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ACCELERATED COMMUNICATION

Loss of Nucleotide Regulation of Epithelial Chloride Transport in the Jejunum of $P2Y_4$-Null Mice

BERNARD ROBAYE, ESAM GHANEM, FRANÇOISE WILKIN, DOMINIQUE FOKAN, WILLY VAN DRIESSCHE, STÉPHANE SCHURMANS, JEAN-MARIE BOEYNAEMS, and RENAUD BEAUWENS

Institute of Interdisciplinary Research, Institute of Biology and Molecular Medicine, Université Libre de Bruxelles, Gosselies, Belgium (B.R., F.W., D.F., S.S., J.-M.B.), Laboratory of Physiopathology, School of Medicine, Université Libre de Bruxelles, Brussels, Belgium (E.G., R.B.), Department of Physiology, Katholiek Universiteit of Leuven, Leuven, Belgium (W.V.D.), Laboratory of Medical Chemistry, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium (J.-M.B.)

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ABSTRACT

The $P2Y_4$ receptor is responsive to UTP in human and to ATP and UTP in rodents. With the aim of identifying its pharmacotherapeutic interest, we generated $P2Y_4$-null mice by a classic gene targeting method. The proportion of genotypes was consistent with X-linked Mendelian transmission. Gene inactivation was checked by the complete disappearance of $P2Y_4$ receptor mRNA from liver, stomach, and intestine. The $P2Y_4$-null mice had a grossly normal behavior, growth, and reproduction. Chloride secretion by the jejunal epithelium was assessed in Ussing chambers by the measurement of the short circuit current in the presence of phlorizin. We show here that the UTP- and ATP-induced chloride secretory responses observed in wild-type mice are abolished in $P2Y_4$-null mice. This is the first clearcut demonstration of a biological role of the $P2Y_4$ receptor.

The $P2Y_4$ receptor is a member of the P2Y family of G-protein-coupled receptors for extracellular nucleotides. Cloning of the human $P2Y_4$ receptor was reported in 1995 (Communi et al., 1995; Nguyen et al., 1995; Stam et al., 1996) and was followed by the cloning of the rat (Bogdanov et al., 1998; Webb et al., 1998) and mouse (Lazarowski et al., 2001; Suarez-Huerta et al., 2001) orthologs. The human $P2Y_4$ receptor is a selective UTP receptor, whereas the rodent ones are activated equipotently by UTP and ATP. No physiological role of the $P2Y_4$ receptor has yet been established, and its pharmacotherapeutic potential thus remains uncertain. $P2Y_4$ mRNA has been detected in human placenta (Communi et al., 1995) and in murine stomach, intestine, and liver (Suarez-Huerta et al., 2001). In the adult rat, they were detected in multiple organs but at a low level, whereas the expression was higher in neonatal animals (Webb et al., 1998). The presence of $P2Y_4$ mRNA and/or protein, combined with functional responses to UTP, supports the expression of the $P2Y_4$ receptor in 6CFSMEo- submucosal cells derived from human lung (Communi et al., 1999), in the vestibular dark cell epithelium of gerbil inner ear (Marcus and Scofield, 2001; Sage and Marcus, 2002), and in rat aortic smooth muscle cells (Harper et al., 1998). Expression in endothelial cells (Jin et al., 1998) has also been reported and might be related to the chemotactic and mitogenic actions of UTP on guinea pig coronary endothelial cells (Satterwhite et al., 1999). In addition, the persistence of a Cl^- secretory response to UTP and ATP in $P2Y_2^{-/-}$ mice led to the suggestion that this response could be mediated by the $P2Y_4$ receptor (Cressman et al., 1999). To evaluate the physiological role and pharmacotherapeutic potential of the $P2Y_4$ receptor we have generated $P2Y_4$-deficient mice. In the work reported here, we focused our attention on the regulation by ATP and UTP of the Cl^- secretion in the jejunal epithelium with the aim of identifying the P2Y receptor mediating this control.

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ABBREVIATIONS: bp, base pair(s); PCR, polymerase chain reaction; RT, reverse transcription; HPRT, hypoxanthine phosphoribosyltransferase; SSC, short-circuit current; 8-*p*-SPT, 8-(*para*-sulfophenyl)theophylline; ES, embryonic stem; CFTR, cystic fibrosis transmembrane conductance regulator.

Materials and Methods

Generation of $P2Y_4$-Deficient Mice. To generate the targeting vector, a 5′-*Sal*I-*Xba*I-3′ 4900-bp fragment, with the *Xba*I site located 700 bp upstream from the initiation codon of the *P2Y₄* gene, was inserted in the pFlox vector upstream from the neomycin gene (Fig. 1A). A 2300-bp fragment, beginning at base 399 of the coding sequence of the gene and obtained by PCR, was inserted in the *Bam*HI and *Xho*I sites of the targeting plasmid (i.e., downstream from the neomycin resistance cassette). Upon integration of the targeting vector, 1100 bp of the recombinant locus containing the first 398 bp of the published *P2Y₄* coding sequence are deleted.

After electroporation of this targeting vector in R1 ES cells, G-418–resistant colonies were isolated. Classically extracted genomic DNA was analyzed by *Eco*RI digestion followed by Southern blotting using a DNA probe located outside the targeting vector, downstream from the *P2Y₄* gene. This probe detected a 7-kilobase pair fragment of the wild-type locus and a 8.6-kilobase pair fragment of the mutated locus.

R1 $P2Y_4^{+/-}$ ES cells were cocultured overnight with CD1 morulae. Blastulae were then transferred into CD1 pseudopregnant female mice to generate chimeric mice. Offspring genotype was analyzed by Southern blotting as described above or by PCR. The actual repartition of genotypes was compared with the theoretical repartition expected from a binomial distribution with equal frequency of each allele.

Total RNA Isolation, Reverse Transcription, and PCR Procedure. The expression of the $P2Y_4$ receptor mRNA was studied on tissues extracted from wild-type and $P2Y_4$-null male mice. Total mouse RNA was isolated from tested organs using TriPure reagent following the manufacturer's instructions. One microgram of total RNA was submitted to OneStep reverse transcription PCR according to the manufacturer's recommendations. Oligonucleotide amplification primers were designed according to the mouse *P2Y₄* sequence: sense primer, 5′-gactccttgctattcacat-3′; antisense primer, 5′-agtagaggttccagtagaaa-3′. OneStep RT-PCR was performed with 0.6 μM concentrations of each primer, with Q solution, and with 0.6 units of RNase inhibitor under the following conditions: 30 min at 50°C for the reverse transcription, 15 min at 95°C for the inactivation of the reverse transcriptase and the initial PCR activation step, followed by 3-step cycles (30 or 40 times) of 60 s at 94°C, 60 s at 52°C, 60 s at 72°C, and a final extension of 10 min at 72°C. Genomic DNA contamination was checked by omitting reverse transcription.

The expression of HPRT mRNA was used for internal control of expression. Oligonucleotide amplification primers were designed according to the mouse HPRT sequence: sense primer, 5′-gctggtgaaaaggacctct-3′; antisense primer, 5′-cacaggactagaacacctgc-3′. The protocol was the same as reported above, except that the PCR conditions were: (30 s at 94°C, 45 s at 57°C, 60 s at 72°C) × 30 cycles, and a final extension of 10 min at 72°C.

Amplification products were resolved on 1.5% (w/v) agarose gel by electrophoresis and visualized under UV light after ethidium bromide coloration.

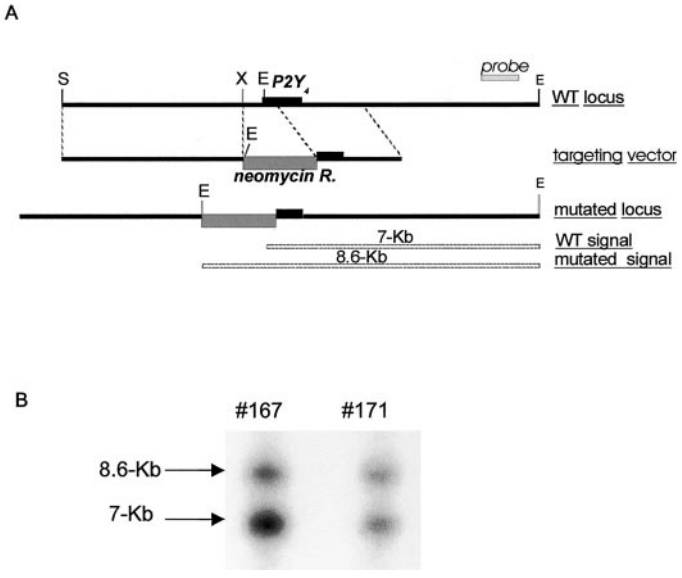

Fig. 1. A, schematic representation of the targeting vector, endogenous allele and mutated allele after homologous integration. X, *Xba*I; E, *Eco*RI; S, *Sal*I. B, Southern blot analysis of genomic DNA extracted from two recombinant clones of ES cells. After extraction, genomic DNA of ES clones was analyzed by *Eco*RI digestion and Southern blotting as described under *Materials and Methods*. The 7-kilobase pair signal corresponds to the wild-type allele and the 8.6-kilobase pair signal to the targeted allele.

Bioelectric Measurements in Isolated Mouse Jejunum. The technique will be fully described in another article that will cover the theoretical aspect of the impedance analysis performed. Briefly, mice aged 2 to 5 months were sacrificed by intraperitoneal pentobarbital (10 mg/kg). The midportion of the jejunum, extending 10 cm after the ligament of Treitz, was dissected, opened, and washed with Krebs bicarbonate solution. The jejunal mucosa was stripped from the adjacent muscularis layer and sealed on the basolateral side to a fixation ring with an opening diameter of 3 mm. This ring was placed between the halves of an Ussing chamber. KCl electrodes connected to the solution via a short agar bridge were used for measuring potential difference and passing current. Impedance analysis was used to determine the resistance of the epithelium and bathing solution between the voltage electrodes. The impedance spectrum represented in a Nyquist diagram consisted of a single semicircle. This behavior is caused by the paracellular conductance related to the structure of the epithelium and/or possible damage of the epithelium caused by stripping the mucosa. Given these parameters, the epithelium can be represented by a lumped model consisting of a parallel circuit of a capacitance and resistance in series with the solution resistance between the voltage electrodes (R_{sol}). The resistance shunting the capacitance represents the transepithelial resistance (R_{epi}). In the Nyquist diagram, the intercepts of the semicircle at high and low frequencies correspond to R_{sol} and $R_{sol} + R_{epi}$, respectively. In this series of experiments, the mean values were: $R_{sol} = 25 \pm 3\ \Omega/cm^2$ and $R_{epi} = 14 \pm 2\ \Omega/cm^2$. R_{sol} attenuates the current recorded by the voltage clamp (SCC_{rec}), and the short circuit current (SCC) expected for an ideal voltage clamp across the epithelium can be calculated as: $SCC = SCC_{rec}\ (R_{epi} + R_{sol})/R_{epi}$. The volume of each compartment bathing the jejunal mucosa was 2 ml, and Krebs bicarbonate solution, pre-equilibrated with a gas mixture of 5% CO_2/95% O_2 at 37°C, was flowing in each compartment at a rate of 20 ml/min. The composition of the Krebs bicarbonate solution was 140 mM Na, 5.2 mM K, 1.2 mM Mg, 1.2 mM Ca, 120 mM Cl, 2.8 mM PO_4, 25 mM HCO_3, and 11.5 mM glucose, pH 7.4.

Study of Cl^- Secretory Response in Jejunum. Preliminary experiments in jejuna from control mice indicated that the short-circuit current can be divided into two components: 1) a sodium absorptive component caused by the operation of the sodium-glucose cotransporter at the apical border of villus enterocytes and 2) a chloride secretory component linked to the existence of apical chloride channels in crypt cells. The first component could be eliminated by addition of 1 mM phlorizin to the apical bath, whereas the latter component was quantitatively accounted for by chloride secretion, because it was abolished in chloride-free solutions. Furthermore, the maneuvers of stripping the mucosa from its adjacent muscularis and mounting it in a small Ussing chamber induce the release of prostaglandins, which constitute a stimulus to chloride secretion, as first demonstrated by Pierce et al. (1971), and could therefore obscure other stimuli. Therefore, jejunal chloride secretion was assessed as the SSC after addition of phlorizin (1 mM) to the mucosal side as well as of indomethacin to both bathing media (100 μM). UTP or ATP were added at 100 μM to the apical solution and in some experiments

after preincubation in the presence of 8-(*para*-sulfophenyl)theophylline (8-*p*-SPT). Forskolin was added to the basolateral solution at the concentration of 10 μM in ethanol 0.1%; this concentration of ethanol does not affect SCC (data not shown). The SCC was expressed in microamperes per square centimeter. Data are expressed as mean ± S.E.M. Statistical analysis was performed using unpaired *t* test.

Materials. OneStep reverse transcription PCR was from QIAGEN (Westburg, Leusden, The Netherlands). RNase inhibitor was from Invitrogen (Merelbeke, Belgium). TriPure kit was from Roche Diagnostics (Basel, Switzerland). UTP, ATP, adenosine, indomethacin, forskolin, phlorizin, and 8-*p*-SPT were purchased from Sigma (Merelbeke, Belgium).

R1 ES cells were a generous gift of Dr. A. Nagy (Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada). CD1 mice were purchased from Iffa Credo Belgium s.a. and Harlan France.

Results

Generation of $P2Y_4$-Null Mice. We generated a targeting vector to inactivate the *P2Y4* gene. Insertion of the neomycin resistance cassette upstream from the gene was associated with the deletion of 700 bp of the promoter region and of the first 399 nucleotides of the coding sequence (Fig. 1A; see *Materials and Methods* for details). Clones of R1 ES cells electroporated with the targeting vector were analyzed for homologous recombination by enzyme restriction and Southern blotting (Fig. 1B). $P2Y_4^{+/-}$ ES cells were then aggregated with morulae and chimeric male mice were crossed with wild-type CD1 female mice. Eighteen F1 newborn mice were obtained from two independent crosses: all female mice were $P2Y_4^{+/-}$ and all male mice were $P2Y_4^{+/+}$ (Fig. 2A). This observation was compatible with a location of the *P2Y4* gene on the X chromosome, as is the case for the human ortholog (Nguyen et al., 1995). Throughout this article, wild-type male mice will be referred to as $P2Y_4^{0/+}$ and mutated male mice as $P2Y_4^{0/-}$. F1 $P2Y_4^{+/-}$ female mice were then crossed with wild-type CD1 male mice. The frequency of the F2 newborn mice in terms of sex and genotype were as expected (Fig. 2B). After a series of crossings between F2 $P2Y_4^{0/-}$ male mice and F1 $P2Y_4^{+/-}$ female mice, the offspring comprised 120 male mice and 79 female mice. Among the male mice, 45.8% had a $P2Y_4^{0/+}$ genotype and 54.2% had a $P2Y_4^{0/-}$ genotype. This frequency was not statistically different from the theoretical 50%/50% repartition. On the other hand, among the female mice, we observed 62% $P2Y_4^{+/-}$ female mice and 38% $P2Y_4^{-/-}$ mice, which is statistically significantly different ($p = 0.0003$) from the expected repartition.

$P2Y_4$-deficient mice were not distinguishable from wild-type mice. Their behavior in the cage seemed normal. They were fertile and their growth was indistinguishable from that of wild-type mice (data not shown). Classic histological examination did not reveal differences in any of tested organs (i.e., brain, heart, lung, intestine, liver, salivary gland, pancreas, stomach, spleen, thymus, thyroid, adrenal gland, kidney, bladder, testis, prostate gland, seminal gland, ovary, uterus, or vagina) (not shown).

To confirm the targeting of the *P2Y4* gene, we assayed the expression of the corresponding mRNA by RT-PCR in liver, stomach, and intestine, the organs in which the highest expression was found previously (Suarez-Huerta et al., 2001). The expected 336-bp signal was detected in tissues of wild-type mice but not when RNA were extracted from $P2Y^{0/-}$ animals, whereas HPRT expression was detected in all tissues independent of mouse genotype (Fig. 3).

Study of Cl^- Secretory Response to Nucleotides in Isolated Jejunum. Extracellular nucleotides regulate chloride secretion in many epithelia, including the jejunum. In particular, UTP and ATP increase chloride secretion in the mouse jejunum; this response is maintained in $P2Y_2^{-/-}$ mice (Cressman et al., 1999). We therefore investigated whether the $P2Y_4$ receptor could be involved in such control. The addition of UTP (100 μM) to the apical solution induced an increase in SCC in 5 of 8 jejuna from $P2Y_4^{0/+}$ mice. But no increase was ever observed in jejuna from $P2Y_4^{0/-}$ mice that were still responsive to forskolin (Fig. 4). This difference between the two groups was statistically significant even though the control group included "nonresponder" tissues (Table 1). In similar experiments, we tested the effect of ATP (100 μM), the other agonist of the $P2Y_4$ receptor. An increase in SCC was similarly observed in jejuna from $P2Y_4^{0/+}$ mice (five of eight responses) but not in jejuna from $P2Y_4^{0/-}$ mice

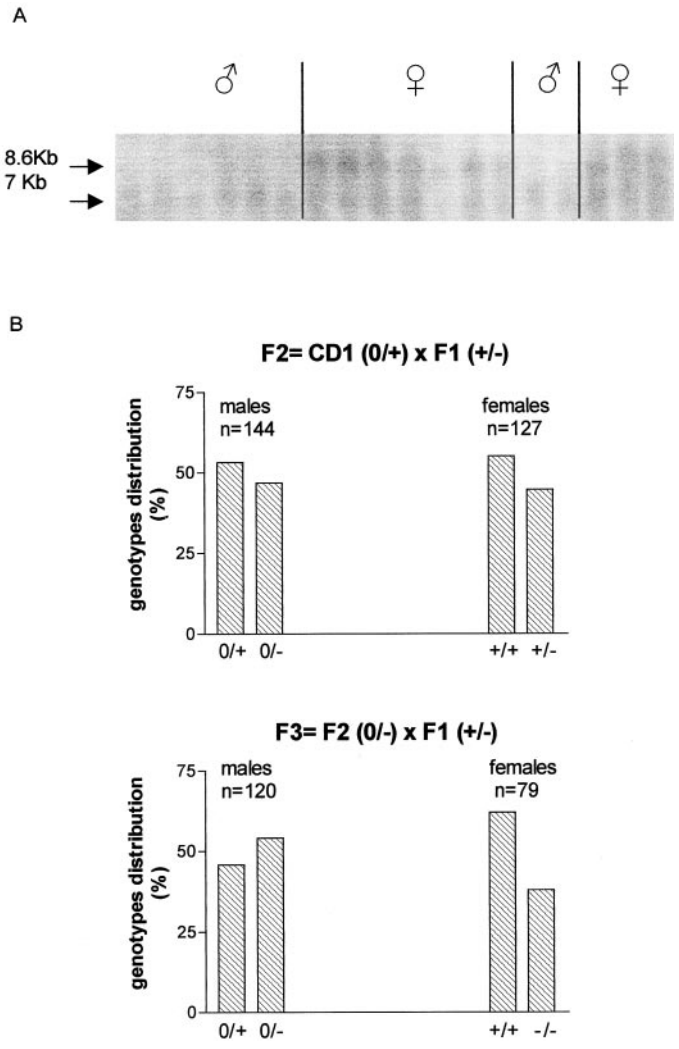

Fig. 2. Repartition of newborn mice in terms of sex and genotype. A, Southern blot analysis of genomic DNA extracted from F1 newborn mice. Chimeric mice were crossed with CD1 wild-type female mice. Genomic DNA of offspring was analyzed as described under *Materials and Methods*. B, histogram of the repartition of sex and genotype of mice as function of crossing. After crossing, the genotype of offspring was determined by analysis of genomic DNA as described under *Materials and Methods*.

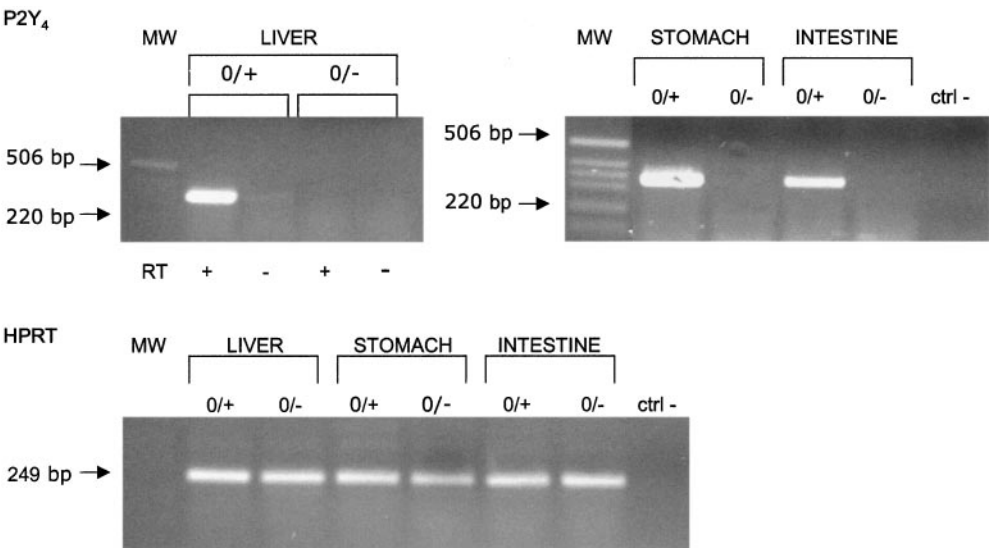

Fig. 3. Detection of $P2Y_4$ mRNA in $P2Y_4^{0/+}$ or $P2Y_4^{0/-}$ mouse tissues by RT-PCR. The extraction of RNA and the OneStep RT-PCR were performed as described under *Materials and Methods*. PCR products of 336 bp for $P2Y_4$ and 249 bp for HPRT are visualized under UV after electrophoresis on a 1.5% agarose gel and ethidium bromide coloration. RT −, controls for genomic DNA contamination (i.e., PCR without reverse transcription).

(Fig. 5 and Table 1). To exclude the possibility that the latter effect could be linked to extracellular degradation of ATP into adenosine by apical 5′-nucleotidase, these experiments were repeated in the presence of 8-*p*-SPT, a nonspecific antagonist of adenosine receptors. In these conditions, apical ATP still induced an increase in SCC in seven of eight jejuna from $P2Y_4^{0/+}$ mice but not in jejuna from null mice (Table 1).

On the other hand, when both groups of jejuna from control and knockout mice (n = 24 in each group) were compared, no statistically significant difference was observed in mean baseline SCC or in mean SCC after addition of the different drugs (phlorizin, indomethacin, and forskolin) except the purinergic agonists (Table 2). This indicates that $P2Y_4$ deletion failed to influence the different components of the SCC other than the chloride secretion response to apical purinergic agonists (i.e., in particular, the Na-glucose component and the various parts of the Cl^- component of SCC) (Table 2).

In control mice, the mean increases in SCC after addition

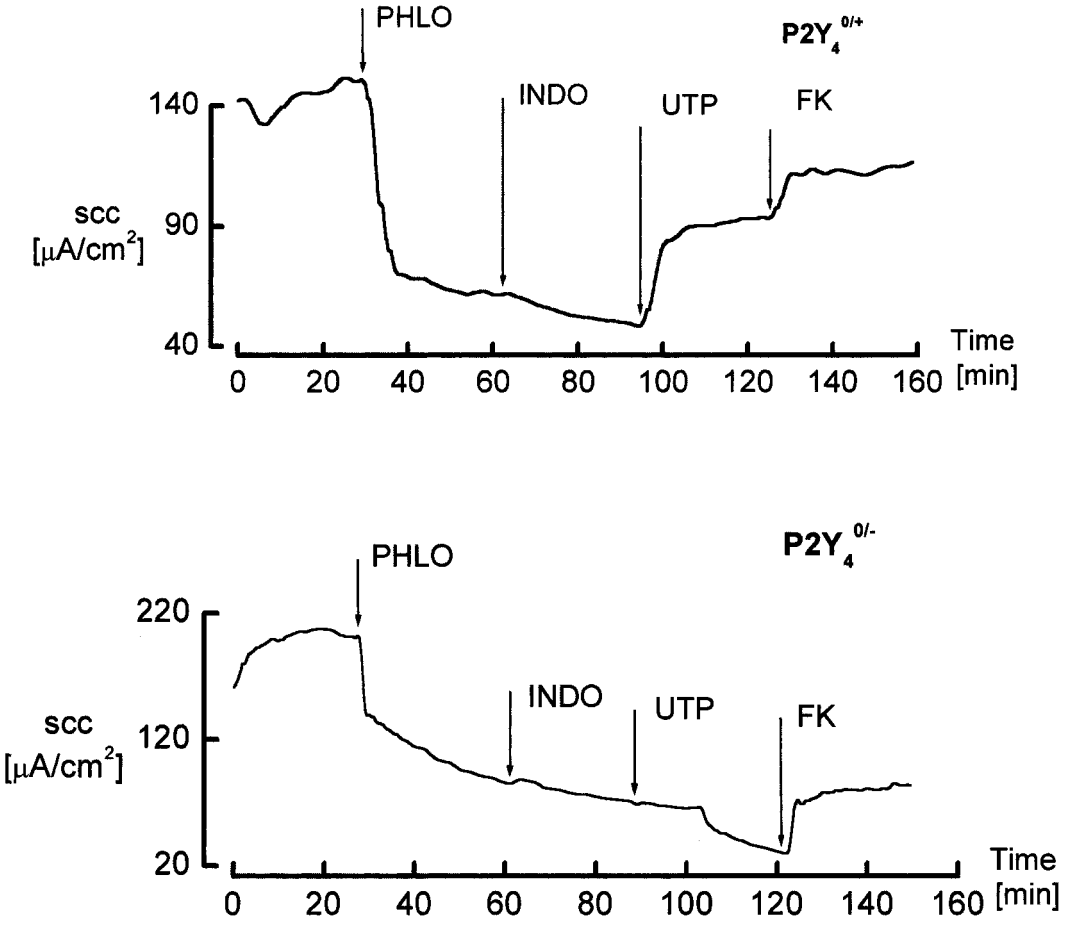

Fig. 4. Stimulation of Cl^- secretion by UTP in the jejunum of control and $P2Y_4$ knockout mice. Sodium reabsorption was inhibited by adding 1 mM phlorizin (PHLO) to the apical solution. Indomethacin (INDO; 100 μM) was added to both sides. Top, time course of SSC in an experiment with a control mouse; bottom, an experiment with a knockout mouse. At the end of the experiment, the stimulation of Cl^- secretion by forskolin (10 μM in the basolateral bath) was tested.

TABLE 1

Increase in SCC in mice jejuna induced by purinergic agonists

The mean increase (± S.E.M.) in SCC ($\mu A/cm^2$) was calculated from recordings illustrated in Figs. 4 and 5 as the difference in SCC immediately before and 20 min after stimulation with the different purinergic agonists The mean response computed for control tissues includes also the "non-responders"; $n = 8$ in each group.

Stimulus	n	$P2Y_4^{0/+}$	$P2Y_4^{0/-}$	Significance
UTP	8	17 ± 5	2 ± 1	$P<0.01$
ATP	8	28 ± 8*	3 ± 2	$P<0.01$
ATP + 8-*p*-SPT	8	27 ± 6*	2 ± 2	$P<0.001$

* $P > 0.2$ compared with the increase in current induced by UTP in $P2Y_4^{0/+}$ mice.

of UTP, ATP, or ATP in the presence of 8-*p*-SPT were not statistically significantly different from each other, in agreement with the known equipotency of these agonists for the murine $P2Y_4$ receptor.

Discussion

The actions of extracellular nucleotides are mediated by two classes of receptors: P2X receptors, which have an intrinsic activity of ion channels, and P2Y receptors coupled to G proteins. Pharmacologically, P2Y receptors can be subdivided into adenine nucleotide-preferring receptors responding mainly to ADP ($P2Y_1$, $P2Y_{12}$, $P2Y_{13}$) and ATP ($P2Y_{11}$), uracil nucleotide-preferring receptors (human $P2Y_4$, $P2Y_6$) responding to UTP and UDP, respectively, and receptors of mixed selectivity activated by both ATP and UTP ($P2Y_2$, rodent $P2Y_4$). One important action of extracellular ATP and UTP is to stimulate the transepithelial secretion of chloride as a result of increased apical permeability (Knowles et al., 1991). This process is mediated by an inositol triphosphate-mediated increase in cytosolic Ca^{2+} that induces the opening of outwardly rectifying chloride channels (Clarke et al., 1994). In some instances, however, it is caused by the activation of the cystic fibrosis transmembrane conductance regulator (CFTR). Calcium-induced activation of CFTR could result from increased insertion into the apical membrane (Cantiello et al., 1994; Atia et al., 1999; Cuffe et al., 2000). Thus, apical chloride permeability is caused by multiple chloride channels and seems controlled by multiple P2Y receptors. For instance, whereas the chloride secretory response to UTP was completely abolished in the trachea of $P2Y_2$-null mice, the response to UTP was maintained partially in the gallbladder and completely in the jejunum (Cressman et al., 1999). Cressman et al. (1999) suggested that the jejunal response to UTP could be transduced by the $P2Y_4$ receptor, the mRNA of which is indeed expressed in small intestine (Lazarowski et al., 2001; Suarez-Huerta et al., 2001). However, an alternative explanation is that the $P2Y_2$ receptor is physiologically involved in the jejunal response but that compensation by $P2Y_4$ occurs after its inactivation. For instance, it has been recently shown that mice lacking all three β-adrenergic receptors (β_1, β_2, β_3) cannot increase thermogenesis and become severely obese during overfeeding, whereas gene inactivation of individual β-adrenergic receptors had no significant effect (Bachman et al., 2002). We have now generated $P2Y_4$-null mice. The invalidation was confirmed by loss of expression of the mRNA in intestine, stomach, and liver. These mutant mice are grossly normal, and the only pheno-

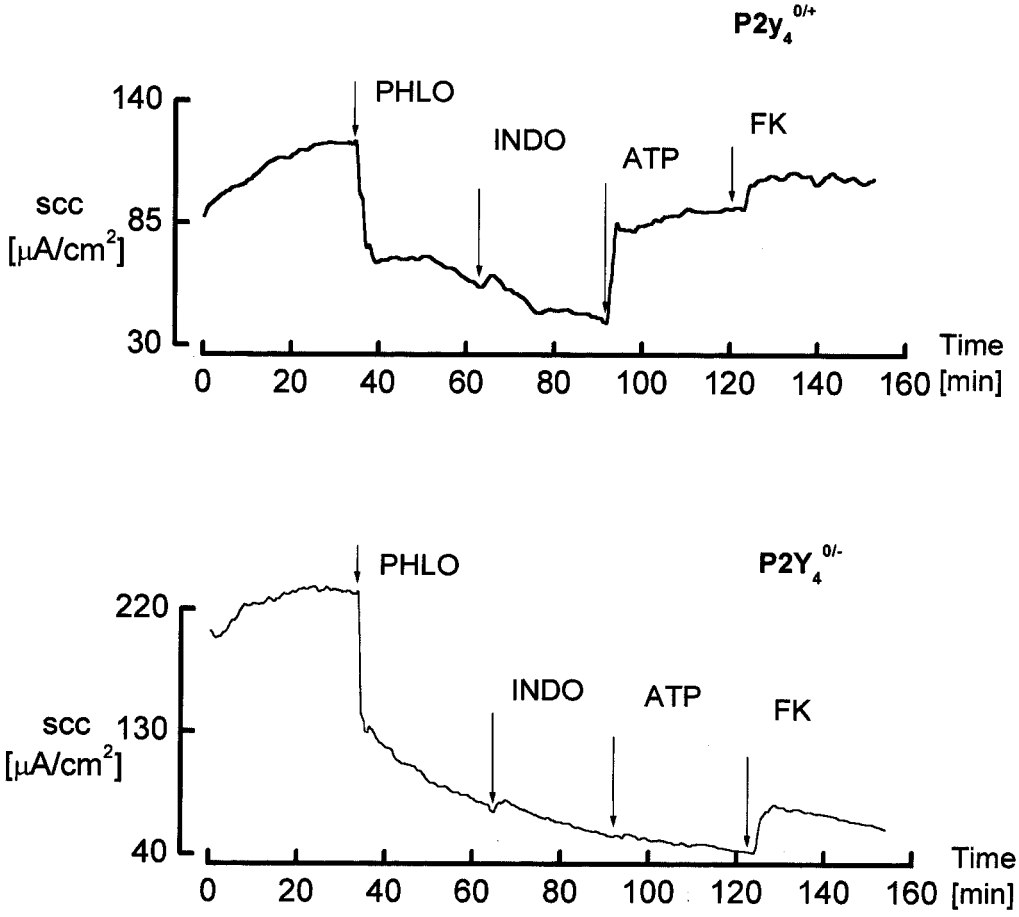

Fig. 5. Stimulation of Cl^- secretion by ATP in the jejunum of control and $P2Y_4$ knockout mice. The protocol was similar to that of Fig. 4, except that ATP (100 μM) instead of UTP was used as stimulatory agent.

TABLE 2
SCC of mice jejuna after addition of the various drugs
The mean SCC ($\mu A/cm^2$) ± S.E.M. was calculated from recordings illustrated in Fig. 4 and 5 as the baseline SCC 20 min after the beginning of the experiment and 20 min after the various drugs added. Because in the case of forskolin, the tissue has been previously exposed to a purinergic agonist with or without an increase in SCC, we provide rather the mean increase in SCC calculated as the difference in SCC immediately before and 20 min after forskolin addition. $n = 24$ for each group.

Drug	$P2Y_4^{0/+}$	$P2Y_4^{0/-}$	Significance
None	181 ± 16	168 ± 13	$P>0.5$
Phlorizin	76 ± 8	63 ± 5	$P>0.1$
Indomethacin	64 ± 6	52 ± 5	$P>0.1$
Forskolin	31 ± 3	40 ± 5	$P>0.1$

typic abnormality that we have detected so far is the disappearance of the jejunal chloride secretory response to UTP and ATP, thus confirming the hypothesis of Cressman et al. (1999). According to these authors, the increased chloride secretion induced by UTP is observed in only about two thirds of the normal mice, perhaps as a result of ATP release during mounting of the tissue in the chamber and receptor occupancy or desensitization. The mean increase in SSC observed here in control mice was similar with UTP and ATP or ATP in the presence of 8-*p*-SPT, although smaller but more sustained than that reported by Cressman et al. (1999). This difference could be related to the different strains of mice used. We have tested the effect of 8-*p*-SPT because ATP has been reported to stimulate CFTR via its local degradation into adenosine with subsequent activation of the A_{2B} receptor (Huang et al., 2001; Matsuoka et al., 2002): the complete disappearance of the ATP response in $P2Y_4^{0/-}$ mice indicates that this mechanism does not operate in mouse jejunum, despite the fact that we observed a response to exogenous adenosine (E. Ghanem and R. Beauwens, unpublished observations) at variance with Cressman et al. (1999).

Provided they can be extrapolated to humans, our results suggest that the $P2Y_4$ receptor should be considered a potential pharmacotherapeutic target for treatment of cystic fibrosis and diarrhea. Indeed cystic fibrosis is associated with gastrointestinal abnormalities. Ten to 15% of CFTR-newborns have meconium ileus. A major problem is long-term malabsorption resulting from both pancreatic insufficiency and the thick layer of highly viscous mucus that coats the absorptive surface and constitutes a diffusion barrier for nutrients. Finally, a significant fraction of adult patients suffer from distal intestinal obstruction. By analogy with the use of aerosolized $P2Y_2$ agonists to hydrate the airway mucus (Kellerman, 2002), oral $P2Y_4$ receptor agonists might be used to stimulate the jejunal secretion of electrolytes and water. The UTP effect on the murine jejunum is abolished in CFTR-null mice, which are completely deficient in that protein (Lazarowski et al., 2001). However the major genetic defect responsible for human cystic fibrosis is the ΔF508 mutation, which is associated with inefficient trafficking to the membrane and decreased activity. It is known, however, that ΔF508 CFTR is significantly expressed in human jejunum (Kalin et al., 1999). The activity of the CFTR can be increased not only by cAMP but also by G proteins and some calcium-dependent protein kinases. In particular, pharmacological activation of the ΔF508 CFTR has been demonstrated in the jejunum of transgenic mice expressing the mutated protein (Steagall and Drumm, 1999). Moreover, thapsigargin, an inhibitor of the endoplasmic reticulum calcium pump, has recently been shown to increase plasma membrane ΔF508 CFTR (Egan et al., 2002). It will therefore be of interest to establish whether the trafficking and/or the activity of the ΔF508 CFTR can be modulated by exposure to UTP. Alternatively, a $P2Y_4$ antagonist might be of value in the treatment of the secretory diarrhea induced by some enteropathogenic bacteria, which have indeed been shown to induce nucleotide release (McNamara et al., 2001; Crane et al., 2002). Additional phenotypic studies of $P2Y_4$-null mice, focused on inner-ear function and angiogenesis, are currently underway.

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References

Atia F, Zeiske W, and Van Driessche W (1999) Secretory apical Cl^- channels in A6 cells: possible control by cell Ca^{2+} and cAMP. *Pflueg Arch Eur J Physiol* **438:**344–353.
Bachman ES, Dhillon H, Zhang C-Y, Cinti S, Bianco AC, Kobilka BK, and Lowell BB (2002) βAR signaling required for diet-induced thermogenesis and obesity resistance. *Science (Wash DC)* **297:**843–845.
Bogdanov YD, Wildman SS, Clements MP, King BF, and Burnstock G (1998) Molecular cloning and characterization of rat $P2Y_4$ nucleotide receptor. *Br J Pharmacol* **124:**428–430.
Cantiello HF, Prat AG, Reisin IL, Ercole LB, Abraham EH, Amara JJF, Gregory RJ, and Ausiello DA (1994) External ATP and its analogs activate the cystic fibrosis transmembrane conductance regulator by a cyclic AMP-independent mechanism. *J Biol Chem* **269:**11224–32.
Clarke LL, Grubb BR, Yankaskas JR, Cotton CU, McKenzie A, and Boucher RC (1994) Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in Cftr(−/−) mice. *Proc Natl Acad Sci USA* **91:**479–483.
Communi D, Pirotton S, Parmentier M, and Boeynaems J-M (1995) Cloning and functional expression of a human uridine nucleotide receptor. *J Biol Chem* **270:**30849–30852.
Communi D, Paindavoine P, Place GA, Parmentier M, and Boeynaems J-M (1999) Expression of P2Y receptors in cell lines derived from the human lung. *Br J Pharmacol* **127:**562–568.
Crane JK, Olson RA, Jones HM, and Duffey ME (2002) Release of ATP during host cell killing by enteropathogenic E. coli and its role as a secretory mediator. *Am J Physiol* **283:**G74–G86.
Cressman VL, Lazarowski E, Homolya L, Boucher RC, Koller BH, and Grubb BR (1999) Effect of loss of $P2Y_2$ receptor gene expression on nucleotide regulation of murine epithelial Cl^- transport. *J Biol Chem* **274:**26461–26468.
Cuffe JE, Bielfeld-Ackermann A, Thomas J, Leipziger J, and Korbmacher C (2000) ATP stimulates Cl^- secretion and reduces amiloride-sensitive Na^+ absorption in M-1 mouse cortical collecting duct cells. *J Physiol (London)* **524:**77–90.
Egan ME, Glockner-Pagel J, Ambrose C, Cahill PA, Pappoe L, Balamuth N, Cho E, Canny S, Wagner CA, Geibel J, et al. (2002) Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. *Nat Med* **8:**485–492.
Harper S, Webb TE, Charlton SJ, Ng LL, and Boarder MR (1998) Evidence that $P2Y_4$ nucleotide receptors are involved in the regulation of rat aortic smooth muscle cells by UTP and ATP. *Br J Pharmacol* **124:**703–710.
Huang P, Lazarowski ER, Tarran R, Milgram SL, Boucher RC, and Stutts J (2001) Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. *Proc Natl Acad Sci USA* **98:**14120–14125.
Jin J, Dasari VR, Sistare FD, and Kunapuli SP (1998) Distribution of P2Y receptor subtypes on haematopoietic cells. *Br J Pharmacol* **123:**789–794.
Kalin N, Claab A, Sommer M, Puchelle E, and Tümmler B (1999) ΔF508CFTR protein expression in tissues from patients with cystic fibrosis. *J Clin Investig* **103:**1379–1389.
Kellerman DJ (2002) $P2Y_2$ receptor agonists A new class of medication targeted at improved mucociliary clearance. *Chest* **121**:201S–205S
Knowles MR, Clarke LL, and Boucher RC (1991) Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N Engl J Med* **325:**533–538.
Lazarowski ER, Rochelle LG, O'Neal WK, Ribeiro CMP, Grubb BR, Zhang V, Harden TK, and Boucher RC (2001) Cloning and functional characterization of two murine uridine nucleotide receptors reveal a potential target for correcting ion transport deficiency in cystic fibrosis gallbladder. *J Pharmacol Exp Ther* **297:**43–49.
McNamara N, Khong A, McKemy D, Caterina M, Boyer J, Julius D, and Basbaum C (2001) ATP transduces signals from ASGM1, a glycolipid that functions as a bacterial receptor. *Proc Natl Acad Sci USA* **98:**9086–9091.
Marcus DC and Scofield MA (2001) Apical $P2Y_4$ purinergic receptor controls K^+ secretion by vestibular dark cell epithelium. *Am J Physiol* **281:**C282–C289.
Matsuoka I, Ohkubo S, Kimura J, and Uezono Y (2002) Adenine nucleotide-induced activation of adenosine A_{2B} receptors expressed in *Xenopus laevis* oocytes: involve-

ment of a rapid and localized adenosine formation by ectonucleotidases. *Mol Pharmacol* **61:**606–613.
Nguyen T, Erb L, Weisman GA, Marchese A, Heng HHQ, Garrad RC, George SR, Turner JT, and O'Dowd BF (1995) Cloning, expression and chromosomal localization of the human uridine nucleotide receptor gene. *J Biol Chem* **270:**30845–30848.
Pierce NF, Carpenter CCj, Elliott HL, Greenough WB III (1971) Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in the canine jejunum. *Gastroentrology* **60:**22–32.
Sage CL and Marcus DC (2002) Immunolocalization of $P2Y_4$ and $P2Y_2$ purinergic receptors in strial marginal cells and vestibular dark cells. *J Membr Biol* **185:**103–115.
Satterwhite CM, Farrelly AM, and Bradley ME (1999) Chemotactic, mitogenic and angiogenic actions of UTP on vascular endothelial cells. *Am J Physiol* **276:**H1091–H1097.
Steagall WK and Drumm ML (1999) Stimulation of cystic fibrosis transmembrane conductance regulator-dependent short-circuit current across ΔF508 murine intestines. *Gastroenterol* **116:**1379–1388.
Stam NJ, Klomp J, van de Heuvel M, and Olijve W (1996) Molecular cloning and characterization of a novel orphan receptor (P_{2P}) expressed in human pancreas that shows high structural homology to the P_{2U} receptor. *FEBS Lett* **384:**260–264.
Suarez-Huerta N, Pouillon V, Boeynaems J-M, and Robaye B (2001) Molecular cloning and characterization of the mouse $P2Y_4$ nucleotide receptor. *Eur J Pharmacol* **416:**197–202.
Webb TE, Henderson DJ, Robets JA, and Barnard EA (1998) Molecular cloning and characterization of the rat $P2Y_4$ receptor. *J Neurochem* **71:**1348–1357.

Address correspondence to: Bernard Robaye, IRIBHM, IBMM, 10, rue A. Bolland, 6041 Gosselies, Belgium. E-mail: brobaye@ulb.ac.be