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Functional characterization of P_{2X}/P_{2Y} receptor in isolated swine renal artery

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Abstract : To understand the role of P_{2X}/P_{2Y} receptor in cortex region of kidney and renal artery, molecular and functional analysis of P_{2X}/P_{2Y} receptor by pharmacophysiological skill in conventional swine tissues were performed. In functional analysis of P_{2Y} receptor for vascular relaxation, 2-methylthio adenosine triphosphate, a strong agonist of P_{2Y} receptor, induced relaxation of noradrenaline (NA)-precontracted renal artery in a dose-dependent manner. Strikingly, relaxative effect of ATP, 2-msATP, agonists of P_{2Y} receptor, abolished by treatment of reactive blue 2, a putative P_{2Y} receptor antagonist. In contrast, no significant differences of gene encoding P_{2X}/P_{2Y} and protein expression in immortalized suprachiasmatic nucleus from brain, primary isolated vascular smooth muscle cells from renal artery of pigs and HEK293 from human embryonic kidney under with/without adenosine triphosphate were observed. Taken together, the relationship between molecular and functional characteristic of P_{2X}/P_{2Y} receptors in conventional pig should be considered that they are another important factor which regulate the kidney function in swine. Based on this study, we propose the purinergic receptor as well as adrenergic and cholinergic receptors is an essential component of the renal homeostasis.

Key words : adenosine triphosphate, 2-methylthio adenosine triphosphate, P_{2X}/P_{2Y} receptor, reactive blue 2, swine renal artery

Introduction

The specificity of neural communication depends on several factors: the signal molecules secreted by neurons, the target cell receptors for these chemicals, and the anatomical conncetions between neurons and their targets, which occur in regions known as synapses. The release of neurotransmitters into the synapse takes place by exocytosis. Neurotransmitters and neuromodulators act as paracrines, with target cells located close to the neuron that secrets them. Neurohormones, on the other hand, are secreted into the blood and distributed throughout the body.

Most important one of described above, purine nucleotides which is one of the neurotransmitters in central nerve system and peripheral nerve system, that is currently called as purinergic nervous system (3th nerve: Non-adrenergic, non-cholinergic, NANC) inclusive of adrenergic and cholinergic nervous system.

Burnstock (1978) [7] proposed a formal classification of receptors for adenosine and adenosine triphosphate (ATP), collectively called purinoceptors. Receptors selective for adenosine and adenosine monophosphate were designated as P_1-purinoceptors and those selective for ATP and adenosine diphosphate (ADP) called P_2-purinoceptors. This classification set the stage for further subdivisions of P_1 receptors, into A_1, A_{2A}, A_{2B} and A_3 and of P_2 receptors into P_{2X} and P_{2Y} families [1, 9, 26].

So far, many studies have attempted to identify the enteric P_2 purinoceptors that are activated by exogenous applied ATP. However, as a consequence of the controversial role of ATP in enteric neurotransmission, the subtypes of P_2 purinoceptors that may be activated by

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endogenously released ATP are hardly explored. In an electrophysiological study on isolated human jejunal muscle strips, Xue *et al.* (1999) [33] reported that the ATP-mediated part of the inhibitory junction potential is mediated by P_{2Y} but not P_{2X} receptors. Similar findings were recently reported for mouse colon [29]. Interestingly, however, there is recent immunohistochemical evidence that the mouse colon contains P_{2X} and P_{2Y} receptors, which can be activated by exogenously added purines [12]. This suggests that there is a discrepancy between the functional effects of endogenously released and exogenously added purines, and supports the hypothesis that endogenous and exogenous purines activate different subtypes of purinoceptors [13, 23].

However, the role of ATP in enteric neurotransmission is complex and much debated. There is good evidence that ATP mediates fast synaptic transmission at neuronal ganglia in the enteric nervous system [11]. Moreover, ATP may also act as a NANC neurotransmitter in the gastrointestinal tract, as first proposed by Burnstock [8]. Since then, the functional evidence that is reported in favour of ATP is well balanced by evidence reported against ATP being a NANC neurotransmitter in the gut [8].

ATP activates P_2 purinoceptors which are subdivided into ion-gated P_{2X} receptors and G-protein-coupled P_{2Y} receptors [25, 26]. The lack of specific antagonists of purinoceptor subtypes makes it difficult to draw a clear conclusion on the subtypes of P_{2X} receptors that mediate purinergic NANC neurotransmission. Although immunohistochemistry failed to show P_{2X1} reactivity in the myenteric plexus of the mouse ileum [12, 32], there is functional evidence for the presence of presynaptic P_{2X1} receptors in the mouse enteric nervous system [32].

At the highest concentration tested, ATP induced a relaxation, which was followed by a rebound contraction of the duodenum, ileum and colon. Previous studies on guinea-pig taenia-coli have demonstrated that rebound contractions are mediated by the release of prostaglandins, since they were blocked by indomethacin, an inhibitor of prostaglandin synthesis [6].

Thus, it is presumable that P_{2X} and P_{2Y} receptor may contribute to the supply of the blood to kidney as primarily and/or secondary regulatory receptor in swine. However, the physiological and pharmacological characterization of P_{2X} and P_{2Y} receptor were not disclosed in swine tissue yet. Also, molecular interrelation between gene expression of P_{2X}/P_{2Y} isoforms and function of their translating products was needed to unscreened. Including above description, another aim of the present study was to clarify these discrepancies by investigating whether ATP which is purinergic neurotransmitter plays a role as an inhibitory neurotransmitter of NANC nerves by binding with P_{2X} or an excitatory one of NANC nerves by by binding with P_{2Y} in the swine tissues and by identifying the subtypes of P_{2X} and P_{2Y} purinoceptor, which are activated by endogenously released and exogenously added purine nucleotide in the swine renal artery.

Materials and Methods

Materials

The drugs used were as follows: All of the chemicals used in this study including Phenylephrine, ATP, 2-methylthio-ATP (2-meSATP), α,β-methylene ATP and tetrodotoxin (TTX), reactive blue 2 (RB-2) were purchased from SigmaAldrich company (USA). Antibodies to P_{2X} and P_{2Y} pAb, ERK 42/44 mAb, and GAPDH mAb were obtained from Santacruz Company (USA). All other materials were obtained from SigmaAldrich and were reagent grade quality. All solutions were prepared in Milli-Q water and filtered (0.45 mm) before use.

Animals

Conventional swines of either sex, 90 ± 5 kg body weight, were used. Throughout this experiment, experimental subjects were free accessed to maternal feeding *ad libitum* after born. All the animals subjected used this experiment had been maintained under clean condition for 12 h prior to sacrifice for experiment or surgery for further molecular analysis at end of this *in vitro* study.

The surgical procedures and pre- and post-operative care of the animals conformed to the Gyeongsang National University Animal Care and Use Committee in accordance with Korean Department of Agriculture guidelines and all efforts were made to minimize animal suffering and to reduce the number of animal used. All of described procedures are based upon ethical principles which have been approved by the Institutional Ethics Committee according to the Helsinki Declaration.

Cell culture

Cell lines including immortalized suprachiasmatic nucleus (SCN) originated from murine brain and kidney cell from human embryonic kidney (HEK293) as well as primary isolated vascular smooth muscle cells (VSMCs)

which were obtained from segments of swine renal artery, were immersed in growth medium consisting of Dulbecco's modified Eagle's medium (Life Technologies, USA) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, USA), penicillin (100 U/ml; SigmaAldrich, USA), and streptomycin (100 μg/ml; SigmaAldrich, USA). After reached around 85% confluency, tissues were treated with ATP at every 2 h for 6 h, the grown tissues were detached by trypsinization. The detached cells were subjected for further molecular analysis.

Preparation of renal artery ring

Renal artery rings for physiological analysis were obtained from male conventional pig of 6~7 months age and weighing 90 ± 5 kg. Under anaesthesia using anaesthetics, experimental subjects were killed by exsanguination of the thoracic aortic artery. Through an abdominal incision, a length of about 3~4 cm of intact renal artery removed and immediately immersed in 4°C ice-cold Krebs solution (Krebs solution; see below). The arterial segment was placed in a dissection dish containing Krebs and the intraluminal contents were flushed using a small cannula filled with Krebs.

Procedures of functional analysis

The prepared segment of swine renal artery ring were flushed and cleared of connective tissue. Tiny silk ligatures were applied to each end of the strip; one end was attached to a rigid support and the other to a Grass FT03 force displacement transducer. Tissues were mounted in 10 ml organ baths, subsequently gassed (95% O_2/ 5% CO_2) and containing Krebs solution of the following composition (mM): NaCl, 133; KCl, 4.7; $NaHCO_3$, 16.4; $MgSO_4$, 0.6; NaH_2PO_4, 1.4; glucose, 7.7 and $CaCl_2$, 2.5; pH 7.2 ± 0.5. Experiments were carried out at 37 ± 0.5°C, as this temperature reduced spontaneous activity. Mechanical activity was displayed on a Grass ink-writing oscillograph. An initial load of 0.5 g was applied to the artery segments; throughout functional studies, tissues were allowed to equilibrate for 60 min prior to the start of experiments.

RNA isolation and RT-PCR

Total RNA extracted from untreated and ATP-treated tissues using by the RNeasy kit (Qiagen, Germany) was treated with RNase free-DNase I to eliminate residual genomic DNA. A total RNA (0.5 μg) was used in a cDNA synthesis reation using 200 units of Moloney murine leukemia virus-reverse transcriptase (Invitrogen, USA) with Retrotransscript Kit reagents (Ambion, Germany) for RT-PCR analysis. Following the manufacturer's protocol, RNA was incubated with 5 μl of oligo (dT) primer (Ambion, Germany) at 70°C for 10 min. RNA was transferred to ice, the Superscript II mixture was added, and cDNA synthesis was allowed to occur at 42°C for 1 hr in the presence of ~20 units of RNasin (Promega, USA). 3 μl of cDNA were used in 50 μl of PCR using recombinant *Taq* polymerase (Invitrogen, USA) at 0.25 units/sample. 1 μl of 10 mM dNTP mixture (Invitrogen, USA) per sample was used for each reaction. PCR was performed for P_{2X1} and P_{2Y1} isoforms, and *GAPDH*. Specific primers designed were described at Table 1.

Primer sequences, the size of amplified products, and GenBank accession numbers and size of PCR products are in Table. PCR conditions were as follows: for P_{2X1} and P_{2Y2}, denaturing was performed at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 2 min, for GAPDH, denaturing was done at 94°C for 1 min, annealing at 68°C for 1 min, and extension at 72°C for 2 min. Following 35 PCR cycles, all samples were allowed to extend at 72°C for 10 min. All PCR were performed in a model LightCycler. 20 μl of each PCR products were electrophoresed in 1% agarose (Invitrogen, USA) gels. The gels were then stained with ethidium bromide, placed on a UV transilluminator, and photographed.

Table 1. Sequence-specific primers used for RT-PCR

Gene	Primer sequence (5'-3')	Gene bank accession No.	Product length (bp)
P_{2X1}	F : GTATGACACTCCCCGGAATGGT R : ACACCCACCCAATGACGTAGA	NM008771	100
P_{2Y1}	F : GCACTGGGACTCGGAAAAAC R : GGCCACAGTCGTGCACATAC	NM008772	100
GAPDH	F : GTATGACTCCACTCACGGCAAA R : GGTCTCGCTCCTGGAAGATG	BC094037	100

Western blot analysis

Extracted proteins from tissues were homogenized in lysis buffer (Cell Signaling Technology, USA), and 50 μg proteins were separated by SDS-PAGE after quantified by the Bradford assay [22, 28]. Then, runned total proteins on the gel were transferred to PVDF membranes. After transfer, PVDF membranes were stained with Ponceau S and blocked with TBST containing 5% Skim milk and 0.1% Tween 20, incubated with nonspecific P_{2X} and P_{2Y} pAb, ERK 42/44 mAb and GAPDH mAb, washed, and incubated with horseradish peroxidase-conjugated rabbit anti-mouse antibody and anti-rabbit antibody, respectively. The ECL western blotting system was used for detection as described previously [22, 28].

Statistical analysis

Data are expressed as mean ± SE "*n*" in Results refers to number of animal preparations on which observations were made. Differences between the means were analyzed by Student's *t*-test (paired or unpaired) for comparison of two groups. *P* value of less than 0.05 was considered significant.

Results

Relaxative effect of P_{2Y} receptor agonists on NA-precontracted renal artery

On NA-precontracted renal artery and in the application of 2-msATP and ATP with concentration gradients, these agonists induced concentration dependent dramatic relaxations. As shown in Fig. 1, 2-msATP, one of the strong agonist among purine nucleotide deritives, induced more potentiated relaxation (**$p <$ 0.01 *vs.* NA-contraction) on NA-precontracted renal artery as concentration dependent manner compared to ATP does (*$p < 0.05$ *vs.* NA-contraction). This result suggests that not only ATP and their deritives as endogenous neurotransmitter but also exogenously applied purine deritives induce relaxative effect in renal artery although do not have effect to molecular levels such as gene (Fig. 3) and protein expression as shown at Fig. 4.

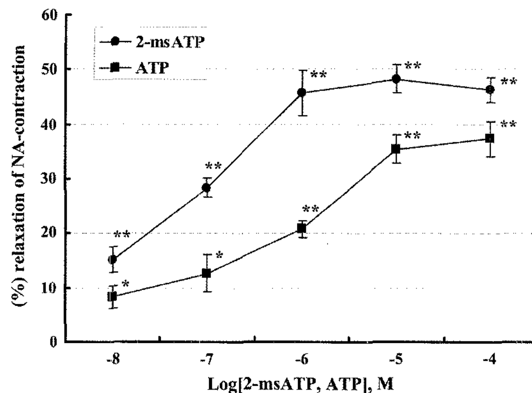

Fig. 1. Cummulative concentration-response curves to ATP and 2-msATP on the NA-precontracted swine renal artery. All symbols represent mean % relaxation ± SE. *$p < 0.05$, **$p < 0.01$ *vs.* NA-contraction.

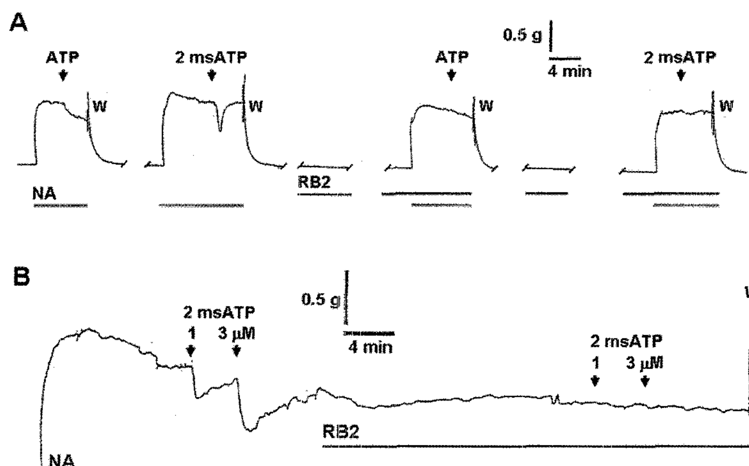

Fig. 2. Typical tracing of renal artery rings showing the ATP and 2-msATP-induced vasodilatations on NA-precontrated swine renal arteries and the deduction or abolishment of relaxation to both strong agonists before (left panels of A, B) and after (right panels of A, B) treatment of the artery ring with reactive blue 2 (RB-2).

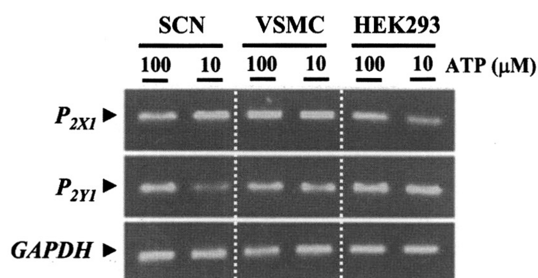

Fig. 3. Analysis of gene expression in SCN, VSMCs and HEK293 cells by RT-PCR. RT-PCR for the expression of P_{2X1} and P_{2Y1} in immortalized suprachiasmatic nucleus (SCN), primary cultured vascular smooth muscle cells (VSMCs) from swine renal artery and human embryonic kidney cells (HEK293) treated with ATP is shown.

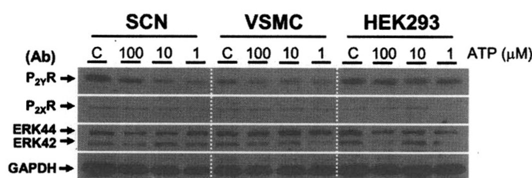

Fig. 4. Western analysis of P_{2X}, P_{2Y} receptor and ERK1/2 protein expression by increasing the concentration of ATP in cultured SCN, VSMCs and HEK293 cells. Cells were harvested and subjected to SDS-PAGE after treating ATP at every 2 h for 6 h as indicated concentrations. Lysed total proteins of 50 μg from the cell were subjected to each lane.

Effect of purinergic receptors and their agonist/antagonist on NA-precontracted renal artery

In previously report, evans blue (10 μM), a putative P_{2X} receptor antagonist in the intestinal muscle [5], slightly inhibited the L-NOARG-resistant relaxations to electrical field stimulation (EFS) and the relaxation to α,β-methylene ATP, (α,β-MeATP) without affecting those to ATP and ADP-âS. NF 279 (1 μM), which blocks rat P_{2X1} and P_{2X2} receptors [10, 21, 27], slightly inhibited the L-NOARG-resistant NANC relaxations to low-frequency EFS and those to α,β-MeATP, but had no effect on NANC relaxations to higher frequency EFS or on the relaxations to ATP and ADP-βS.

As same idea, we tried to figure out that functional relationship between purine nucleotide and their putative inhibitor, specifically RB-2. On NA-precontracted renal artery, ATP (1 μM) and 2-msATP (1 μM) induced NANC relaxations. On the other hand, we performed inhibitory effect of a putative antagonist RB-2 in purinergic nerve, on NA-precontracted renal artery and in the presence of RB-2 (10 μM), all relaxations to ATP (1 μM) and 2-msATP were abolished as like apply by the nerve-conductance blocker tetrodotoxin (1 μM, results not shown) (Fig. 2A). A putative blocker of purinergic nerve, RB-2 does inhibit the potentiated relaxative effect by 2-msATP treating dose gradient concentration (1~3 μM). This inhibitory effect was sustainable and potentiated as shown in Fig. 2B.

Gene expression of P_{2X1}/P_{2Y1} treated with ATP in cultivated tissues

Xue *et al.* [33] reported that the ATP-mediated part of the inhibitory junction potential is mediated by P_{2Y} but not P_{2X} receptors using an electrophysiological study on isolated human jejunal muscle strips. Similar findings were recently reported for mouse colon [29]. Interestingly, however, there is recent immunohistochemical evidence that the mouse colon contains P_{2X} and P_{2Y} receptors, which can be activated by exogenously added purines [12]. This suggests that there is a discrepancy between the functional effects of P_{2X} and P_{2Y} receptors and protein or gene expression of their isoforms in varies swine tissues at developmental stages.

Thus, to examine the differential expression of P_{2X}/P_{2Y} gene in ATP applied cultivated tissues including SCN, VSMC and HEL293, we extracted the total RNA and subjected to real-time RT-PCR to analyze of gene expression in each tissue as described at "Materials & Methods". As shown at Fig. 3, P_{2X1} and P_{2Y1} genes were clearly detected in all tissues, but no differential expressing signal of these genes by incubation with ATP at every 2 h for 6 h (Fig. 3). This result suggests that although it is important not only functional role of purinergic receptors in circulatory tissues but also their gene expression in various tissues, ATP does not affect to gene expression of P_{2X1} and P_{2Y1}.

P_{2X}/P_{2Y} receptor expression with ATP in cultivated tissues

To assess the role of P_{2X}/P_{2Y} receptor and their characteristic in protein level in a different way of gene expression, we extracted protein from cultivated tissues then subjected to western blot analysis described at "Materials & Methods" in detail. Non-specific polyclonal P_{2X}/P_{2Y} antibody detected their protein from subjected tissues including SCN, VSMCs as well as HEK293 cells. Especially, constitutively expression of P_{2Y} receptor was predominant in HEK293 cells compared to SCN and VSMCs but not shown any changing their

expression on ATP treatment (Fig. 4). This result strongly suggests that even though ATP is a candidate of regulating purinergic receptor function, it never affect to expression of gene (Fig. 3) and protein (Fig. 4) in any way which means that endogenously and exogenously applied ATP as neurotransmitter works at these receptors whatever in peripheral and central nerve system.

Discussion

This investigation revealed that the expression of P_{2X} /P_{2Y} receptors in molecular level, are completely independent on physiological function with ATP which act as neurotransmitter. The largest variety of neurotransmitters is found within the CNS. These neurotransmitters include many polypeptides known mostly for their hormonal activity. In contrast, three primary neurotransmitters are used within the peripheral nervous system: acetylcholine, norepinephrine, and epinephrine.

The purine nucleotide ATP released from the enteric neurines is often cited as a NANC inhibitory transmitter [4, 8, 5] but there are date indicating excitatory effects of ATP [24]. The pharmacological action of ATP is complicated by its breakdown by ecto-nucleotidases to adenosine, which retains its own effects by acting at P_1 receptors [17, 24]. Adenosine-induced relaxation suggests the presence of inhibitory P_1 receptors in each of the regions examined. Indeed, the sensitivity of ATP-induced relaxations to the P_1 receptor antagonist 8*p*SPT suggests that the effect of ATP may be partly mediated through P_1 receptor activation.

Under NANC stimulation conditions, the cholinergic transmitter pathway was blocked by atropine. For this reason the mediation of the action of applied ATP or modulation of the EFS-elicited responses exerted by prejunctional P_2-purinoceptors [2] located on cholinergic nerves [18] could not be demonstrated. This suggests that the excitatory ATP action observed in the present experiments was mediated via P_2-post-junctional purinoceptors. It has been proposed that the intestinal smooth muscle cells are endowed with two subtypes of P_{2Y}-purinoceptors: a relaxation-mediating P_{2Y}-receptor [16] and a concentration-mediating P_{2Y}-receptor [18]. It could be suggested that the excitatory action of ATP observed in the present study was effected via contraction-mediated smooth muscle P_{2Y}-purinoceptors. In this study, at either $P_{2X}R$ or $P_{2Y}R$, none of the tested agonists such as ATP and 2-msATP showed potency for gene and protein expression in SCN, VSMCs and HEK293 cells.

In previously report, evans blue (10 μM), a putative P_{2X} receptor antagonist in the intestinal muscle [5], slightly inhibited the L-NOARG-resistant relaxations to EFS and the relaxation to α,β-MeATP, without affecting those to ATP and ADP-βS. NF 279 (1 μM), which blocks rat P_{2X1}, P_{2X2} and P_{2X3} receptors [10, 21, 27]. Brilliant blue G (110 μM), which blocks P_{2X5} and P_{2X7} receptors [3, 14, 19, 31], had no effect on the relaxations to EFS or ATP. Also, RB-2 remains the most potent (mean IC_{50}, 0.36 μM) at the rat $P_{2X2}R$ [20] and A317491 is one of the more potent (mean IC50, 0.1 μM) and highely selective antaginists at rat $P_{2X3}R$ [15].

2-msATP has been shown to elicit non-selective inward cation currents with high potency, whereas α,β-MeATP is inactive as an agonist at the recombinant P_{2X} receptor. The lack of sensitivity of ATP and 2-msATP-induced contractions to TTX (data not shown), suggests that a majority of excitatory P_2 receptors are located on smooth muscle of swine renal artery. This hypothesis is substantiated by the presence of immunoreactivity to P_{2X2} receptors on smooth muscle cell layer. However, no significant differences of P_{2X}/P_{2Y} encoding gene and protein expression in SCN, VSMCs and HEK293 under with/without ATP, a P_2 receptor agonist were observed. In functional analysis of P_{2Y} receptor for vascular relaxation, 2-msATP, a strong agonist of P_{2Y} receptor, induced relaxation of NA-precontracted renal artery in a dose-dependent manner. Strikingly, vascular relaxative effect of ATP, 2-msATP, agonists of P_{2Y} receptor, abolished by treatment of RB-2, a putative P_{2Y} receptor antagonist. Taken together, the relationship between molecular and functional characteristic of P_{2X}/ P_{2Y} receptors in conventional pig should be considered that they are another important factor which regulate the kidney function in swine. Based on this study, we propose the purinergic receptor as well as adrenergic and cholinergic receptors is an essential component of the renal homeostasis. Finally, the relationship between molecular and functional characteristic of P_{2X}/P_{2Y} receptors in smooth muscle cell of renal artery as well as nerve and kidney cells of conventional pig should be applied to clinical fields.

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References

1. **Abbracchio MP, Burnstock G.** Purinoreceptors: are there families of P_{2X} and P_{2Y} Purinoreceptor? Pharmacol Ther 1994, **64**, 445-475.
2. **Barthó L, Lénárd L Jr, Maggi CA.** Evidence for the involvement of P_2-purinoceptors in the cholinergic contraction of the guinea-pig ileum. Br J Pharmacol 1997, **121**, 1507-1508.
3. **Bo X, Jiang LH, Wilson HL, Kim M, Burnstock G, Surprenant A, North RA.** Pharmacological and biophysical properties of the human P_{2X5} receptor. Mol Pharmacol 2003, **63**, 1407-1416.
4. **Boeckxstaens GE, Pelckmans PA, Bult H, De Man JG, Herman AG, van Maercke YM.** Evidence for nitric oxide as mediator of non-adrenergic non-cholinergic relaxation induced by ATP and GABA in the canine gut. Br J Pharmacol 1991, **102**, 434-438.
5. **Bültmann R, Tuluc F, Starke K.** On the suitability of adenosine 3-phosphate 5-phosphosulphate as a selective P_{2Y} receptor antagonist in intact tissues. Eur J Pharmacol 1998, **351**, 209-215.
6. **Burnstock G.** Comparative studies of purinergic nerves. J Exp Zool 1975, **194**, 103-133.
7. **Burnstock G.** A basis for distinguishing two types of purinergic receptor. In: Straub RW, Bolis L. (eds.). Cell Membrane Receptors for Drugs and Hormones. A Multidisciplinary Appoach. pp. 107-118, Raven Press, New York, 1978.
8. **Burnstock G, Campbell G, Satchell D, Smythe A.** Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. Br J Pharmacol 1970, **40**, 668-688.
9. **Burnstock G, Kennedy C.** Is there a basis for distinguishing two types of P_2-purinoceptor? Gen Pharmacol 1985, **16**, 433-440.
10. **Damer S, Niebel B, Czeche S, Nickel P, Ardanuy U, Schmalzing G, Rettinger J, Mutschler E, Lambrecht G.** NF279: A novel potent and selective antagonist of P_{2X} receptor-mediated responses. Eur J Pharmacol 1998, **350**, R5-6.
11. **Galligan JJ, LePard KJ, Schneider DA, Zhou X.** Multiple mechanisms of fast excitatory synaptic transmission in the enteric nervous system. J Auton Ner Syst 2000, **81**, 97-103.
12. **Giaroni C, Knight GE, Ruan HZ, Glass R, Bardini M, Lecchini S, Frigo G, Burnstock G.** P_2 receptors in the murine gastrointestinal tract. Neuropharmacology 2002, **43**, 1313-1323.
13. **Heinemann Á, Shahbazian A, Barthó L, Holzer P.** Different receptors mediating the inhibitory action of exogenous ATP and endogenously released purines on guinea-pig intestinal peristalsis. Br J Pharmacol 1999, **128**, 313-320.
14. **Hu B, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ.** P_{2X} receptors in trigeminal subnucleus caudalis modulate central sensitization in trigeminal subnucleus oralis. J Neurophysiol 2002, **88**, 1614-1624.
15. **Jarvis MF, Burgard EC, Mcgaraughty S, Honore P, Lynch K, Brennan TJ, Subieta A, Van Biesen T, Cartmell J, Bianchi B, Niforatos W, Kage K, Yu H, Mikusa J, Wismer CT, Zhu CZ, Chu K, Lee CH, Stewart AO, Polakowski J, Cox BF, Kowaluk E, Williams M, Sullivan J, Faltynek C.** A-317491, a novel potent and selective non-nucleotide antagonist of P_{2X3} and $P_{2X2/3}$ receptors, reduces chronic inflammatory and neuropathic pain in the rat. Proc Natl Acad Sci USA 2002, **99**, 17179-17184.
16. **Johnson CR, Charlton SJ, Hourani SM.** Responses of the longitudinal muscle and the muscularis mucosae of the rat duodenum to adenine and uracil nucleotides. Br J Pharmacol 1996, **117**, 823-830.
17. **Kadowaki M, Nagakura Y, Tokita K, Hanaoka K, Tomoi M.** Adenosine A_1 receptor blockade reverses experimental postoperative ileus in rat colon. Eur J Pharmacol 2003, **458**, 197-200.
18. **Kennedy I, Humphrey PPA.** Evidence for the presence of two types of P_2 purinoceptor in the guinea-pig ileal longitudinal smooth muscle preparation. Eur J Pharmacol 1994, **261**, 273-280.
19. **King B, Chen CC, Akopian AN, Burnstock G, Wood JN.** A role for calcineurin in the desensitization of the P_{2X3} receptor. Neuroreport 1997, **8**, 1099-1102.
20. **King BF, Wildman SS, Ziganshina LE, Pintor J, Burnstock G.** Effects of extracellular pH on agonism and antagonism at a recombinant P_{2X2} receptor. Br J Pharmacol 1997, **121**, 1445-1453.
21. **Klapperstück M, Büttner C, Nickel P, Schmalzing G, Lambrecht G, Markwardt F.** Antagonism by the suramin analogue NF279 on human P_{2X1} and P_{2X7}

receptors. Eur J Pharmacol 2000, **387**, 245-252.

22. **Mastick CC, Saltiel AR.** Insulin-stimulated tyrosine phosphorylation of caveolin is specific for the differentiated adipocyte phenotype in 3T3-L1 cells. J Biol Chem 1997, **272**, 20706-20714.
23. **Matsuo K, Katsuragi T, Fujiki S, Sato C, Furukawa T.** ATP release and contraction mediated by different P_2-receptor subtypes in guinea-pig ileal smooth muscle. Br J Pharmacol 1997, **121**, 1744-1748.
24. **Moody CJ, Burnstock G.** Evidence for the presence of P1-purinoceptors on cholinergic nerve terminals in the guinea-pig ileum. Eur J Pharmacol 1982, 77, 1-9.
25. **North RA.** Molecular physiology of P_{2X} receptors. Physiol Rev 2002, **82**, 1013-1067.
26. **Ralevic V, Burnstock G.** Receptors for purines and pyrimidines. Pharmacol Rev 1998, **50**, 413-492.
27. **Rettinger J, Aschrafi A, Schmalzing G.** Roles of individual N-glycans for ATP potency and expression of the rat P_{2X1} receptor. J Biol Chem 2000, **275**, 33542-33547.
28. **Schlegel A, Lisanti MP.** A molecular dissection of caveolin-1 membrane attachment and oligomerization. J Biol Chem 2000, **275**, 21605-21617.
29. **Serio R, Alessandro M, Zizzo MG, Tamburello MP, Mulè F.** Neurotransmitters involved in the fast inhibitory junction potentials in mouse distal colon. Eur J Pharmacol 2003, **460**, 183-190.
30. **Soediono P, Burnstock G.** Contribution of ATP and nitric oxide to NANC inhibitory transmission in rat pyloric sphincter. Br J Pharmacol 1994, **113**, 681-686.
31. **perlágh B, Illes P, Gerevich Z, Köfalvi A.** Distinct mechanisms underlying α_1-adrenoceptor and P_{2x} purinoceptor operated ATP release and contraction in the guinea-pig vas deferens. Neurochem Res 2002, **26**, 951-957.
32. **Vial C, Evans RJ.** Smooth muscle does not have a common P_{2x} receptor phenotype: expression, ontogeny and function of P_{2x1} receptors in mouse ileum, bladder and reproductive systems. Auton Neurosci 2001, **92**, 56-64.
33. **Xue L, Farrugia G, Sarr MG, Szurszewski JH.** ATP is a mediator of the fast inhibitory junction potential in human jejunal circular smooth muscle. Am J Physiol 1999, **276**, G1373-1379.