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## TEMPERATURE AND PH SENSITIVITY OF P2X<sub>3</sub> RECEPTOR DESENSITIZATION

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## Introduction

P2X<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> subunits may play their strong physiological roles only as heteromeric forms with P2X<sub>2</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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 ( $\tau_{\text{fast}} = 14.7 \pm 1$  ms and  $\tau_{\text{slow}} = 231 \pm 20$  ms,  $n = 23$ ). On the contrary, the recovery from desensitization measured at 25 °C was extremely slow and sigmoid with the characteristic time constant  $\tau_{\text{rec}}$  of  $6.77 \pm 0.55$  min ( $n = 8$ ) and at least 25 min were necessary to obtain a similar response after the first ATP application.

## Temperature sensitivity of P2X<sub>3</sub> 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<sub>3</sub> mediated currents with the corresponding energy of activation of  $30 \pm 1.31$  kJ/M.

The  $Q_{10}$  value for the increase in current amplitude was  $1.52 \pm 0.026$  ( $n = 23$ ; 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<sub>3</sub> receptors to ATP was temperature-independent within the observed range from 25 °C to 40 °C: the  $EC_{50}$  was close to 1  $\mu$ M at all temperatures ( $n = 12$ ).

To characterize the temperature dependence of recovery from desensitization, we performed a series of 10  $\mu$ M ATP applications at different time intervals after control application. The characteristic time constants of recovery ( $\tau_{rec}$ ) were (in min):  $2.1 \pm 0.26$ ,  $n = 5$  at 30 °C;  $0.73 \pm 0.065$ ,  $n = 6$  at 35 °C, and  $0.26 \pm 0.019$ ,  $n = 5$  at 40 °C. The recovery from desensitization calculated from Arrhenius plot requires a considerable energy of activation of  $167 \pm 1.39$  kJ/M indicating possible bonding cleavage process. The corresponding  $Q_{10}$  for the temperature dependence of  $\tau_{rec}$  was  $9.03 \pm 0.016$  (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<sub>3</sub> receptors is dependent on extracellular pH**

Increased temperature and slight acidosis are typical phenomena for inflammatory processes, and P2X<sub>3</sub> receptors are crucial elements in the induction of inflammatory pain. So, we decided to test the action of protons on the P2X<sub>3</sub> receptor desensitization recovery. We found that the recovery from desensitization of native P2X<sub>3</sub> 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<sub>3</sub> receptor function should be strongly facilitated.

## Discussion

Our data suggest that increased temperature and acidosis provide strong up-regulation of the function of P2X<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> receptors resulted in a serious warm-coding deficits, when P2X<sub>3</sub> deficient mice were unable to code the intensity of non-noxious "warming" stimuli (Souslova et al., 2000a). The P2X<sub>3</sub> 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.

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