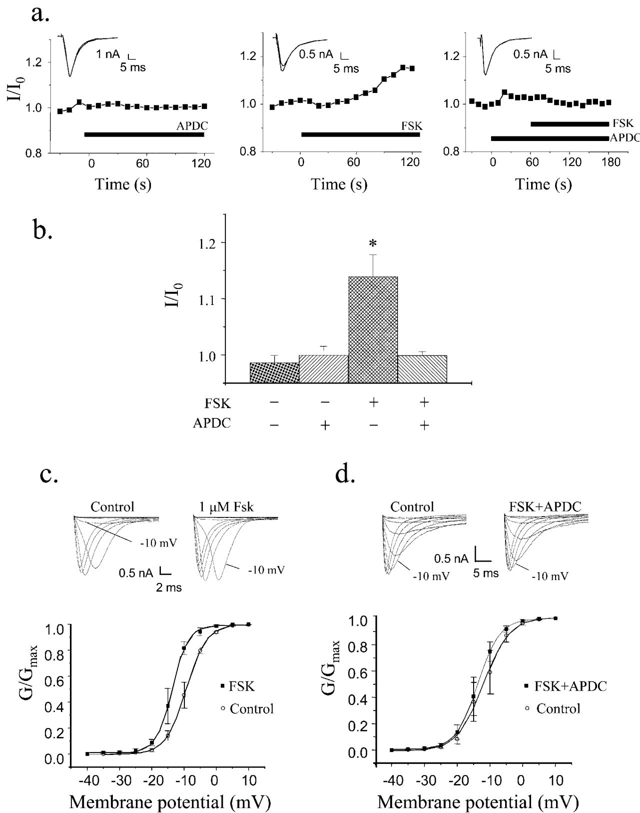

Fig. 2. Activation of group II mGluRs blocks forskolin-induced sensitization of TTX-R. (a) The effect of APDC, forskolin (FSK) and APDC + FSK on TTX-R currents. Cells were given 2 min for stabilization before recording. Traces from before and 2 min after APDC or FSK application are shown. Bars represent drug perfusion. The average of three current amplitudes before drug perfusion was taken as I_0 to which all current amplitudes were normalized. (b) Mean ± S.E.M. of normalized current amplitude. Application of APDC (5 μM, 2 min) alone did not change current amplitude. Application of forskolin (1 μM, 2 min) increased the current amplitude and 5 μM APDC completely blocked the effect of forskolin. $n = 6–11$, $*P < 0.05$, significantly different from control (one-way ANOVA). (c) Forskolin induced a leftward shift of the activation curve. (d) APDC blocked the shift of the activation curve, $P < 0.05$.

currents in cultured mouse DRG neurons. Application of the selective group II mGluR agonist, APDC (5 μM), did not alter basal TTX-R currents (Fig. 2a,b). However, when the APDC was applied 1 min in advance and together with forskolin, the forskolin-induced increase of TTX-R amplitude was totally blocked (Fig. 2b). Application of APDC also blocked the forskolin-induced increase in G_{max} and the leftward shift of the activation curve. G_{max} change and $V_{0.5}$ change were −1 ± 2 μSiemens and 1.8 ± 0.8 mV ($n = 6$), respectively, as compared to 3 ± 1 μSiemens and 4.7 ± 0.7 mV ($n = 7$) in forskolin-treated cells (Fig. 2d). To verify that the effects we observed with APDC were due specifically to activation of group II mGluRs, we used a potent group II mGluR antagonist, LY341495. LY341495 (250 nM) blocked the APDC effects on the forskolin-induced enhancement of TTX-R, confirming the specific involvement of group II mGluRs (Fig. 3).

Both APDC and LY341495 can affect other groups of

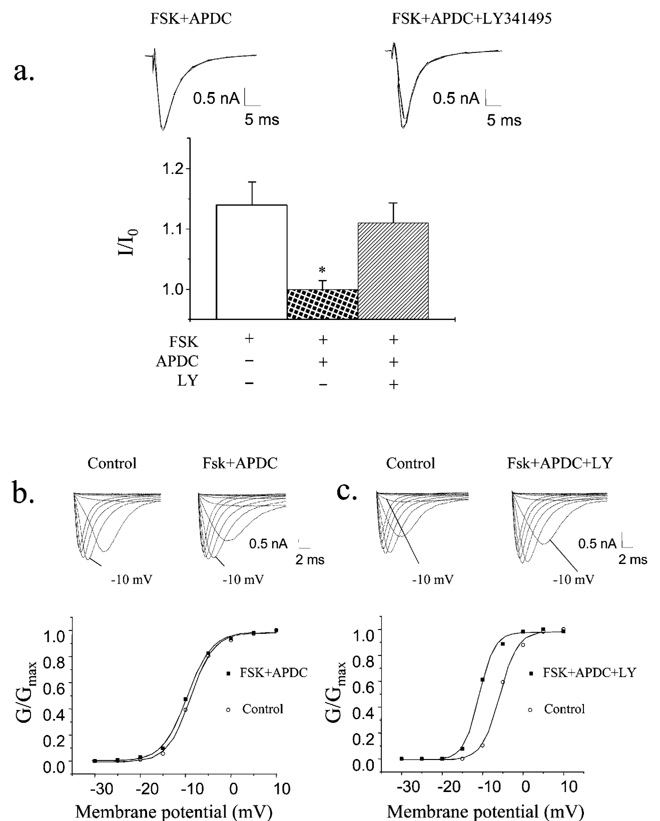

Fig. 3. The selective group II mGluR antagonist LY341495 blocks the effects of APDC. (a) LY341495 (250 nM) prevented APDC from blocking the forskolin-induced increase of TTX-R current amplitude $*P < 0.05$; ANOVA. (b) Currents recorded from a series of membrane depolarizations and the corresponding activation curve showing that LY31495 prevented APDC from blocking the forskolin-induced shift of activation curve.

mGluRs at high concentrations. APDC can activate group I and III mGluRs with $EC_{50} > 100$ μM and > 300 μM, respectively [15]. The concentration used here was 5 μM. Therefore, the effects we reported are unlikely due to activation of group I or III mGluRs. Application of 250 nM LY341495, which acts as a group II mGluR antagonist with an IC_{50} of < 30 nM, completely blocked the APDC effects, further supporting a specific involvement of group II mGluRs. However, the IC_{50} of LY341495 at some group III mGluRs is in the nanomolar range and we cannot rule out a possible involvement of group III mGluRs.

We showed that 1 μM forskolin significantly enhanced TTX-R current, inducing an increase of current amplitude elicited by a step from −80 mV to 0 mV, and a leftward shift of activation curve. The percentage increase of current amplitude we observed with 1 μM forskolin is smaller than what has been previously reported [6,10], likely due to the choice of depolarizing membrane potential. At more hyperpolarized potentials, where the activation curve is steeper, a larger effect is seen (Fig. 2c). A forskolin-induced change in activation kinetics has been reported previously [6,10]. We did not see significant change of activation kinetics in the present study. This may be due in part to the

fact that the membrane voltages of some neurons were not clamped well.

In conclusion, we demonstrate here that activation of group II mGluRs in mouse DRG neurons inhibits forskolin-induced cAMP/PKA-mediated enhancement of TTX-R sodium currents. These results are consistent with previous studies showing that TTX-R sodium currents are important for inflammation-induced pain sensitization (reviewed in [8]) and that activation of peripheral group II mGluRs inhibits inflammatory pain [18,19]. In addition to negative regulation of TRPV1 receptors, negative regulation of TTX-R sodium currents may represent a mechanism by which activation of peripheral group II mGluRs can inhibit inflammation-induced pain hypersensitivity. However, these are unlikely the only targets of group II mGluRs and we expect that more mechanisms for the anti-nociceptive effects of group II mGluRs will be found.

Acknowledgements

This work was funded by grants to RG from the National Alliance for Research on Schizophrenia and Depression and the NINDS (NS42595).

References

[1] A.N. Akopian, V. Souslova, S. England, K. Okuse, N. Ogata, J. Ure, A. Smith, B.J. Kerr, S.B. McMahon, S. Boyce, R. Hill, L.C. Stanfa, A.H. Dickenson, J.N. Wood, The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways, Nat. Neurosci. 2 (1999) 541–548.

[2] D.A. Baxter, J.H. Byrne, Reduction of voltage-activated K + currents by forskolin is not mediated via cAMP in pleural sensory neurons of Aplysia, J. Neurophysiol. 64 (1990) 1474–1483.

[3] L.M. Cardenas, C.G. Cardenas, R.S. Scroggs, 5HT increases excitability of nociceptor-like rat dorsal root ganglion neurons via cAMP-coupled TTX-resistant Na(+) channels, J. Neurophysiol. 86 (2001) 241–248.

[4] N.A. Castle, Inhibition of voltage-dependent Na + and K + currents by forskolin in nodes of Ranvier, Pflugers Arch. 415 (1989) 322–329.

[5] A.A. Elliott, J.R. Elliott, Characterization of TTX-sensitive and TTX-resistant sodium currents in small cells from adult rat dorsal root ganglia, J. Physiol. 463 (1993) 39–56.

[6] S. England, S. Bevan, R.J. Docherty, PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade, J. Physiol. 495 (Pt. 2) (1996) 429–440.

[7] K. Fisher, C. Lefebvre, T.J. Coderre, Antinociceptive effects following intrathecal pretreatment with selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain, Pharmacol. Biochem. Behav. 73 (2002) 411–418.

[8] M.S. Gold, Tetrodotoxin-resistant Na + currents and inflammatory hyperalgesia, Proc. Natl. Acad. Sci. USA 96 (1999) 7645–7649.

[9] M.S. Gold, J.D. Levine, DAMGO inhibits prostaglandin E2-induced potentiation of a TTX-resistant Na + current in rat sensory neurons in vitro, Neurosci. Lett. 212 (1996) 83–86.

[10] M.S. Gold, J.D. Levine, A.M. Correa, Modulation of TTX-R I_{NA} by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro, J. Neurosci. 18 (1998) 10345–10355.

[11] M.S. Gold, D.B. Reichling, M.J. Shuster, J.D. Levine, Hyperalgesic agents increase a tetrodotoxin-resistant Na + current in nociceptors, Proc. Natl. Acad. Sci. USA 93 (1996) 1108–1112.

[12] S.G. Khasar, M.S. Gold, J.D. Levine, A tetrodotoxin-resistant sodium current mediates inflammatory pain in the rat, Neurosci. Lett. 256 (1998) 17–20.

[13] J.M. Laird, V. Souslova, J.N. Wood, F. Cervero, Deficits in visceral pain and referred hyperalgesia in Nav1.8 (SNS/PN3)-null mice, J. Neurosci. 22 (2002) 8352–8356.

[14] F. Porreca, J. Lai, D. Bian, S. Wegert, M.H. Ossipov, R.M. Eglen, L. Kassotakis, S. Novakovic, D.K. Rabert, L. Sangameswaran, J.C. Hunter, A comparison of the potential role of the tetrodotoxin-insensitive sodium channels, PN3/SNS and NaN/SNS2, in rat models of chronic pain, Proc. Natl. Acad. Sci. USA 96 (1999) 7640–7644.

[15] D.D. Schoepp, D.E. Jane, J.A. Monn, Pharmacological agents acting at subtypes of metabotropic glutamate receptors, Neuropharmacology 38 (1999) 1431–1476.

[16] E.F. Sharpe, A.E. Kingston, D. Lodge, J.A. Monn, P.M. Headley, Systemic pre-treatment with a group II mGlu agonist, LY379268, reduces hyperalgesia in vivo, Br. J. Pharmacol. 135 (2002) 1255–1262.

[17] R.M. Simmons, A.A. Webster, A.B. Kalra, S. Iyengar, Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats, Pharmacol. Biochem. Behav. 73 (2002) 419–427.

[18] D. Yang, R.W. Gereau, Peripheral group II metabotropic glutamate receptors (mGluR2/3) regulate prostaglandin E2-mediated sensitization of capsaicin responses and thermal nociception, J. Neurosci. 22 (2002) 6388–6393.

[19] D. Yang, R.W. Gereau, Peripheral group II metabotropic glutamate receptors mediate endogenous anti-allodynia in inflammation, Pain 106 (2003) 411–417.

[20] N. Yoshimura, S. Seki, S.D. Novakovic, E. Tzoumaka, V.L. Erickson, K.A. Erickson, M.B. Chancellor, W.C. de Groat, The involvement of the tetrodotoxin-resistant sodium channel Na(v)1.8 (PN3/SNS) in a rat model of visceral pain, J. Neurosci. 21 (2001) 8690–8696.

[21] Z. Zhou, G. Davar, G. Strichartz, Endothelin-1 (ET-1) selectively enhances the activation gating of slowly inactivating tetrodotoxin-resistant sodium currents in rat sensory neurons: a mechanism for the pain-inducing actions of ET-1, J. Neurosci. 22 (2002) 6325–6330.