Evidence for the presence of both pre- and postjunctional P₂-purinoceptor subtypes in human isolated urinary bladder

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1 In order to characterize P₂-purinoceptor(s) in human urinary bladder the contractile effects of ATP and its slowly-hydrolyzable analogues α,β -methylene ATP (α,β -MeATP) and β,γ -methylene ATP (β,γ -MeATP) were investigated on human detrusor strips taken from patients undergoing cystectomy for bladder carcinoma.

2 Serial concentration-response curves (SCRC) for ATP, α,β -MeATP and β,γ -MeATP were constructed with an interval of 25 min between two successive doses to avoid tachyphylaxis. ATP (10 μ M–10 mM) induced a phasic contraction, which was very rapid in onset. The dose-response curve to ATP appeared not to be monophasic: at the lower concentrations (10–300 μ M) the curve was shallow, whilst at high concentrations (1–10 mM) the curve was steeper. The magnitude of the response obtained at the highest concentration tested (10 mM) was only $21.1 \pm 2.8\%$ (mean $\pm$ s.e.mean; $n = 4$) of the KCl (100 mM)-induced contraction.

3 α,β -MeATP (0.3 μ M–1 mM) and β,γ -MeATP (10 μ M–1 mM) elicited a phasic contraction with a time course similar to that exhibited by ATP. The magnitude of the response obtained at the highest concentration tested (1 mM) was $70.3 \pm 6.3\%$ for α,β -MeATP ($n = 10$) and $27.9 \pm 4.5\%$ for β,γ -MeATP ($n = 8$) of KCl (100 mM)-induced contraction. The rank order of potency was α,β -MeATP > β,γ -MeATP > ATP. A plateau of response could not be achieved by any of these agonists.

4 The P₂-purinoceptor antagonist, suramin (10–300 μ M), dose-dependently antagonized only the lower part of α,β -MeATP dose-response curve. Data were analysed in terms of dose-ratio estimated at two levels of response (10% and 35% of KCl 100 mM-induced contraction). At 10% of KCl response the Schild plot slope was 0.98 and the estimated pK_B was 5.85, whereas using the dose-ratio at the 35% level of the KCl response, the Schild plot was not linear suggesting an interaction of α,β -MeATP with a heterogeneous receptor population.

5 The putative P₂-purinoceptor antagonist, Coomassie Brilliant Blue G (CB-G) at 0.3 and 1 μ M ($n = 5$), shifted to the left the α,β -MeATP SCRC. The response at the highest concentration of agonist was potentiated, being equal to $78.8 \pm 11.7\%$ of the KCl (100 mM) response ($n = 5$). CB-G at 0.3 μ M also shifted to the left the β,γ -MeATP SCRC and significantly potentiated the response at 1 mM up to $46.3 \pm 5.6\%$ of KCl 100 mM response ($n = 4$).

6 Pretreatment with terodotoxin (TTX) at 1 μ M shifted to the left the α,β -MeATP SCRC but the response to the highest concentration of the agonist was not potentiated, being $73.6 \pm 9.9\%$ of the KCl (100 mM) response ($n = 5$). TTX (1 μ M) shifted to the left the β,γ -MeATP SCRC and significantly potentiated the response at 1 mM ($61.6 \pm 3.1\%$ of KCl response; $n = 4$).

7 The NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) at 100 μ M did not modify the SCRC to either α,β or β,γ -MeATP.

8 We conclude that in human detrusor muscle there is a heterogeneity of purinoceptors. The complex antagonism exhibited by suramin suggests the presence not only of P_{2X}-purinoceptors but also of another contractile P₂-purinoceptor subtype insensitive to suramin. Moreover, the activity of CB-G and TTX seems to support the existence of a prejunctional P₂-purinoceptor subtype inducing the release of one or more inhibitor neurotransmitters.

Keywords: Human isolated urinary bladder; adenosine triphosphate; P₂-purinoceptors; contraction; suramin; coomassie brilliant blue G

Introduction

The pharmacological effects of extracellular adenosine and adenine nucleotides are mediated through two distinct classes of receptors named P₁ and P₂. At the P₁-purinoceptors, the rank order of agonist potency is adenosine > ATP and methylxanthines are selective antagonists, whereas at the P₂-purinoceptors the rank order of potency is ATP > adenosine and methylxanthines are without effect. It is well established that P₁ and P₂ purinoceptors are heterogeneous, P₁ being subdivided into A₁ and A₂ subtypes and P₂ into P_{2X}, P_{2Y}, P_{2T} and P_{2Z} subtypes (Burnstock, 1991). However, this classification is not definitive because it is based only on

agonist potencies and because of a lack of selective and potent antagonists for the proposed subtypes.

It was recently reported that the trypanocidal drug, suramin, behaves as a competitive antagonist on contractile responses induced by the P_{2X} selective agonist, α,β -methylene ATP (α,β -MeATP) in rabbit isolated ear artery (Leff *et al.*, 1990). On the other hand, Hoyle and collaborators (1990) reported that suramin did not discriminate between the P_{2X} subtype mediating contraction of the guinea-pig urinary bladder and the P_{2Y} subtype mediating relaxation of guinea-pig taenia-coli. Another compound, reactive blue 2 (RB-2) was claimed to be a selective antagonist of P_{2Y}-mediated effects in vascular and visceral smooth muscles (Burnstock & Warland, 1987; Matharu & Hollingsworth, 1992). However,

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insufficient selectivity between muscarinic and purinoceptors in guinea-pig urinary bladder has been reported for this compound (Choo, 1981; Crema *et al.*, 1983). Furthermore, the sulphonic dye, Coomassie Brilliant Blue G (CB-G), was found to be a more potent and selective P_{2Y} antagonist with respect to RB-2 in antagonizing the ATP-induced ⁴⁵Ca²⁺ entry in rat parotid acinar cells (Soltoff *et al.*, 1989).

In guinea-pig urinary bladder ATP and its slowly degradable analogue, α,β -MeATP, contract the detrusor muscle acting on P_{2X}-purinoceptors (Hoyle *et al.*, 1990). In human isolated urinary bladder the presence of P_{2X}-purinoceptors mediating contraction was proposed on the basis of the rank order of potency exhibited by ATP and α,β -MeATP (Hoyle *et al.*, 1989) but more detailed studies were not performed. Hence, we decided to evaluate the effect of some purinoceptor agonists and of two putative P₂ antagonists, namely suramin and CB-G, in human isolated detrusor muscle strips. Furthermore, because it was recently reported that the putative P_{2X} selective purinoceptor agonist β,γ -methylene ATP (β,γ -MeATP) has a direct contractile effect on P₁-purinoceptor of the A₁ subtype (Bailey *et al.*, 1992), the effect of the potent and selective A₁ antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; Collis *et al.*, 1989) was also evaluated.

Some of these results were presented at the Symposium 'Purines '92: Pharmacology and Clinical Applications', Milan, June 21–24, 1992 (Palea *et al.*, 1992).

Methods

Specimens from the anterior part of the dome of the urinary bladder were obtained from male patients undergoing radical cystectomy because of bladder malignancy. Immediately after surgical removal a bladder specimen (macroscopically normal, with no sign of tumour or inflammation) was taken from the anterior part of the dome and immediately transported to the laboratory. The serosal and mucosal layers were dissected away and muscular strips were cut off. Each bladder strip was mounted vertically in a 2 ml glass organ bath containing a modified Krebs solution, gassed with 5% CO₂ in O₂ and maintained at 37°C. The composition of the modified Krebs solution was (mM): NaCl 120, KCl 4.7, MgSO₄ · 7H₂O 0.6, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2, glucose 10 and indomethacin 0.001. Strips were initially placed under a tension of 2 g and allowed to equilibrate for 1 h. The tension generated was measured with a Grass Instrument FT03 isometric force displacement transducer, connected to a polygraph (Linseis model 7025).

Concentration-response curves to purinoceptor agonists

Serial concentration-response curves (SCRC) to α,β -MeATP, β,γ -MeATP and ATP were constructed by adding to the organ bath a single dose of the agonist and washing it out immediately after the maximal response was obtained. Twenty five minutes were allowed between two consecutive applications of the agonist and in this interval the tissue was washed every 5 min. This protocol was used to avoid desensitization of P_{2X}-purinoceptors, according to other authors (Hoyle *et al.*, 1989). SCRC to α,β -MeATP and β,γ -MeATP were also performed in the presence of 1 μ M tetrodotoxin (TTX) using the same interval between two consecutive concentrations plus 15 min of exposure to TTX.

Studies with purinoceptor antagonists

SCRC to α,β -MeATP were performed in the absence and presence of increasing concentrations of suramin. The incubation times of this antagonist were derived from the study of Leff *et al.* (1990) and were inversely related to the concentrations used. The following pairs of concentrations of suramin and time were used: 10 μ M for 4 h; 30 μ M for 2 h,

100 μ M for 90 min and 300 μ M for 45 min. SCRC to β,γ -MeATP were carried out in the absence and presence of 30 μ M suramin by using 2 h as incubation time. Incubation time of 90 min and 45 min for CB-G (0.003–0.3 μ M) and DPCPX (1 μ M) were used, respectively. Antagonists were dissolved in the appropriate solvent (see *Drugs and solutions*) then diluted in the modified Krebs solution which was used to wash out the strips between two doses of the agonist. Because of the sensitivity to light of suramin, experiments with this antagonist were performed in the absence of a direct light source.

Effect of suramin and CB-G on contraction induced by exogenous acetylcholine

In order to show nonspecific effects of suramin and CB-G on cholinergic contractility, a cumulative dose-response curve to acetylcholine (ACh) was constructed on each bladder strip to determine the dose that elicited a contraction approximately equal to half of the maximal response produced by ACh. Then, this concentration (normally 10 or 30 μ M) was applied two or three times to each bladder strip in order to obtain a constant response and when it was achieved, suramin or CB-G, both at 1 mM, were incubated in the organ bath for 30 and 45 min respectively; the ACh effect was then evaluated again.

Drugs and solutions

α,β -MeATP, β,γ -MeATP and ATP (Sigma, St., Louis, Missouri, U.S.A.) were dissolved in distilled water on the day of the experiment and then diluted in Krebs solution. CB-G, L-NAME (Sigma), suramin (kindly donated by Bayer, Wuppertal, Germany) and TTX were dissolved in distilled water. DPCPX (Cookson Chemicals Ltd, Nursling, Southampton, U.K.) was dissolved in DMSO at 1 mM.

Analysis of agonist concentration-response curves

Since the agonists under study did not reach a maximal effect at the highest concentrations used, to measure the agonists' potency the concentration of the agonist required to produce the equivalent of 35% of the KCl (100 mM) response was estimated. This value has been referred as $-\log A_{35}$ and has been obtained by evaluating the linear part of the concentration-response curves to the agonists. $-\log A_{35}$ are expressed as single values with 95% confidence interval (c.i.).

Analysis of the antagonism of suramin

The antagonism of α,β -MeATP-induced contractions by suramin (at 10–30–100–300 μ M) was quantified by use of the Schild plot regression analysis (Arunlakshana & Schild, 1959): $\log(DR - 1) = m \log[B] - \log K_B$.

Where, DR is the equiactive dose-ratio of the agonist in the presence and absence of the antagonist, [B] the concentrations of the antagonist and K_B the equilibrium dissociation constant for the antagonist when the slope of the regression line (m) is equal to one.

However, two plots were constructed by utilising the dose-ratios between treated and control-curves estimated at two levels of KCl 100 mM response: 10 and 35% versus the concentrations of suramin. The corresponding A_{10} and A_{35} (concentration of the agonist that induces 10 and 35% of the KCl (100 mM) response) were obtained by linear regression analysis as previously reported and they were utilized to calculate the DR at 10 and 35% of response, respectively. When the Schild plot slope was found not to be significantly different from one ($P > 0.05$) a regression analysis was performed with the slope equal to one, to estimate the pK_B ($-\log K_B$) of suramin.

The antagonism of β,γ -MeATP (where suramin was tested only at 30 μ M) was quantified by using the equation:

$pK_B = -\log ([B]/DR-1)$ where [B] is the suramin concentration used (30 μ M) and DR is as defined before (detected by linear regression analysis at 10% of the KCl 100 mM response in the presence and absence of the antagonist).

Statistical analysis

All data are expressed as means $\pm$ s.e.mean. Differences in the response induced by the highest concentration tested of α,β -MeATP and β,γ -MeATP in control conditions versus preincubation with TTX, L-NAME or CB-G were analysed by means of a Student's *t* test for unpaired data (significant level = $P < 0.05$).

Results

Effect of α,β -MeATP, β,γ -MeATP and ATP

The selective P_{2X} agonists, α,β -MeATP (0.3 μ M–1 mM; $n = 10$) and β,γ -MeATP (10 μ M–1 mM; $n = 8$) and the non selective purinergic agonist ATP (10 μ M–10 mM; $n = 4$) all contracted the detrusor muscle strip (Figure 1). A plateau of response could not be reached at the highest concentration tested. Agonist potencies are expressed as $-\log A_{35}$ values and were calculated as described in the Methods. The rank order of potency obtained was as follows: α,β -MeATP $>$ β,γ -MeATP $>$ ATP.

SCRC to ATP seemed non-monophasic since at the lower concentrations (10–300 μ M) the curve was shallow, whilst at higher concentrations (1–10 mM) the curve was steeper. In the presence of TTX (1 μ M) SCRC for α,β -MeATP was shifted to the left and the $-\log A_{35}$ value of the TTX-treated strips (4.96; 95% c.i. = 4.87–5.06) was significantly higher than that of controls (4.56; 95% c.i. = 4.39–4.71). However, the response at the highest concentration of agonist was unchanged (70.3% of KCl 100 mM response in controls versus 73.6% in TTX-treated; Figure 2a). The contractile response to β,γ -MeATP at the highest concentration tested was too low to calculate a $-\log A_{35}$ value but, in TTX-treated strips, responses to 1 mM β,γ -MeATP were greatly potentiated being 61.6% of KCl 100 mM response versus 27.7% of controls ($P < 0.01$; Figure 3). A $-\log A_{35}$ value of 3.95 (c.i. = 3.75–4.13) was estimated. These results are summarized in Table 1.

Effect of CB-G on α,β -MeATP and α,β -MeATP SCRC

CB-G was tested at 0.003, 0.3 and 1 μ M against α,β -MeATP-induced contractions. CB-G at 0.003 μ M was without effect

Figure 1 Serial concentration response curves to α,β -methylene ATP (●), β,γ -methylene ATP (▲) and ATP (◆). Each point represents the mean $\pm$ s.e.mean of 4–10 replications.

($n = 3$), but at 0.3 and 1 μ M ($n = 5$) shifted to the left the SCRC to α,β -MeATP without potentiating significantly the response at the highest concentration of the agonist (Figure 2b and Table 1). The $-\log A_{35}$ value for CB-G-treated strips was 5.18 (c.i. = 5.03–5.33) and it was significantly higher than the corresponding value for controls (4.56; c.i. = 4.39–4.71). Furthermore, CB-G at 0.3 μ M slightly shifted the SCRC to β,γ -MeATP to the left (Figure 3) and significantly potentiated the response at 1 mM ($46.3 \pm 5.6\%$

Figure 2 Serial concentration response curves to α,β -methylene ATP alone (●) or in presence of tetrodotoxin at 1 μ M (△); a), Coomassie Brilliant Blue G at 0.003 μ M (▲), 0.3 μ M (◆) and 1 μ M (■) (b) and L-NAME at 100 μ M (■); c). Each point represents the mean $\pm$ s.e.mean of 3–5 replications except for the control curve ($n = 10$). In (b) error bars have been omitted for clarity.

in CB-G-treated vs $27.9 \pm 4.5\%$ in controls, $P = 0.034$). These results are summarized in Table 1.

Effect of L-NAME on SCRC to α,β -MeATP and β,γ -MeATP

The nitric oxide synthase inhibitor L-NAME at $100 \mu\text{M}$ did not modify the α,β -MeATP SCRC (Figure 2c). However, L-NAME, at $100 \mu\text{M}$, slightly shifted the SCRC to β,γ -MeATP to the left (Figure 3) with a $-\log A_{35}$ value of 3.21 (c.i. = 3.00–3.41) and potentiated, although not significantly, the response to the highest dose of β,γ -MeATP ($41.4 \pm 2.4\%$ of KCl 100 mM response in treated strips versus $27.9 \pm 4.5\%$ in controls; $P = 0.068$).

Antagonism by suramin

Suramin (10 – $300 \mu\text{M}$; $n = 4$ – 7) dose-dependently antagonized the lower part of α,β -MeATP SCRC, whereas the upper part was much less affected (Figure 4). The antagonism was analysed by using dose-ratios at two different magnitude of agonist's effect as described in Methods. By using the dose-ratio at 10% of KCl response there was a good correlation between the right shifts and doses of suramin. The Schild plot slope was 0.98 ± 0.07 and it was not significantly different from one ($P > 0.05$; Figure 5). A pK_B value of 5.85 ± 0.04 (c.i. = 5.73–5.96) was estimated. On the other hand, the Schild plot using the dose-ratio at 35% of KCl 100 mM response was not linear and a lack of correlation between the concentrations of suramin tested and its effect was observed. In fact, 10 and $30 \mu\text{M}$ suramin gave a similar dose-ratio (DR = 1.88), as did 100 and $300 \mu\text{M}$ (DR = 10). This non-competitive antagonism of suramin on the upper

portion of the agonist SCRC did not allow us to estimate a pK_B .

Suramin at $30 \mu\text{M}$ ($n = 3$) clearly antagonized β,γ -MeATP SCRC but the low efficacy of this agonist precluded the use of larger concentrations of suramin to evaluate further the

Figure 4 Serial concentration response curves to α,β -methylene ATP alone (●) or in the presence of suramin at $10 \mu\text{M}$ (▲), $30 \mu\text{M}$ (◆), $100 \mu\text{M}$ (▽) and $300 \mu\text{M}$ (■). Each point represents the mean $\pm$ s.e.mean of 4–7 replications except for the control curve ($n = 10$).

Figure 3 Serial concentration response curves to β,γ -methylene ATP alone (●) or in the presence of tetrodotoxin at $1 \mu\text{M}$ (▲), Coomassie Brilliant Blue G at $0.3 \mu\text{M}$ (■) and L-NAME at $100 \mu\text{M}$ (◆). Each point represents the mean $\pm$ s.e.mean of 3–6 replications.

Figure 5 Schild regression analysis for suramin versus α,β -methylene ATP-induced contractions using the dose-ratios obtained at the level of agonist corresponding to 10% (●) and 35% (■) of KCl (100 mM)-effect.

Table 1 $-\log A_{35}$, confidence intervals (95% c.i.) and responses at the highest concentration tested (expressed as % of the contractile response to KCl 100 mM) (mean $\pm$ s.e.) induced by P_{2X}-purinoceptor agonists alone or in the presence of tetrodotoxin (TTX) at $1 \mu\text{M}$ or Coomassie Brilliant Blue G (CB-G) at $0.3 \mu\text{M}$ to contract human isolated urinary bladder

	Control		TTX-treated		CB-G-treated	
	$-\log A_{35}$	Response	$-\log A_{35}$	Response	$-\log A_{35}$	Response
α,β -MeATP	4.56 (4.39–4.71)	70.3 ± 6.3	4.96 (4.87–5.06)	73.6 ± 9.9	5.18 (5.03–5.33)	78.8 ± 11.7
β,γ -MeATP	< 3.0	27.7 ± 5.8	3.95 (3.75–4.13)	61.6 ± 3.1	3.54 (3.43–3.63)	46.3 ± 5.6
ATP	< 2.0	21.1 ± 2.8	NT		NT	

$-\log A_{35}$ values were calculated as described in Methods.
NT = not tested.

nature of the antagonism (Figure 6). Nevertheless, a dose-ratio analysis gave an estimated pK_B of 5.76, a value similar to that obtained with α,β -MeATP as agonist.

Effect of the selective A₁ antagonist DPCPX on α,β -MeATP and β,γ -MeATP SCRC

DPCPX at 1 μ M ($n = 5$) elicited a slight shift of the dose-response curve to β,γ -MeATP to the right (Figure 6) and was completely ineffective versus α,β -MeATP SCRC ($n = 3$; Figure 7).

Effects of suramin and CB-G on acetylcholine-induced contractions

Suramin and CB-G, both at 1 mM, did not induce a significant variation in the magnitude of the response to ACh ($n = 3$ for both antagonists).

Discussion

The rank order of potency for purinoceptor agonist found in this study seems to be consistent with the presence in the

human urinary bladder of the P_{2X}-purinoceptor subtype (Burnstock, 1991). However, the results obtained with the antagonists, suramin and CB-G and with the neuroblocking agent, TTX, also reveal that the purinoceptor population in human urinary bladder is not homogeneous. In fact, the antagonism by suramin was not identical on the entire SCRC to α,β -MeATP. This implies that the system may not be constituted of a single receptor population. It was found that suramin was a competitive antagonist only of the lower portion of the α,β -MeATP SCRC, whereas the upper part was much less affected. The Schild analysis using dose-ratios at 35% of the effect allowed us to draw a plot that was flat and therefore it did not satisfy the condition of competitive interaction between agonist and antagonist (Kenakin, 1987). On the other hand the Schild plot obtained at 10% of response was linear with a slope not statistically different from one, indicating that suramin was competitive in that portion of the α,β -MeATP SCRC and that the incubation times used were adequate. Hence two receptor populations with a different sensitivity to suramin, may be activated by α,β -MeATP and on the most sensitive receptor suramin showed a pK_B value of 5.85. Interestingly enough, a similar potency for suramin was found when the other agonist β,γ -MeATP was used ($pK_B = 5.76$).

However, such pK_B values are considerably greater than those reported for the same antagonist on P_{2X} receptors in rabbit isolated ear artery (Leff *et al.*, 1990). Thus we cannot exclude the existence of either P_{2X} receptor subtypes or some forms of species-differences. It is interesting to point out that other investigators recently proposed the presence of a population of P_{2X} purinoceptors insensitive to suramin in cat colon circular muscle (Venkova & Krier, 1993). Moreover, a non P_{2X} non P_{2Y} purinoceptor subtype not blocked by suramin and mediating the contraction of the rat vas deferens was postulated by Bultmann & Starke (1994). Again, it was recently shown that [³H]- α,β -MeATP labelled two populations of binding sites in several peripheral organ and brain regions of the rat, thus suggesting the existence of two different P₂-purinoceptors both activated by α,β -MeATP (Michel & Humphrey, 1993).

The nature of the purinoceptor insensitive to suramin needs to be further elucidated. However, it may not be an A₁ type because DPCPX was without effect on α,β -MeATP-induced contractions. We can also exclude that this receptor is the same as that activated by ADP β S recently proposed in human detrusor (Palea *et al.*, 1994). In fact ADP β S-induced contractions, in contrast to those induced by α,β -MeATP, were antagonized by *p*-chloro-mercuribenzenesulphonic acid.

The antagonist, CB-G, and the neuroblocking agent, TTX, shifted to the left both the two agonists SCRC. This effect was not nonspecific because CB-G did not enhance the contractility to exogenous ACh. Consequently, it might be that α,β -MeATP and β,γ -MeATP interact also with a prejunctional purinoceptor which induces the release of one or more inhibitory neurotransmitter(s). This latter, acting on the smooth muscle cell, counteracts the contractile effect evoked by P_{2X}-purinoceptor activation. To test the hypothesis that NO could be released as the inhibitory neurotransmitter by α,β -MeATP and β,γ -MeATP, experiments were carried out by using the NO synthase inhibitor L-NAME. Unfortunately, L-NAME had only a weak potentiating effect on β,γ -MeATP and α,β -MeATP SCRC, thus suggesting a modest role in the presynaptic effect of the two purine agonists.

The existence of prejunctional purinoceptors having some characteristics of the P_{2Y} subtype was proposed in the mouse isolated vas deferens (Von Kugelgen *et al.*, 1989). Furthermore, it was claimed that Reactive Blue 2 and CB-G were able to antagonize the inhibitory effect of β,γ -MeATP on urinary bladder contractions induced by pelvic nerve stimulation *in vivo* (Theobald, 1992), still suggesting the existence in the mammalian urinary bladder of a presynaptic inhibitory purinoceptor activated by β,γ -MeATP and antagonised by CB-G. Reactive Blue 2 is considered a relatively selective P_{2Y}

Figure 6 Serial concentration-response curves to β,γ -methylene ATP alone (●) or in the presence of DPCPX 1 μ M (■) and suramin 30 μ M (▲). Each point represents the mean $\pm$ s.e.mean of 3 replications except for the control curve ($n = 8$).

Figure 7 Serial concentration response curves to α,β -methylene ATP alone (●) or in the presence of DPCPX 1 μ M (▲). Each point represents the mean $\pm$ s.e.mean of 5 replications except for the control curve ($n = 10$).

antagonist although in a small concentration-range, so by analogy we can speculate that the prejunctional purinoceptor in our preparation is a P_{2Y} subtype. However, the fact that α,β -MeATP and β,γ -MeATP are reported to interact poorly with the P_{2Y} purinoceptor (Ralevic *et al.*, 1991; Boland *et al.*, 1992) casts some doubt about this hypothesis.

In conclusion, in human detrusor α,β -MeATP and β,γ -MeATP both act on an excitatory and an inhibitory purinoceptor. The physiological significance of the former could be the mediation of neurogenic contraction following ATP and ACh release from parasympathetic nerve terminals. In fact, a non cholinergic neurogenic response, inhibited by P_{2X} desensitization with α,β -MeATP was detected in human detrusor strips taken from patients undergoing cystectomy

for an intractable form of interstitial cystitis (Palea *et al.*, 1993). Moreover it was occasionally found also in muscle strips taken from patients undergoing cystectomy for bladder malignancy or surgery for bladder reconstruction (Hoyle *et al.*, 1989). The physiological role of the inhibitory purinoceptors may be found in the hypothesis that a basal release of ATP or a related compound (Burnstock *et al.*, 1978) activates this purinoceptor which, in turn, releases some inhibitory neurotransmitters in order to prevent detrusor muscle contraction during urinary bladder filling. This effect could be physiologically important in assuring the necessary compliance of the urinary bladder in response to bladder filling, a property which is fundamental for continence.

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