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P2X receptor expression in mouse urinary bladder and the requirement of $P2X_1$ receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle

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1 We have used subtype selective P2X receptor antibodies to determine the expression of $P2X_{1-7}$ receptor subunits in the mouse urinary bladder. In addition we have compared P2X receptor mediated responses in normal and $P2X_1$ receptor deficient mice to determine the contribution of the $P2X_1$ receptor to the mouse bladder smooth muscle P2X receptor phenotype.

2 $P2X_1$ receptor immunoreactivity was restricted to smooth muscle of the bladder and arteries and was predominantly associated with the extracellular membrane. Diffuse $P2X_2$ and $P2X_4$ receptor immunoreactivity not associated with the extracellular membrane was detected in the smooth muscle and epithelial layers. Immunoreactivity for the $P2X_7$ receptor was associated with the innermost epithelial layers and some diffuse staining was seen in the smooth muscle layer. $P2X_3$, $P2X_5$ and $P2X_6$ receptor immunoreactivity was not detected.

3 P2X receptor mediated inward currents and contractions were abolished in bladder smooth muscle from $P2X_1$ receptor deficient mice. In normal bladder nerve stimulation evoked contractions with P2X and muscarinic acetylcholine (mACh) receptor mediated components. In bladder from the $P2X_1$ receptor deficient mouse the contraction was mediated solely by mACh receptors. Contractions to carbachol were unaffected in $P2X_1$ receptor deficient mice demonstrating that there had been no compensatory effect on mACh receptors.

4 These results indicate that homomeric $P2X_1$ receptors underlie the bladder smooth muscle P2X receptor phenotype and suggest that mouse bladder from $P2X_1$ receptor deficient and normal animals may be models of human bladder function in normal and diseased states.
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Abbreviations: α,β-meATP, α,β-methylene ATP; 1-β,γ-meATP, 1-β,γ methylene ATP; mACh, muscarinic acetylcholine

Introduction

The reflex activation of the parasympathetic nerve supply is thought to be responsible for bladder emptying. ATP is co-stored and co-released with acetylcholine (Dowdall *et al*., 1974; Silinsky & Redman, 1996) and the neurogenic contraction of the bladder can be divided into atropine sensitive muscarinic ACh (mACh) receptor and atropine resistant P2X receptor mediated components (Kasakov & Burnstock, 1983). *In vitro* and *in vivo* studies on rodents have shown the P2X receptor response can account for a considerable component (up to 50%) of the neurogenic bladder contraction (Brading & Williams, 1990; Hegde *et al*., 1998; Igawa *et al*., 1993). P2X receptors are expressed on normal human bladder (Hoyle *et al*., 1989; Inoue & Brading, 1991) however they do not appear to contribute to the neurogenic response (Palea *et al*., 1993; Sibley, 1984). In contrast, a considerable component of the neurogenic contraction is mediated by P2X receptors in tissues from patients with bladder disorders, e.g. carcinoma or intestitial cystitis (Hoyle *et al*., 1989; Luheshi & Zar, 1990; Palea *et al*., 1993; Sjogren *et al*., 1982) suggesting that P2X receptors may play a role in disease states.

P2X receptors for ATP are a family of ligand gated cation channels. Seven isoforms of the receptor ($P2X_{1-7}$) have been isolated at the molecular level (Burnstock, 1997). P2X receptors are formed by the multimeric assembly of channel subunits and can associate as either homo-multimers or hetero-multimers (Torres *et al*., 1999). Heteromeric channels often have composite phenotypes derived from a combination of the properties of the constituent subunits e.g $P2X_{2/3}$ (Lewis *et al*., 1995), $P2X_{4/6}$ (Le *et al*., 1998) and $P2X_{1/5}$ receptors (Haines *et al*., 1999; Le *et al*., 1999; Torres *et al*., 1998). Immunohistochemical studies in rats have described the distribution of P2X receptors within the bladder (Dutton *et al*., 1999; Lee *et al*., 2000). Dutton *et al*. (1999) reported large numbers of homomeric and mixed sub-type clusters of $P2X_{1-6}$ receptors expressed on smooth muscle cells. In contrast Lee *et al*. (2000) reported $P2X_1$ receptor immunoreactivity associated with muscle membranes and non-membrane associated staining of $P2X_{2,5\&6}$ receptors. Thus the expression of P2X receptor subunits in bladder remains unclear and raises the possibility that urinary bladder smooth muscle cells may express a variety of different P2X receptor combinations. The functional properties of P2X receptors in urinary bladder smooth muscle, desensitising α,β-methylene ATP (α,β-meATP) sensitive responses, correspond closely to those of recombinant homomeric $P2X_1$ receptors (Inoue & Brading, 1990; Kasakov & Burnstock, 1983). However due to the lack of subtype selective P2X receptor agonists it is difficult to exclude the possibility that other P2X receptor subunits may also contribute to a bladder smooth muscle phenotype that is dominated by the expression of $P2X_1$ receptor subunits. We have recently shown using $P2X_1$ receptor deficient transgenic mice that the $P2X_1$ receptor is essential for functional P2X

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receptor responses in the smooth muscle of the vas deferens (Mulryan *et al*., 2000).

In order to investigate the role(s) of P2X receptor isoforms in control of mouse urinary bladder we have (1) used immunohistochemical techniques to characterize the expression pattern of P2X receptor subunits in the bladder and (2) compared the functional response of bladder smooth muscle from normal (+/+) and $P2X_1$ receptor deficient (−/−) mice to determine whether P2X receptors other than the $P2X_1$ receptor subtype contribute to the bladder smooth muscle phenotype.

Methods

Adult male $P2X_1$-receptor-deficient (−/−), heterozygote (+/−) or wild type (+/+) mice (Mulryan *et al*., 2000) were killed by cervical dislocation, exsanguinated and the urinary bladder was dissected. For immunohistochemical studies the bladder was rapidly frozen in Tissue-Tek® (Sakuna, The Netherlands), cut into section of 12 μm thick, and thaw-mounted on 3-aminopropyltriethoxysilane (Sigma-Aldrich, U.K.) coated slides. The sections were fixed in 2% paraformaldehyde (Sigma-Aldrich, U.K.), 80 mM Na_2HPO_4, 20 mM NaH_2PO_4 for 10 min and washed in PBS. The sections were then incubated in 0.5% Triton X-100 (Sigma-Aldrich, U.K.), 10% normal donkey serum (Jackson Immunoresearch Laboratories, U.S.A.) in PBS for 30 min at room temperature, washed in PBS and incubated overnight at 4°C in primary antisera in the absence or presence of the control antigen blocking peptide. Primary antibodies directed against different P2X receptor subtypes (anti-$P2X_1$, anti-$P2X_2$, anti-$P2X_4$ and anti $P2X_7$ Alomone Laboratories, Israel, anti-$P2X_3$ from Dr L. Vulchanova and Prof R. Elde, U. Minnesota, U.S.A and anti-$P2X_5$ and anti-$P2X_6$ from Roche Bioscience, U.S.A.) have been used. These antibodies were derived for rat epitopes; where amino acid sequences for P2X receptor subtypes are available for mouse there is complete conservation at the amino acid level for the $P2X_3$ receptor epitope and 18/19, 14/16 and 18/20 amino acids are identical for $P2X_1$, $P2X_2$ and $P2X_7$ receptors, the amino acid sequence for mouse $P2X_4$, $P2X_5$ and $P2X_6$ receptors are not available, however specific staining that is reduced by the appropriate blocking peptide is seen for these antibodies in mouse tissues (this study and unpublished observations). The antibodies were used at a dilution of 1 : 200 with 10% normal donkey serum in PBS. For negative control, 1 μg of each primary antibody was pre-incubated with 1 μg of its corresponding antigen peptide for 1 h at room temperature. The sections were subsequently washed in PBS, incubated with Texas-Red® dye-conjugated donkey anti-rabbit IgG (dilution 1 : 100) (Jackson Immunoresearch Laboratories, U.S.A.) in presence of 10% normal donkey serum in PBS for 2 h at room temperature, washed in PBS and then mounted in Citifluor (UKC. Chem Lab, U.K.). Finally, bladder sections were examined under epifluorescence microscope with neutral density filters. Images were captured using Scionimage software.

For physiological studies bladders were dissected and placed in Ringers solution and epithelium-free strips of bladder (as described previously e.g Bailey & Hourani 1994) were cut in the longitudinal axis of the detrusor. In contraction experiments silk ligatures were attached to each end. Strips were then mounted in Ringers solution at 35°C in 15 ml organ bath under an initial load of 1 g and the tension was monitored isometrically. Agonists were adding to the organ bath at 30 min intervals for purine compounds and 10 min intervals for carbachol, and removed by three successive washings following the maximum response. Trains of electrical field stimulation (10 and 100 pulses at 10 Hz, 45 V 0.5 ms pulse width) were given at 5 and 10 min intervals for 10 and 100 pulses respectively.

Concentration response relationships are expressed as per cent of the maximum response to carbachol and fitted by the least squares method using Origin software (Microsoft, U.S.A.) with the equation;

$$\text{response} = \alpha[A]^H/([A]^H + [EC_{50}]^H).$$

α and H are the asymptote and Hill coefficient, [A] is the agonist concentration and EC_{50} is the agonist concentration producing 50% of the maximum agonist response, pEC_{50} is $-\log_{10}$ (EC_{50}). Data are presented throughout as mean ± s.e.mean, n = number of observations. Differences between means were determined by the appropriate Student's t-test and were considered significant when $P<0.05$.

Bladder smooth muscle cells were dissociated by using a two-step papain and collagenase/hyaluronidase enzymatic digestion (Quayle *et al*., 1996) for patch clamp studies. Cells were plated onto glass coverslips, stored at 4°C and used within 1–7 h. For electrophysiological recordings, smooth muscle cells were superfused at 2 ml min^{-1} with a physiological solution containing (in mM): NaCl 150, KCl 2.5, HEPES 10, $CaCl_2$ 2.5 and $MgCl_2$ 1 (pH adjusted to 7.3 with NaOH). Drugs were applied rapidly using a U-tube perfusion system (Evans & Kennedy, 1994). Experiments were performed at a holding potential of −60 mV at room temperature. Currents were recorded with an Axopatch 200B amplifier and data was collected using pClamp7 software (Axon Instruments, U.S.A.). Patch electrodes (2–5 MΩ) were filled with a solution containing (in mM): potassium gluconate 140, NaCl 5, HEPES 10, EGTA 9, (pH adjusted to 7.3 with KOH).

Drugs: Papain, dithioerythritol, collagenase, hyaluronidase, atropine, carbachol, α,β methylene ATP (α,β-meATP), ATP, 1-β, γ methylene ATP (1-β, γ-meATP) (Sigma-Aldrich, UK).

Results

To determine the distribution of P2X receptor subunits in the bladder we have used subtype selective antibodies raised against $P2X_{1-7}$ receptors. In sections of wild type (+/+) mouse bladder, $P2X_1$ receptor immunoreactivity was strongly detected in the detrusor and the blood vessels (including those in the epithelium), whereas no staining was associated directly with the epithelium (Figure 1a). $P2X_1$ receptor immunoreactivity appeared to be localized on the membranes of the smooth muscle cells (Figure 1b). In $P2X_1$ receptor deficient mice (−/−), $P2X_1$ receptor immunoreactivity was absent in bladder smooth muscle cells and blood vessels (Figure 1c). $P2X_2$ receptor immunoreactivity was associated with the detrusor muscle (Figure 1d) and the epithelium (Figure 1f) and appeared to be diffuse with no obvious localization at the extracellular membrane. In the bladder wall, the $P2X_2$ antibody reacted with interstitial cells localized between the smooth muscle cells (Figure 1d) and in the epithelium the staining appeared to be restricted to a small number of cells (Figure 1f). A similar staining pattern was seen for the $P2X_4$ receptor with subtype weak diffuse specific immunoreactivity associated with the detrusor muscle (Figure 1h) and epithelium (Figure 1j). $P2X_7$ receptor specific immunoreactivity was clearly localized on the luminal side

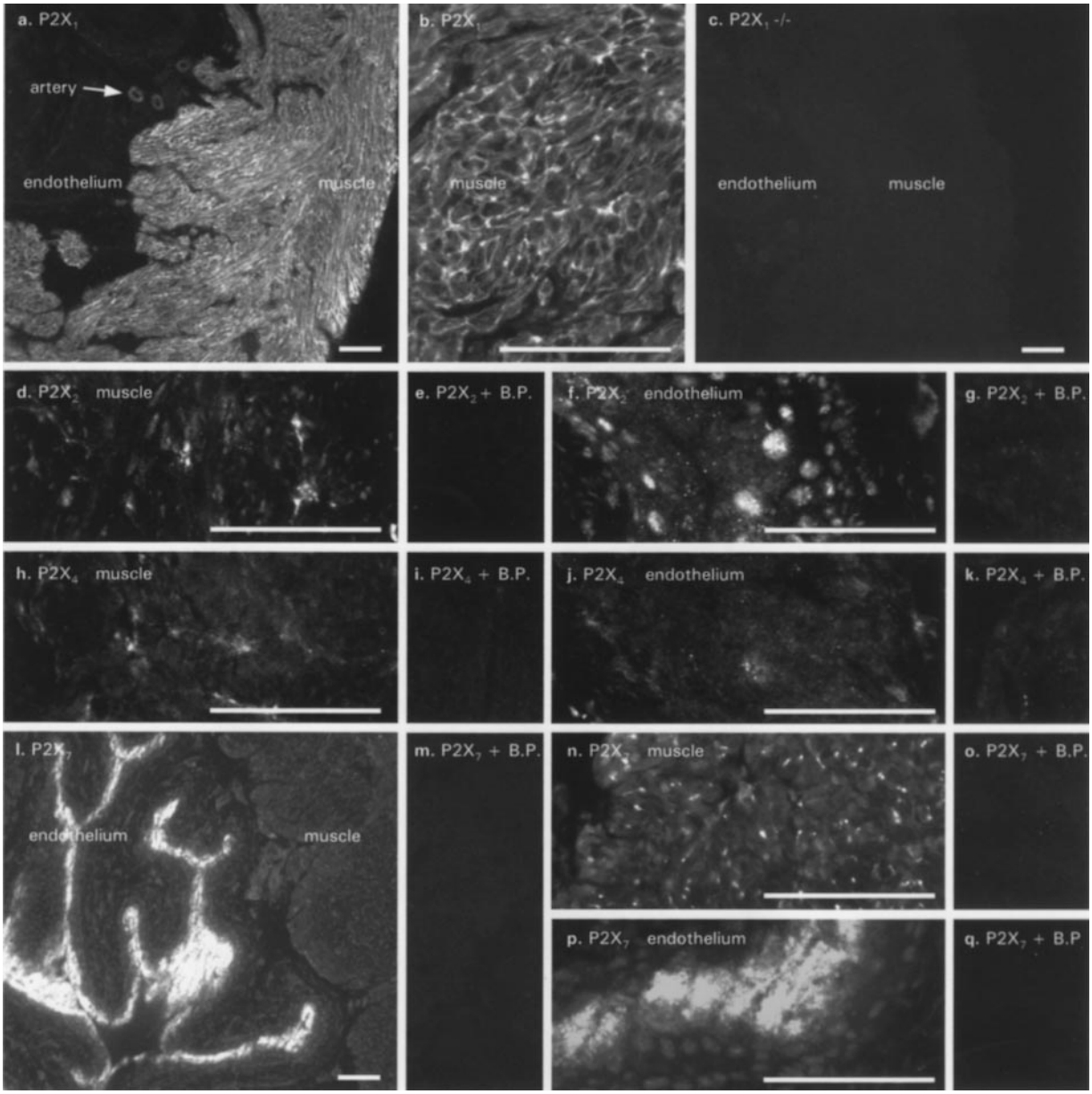

Figure 1 Immunohistochemical detection of $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_7$ receptor subunits in mouse bladder. $P2X_1$ receptor immunoreactivity (+/+) in mouse bladder (a) with detail of the detrusor smooth muscle (b) and in (−/−) mouse bladder (c). $P2X_2$ receptor immunoreactivity in mouse bladder detrusor (d) with control antigen blocking peptide (B.P.) (e) and in epithelium (f) with B.P. (g). $P2X_4$ receptor immunoreactivity in mouse bladder detrusor (h) with B.P. (i) and in epithelium (j) with B.P. (k). $P2X_7$ receptor immunoreactivity in mouse bladder detrusor (l) with B.P. (m). Details of the detrusor (n) and of the epithelium (p), respectively with B.P. (o) and (q). Magnification 10× (a,c,l,m). Magnification 40× (b,d,e,f,g,h,i,j,k,n,o,p,q). Bar = 100 μm.

of epithelial cells (Figure 1l) but the subcellular localization of the immunoreactivity was unclear (Figure 1p). At high magnification, a homogenous $P2X_7$ receptor labelling was shown in the bladder wall, in interstitial cells localized between the smooth muscles cells (Figure 1n). $P2X_{3,5\&6}$ receptor specific immunoreactivity was not detected in the bladder.

The immunohistochemical studies indicate that $P2X_1$, $P2X_2$ and $P2X_4$ receptors are expressed in the smooth muscle layer of the bladder, however only the $P2X_1$ receptor appears to be localized to the extracellular membrane. To determine the role of $P2X_1$ receptors in the smooth muscle of the bladder we have compared responses to exogenously applied agonists and nerve stimulation in normal and $P2X_1$ receptor deficient mice. In addition this approach will allow the contribution of non-$P2X_1$ receptor subtypes to the bladder smooth muscle P2X phenotype to be examined. ATP (1 mM) and the metabolically stable purinergic agonists α,β-meATP and L-β,γ-meATP (both 100 μM) evoked transient contractions of the mouse urinary bladder that decayed back to baseline during the continued presence of agonist (Figure 2a). ATP was considerably less potent than α,β-meATP at evoking contractions in +/+ mice. This probably reflects the breakdown of ATP by ectonucleotidases in whole tissue studies (Inoue & Brading, 1990). The agonists failed to evoke contractions in $P2X_1$ receptor deficient −/− mice (Figure 2b). To determine whether there is any residual ATP sensitive P2X receptor mediated response we have used patch clamp studies where agonists can be applied rapidly under concentration clamp conditions. ATP and α,β-meATP (10 μM) evoked rapid transient inward currents in acutely dissociated bladder smooth muscle cells (peak amplitude 2947 ± 926 and 3713 ± 915 respectively $n = 7-8$) (Figure 3a). In contrast ATP (100 μM) and α,β-meATP (10 μM) had no

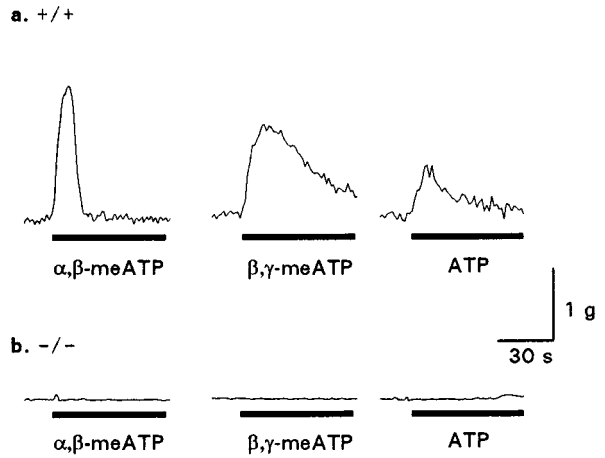

Figure 2 Effects of the $P2X_1$ receptor deficiency on mouse bladder contractions in response to α,β-maATP, β,γ-meATP and ATP. α,β-maATP (100 μM), β,γ-meATP (100 μM) and ATP (100 μM) evoked transient contractions of the (+/+) mouse bladder smooth muscle (a), no response was evoked by these three nucleotides in (−/−) mouse bladder smooth muscle (b). Drugs were applied for the time indicated by the bar.

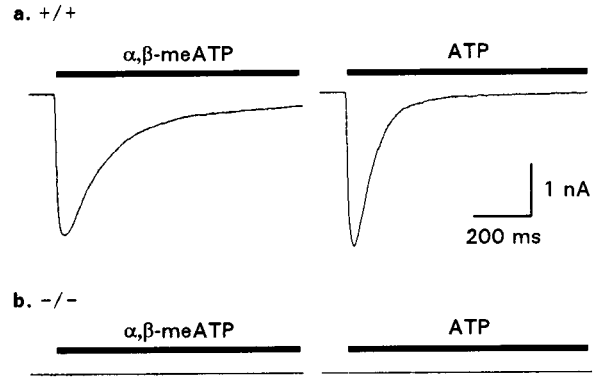

Figure 3 P2X receptor mediated inward currents are abolished in $P2X_1$ receptor deficient mice. Representative traces of inward currents evoked by α,β-meATP (10 μM) and ATP (10 μM) in acutely dissociated (+/+) mouse bladder smooth muscle cells (a). In dissociated (−/−) mouse bladder smooth muscle cells, α,β-meATP (10 μM) and ATP (100 μM) failed to evoke inward currents (b). Drugs were applied for the time indicated by the bar.

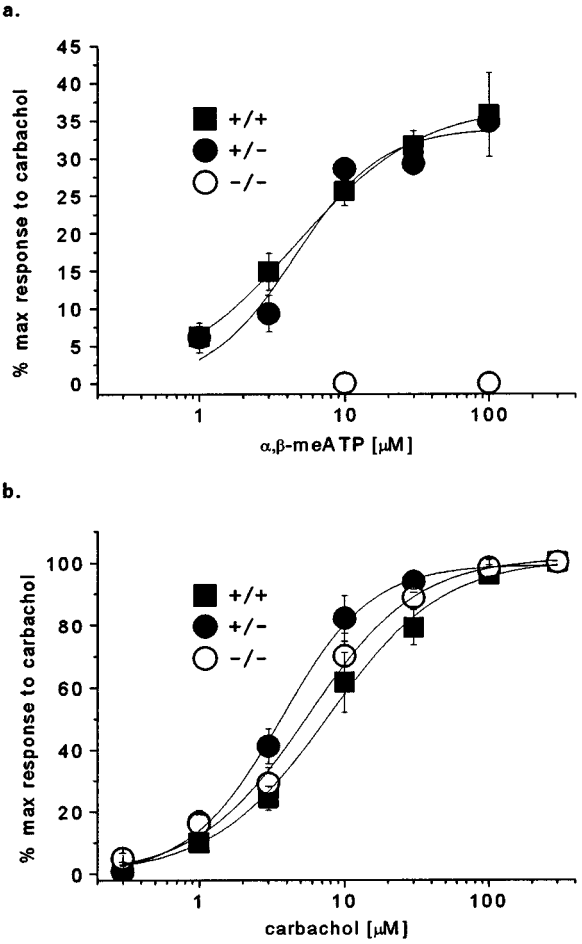

Figure 4 Concentration response relationships for $P2X_1$ receptor (+/+), (+/−) and (−/−) mouse detrusor contractions evoked by α,β-meATP (a) or carbachol (b). Each point is the mean percentage of the maximum response evoked by 100 μM carbachol ($n=6-8$ for each point).

effect on the holding current on bladder smooth muscle cells from −/− mice ($n=7-8$) (Figure 3b).

There was no difference in the amplitude or concentration dependence of α,β-meATP evoked contractions between mice +/+ and +/− for the $P2X_1$ receptor (the pEC_{50} values were 5.39 ± 0.09 and 5.31 ± 0.04 respectively, $n=7-8$, Figure 4a). This indicates that like for the vas deferens (Mulryan *et al.*, 2000) only one copy of the $P2X_1$ receptor gene is required for normal expression of P2X receptors in bladder smooth muscle. The peak amplitude of contractions to a maximal concentration of carbachol were the same for +/+, +/− and −/− mice (4.3 ± 0.5, 4.4 ± 0.4 and 4.5 ± 0.3 respectively) and so data have been normalized to the response to the maximal carbachol response. In the bladder the maximal response and pEC_{50} for carbachol is the same for +/+, +/− and −/− mice (pEC_{50}s of 5.07 ± 0.14, 5.4 ± 0.09 and 5.28 ± 0.09 respectively, $n=4-10$ Figure 4b) demonstrating that the lack of contraction to purinergic agonists of the −/− mouse bladder is not due to an effect on contractile function. In addition to the lack of effect on contractions to carbachol there was no obvious difference in bladder size or function between +/+ and −/− mice indicating that the $P2X_1$ receptor deficiency had no effect on the development or morphology of the bladder, and as reported previously there is no obvious effect of the $P2X_1$ receptor deficiency on bladder filling (Mulryan *et al.*, 2000).

Nerve stimulation (10 Hz) evoked bladder contractions, the amplitude of the response was dependent on the length of stimulation and the $P2X_1$ receptor genotype of the mouse (Figures 5 and 6). The peak amplitude of the response for $P2X_1$ receptor −/− mice was ~30 and 50% of that for +/+ or +/− mice for 10 and 100 pulses respectively. After treatment with α,β-meATP to desensitize the P2X receptors on the smooth muscle contractions to nerve stimulation of $P2X_1$ receptor +/+ or +/− mice were the same as those for −/− mice. α,β-meATP treatment had no effect on the amplitude of contractions in $P2X_1$ −/− mice. Atropine (10 μM) treatment revealed the presence of endogenous muscarinic acetylcholine receptor mediated tone in the bladder (0.22 ± 0.06 and 0.34 ± 0.12 g for +/+ or +/− and −/− respectively, $n=$, Figure 5). The residual contractions in the presence of α,β-meATP were abolished by atropine (10 μM) (Figures 5 and 6).

Discussion

In this study we have shown that there is a heterogeneous distribution of P2X receptor subunits in the bladder. $P2X_1$

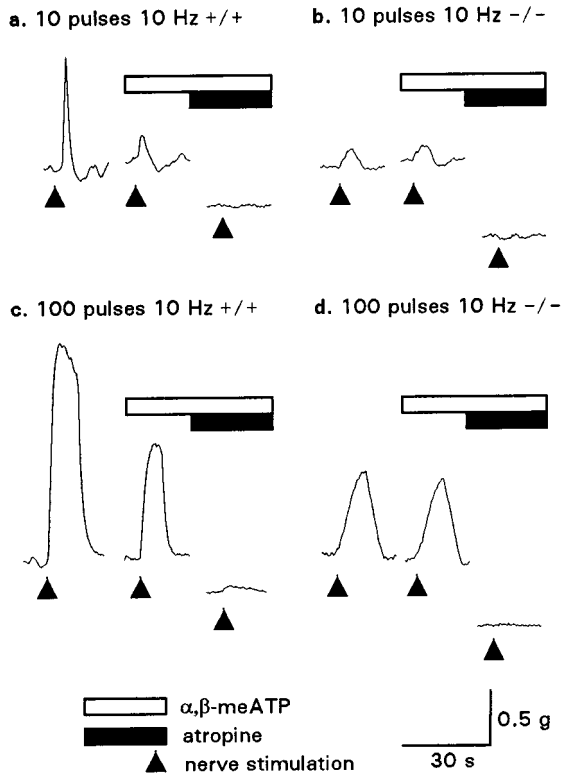

Figure 5 Contraction of the mouse bladder detrusor muscle to electrical field stimulation. A train of 10 pulses at 10 Hz applied to normal mouse detrusor evoked a contraction. The amplitude of the contraction decreased when α,β-meATP (100 μM) was applied before the stimulation and was abolished by the concomitant the addition of atropine (10 μM) (a). For $P2X_1$ receptor deficient (−/−) mouse bladder smooth muscle, the contraction was reduced in amplitude, and was unaffected by the addition of α,β-meATP. Atropine abolished the nerve evoked contraction (b). Similar responses to (a) and (b) are obtained for a train of 100 pulses at 10 Hz respectively in normal (+/+) (c) and $P2X_1$ receptor deficient (−/−) (d) mouse detrusor. Drugs were applied for the time indicated by the bar.

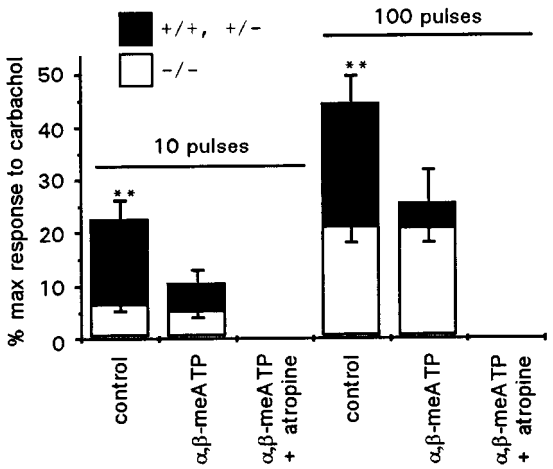

Figure 6 Comparison between the amplitude of nerve evoked contractions in normal (+/+) and $P2X_1$ receptor defieicent (−/−) mouse bladder smooth muscle. Contractions were evoked by trains pulses of 10 or 100 pulses at 10 Hz, the effects of α,β-meATP (100 μM) and atropine (10 μM) on the amplitude of contractions are shown. Each column is the mean response expressed as a percentage of the maximum contraction response to 100 μM carbachol ($n=6-8$) (**).

receptor immunoreactivity was localized to the smooth muscle membranes of the bladder and associated arteries and ATP mediated bladder responses were absent in $P2X_1$ receptor deficient mice indicating that this receptor underlies the bladder smooth muscle P2X receptor phenotype.

The presence of multiple P2X receptor isoforms in the bladder has been shown previously in rat tissues by Northern analysis (Bo *et al*., 1995; Brake *et al*., 1994; Valera *et al*., 1994). However it is only in immunohistochemical studies (Dutton *et al*., 1999; Lee *et al*., 2000; Vulchanova *et al*., 1996) that the cellular localization of P2X receptor isoforms has been described. The distribution of immunoreactivity in the mouse is similar to that reported recently by Lee *et al*. (2000) for the rat bladder, particularly with regard to only $P2X_1$ receptor immunoreactivity being localized to the smooth muscle membranes (like the present study antibodies were raised against the carboxy termini of the intracellular loop). This is in marked contrast to the study of Dutton *et al*. (1999), where large numbers of homomeric and heteromeric P2X receptor clusters were detected using antibodies generated against variant regions of the extracellular loop of the receptors. The results from the use of $P2X_1$ receptor deficient mice are consistent with the membrane localization of only $P2X_1$ receptors and we have no evidence for the expression of other functional P2X receptor subtypes on the smooth muscle of the mouse bladder. The role of the $P2X_2$, $P2X_4$ and $P2X_7$ receptors associated with cell types other than smooth muscle in the bladder function remains unclear.

The transient α,β-meATP sensitive P2X receptor mediated inward currents and associated contractions of mouse bladder smooth muscle were essentially the same as those reported previously for rabbit, rat, guinea-pig and human urinary bladder (Bailey & Hourani, 1994; Hoyle *et al*., 1989; Inoue & Brading, 1991; Ziganshin *et al*., 1993). The similarity between these species suggests that bladder smooth muscle has a common P2X receptor phenotype. P2X receptor mediated responses, either inward currents or contractions, were not detected in $P2X_1$ receptor deficient mice. These results indicate that the $P2X_1$ receptor is essential for the expression of functional P2X receptors in the smooth muscle of the bladder. In the immunohistochemical studies diffuse, non-membrane associated $P2X_2$ and $P2X_4$ receptor specific immunoreactivity was recorded in the smooth muscle layer. $P2X_2$ and $P2X_4$ receptors are capable as forming as homomeric receptors (Brake *et al*., 1994; Buell *et al*., 1996), when these subunits are co-expressed they do not appear to form heteromeric $P2X_{2/4}$ receptor channels (Torres *et al*., 1999). The lack of a residual P2X receptor mediated response in $P2X_1$ receptor deficient mice confirms that $P2X_2$ or $P2X_4$ receptor subunits are not expressed functionally at the smooth muscle membrane. These results indicate that homomeric $P2X_1$ receptors underlie the bladder smooth muscle P2X receptor phenotype as has been reported for the vas deferens smooth muscle (Mulryan *et al*., 2000; Valera *et al*., 1994).

A substantial component of the nerve evoked bladder contraction is mediated through the $P2X_1$ receptor with the muscarinic acetylcholine receptors accounting for the residual response. The P2X receptor component was particularly apparent with short trains of stimuli (~70% with 1 s trains of stimuli). This may correspond to the rapid activation of ligand gated P2X receptors compared to the slower activation of G-protein coupled muscarinic acetylcholine receptors. Similar results have been reported for other rodent bladders (Brading & Williams, 1990; Kasakov & Burnstock, 1983; Ziganshin *et al*., 1993). Bladder function in $P2X_1$ receptor deficient mice appeared normal (Mulryan *et al*., 2000). This

suggests that the residual muscarinic acetylcholine receptor mediated response is sufficient for normal bladder function. *In vivo* studies on rats have shown that either the muscarinic or purinergic component can be blocked and have no major effect on bladder function (Igawa *et al*., 1993). These results suggest that the dual purinergic and cholinergic systems act as a failsafe mechanisms for bladder contractile function. Further support for this assumption comes from the lack of compensatory mechanisms in the mouse bladder smooth muscle. There was no change in the magnitude or concentration dependence of contractions to the muscarinic receptor agonist carbachol in $P2X_1$ receptor deficient mice. Similarly there was no difference between the amplitude of nerve evoked contractions between $P2X_1$ receptor deficient mice and the residual cholinergic component in normal mice. This is in contrast to studies on the vas deferens where the $P2X_1$ receptor deficiency resulted in an increased sensitivity to noradrenaline and an increase in the α-adrenoceptor mediated component of contraction following sympathetic nerve stimulation (Mulryan *et al*., 2000).

There is a marked difference between rodent and human bladders in the contribution of P2X receptors to neurogenic contractions of the bladder. In normal human bladder the nerve evoked contraction is essentially abolished by the muscarinic receptor antagonist atropine (Kinder & Mundy, 1985; Palea *et al*., 1993; Sibley, 1984) even though P2X receptors are expressed by human bladder and mediate contraction to exogenously applied agonists (Hoyle *et al*., 1989; Inoue & Brading, 1991; Palea *et al*., 1993). However in disease states, e.g. carcinoma or intistitial cystitis a residual P2X receptor mediated component of up to 40% may be found. Thus it is possible that P2X receptors may play a role in the aetiology of human bladder disease. In the present study we have shown that $P2X_1$ receptors play a substantial role in the neurogenic control of bladder smooth muscle. The lack of a neurogenic P2X receptor mediated bladder contraction in humans has meant that rodent bladder has not been a good model to study normal human bladder function. This study suggests that the $P2X_1$ receptor deficient mouse may provide a model for normal human (cholinergic) bladder and the normal mouse can be used as a model of diseased (mixed purinergic-cholinergic) bladder control.

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