TEMPERATURE AND PH SENSITIVITY OF $P2X_3$ RECEPTOR DESENSITIZATION

O.A. KRISHTAL, V.V. KHMYZ, O.P. MAKSYMIUK

Bogomoletz Institute of Physiology, Ukraine
krishtal@biph.kiev.ua

***Oleh Krishtal**, Ph.D. (1971), D. Sc. (1978), born on July 5, 1945. He received his University degree in Molecular Physics (1968) from Taras Shevchenko Kyiv State University. Since then he has been working at h Institute of Physiology starting as a post-graduate student. Now Prof. O. Kryshtal is the head of the Department of Cellular Membranology (since 1985) and first deputy director (since 2003).*

He is Full Member of the Ukrainian Academy of Sciences (1997), Corresponding Member of the Russian Academy of Sciences (1987), Member of Academia Europaea (1990). O. Kryshtal has served as a visiting professor of University of Kyushu, Japan (1983, 1986), Harvard University, USA (1989), Complutense University, Madrid, Spain (1993), University of Pennsylvania, USA (1994). He served as an expert panel member of international scientific foundation "Welcome Trust" (2002-2005). Since 2000 Prof. Kryshtal has been President of Ukrainian National Society for Neuroscience and since 2009 Member of Governing Council of IBRO. He is also a Member of Supervisory Board of the Institute of Molecular and Cellular Biology (Warsaw, Poland). He is a laureate of the State Prize of the USSR for Science (1983) and State Prize of Ukraine for Science (2003). From 1995 to 2000 O. Kryshtal was a foreign investigator at Howard Hughes Medical Institute. He is Member of Dana Alliance.

Prof. O. Kryshtal is a member of Editorial Board in the following international scientific journals: Neuroscience, Autonomic Neuroscience, Purinergic Signaling, Central European Journal of Biology, Frontiers in Neuroscience. He has above 300 scientific publications including more than 100 in leading peer-reviewed journals. He supervised more than 20 PhDs who nowadays work at leading international universities and institutions.

***V. Khmyz** was born on March 20, 1978. In 2001, he graduated from Taras Shevchenko National University. Since 2004, he has been a scientific researcher of the Department of Cellular Membranology at the Bogomoletz Institute of Physiology. He has scientific publications in leading Ukrainian and international journals.*

***O. Maksymiuk**, Ph.D. (2004), was born on April 8, 1975. He got his University degree in Radiophysics (1997) from Taras Shevchenko National University. Since then he has been working all the time at Bogomoletz Institute of Physiology starting as a post-graduate student. Now Dr. O. Maxymiuk is a leading scientific researcher of the Department of Cellular Membranology at the same institute. He has 15 scientific publications in leading Ukrainian and international journals.*

Introduction

$P2X_3$ receptors are widely expressed in the dorsal root ganglion (DRG) neurons responsible for primary sensory functions including nociception (Vulchanova et al., 1998). Knockout studies strongly linked $P2X_3$ receptors to inflammatory pain, warm-coding and volume reflexes of the urinary bladder (Cockayne et al., 2000; Souslova et al., 2000b; Shimizu et al., 2005b). In response to ATP application, $P2X_3$ receptors desensitize within tens of milliseconds; at the same time, up to 30 min are required for the recovery. Recent data indicate that desensitization of $P2X_3$ receptors is use-dependent and occurs within a nanomolar range of background ATP concentrations (Pratt et al., 2005a), while in the tissues the ATP level is 20-100 nM, especially rising in inflammation, ischemia, muscle functioning, renal failure, *etc.* (Vassort, 2001; Li et al., 2003). These findings may suggest that $P2X_3$ subunits may play their strong physiological roles only as heteromeric forms with $P2X_2$ subunits, but not as homomeric channels. Furthermore, our data demonstrate that only 6 ms long application of ATP is sufficient to induce almost complete desensitization of $P2X_3$ receptors. This result confirms a high-affinity site hypothesis suggested by Pratt *et al.* (Pratt et al., 2005b). However, in accordance with the earlier studies, $P2X_3$ receptors are the main subtype of P2X receptors that expressed in nociceptive DRG neurones. Correspondingly, the question arises: how can these receptors be functional?

By combining patch-clamp with extracellular temperature-clamp techniques, we examined the biophysical behavior of $P2X_3$ receptors in sensory neurons isolated from DRGs of rats at different temperatures ranging from 25 °C to 40 °C. In about 80% of cultured DRG neurons voltage-clamped at –60 mV 3 s long application of 10 μM ATP evoked a large inward current, which completely decayed to the baseline in the presence of the agonist, indicating full desensitization. A rapid current decline, which reflects the onset of desensitization, was best fitted with the sum of two exponentials (τ_{fast} = 14.7 ± 1 ms and τ_{slow} = 231 ± 20 ms, n = = 23). On the contrary, the recovery from desensitization measured at 25 °C was extremely slow and sigmoid with the characteristic time constant τ_{rec} of 6.77 ± 0.55 min (n = 8) and at least 25 min were necessary to obtain a similar response after the first ATP application.

Temperature sensitivity of $P2X_3$ receptors recovery from desensitization

Our preliminary observations showed that only one-minute long exposure of the cell at 35 °C resulted in the complete recovery of previously desensitized ATP response. An increase in temperature also increased the amplitudes of $P2X_3$ mediated currents with the corresponding energy of activation of 30 ± 1.31 kJ/M.

The Q_{10} value for the increase in current amplitude was 1.52 ± 0.026 (n = 23; between 25 °C and 35 °C). It coincides with typical values of Q_{10} (1.3-1.6) reported for ion channels (Hille, 2001). However, the sensitivity of $P2X_3$ receptors to ATP was temperature-independent within the observed range from 25 °C to 40 °C: the EC_{50} was close to 1 μM at all temperatures (n = 12).

To characterize the temperature dependence of recovery from desensitization, we performed a series of 10 μM ATP applications at different time intervals after control application. The characteristic time constants of recovery (τ_{rec}) were (in min): 2.1 ± 0.26, n = 5 at 30 °C; 0.73 ± 0.065, n = 6 at 35 °C, and 0.26 ± 0.019, n = 5 at 40 °C. The recovery from desensitization calculated from Arrhenius plot requires a considerable energy of activation of 167 ± 1.39 kJ/M indicating possible bonding cleavage process. The corresponding Q_{10} for the temperature dependence of τ_{rec} was 9.03 ± 0.016 (between 25°C and 35°C; n = 24). Conversely, the onset of desensitization, which was reflected in current decline, was absolutely temperature independent. The two-exponential fit of the current decay did not reveal significant differences in the fast and slow time constants (p = 0.12 and p = 0.49, respectively; one-way ANOVA test).

The absence of temperature dependence is highly unusual phenomenon in biology. The temperature independence (or rather a weak dependence) has been reported for phosphorylation-dephosphorylation processes, when the rates of opposite reactions have similar temperature dependences, thus producing a net compensation (Youn et al., 1998). Another example of the temperature independence is represented by temperature-compensated circadian rhythms (Tsuchiya et al., 2003). In all these cases, the independence of a certain parameter of temperature is a result of interaction of several complex processes providing cross-compensation.

The recovery from desensitization of $P2X_3$ receptors is dependent on extracellular pH

Increased temperature and slight acidosis are typical phenomena for inflammatory processes, and $P2X_3$ receptors are crucial elements in the induction of inflammatory pain. So, we decided to test the action of protons on the $P2X_3$ receptor desensitization recovery. We found that the recovery from desensitization of native $P2X_3$ receptors expressed in DRG neurons is accelerated by a drop in extracellular pH. In our experiments, moderate acidic conditions (pH = 6.4) between ATP applications produced a twofold increase in desensitization recovery. Under strong acidic conditions (pH = 4.4) only 1 min was necessary for the complete current recovery. In the case of vesicular release, ATP molecules are accompanied with a dozen of protons (pH ~ 4-5). So, we can expect that the $P2X_3$ receptor function should be strongly facilitated.

Discussion

Our data suggest that increased temperature and acidosis provide strong up-regulation of the function of $P2X_3$ receptor. The observed increase in desensitization recovery may result in functional receptors even in the presence of low nanomolar background ATP. A strong temperature dependence of $P2X_3$ receptor desensitization recovery is a good candidate for the major mechanism of cold-mediated analgesia. The temperature-independence of desensitization onset (or compensation, whatever its mechanism) and the high temperature-dependence of recovery can be important for some specific functions of $P2X_3$ receptors. These receptors may provide a specifically calibrated temperature-sensing system, which produces standardized duration bursts of signals irrespectively to the temperature changes. Indeed, genetic deletion of $P2X_3$ receptors resulted in a serious warm-coding deficits, when $P2X_3$ deficient mice were unable to code the intensity of non-noxious "warming" stimuli (Souslova et al., 2000a). The $P2X_3$ deficient animals also showed a considerably enhanced thermal avoidance (Shimizu et al., 2005a). The temperature independence of relevant receptor molecules, which could be important to produce sensitive and calibrated sensors, may employ certain specific form of gating, such as, e.g., electron transfer, which is barely affected by temperature changes.

Acknowledgements. Research in the authors' laboratory is supported by grants from CRDF and HHMI.

REFERENCES

Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP (Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407: 1011-1015.2000).

Hille B (2001) Ion channels of excitable membranes: Sunderland, Mass.: Sinauer.

Li J, King NC, Sinoway LI (ATP concentrations and muscle tension increase linearly with muscle contraction. J Appl Physiol 95: 577-583.2003).

Pratt EB, Brink TS, Bergson P, Voigt MM, Cook SP (Use-dependent inhibition of P2X3 receptors by nanomolar agonist. J Neurosci 25: 7359-7365.2005b).

Pratt EB, Brink TS, Bergson P, Voigt MM, Cook SP (Use-dependent inhibition of P2X3 receptors by nanomolar agonist. J Neurosci 25: 7359-7365.2005a).

Shimizu I, Iida T, Guan Y, Zhao C, Raja SN, Jarvis MF, Cockayne DA, Caterina MJ (Enhanced thermal avoidance in mice lacking the ATP receptor $P2X_3$. Pain 116: 96-108.2005b).

Shimizu I, Iida T, Guan Y, Zhao C, Raja SN, Jarvis MF, Cockayne DA, Caterina MJ (Enhanced thermal avoidance in mice lacking the ATP receptor $P2X_3$. Pain 116: 96-108.2005a).

Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebenuis-Oosthuizen D, Smith AJ, Kidd EJ, Wood JN (Warm-coding deficits and aberrant inflammatory pain in mice lacking $P2X_3$ receptors. Nature 407: 1015-1017.2000a).

Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebenuis-Oosthuizen D, Smith AJ, Kidd EJ, Wood JN (Warm-coding deficits and aberrant inflammatory pain in mice lacking $P2X_3$ receptors. Nature 407: 1015-1017.2000b).

Tsuchiya Y, Akashi M, Nishida E (Temperature compensation and temperature resetting of circadian rhythms in mammalian cultured fibroblasts. Genes Cells 8: 713-720.2003).

Vassort G (Adenosine 5‘-triphosphate: a P2-purinergic agonist in the myocardium. Physiol Rev 81: 767-806.2001).

Vulchanova L, Riedl MS, Shuster SJ, Stone LS, Hargreaves KM, Buell G, Surprenant A, North RA, Elde R (P2X3 is expressed by DRG neurons that terminate in inner lamina II. Eur J Neurosci 10: 3470-3478.1998).

Youn T, Kim SA, Hai CM (Length-dependent modulation of smooth muscle activation: effects of agonist, cytochalasin, and temperature. Am J Physiol 274: C1601-C1607. 1998).