PHARMACOLOGY AND TOXICOLOGY

Effect of Temperature on Guinea Pig Urinary Bladder Contraction Mediated via P2X-Receptors

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In vitro experiments showed that P2X-receptor agonist α,β-methylene-ATP and electrical field stimulation in the presence of muscarinic and α-adrenoreceptors blockers induced contractile responses of isolated guinea pig bladder, which were more pronounced at 30°C than at 37°C or 42°C. P2X-receptor antagonist pyridoxal-6-phosphate-2',4'-disulfonic acid, produced a more potent inhibitory effect on contractions induced by electrical field stimulation at 30°C in comparison with that at 37°C or 42°C, while the contractions induced by α,β-methylene-ATP were similarly suppressed at all examined temperatures.

Key Words: *P2X-receptors; bladder; temperature dependence*

ATP receptors (P2-receptors) were revealed in various tissues, the bladder of guinea pig included [1]. Activation of these receptors with α,β-methylene-ATP, an agonist of P2X-receptors, or electric field induces phasic contractions, which are inhibited with P2X-receptor antagonists suramin and pyridoxal-6-phosphate-2',4'-disulfonic acid (PPADS) [6,12,13]. Most experiments with P2-receptors were carried out under standard physiological conditions; however, there is evidence that the responses mediated by P2-receptors depend on pH [7,10]. Our aim was to study the effect of temperature on the contractile responses mediated by P2X-receptors.

MATERIALS AND METHODS

Guinea pigs weighing 350-550 g were euthanized and dehematized. The bladder was isolated, 2×10-mm smooth muscle preparations were prepared and placed in 10-ml thermostabilized vials to measure isomeric contraction. The tissue was rested under a load of 1 g for about 1 h to adapt to the medium. Electrical field stimulation (EFS) was performed with two platinum rings with a diameter of 2.5 mm, through which the muscle strip was threaded. The modified Krebs solution contained (in mM): 133 NaCl, 4.7 KCl, 16.3 $NaHCO_3$, 0.6 $MgCl_2$, 1.35 $NaHPO_4$, 2.5 $CaCl_2$, 7.8 $C_2H_{12}O_6$, oxygenated with 95% O_2 and 5% CO_2 (pH 7.3-7.4). In EFS experiments, atropine (3×10^{-7} M) and phentolamine (10^{-6} M) were added. The contractions were measured with an FSG-01 transducer (Linton) coupled with a Biopack digitizer and imputed to PC. EFS was performed with a Grass S9 stimulator. The duration, amplitude, and frequency of pulses were 0.5 msec, 100 V, and 1-64 Hz, respectively. α,β-Methylene-ATP (10^{-8}-3×10^{-5} M) was added to vials, and after attaining the maximum contraction the preparation was rapidly washed several times in fresh Krebs solution to prevent desensitization of P2X-receptors. All contractile responses were calculated as the percentage of maximum response to KCl (240 mM), which was introduced into the solution at the end of the experiment.

The temperature was regulated with a Techne TE-8A pump by changing the temperature of circulating fluid. In all experiments, the initial stages and adaptation were carried out at 37±1°C. Two successive contractions induced by α,β-methylene-ATP (3×10^{-6}

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M) or EFS were recorded at the same temperature to test the stability of the contractile responses. Then in some experiments the temperature of the incubation solution was decreased to 30±1°C, while in others it was increased 42±1°C. The samples were adapted to the chosen temperature during 10-15 min, and then contractions in response to α,β-methylene-ATP or EFS were recorded. These contractions were repeated at 37±1°C and at 42±1°C or 30±1°C. In some experiments, the contractile responses were recorded only in two temperature regiments, one of which was 37°C. At the end of the experiment, the response to KCl was measured at 37°C.

The effect PPADS on the contractile responses induced by α,β-methylene-ATP or EFS was assessed only at one temperature (30, 37, or 42°C). Initial adaptation and KCl-induced contractions at the end of experiment were always recorded at 37°C. The responses to α,β-methylene-ATP or EFS were always recorded before and after successive incubation with PPADS in concentrations of 10^{-6} M, 3×10^{-6} M, 10^{-5} M, and 3×10^{-5} M for at least 25 min.

The experimental dose-response or frequency-response curves were analyzed using nonlinear regression analysis. The maximum response was determined, and the negative logarithm of α,β-methylene-ATP molar concentration (pD_2) or logarithm of EFS frequency producing the half-maximum response ($logEF_{50}$) were calculated. The data were processed statistically using Student's *t* test and presented as $M\pm m$.

RESULTS

At normal temperature (37°C), α,β-methylene-ATP induced concentration-dependent contractions of isolated bladder smooth muscle preparation (Fig. 1, *a*). The highest of the test concentrations (3×10^{-5} M) produced contractions constituting 21.3±5.1% of those induced by 240 M KCl. At 30°C, α,β-methylene-ATP induced a more potent contraction than at 37°C, which manifested by a shift of the dose-response curve to the left. At this temperature, the maximum response was observed at agonist concentration of 10^{-5} M (37.4± 4.1%, n=8). Increasing the temperature to 42eC slightly inhibited the contractile responses induced by α,β-methylene-ATP in comparison with that at 37°C. pD_2 for α,β-methylene-ATP were 6.68±0.11 (n=8), 6.24± 0.10 (n=8), and 5.91±0.08 (n=6) at 30, 37, and 42°C, respectively (all differences are significant).

In the presence of atropine and phentolamine, EFS performed at 37°C induced frequency-dependent contractile responses of isolated bladder preparations. The maximum contractions were observed at 16 Hz (48.5± 3.9%, n=14). Decreasing the temperature to 30°C shifted the frequency-response curve to the left (Fig. 1, *b*), which was accompanied by a significant increase in the maximum response to 53.9±5.8% (n=10). At 42°C, EFS induced weaker responses compared to those at 30 and 37°C: the maximum response decreased to 29.0±2.2% (n=14). The values of $logEF_{50}$ at 30, 37, and 42°C were 0.36±0.15 (n=10), 0.60±0.03 (n=13), and 0.59±0.04 (n=14), respectively (the differences with contractions at 30°C are significant).

At all tests temperatures, P2-receptor antagonist PPADS produced concentration-dependent inhibition of bladder contractions induced by α,β-methylene-ATP and significantly changed pD_2 of this agonist: pD2 calculated for PPADS in a concentration of 10^{-5} M were 5.60±0.06 (n=4), 5.64±0.17 (n=4), and 5.22±0.42 (n=3) for 30, 37, and 42°C, respectively (all differences are insignificant).

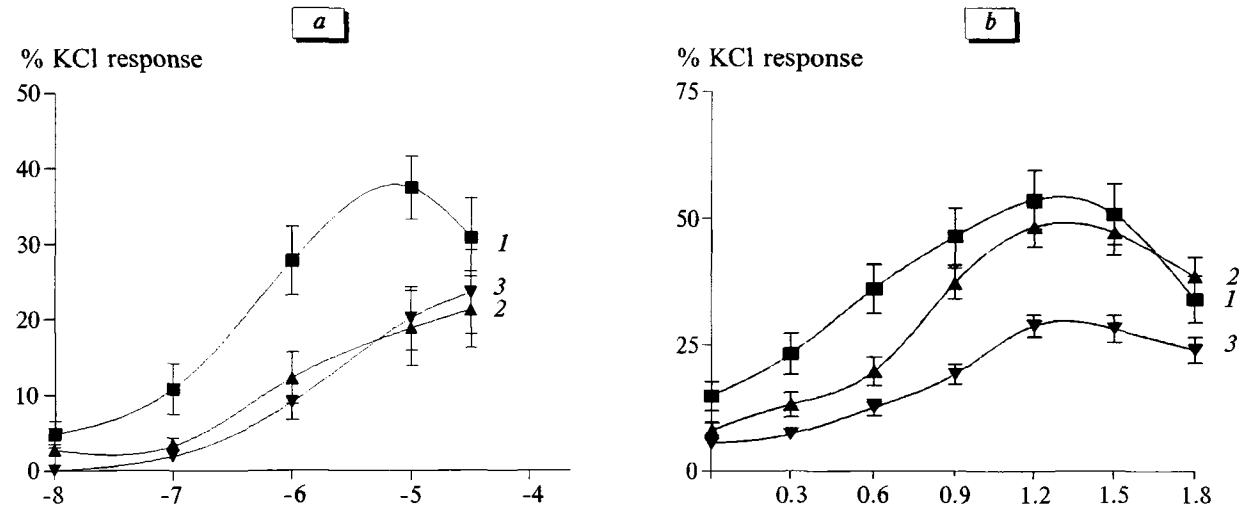

Fig. 1. Effect of α,β-methylene-ATP (*a*) and electrical field stimulation (*b*) on contractile responses of isolated guinea pig bladder in the presence of atropine (0.3 μM) and phentolamine (1 μM) at 30°C (*1*, n=8), 37°C (*2*, n=8), and 42°C (*3*, n=6). Abscissa: logarithm of molar concentration of α,β-methylene-ATP (*a*) and logarithm of frequency of electrical field stimulation (*b*) inducing half-maximum response.

Moreover, PPADS inhibited the contractile responses of the bladder to EFS. pD_2 for PPADS in a concentration of 10^{-5} M was 4.96±0.07 (n=4) at 30°C, which significantly differed from pD_2 measured at 37 and 42°C: 4.15±0.22 (n=4) and 4.00±0.35 (n=4), respectively.

The temperature dependence of some receptor-mediated processes was measured in various human and animal tissues. For example, temperature drop to 24°C diminishes histamine-induced contractions of the central auricular, but not femoral artery in rabbits [3], and promotes relaxation induced by cholinoreceptor stimulation [9]. It was also shown that at 30°C the α-adrenoreceptor-mediated responses of the central auricular artery decreased, while P2-mediated responses became more pronounced [4].

Enhancement of bladder contractility at low temperature can be related to excitation of the cold receptors in human and animal bladder [5,8]. However, we hypothesize that these receptors only little contribute to the effects described here, because the threshold temperature of their activation in the bladder is below 30°C, while their maximum stimulation occurs at 20°C [5].

We found that EFS-induced activation of P2-receptors induces more stronger responses at low temperature than at the normal temperature. It can be explained by reduced activity of enzymes involved in degradation of P2-receptor transmitter (*i.e.*, ATP) at low temperatures, which decelerates the transmitter metabolism and potentiates the response. It is widely accepted that the major enzyme involved in this process is ectoATPase, whose activity in mammals is maximum at normal temperature [1]. P2-receptors agonist α,β-methylene-ATP is resistant to enzymatic degradation [14]. We found that contractions induced by this agent also increased at low temperatures. Therefore, enhancement of bladder contractile responses induced by stimulation of P2-receptors cannot be explained only by a decrease in ectoATPase activity, but results from specific intrinsic properties of the receptor.

We showed that the antagonistic effect of PPADS on contractile responses induced by α,β-methylene-ATP did not change at various temperatures. However, in experiments with EFS, the effect of PPADS was significantly stronger at low (30°C) temperature. Previously we hypothesized that PPADS is a selective antagonist of P2X-receptors [12,13], although recent studies demonstrated that this antagonist inhibits also P2Y-receptors [11]. The differences in the effects of PPADS on endogenous and exogenous stimulation of P2X-receptors also confirms low selectivity of this agent.

P2-receptors were found in many tissues of various animals, including lower vertebrates and invertebrates [2]. Our results suggest that P2X-mediated responses are more pronounced in cold-blooded animals than in warm-blooded mammals. However, this hypothesis should be tested in comparative experiments on various animal species.

Therefore, P2X-receptor-mediated contractile responses of guinea pig bladder are characterized by a clear-cut temperature dependence: temperature drop enhances contractions induced by endogenous and exogenous stimulation of P2X-receptors, while temperature rise produces an opposite effect.

REFERENCES

1. A. U. Ziganshin and L. E. Ziganshina, *Pharmacology of ATP Receptors* [in Russian], Moscow (1999).
2. G. Burnstock, *Drug Dev. Res.*, **39**, 204-242 (1996).
3. N. Fernandez, A. L. Garcia-Villalon, J. Borbugjo, *et al.*, *Acta Physiol. Scand.*, **151**, 441-451 (1994).
4. A. L. Garcia-Villalon, J. Padilla, L. Monge, *et al.*, *Br. J. Pharmacol.*, **122**, 172-178 (1997).
5. G. Geirson, S. Lindstrom, and M. Fall, *Br. J. Urol.*, **71**, 675-680 (1993).
6. C. H. V. Hoyle, G. E. Knight, and G. Burnstock, *Br. J. Pharmacol.*, **99**, 617-621 (1990).
7. B. F. King, L. E. Ziganshina, J. Pintor, *et al.*, *Ibid.*, **117**, 1371-1373 (1996).
8. S. Lindstrom and L. Mazieres, *Acta Physiol. Scand.*, **141**, 1-10 (1991).
9. L. Monge, A. L. Garcia-Villalon, J. J. Montoya, *et al.*, *Br. J. Pharmacol.*, **109**, 61-67 (1993).
10. H. Nakanishi, I. Matsuoka, T. Ono, *et al.*, *Clin. Exp. Pharmacol. Physiol.*, **26**, 35-38 (1999).
11. U. Windscheif, O. Pfaff, A. U. Ziganshin, *et al.*, *Br. J. Pharmacol.*, **115**, 1509-1517 (1995).
12. A. U. Ziganshin, C. H. V. Hoyle, X. Bo, *et al.*, *Ibid.* **110**, 1491-1495 (1993).
13. A. U. Ziganshin, C. H. V. Hoyle, G. Lambrecht, *et al.*, *Ibid.*, **111**, 923-929 (1994).
14. A. U. Ziganshin, L. E. Ziganshina, P. Bodin, *et al.*, *Biochem. Mol. Biol. Int.*, **36**, 864-869 (1995).