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Temperature dependency of P2 receptor-mediated responses

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Abstract

The P2 receptor-mediated responses of isolated guinea pig urinary bladder and vas deferens (P2X receptors) and taenia caeci (P2Y receptors) were registered at the three temperature conditions of 30, 37 and 42 °C. The contractile responses of both urinary bladder and vas deferens to a P2X receptor agonist α,β-methylene ATP (α,β-meATP; 0.01–30 μM) and to electrical field stimulation (1–64 Hz, 0.1 ms, supramaximal voltage) in the presence of atropine (0.1 μM) and phentolamine (1 μM) were markedly more prominent at a temperature of 30 °C than at 37 or 42 °C. Similarly, relaxation of carbachol-precontracted taenia caeci caused by electrical field stimulation (0.5–8 Hz, 0.1 ms, supramaximal voltage) temperature-dependently increased with decrease of temperature, while relaxation of this tissue by exogenous ATP (1–100 μM) was not affected by the temperature. A P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, 1–30 μM) at all three temperature conditions concentration-dependently antagonised contractile responses to α,β-methylene ATP and electrical field stimulation in both urinary bladder and vas deferens. PPADS, even at the highest concentration tested (30 μM), had no effect on the relaxant responses of the taenia caeci either to electrical field stimulation or ATP and its action was not affected by the change of temperature. It is concluded from this study that the effectiveness of P2 receptor-mediated responses in guinea pig urinary bladder, vas deferens and taenia caeci increases by decrease of temperature.

Keywords: P2X receptor; P2Y receptor; Temperature dependency; PPADS

1. Introduction

The presence of P2 receptors has been shown in many animal tissues including guinea pig urinary bladder, vas deferens and taenia caeci (Hoyle, 1992). In the first two tissues the predominant subtype of P2 receptors was characterised as $P2X_1$ (Ralevic and Burnstock, 1998; Burnstock, 2001a) stimulation of which by the $P2X_1$ receptor agonist α,β-methylene ATP or by applying electrical field caused rapid transient contraction which was antagonised in a concentration-dependent manner by P2 receptor antagonists such as suramin or pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, Hoyle et al., 1990; Ziganshin et al., 1993). In the guinea pig taenia caeci P2Y receptor agonists 2-methylthio ATP or ATP and low-frequency electrical field stimulation cause relaxation of tone-raised preparations (Windscheif et al., 1995; Bültmann et al., 1996). Although subtypes of P2Y receptors in the guinea pig taenia caeci have not been clearly identified, it is believed that they belong to $P2Y_1$ type (Hourani et al., 1998).

Most experiments on P2 receptors were carried out in normal conditions; however, it has been shown that responses mediated via some subtypes of P2 receptor are significantly affected by the change of pH level (King et al., 1996; Nakanishi et al., 1999) or change of some ion concentrations (Wildmann et al., 1999). There are also a few observations about the effect of temperature on some P2 receptor-mediated responses. It has been shown for example that in the rabbit central ear artery P2 receptor-mediated contractions become more prominent at low temperature compared to that at normal temperature condition (Garcia-Villalon et al., 1997).

Mild hypothermia (32–30 °C) is a routine methodology used during cardio and brain surgery with the aim to increase the tissue resistance to hypoxia (Zindler, 2000). It

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is also known that artificial blood circulation damages red blood cells (Osborn et al., 1962; Thompson, 1977), which could result in a marked elevation of ATP plasma concentrations. On the other hand, hypertermia is one of the most common features of the inflammation, when extracellular concentration of purine nucleotides could significantly increase due to breach of the cell integrity (Gordon, 1986). Thus, there are certain clinical situations when, at altered temperature conditions, extracellular ATP concentrations could be high enough to act on P2 receptors. However, the systematic study of temperature dependency of different P2 receptor-mediated responses has not been carried out yet. The aim of the present study was to test the dependency of P2X receptor-mediated contractions and P2Y receptor-mediated relaxation on bath temperature conditions using classical tissues for those receptors—guinea pig urinary bladder, vas deferens and taenia caeci.

2. Methods

2.1. General procedures

Male white guinea pigs (300–550 g) were killed by a blow to the head and exsanguination. The urinary bladder, both vas deferens and ventral taenia caeci were removed and strips of smooth muscle, approximately 2 by 10 mm, were prepared and suspended vertically in 10 ml organ baths for isometric recording of mechanical activity. An initial load of 1 g was applied to the strips, which were then allowed to equilibrate for at least 60 min. Electrical field stimulation was provided by a Grass S9 stimulator and was applied via two platinum wire rings 2.5 mm in diameter, 10 mm apart, through which the strips was threaded. The modified Krebs solution used in these experiments had the following composition (mM): NaCl 133, KCl 4.7, $NaHCO_3$ 16.3, $MgCl_2$ 0.6, NaH_2PO_4 1.35, $CaCl_2$ 2.5 and glucose 7.8, gassed with 95% O_2 and 5%CO_2 (pH 7.3–7.4). Mechanical activity of the tissues were recorded with a Linton FSG-01 (Great Britain) force displacement transducer, acquired by Biopack MP100WSW Data Acquisition System and displayed on a computer screen.

2.2. Urinary bladder and vas deferens

Electrical field stimulation was applied at a given frequency (1–64 Hz) with a pulse width of 0.5 ms and supramaximal voltage until a maximal contraction was reached and the tone of the tissue declined by approximately 30%. Intervals of 1 min were maintained between stimulations. Atropine (0.3 μM) and phentolamine (1 μM) were present in Krebs solution throughout the experiments with electrical field stimulation.

α,β-Methylene ATP (α,β-meATP, 10 nM–30 μM) was added directly to the organ bath and the tissue was washed out several times with fresh Krebs solution after maximal contraction was reached. Intervals of 25–30 min were allowed between contractions in order to prevent desensitisation of the P2X receptors.

All contractile responses were calculated as a percentage of the response evoked by KCl at a concentration of 240 mM which was added at the end of the experiments.

2.3. Taenia caeci

A standard tone of taenia caeci was induced by carbachol (0.1 μM), which maintained constant precontraction of the tissue. When the carbachol contraction reached plateau, a single concentration of ATP (1–100 μM) or electrical field stimulation (0.5–8 Hz, a pulse width of 0.5 ms and supramaximal voltage) was applied. After maximal relaxation had been reached, the tissue was washed out several times with fresh Krebs solution. The testing of the next concentration of ATP on carbachol-precontracted tissue was made not earlier than 10 min after the previous washout.

The relaxant responses were calculated as a percentage of the maximal possible relaxation of the tissue in a given precontraction condition.

2.4. Temperature dependency

In all experiments, initial procedures of setting up the tissues, equilibration period and the final challenge of the tissue by KCl at a concentration of 240 mM were carried out at a temperature of 37 ± 1 °C. Then at the same temperature, tissue was challenged twice by α,β-meATP at a concentration of 3 μM (urinary bladder and vas deferens) or by ATP at a concentration of 10 μM (carbachol-precontracted taenia caeci) to make sure that the responses of the tissue are stable. After that, in half of the experiments, temperature of the bath solution was lowered down to 30 ± 1 °C, and in another half of the experiments the temperature was raised up to 42 ± 1 °C. Tissues equilibrated to the given temperature for about 10–15 min and then concentration–response or frequency–response curve was constructed for α,β-meATP, ATP or electrical field stimulation, respectively. The second similar curve was obtained at the same tissue at a temperature of 37 °C and the third one was obtained either at 42 or 30 °C depending on at what temperature was the first curve constructed. In some experiments only two curves were obtained on the same tissues, one of which was taken at 37 °C.

2.5. Experiments with PPADS

After the initial contractions of the tissue were obtained at 37 °C, bath temperature was changed to 30 or 42 °C or kept at 37 °C and all the testing in a given tissue was carried out at that one temperature. Response of the tissue to α,β-meATP, ATP or electrical field stimulation were examined before and after incubation with PPADS at concentrations of 1, 3, 10 and 30 μM for at least 25 min. Time control

preparations were maintained in parallel and it has been established that there was no significant changes in response to either α,β-meATP, ATP or electrical field stimulation throughout experiments.

2.6. Drugs used

Atropine sulphate, phentolamine, carbamylcholine chloride (carbachol), adenosine 5′-triphosphate sodium salt (ATP), α,β-methylene ATP disodium salt (α,β-meATP) were obtained from Sigma. PPADS tetrasodium salt was supplied by Tocris Cookson.

2.7. Analysis of results

For evaluation of concentration–effect or frequency–effect relationships, experimental curves were fitted to a nonlinear regression analysis. The calculation resulted in the maximal response and the negative log molar values ($-\log EC_{50} = pD_2$) or log frequency values ($\log EF_{50}$) inducing 50% of the maximal response. Means were compared by Student's paired and unpaired *t*-test as well as by two-way ANOVA test. A probability of less than or equal to 0.05 was considered significant. Data are presented as mean ± S.E.M. (*n*).

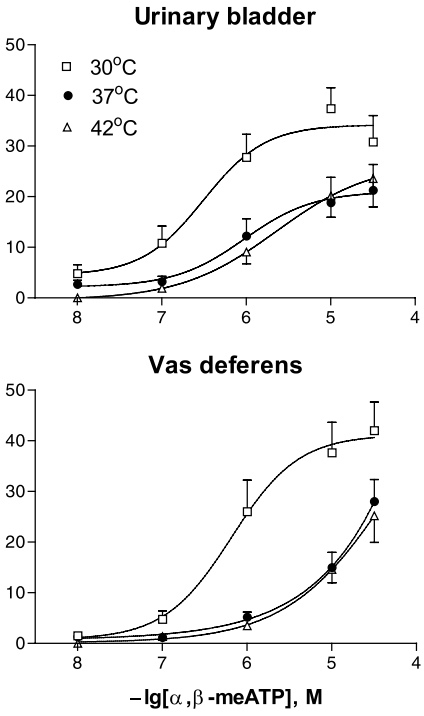

Fig. 1. Contractile responses of isolated guinea pig urinary bladder (upper panel) and vas deferens (lower panel) to α,β-methylene ATP (0.1–30 μM) recorded at temperatures 30, 37 and 42 °C. Data presented as a percentage of maximal tissue response to KCl (240 mM). Data shown are means and vertical bars indicate S.E.M., $n = 6–8$.

3. Results

3.1. P2X receptor-mediated contractions

At a temperature of 37 °C, α,β-meATP caused concentration-dependent contractions of the guinea pig isolated urinary bladder (Fig. 1, upper panel). At the highest concentration of the agonist tested (30 μM), the contraction of the tissue was 21.3 ± 5.1% relative to the contraction evoked by KCl at a concentration of 240 mM ($n = 8$). At a temperature of 30 °C, the contractions evoked by α,β-meATP was significantly higher than that at a temperature of 37 °C, concentration–response curve being shifted to the left. The maximal response of the tissue was obtained at the agonist concentration of 10 μM which was 37.4 ± 4.1% ($n = 8$). The increase of temperature to 42 °C slightly shifted to the right the concentration–response curve for α,β-meATP compared to that at 37 °C. The estimated pD_2 values for α,β-meATP were 6.58 ± 0.11 ($n = 8$), 6.24 ± 0.10 ($n = 8$) and 5.91 ± 0.08 ($n = 6$) at temperatures 30, 37 and 42 °C, respectively, and all these values were significantly different from each other.

Similarly, in the vas deferens at a temperature of 30 °C the contractions evoked by α,β-meATP were significantly higher than those at a temperature of 37 °C, concentration–response curve being shifted to the left (Fig. 1, lower panel). At the maximal agonist concentration tested (30 μM), the response of the tissue was 42.0 ± 5.7% ($n = 8$). The increase of temperature to 42 °C did not significantly change the concentration–response curve for α,β-meATP comparing to that at 37 °C. The estimated pD_2 values for α,β-meATP were 6.28 ± 0.08 ($n = 8$), 5.57 ± 0.14 ($n = 8$) and 5.58 ± 0.11 ($n = 6$) at temperatures 30, 37 and 42 °C, respectively, last two figures being significantly different from the first one.

At a temperature of 37 °C, the isolated guinea pig urinary bladder in the presence of cholino- and adrenoblockers contracts to electrical field stimulation in a frequency-dependent manner (Fig. 2, upper panel). The maximum contraction to electrical field stimulation has been obtained at a frequency of 16 Hz (48.5 ± 3.9% relative to contraction caused by KCl at a concentration of 240 mM, $n = 14$). The decrease of the temperature down to 30 °C caused the shift to the left of the frequency–response curve with no significant change of the maximal response (53.9 ± 5.8%, $n = 10$). At a temperature of 42 °C, the frequency–response curve was lowered and the maximal response to electrical field stimulation was significantly less than at 37 °C (29.0 ± 2.2%, $n = 14$). The log EF_{50} values (i.e. the frequency that cause a 50% contraction of the maximum response) were 0.36 ± 0.15 ($n = 10$), 0.60 ± 0.03 ($n = 13$) and 0.59 ± 0.04 ($n = 14$) at temperatures 30, 37 and 42 °C, respectively, last two values being significantly different from the first one.

In the vas deferens, maximal response to electrical field stimulation at a temperature of 37 °C was observed at a frequency of 32–64 Hz. At a temperature of 30 °C,

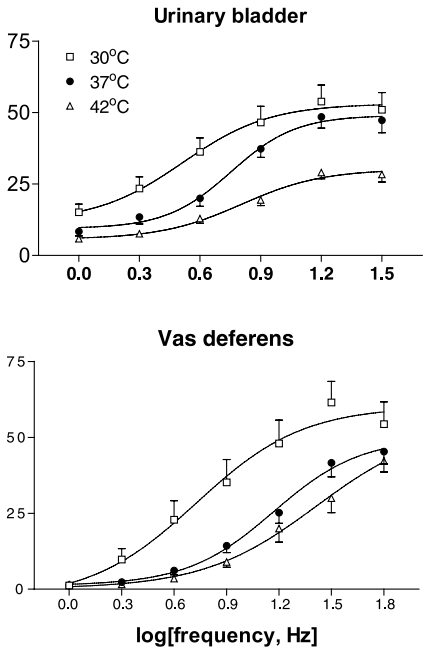

Fig. 2. Contractile responses of isolated guinea pig urinary bladder (upper panel) and vas deferens (lower panel) to electrical field stimulation (0.5 ms, supramaximal voltage, 1–64 Hz) in the presence of atropine (0.3 μM) and phentolamine (1 μM) recorded at temperatures 30, 37 and 42 °C. Data presented as a percentage of maximal tissue response to KCl (240 mM). Data shown are means and vertical bars indicate S.E.M., $n = 10–14$.

maximal response was significantly higher than that at 37 °C and the frequency–response curve was markedly shifted to the left (Fig. 2, lower panel).

3.2. P2Y receptor-mediated relaxation

The used temperature conditions did not affect significantly the amplitude of precontraction caused by carbachol which were 4.87 ± 0.67 g ($n = 12$), 5.11 ± 0.56 g ($n = 20$) and 5.21 ± 0.73 g ($n = 12$) at temperatures 30, 37 and 42 °C, respectively.

At a temperature of 37 °C, ATP caused concentration-dependent relaxation of carbachol-precontracted taenia caeci, which was close to the maximal relaxation at the highest agonist concentration tested (100 μM). The decrease or the increase of the bath temperature did not affect the response to ATP; concentration–response curves for this agonist in taenia caeci at 30 and 42 °C were almost identical to that at 37 °C (Fig. 3, upper panel). The pD_2 values for ATP were 6.15 ± 0.39 ($n = 12$), 6.14 ± 0.38 ($n = 24$) and 6.38 ± 0.24 ($n = 12$) for a temperature of 30, 37 and 42 °C, respectively; all these figures are not significantly different from each other.

In the experiments with electrical field stimulation at a temperature of 37 °C, low-frequency stimuli caused frequency-dependent relaxation of the taenia caeci (Fig. 3, lower panel). The decrease of the bath temperature down to 30 °C significantly enhanced the relaxant response of the tissue at all frequencies tested while the increase of temperature to 42 °C reduced the relaxant action of electrical field stimulation. The log EF_{50} values (i.e. the frequency that cause a 50% relaxation of the maximum response) were -1.06 ± 0.21 ($n = 12$), -0.48 ± 0.17 ($n = 20$) and -0.11 ± 0.09 ($n = 12$) at temperatures 30, 37 and 42 °C, respectively, all values being significantly different from each other.

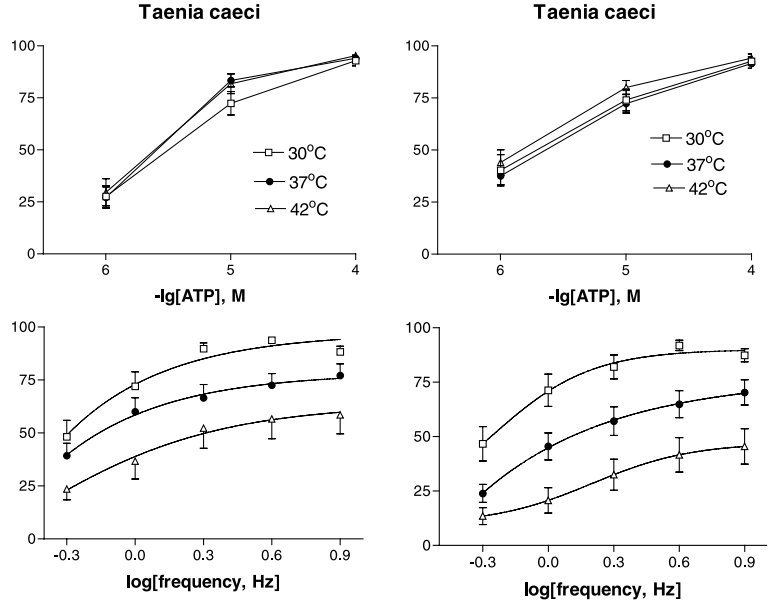

Fig. 3. Relaxant responses of isolated guinea pig taenia caeci to ATP (1–100 μM, upper panels) and to electrical field stimulation (0.5 ms, supramaximal voltage, 0.5–8 Hz, lower panels) recorded in the absence (left side panels) and presence (right side panels) of PPADS (30 μM) at temperatures 30, 37 and 42 °C. Data presented as a percentage of maximal tissue relaxation. Data shown are means and vertical bars indicate S.E.M., $n = 12–20$.

3.3. Effects of PPADS

A P2 receptor antagonist PPADS concentration-dependently antagonised contractile responses of the guinea pig vas deferens and urinary bladder to α,β-meATP; however, the antagonism by PPADS was clearly not competitive since there was a marked depression of the agonist maximal response (Fig. 4). Statistical analysis revealed that in both tissues at all three tested temperature conditions there were significant differences between control responses to the agonist and those in the presence of PPADS at concentrations of 1 μM and above ($P < 0.05$). Also, in the vas deference at any given concentration of PPADS (except 30 μM), there were significantly higher contractions to α,β-meATP at a temperature of 30 °C than at 37 or 42 °C ($P < 0.05$). In the urinary bladder, only at a concentration of PPADS of 1 μM was there a statistically significant difference between responses to the agonist at 30 °C and those at 37 or 42 °C ($P < 0.05$). In both tissues in the presence of PPADS there were no significant differences between agonist activity at temperatures of 37 and 42 °C. Interestingly, in the vas deferens at temperatures 37 and 42 °C, PPADS already at a concentration of 3 μM completely abolished contractions of the tissue to α,β-meATP, while at 30 °C PPADS at this given concentration antagonised responses of the vas deferens to α,β-meATP only approximately by half.

PPADS at concentrations of 1–30 μM antagonised contractile responses of vas deferens and urinary bladder to electrical field stimulation at all temperatures tested, clearly depressing maximal responses in both tissues thus indicating the presence of noncompetitive antagonism (Fig. 5). In both tissues at temperatures 37 and 42 °C, responses to electrical field stimulation in the presence of all tested concentrations of PPADS were significantly lower than control ones; at a temperature of 30 °C significant inhibition took place at antagonist concentrations not less than 3 μM ($P < 0.05$). Also, in both tissues at any given concentration of PPADS contractile responses to electrical field stimulation were higher at 30 °C than those at 37 or 42 °C. In the vas deferens, in the presence of PPADS there were no

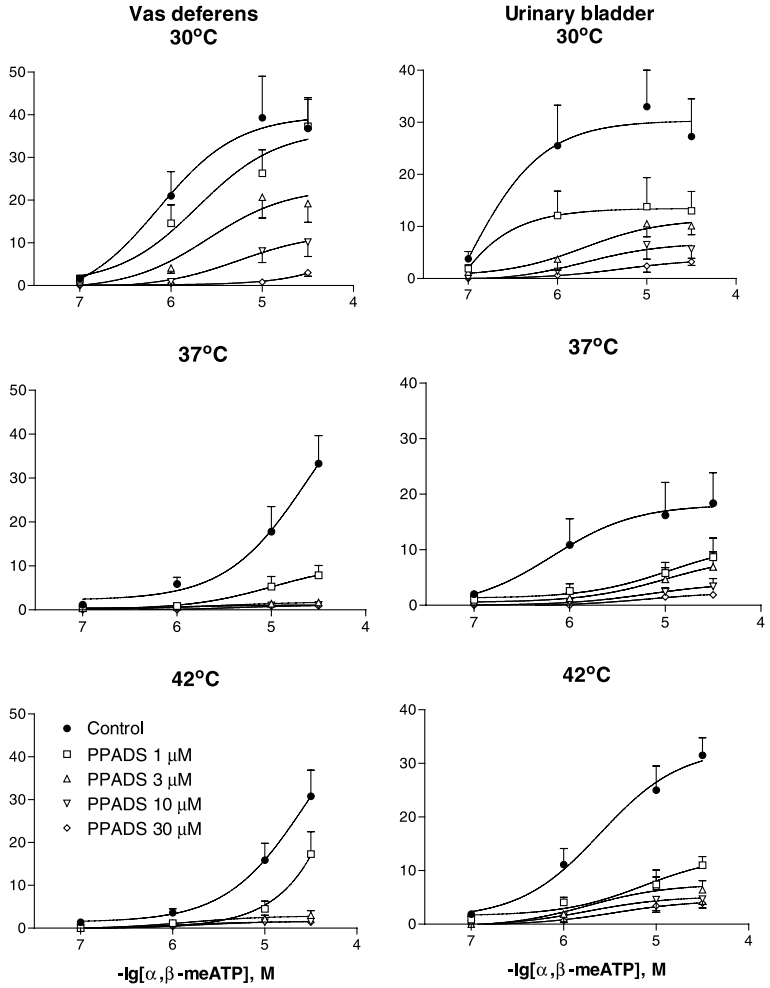

Fig. 4. Effect of PPADS (1–30 μM) on contractile responses of isolated guinea pig vas deferens (left side panels) and urinary bladder (right side panels) to α,β-methylene ATP (0.1–30 μM) recorded at temperatures 30, 37 and 42 °C. Data presented as a percentage of maximal tissue response to KCl (240 mM). Data shown are means and vertical bars indicate S.E.M., $n = 4–6$.

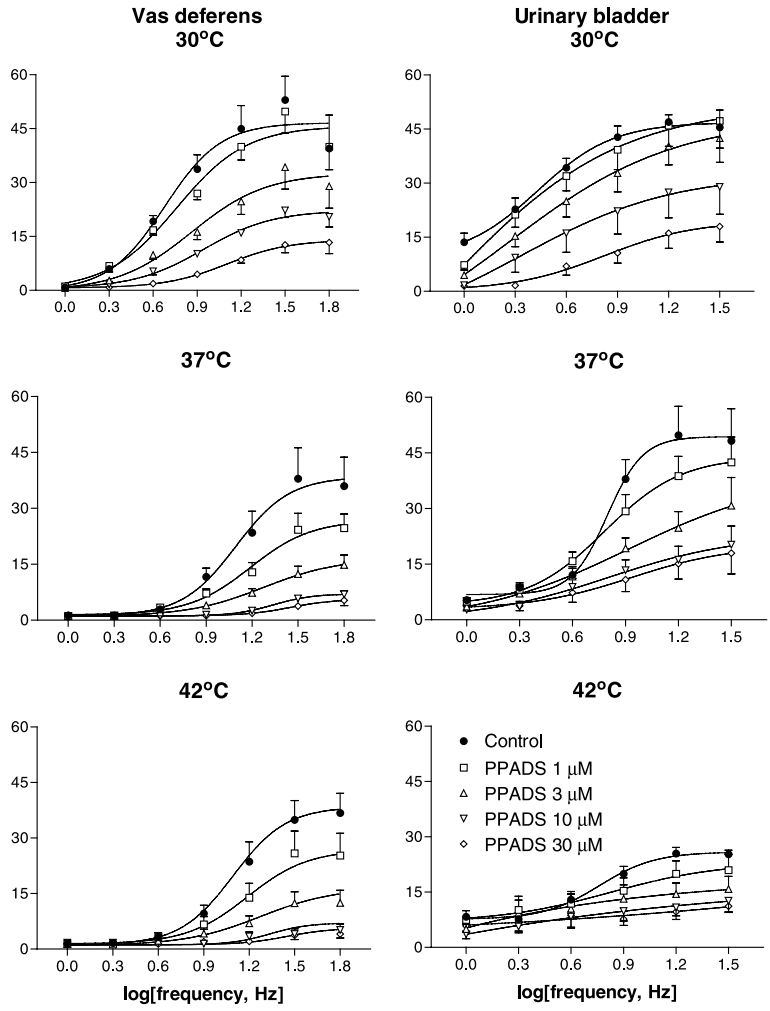

Fig. 5. Effect of PPADS (1–30 μM) on contractile responses of isolated guinea pig vas deferens (left side panels) and urinary bladder (right side panels) to electrical field stimulation (0.5 ms, supramaximal voltage, 1–64 Hz) in the presence of atropine (0.3 μM) and phentolamine (1 μM) recorded at temperatures 30, 37 and 42 °C. Data presented as a percentage of maximal tissue response to KCl (240 mM). Data shown are means and vertical bars indicate S.E.M., $n=4–6$.

significant differences between responses to electrical field stimulation at 37 and 42 °C, while in the urinary bladder contractions at a temperature of 42 °C were generally lower than responses at 37 °C.

In the taenia caeci, PPADS even at the highest concentration tested (30 μM) did not significantly change any point at either concentration–response curve for ATP or frequency–response curve for electrical field stimulation comparing with those obtained without this antagonist (Fig. 3).

4. Discussion

In the present study, we found that in guinea pig tissues both P2X and P2Y receptor-mediated responses are clearly temperature-dependent. Typically, the lowering of the temperature significantly increases the P2 receptor-mediated responses caused by endogenous or exogenous stimulations compared to normal temperature, while the opposite effect is usually observed with the increase of the temperature.

Temperature dependency for some receptor-mediated responses has been tested earlier on several animal and human tissues. Using guinea pig ileum and trachea and rat vas deferens and atria preparations hypothermia-induced supersensitivity to adenosine has been established for responses mediated via adenosine A_1- but not adenosine A_2-receptors (Broadley et al., 1985). It has been shown that in the rabbit central ear artery, but not femoral artery, cooling to 24 °C reduces contraction and increases the relaxation caused by histamine (Fernandez et al., 1994) and enhances the relaxation caused by cholinoceptor stimulation (Monge et al., 1993). Later in the study from the same laboratory it was shown that in rabbit central ear artery at 30 °C α_1-adrenoceptor-mediated response is reduced and the P2 receptor-mediated component becomes more prominent (Garcia-Villalon et al., 1997). On the other hand, it was found that the release of ATP from rabbit pulmonary

artery induced by methoxamine, an α_1-adrenoceptor agonist, being observed at 37 °C, was completely eliminated at a temperature of 27 °C (Takeuchi et al., 1994).

Similar somehow obscure results were obtained in one of our laboratories in recent experiments on hibernated animals. We found that in vas deferens taken from hibernated golden hamsters P2X receptor-mediated responses were significantly higher than that of nonhibernated age-matched control animals (Knight and Burnstock, 1998) while in the urinary bladder (Pinna et al., 1998) and in mesenteric arteries (Ralevic et al., 1997) of the same animals, opposite results were registered. Thus it seems that the temperature differently acts on differently localised P2 receptor.

The increase of bladder contractility at low temperature might be due to activation of cold receptors in the bladder, the presence of which has been shown both in animal and human urinary bladder (Lindström and Mazieres, 1991; Geirson et al., 1993). However, it is unlikely that cold receptors are involved in the effects which we registered in the present study since the threshold temperature to stimulate these receptors was found to be less than 30 °C and the maximum effect was registered at around 20 °C (Geirson et al., 1993).

It is generally accepted that in the presence of adreno- and cholinoceptor blockers the contractions of guinea pig vas deferens and urinary bladder is mediated by P2X receptors, while in guinea pig taenia caeci low-frequency electrical field stimulation evoke the relaxation via P2Y receptors (Hoyle et al., 1990; McLaren et al., 1994, Stjarne and Stjarne, 1997). We have found that both P2X and P2Y receptor-mediated responses elicited by electrical field stimulation are increased at low temperature. It could be suggested that this effect occurs due to decrease of activity of the transmitter-metabolising enzymes, namely ecto-ATPase and ecto-nucleotidases during cooling, since it is generally accepted that ecto-ATPase activity is a temperature-dependent, with the optimum temperature of 37 °C for warm-blood temperature animals (Ziganshin and Ziganshina, 1999). However, this cannot explain results with the enzymatically stable P2X receptor agonist α,β-meATP, the effects of which are not affected by ecto-ATPases. Moreover, in taenia caeci when we used ATP, which is readily degraded by ecto-ATPases, we did not find any temperature dependency in agonist activity. Thus it seems that supersensitivity of P2 receptors at a low temperature is a feature of receptor itself and is not dependent on ecto-ATPase activity.

In our experiments, we have found that antagonistic activity of PPADS against a $P2X_1$ receptor agonist α,β-meATP and electrical field stimulation of non-cholinergic, non-adrenergic nerves was noncompetitive in manner since in all cases there was clear depression of maximal response of the tissues. Thus we were unable to calculate the pA_2 values for PPADS at used experimental conditions. However, ANOVA test showed extremely significant difference between concentration–response or frequency–response curves constructed at different antagonist concentrations at all temperatures tested. This indicates that PPADS maintains its P2X receptor antagonistic activity at a good range of temperature conditions.

It was believed initially that PPADS was a selective P2X receptor antagonist (Ziganshin et al., 1993, 1994) although later antagonism of recombinant P2Y receptors by PPADS was reported (Boyer et al., 1994). In our earlier study we established that in the guinea pig taenia caeci, substantial antagonism against P2Y receptor-mediated relaxation was obtained only at a concentration of 100 μM of PPADS (Windscheif et al., 1995). Similarly, in the experiments we discuss in this paper we did not find any antagonism at P2Y receptors of PPADS at concentrations up to 30 μM on taenia caeci. Thus, it supports the view that at least in the pharmacological organ bath experiments PPADS shows relatively good selectivity to P2X receptors.

It was an interesting finding that in taenia caeci responses to electrical field stimulation were clearly temperature-dependent while the relaxation caused by exogenous ATP was statistically identical at different temperature conditions. Since it has been clearly shown that ATP is a transmitter which is released during electrical field stimulation of guinea pig teania caeci to act on P2Y receptors (see Burnstock, 2001b), it seems that in this tissue only prejunctional mechanisms of transduction are sensitive to the shifts of the temperature while post-junctional processes are not.

In conclusion, in this study we established that P2 receptor-mediated responses of the guinea pig urinary bladder and vas deferens significantly bigger at hypothermia (30 °C) than at normal or high temperature conditions. Similar results were obtained for P2Y-mediated relaxation of taenia caeci caused by electrical field stimulation, although relaxation of this tissues to ATP are not significantly affected by temperature.

Acknowledgements

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