REVIEWS

P2 Receptors: Theoretical Background for the Use in Clinical Practice

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Here we review the results of physiological, pharmacological, and pathophysiological studies of ATP receptors, P2 type receptors. Characteristics of two families of P2 receptors (P2X and P2Y) are presented and the possibility of using preparations affecting these receptors is discussed.

Key Words: *P2 receptors; agonists; antagonists; use in clinical practice*

Over many years ATP was considered only as the intracellular source of energy. At the beginning of the 1970s a hypothesis on a transmitter role of ATP was put forward [11]. Recent studies confirmed this hypothesis. ATP and other nucleotides regulate various intracellular processes via specific P2 purinergic receptors. In the modern classification they are designated as P2 receptors, since not only purine, but also pyrimidine derivatives act as ligands for these receptors [1,2,12,16].

According to modern classification of the Nomenclature Committee of the International Pharmacological Society, P2 receptors are divided into P2X and P2Y families (Fig. 1). Each family includes several subtypes of receptors designated by the corresponding numbers. These numbers are assigned when the molecular structure of receptors is determined, and their molecules are cloned. There are 7 subtypes of P2X receptors and 6 subtypes of P2Y receptors [37,43].

By the mechanism of action, P2X receptors are ligand-operated ion channels controlling the influx of Na^+, K^+, Ca^{2+}, and Cl^-. Most part of the protein molecule is localized extracellularly and forms a large loop, while both terminal fragments lie intracellularly. Subtypes of receptors differ in the length of C-terminal fragments [29]. In the 1990s the structure of these receptors was determined, and they were cloned [10, 36]. However, recent studies showed that recombinant receptors expressed on frog oocytes and native receptors differ in their pharmacological characteristics. This can be explained by the fact that under physiological conditions ion channel is formed by 3 or 4 subunits of P2X receptors [10]. Homomultimeric ion channels contain only 1 subtype of P2X receptors, while heteromultimers include various subtypes of these receptors ($P2X_{2/3}$, $P2X_{4/6}$, and $P2X_{1/5}$ receptors). $P2X_7$ receptors form only homomultimers [16]. Moreover, the existence of the potent ectoenzyme system cleaving extracellular purine and pyrimidine nucleotides [63] is often ignored in experiments with recombinant receptors. The presence of structurally abnormal receptors possessing no functional activity, but modulating the effects of normal P2X receptors under physiological conditions cannot also be excluded [59].

These data show that *in vitro* experiments are not optimal for studying P2X receptors. The physiological role of receptors should be evaluated at the tissue and organ levels.

$P2X_1$ receptors are present in various smooth muscle organs (Table 1). Stimulation of these receptors induces contractions, while blockade inhibits contrac-

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tile activity in these organs [35]. Knockout male mice with genetic defects in these receptors are characterized by decreased contractile activity of the ejaculatory duct. Fertility in these mice decreases by 90%, while spermatogenesis and erectile function remain unchanged [47].

There are data on the role of $P2X_3$ receptors in nociceptive transmission. Immunohistochemical and radioligand assays revealed these receptors in peripheral endings of sensory nerves and sensory neurons of the spinal cord dorsal horns [15,18,20].

$P2X_7$ receptor is the largest molecule among receptors of this family. The homomultimer consisting of 2 or 3 molecules of $P2X_7$ receptors forms a large channel in the cell membrane (up to 4 nm) permeable for substances with a molecular weight up to 900 Da. These channels are formed during ischemia and inflammation and their formation triggers apoptosis in cells [49,57].

By the mechanism of action, P2Y receptors are G protein-mediated receptors. The protein has 7 transmembrane fragments and forms 3 intracellular and 3 extracellular loops. Subtypes of these receptors differ by the transmembrane fragments. The role of a second messenger is usually played by inositol triphosphate

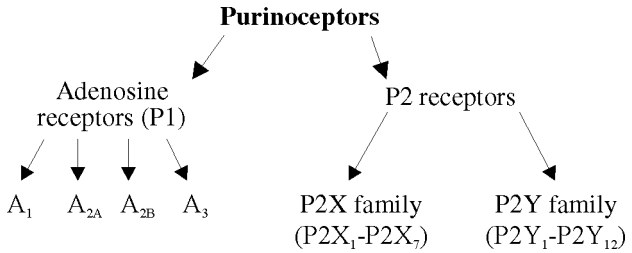

Fig. 1. Classification of purinoceptors [37,43].

produced after activation of phospholipase C, but activation of phospholipase A_2 (with the formation of arachidonic acid metabolites) and adenylate cyclase (followed by changes in cAMP level) can also result from stimulation of P2Y receptors [13,16,42].

Many subtypes of P2Y receptors were described and cloned. However, in the past 2-3 years some subtypes of P2Y receptors were excluded from the classification. These receptors had no functional activity or were analogs of existing receptors [43]. Therefore, numeration of P2Y receptors is not consecutive.

The last subtype of P2Y receptors is of particular interest (Table 2). ADP acts as a selective agonist, while ATP is an antagonist of these receptors. $P2Y_{12}$ receptors are localized in platelets. Their stimulation

TABLE 1. Subtypes of P2X Receptors

Subtype of receptors	Localization	Specific features	Agonists	Antagonists
$P2X_1$	Smooth muscles, platelets, cerebellum, dorsal horns of the spinal cord	Rapid desensitization	α,β-meATP= ATP=2meSATP	TNP-ATP, IP_5I, NFO23
$P2X_2$	Smooth muscles, CNS, chromaffin cells, autonomic and sensory ganglia	Strong sensitivity to pH and Zn^{2+}	ATP≥ATPγS≥2meSATP>> α,β-meATP	Suramin, PPADS
$P2X_3$	Sensory sympathetic neurons	Rapid desensitization	2meSATP≥ATP≥ α,β-meATP	TNP-ATP, suramin, PPADS
$P2X_4$	CNS, testes, large intestine	Effect is potentiated with suramin, PPADS, and RB2	ATP>>α,β-meATP	—
$P2X_5$	Proliferating cells and cells of the skin, intestine, urinary bladder, and spinal cord	Association with cell proliferation and differentiation	ATP>>α,β-meATP	Suramin, PPADS
$P2X_6$	CNS, motor neurons of the spinal cord	—	—	—
$P2X_7$	Cells of the immune system, skin, and pancreas	Formation of large channels and initiation of apoptosis	BzATP>ATP≥2meSATP>> α,β-meATP	Coomassie brilliant blue

Note. Here and in Table 2: α,β-meATP) α,β-methylene-ATP; BzATP) 2'-3'-O-(4-benzoylbenzoyl)-ATP; 2meSATP) 2-methylthio-ATP; 2meSADP) 2-methylthio-ADP; IP_5I) diinosine pentaphosphate; RB2) reactive blue 2; TNP-ATP) 2',3'-O-(2,4,6-trinitrophenyl)-ATP; MRS 2279) N-methanocarbo-N^6-methyl-2-chloro-2'-deoxyadenosine 3',5'-diphosphate; NFO23, pyridoxal 5'-phosphate 6-azophenyl-4'-carboxylate; and PPADS) pyridoxal phosphate 6-azophenyl-2',4'-disulfonic acid. Mathematical symbols between agonists reflect their comparative activity.

is followed by platelet aggregation, while blockade abolishes this effect [46].

Recent advances in molecular genetics (creation of knockout models lacking certain genes) opened new prospects in evaluating the physiological role of P2 receptors. Male mice with genetic deficiency of $P2X_1$ receptors are infertile [47]. $P2X_3$ receptor deficiency determines reduced pain sensitivity [21], deficiency of $P2Y_1$ receptors determines impaired platelet aggregation and prolongs bleeding time [30].

Thus, ample experimental data confirm the possibility of pharmacological modulation of P2 receptors, which forms the basis for creation of novel drugs with principally new mechanisms of action [3,19,35]. Moreover, a variety of P2 receptor subtypes provide the basis for creation of organ- and tissue-specific preparations effective in the therapy of various diseases (Table 3).

A recent achievement of pharmacology in modulation of P2 receptors is introduction of clopidogrel into medical practice. Clopidogrel, an antagonist of P2Y receptors, prevents platelet aggregation. This drug has some advantages over aspirin (*e.g.*, in patients with atherosclerosis) [9,40]. Now several preparations with the same mechanism of action undergo clinical tests.

Extracellular ATP in high concentrations suppresses the growth of cultured tumor cells and produces cytostatic and cytotoxic effects on murine tumors [54]. Therefore, this agent holds much promise for the therapy of tumors. Intravenous infusions of ATP to patients with lung cancer reduce weight loss, normalize blood albumin concentration, decrease mortality rate, and improve the quality of life in these patients [4,55]. The antitumor effect of ATP is probably realized via stimulation of $P2X_7$ receptors followed by opening of nonselective channels in the cell membrane and induction of apoptosis [26].

The positive effect of ATP in patients with cardiovascular diseases is associated with its degradation to adenosine, which causes bradycardia, produces the negative inotropic effect, and decreases blood pressure [56,62]. It should be emphasized that ATP produces a positive effect in patients with paroxysmal supraventricular tachycardia [60], cerebral ischemia [38, 48], and cardiac ischemia [44,45]. Controlled hypotension induced by intravenous infusion of ATP is used in medical practice [5,7], but unlike sodium nitroprusside, ATP does not induce the rise of blood catecholamine concentration and hypertension upon withdrawal [6]. Recent studies showed that specific changes in the cardiovascular system are observed even after peroral treatment with ATP [41].

Over many years the central effects of purines were considered to be associated with adenosine [33, 52]. However, recent studies showed that ATP plays an important role in the central nervous system (CNS). It regulates and modulates the release of other neurotransmitters [24]. ATP acts as a messenger of rapid excitatory impulses in interneuronal contacts in CNS [32]. The physiological significance of this effect is poorly understood. ATP produces a dual effect on impulse conduction in synapses: rapid stimulatory influence of ATP is followed by long-term inhibition induced by its metabolite adenosine [14]. This promising approach attracts much attention.

P2Y receptors were identified on insulin-secreting pancreatic β-cells [61]. Stimulation of these receptors leads to mobilization of intracellular Ca^{2+}, which provides secretion of insulin and resistance to hyperglycemia. It is interesting that the insulin-stimulating effect was observed after peroral administration of P2Y receptor agonist ADPβS [34]. These findings open new vistas in creation of peroral drugs reducing blood glucose level.

The inflammatory reaction is accompanied by massive ATP release into the extracellular space, which

TABLE 2. Subtypes of P2Y Receptors

Subtype of receptors	Localization	Agonists	Antagonists
$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts	2meSADP>2meSATP>ADP=ATP	MRS 2279
$P2Y_2$	Immune cells, epithelial and endothelial cells, renal tubules, osteoclasts	UTP=ATP	Suramin
$P2Y_4$	Endothelial cells	UTP>ATP	RB2, PPADS
$P2Y_6$	Individual epithelial cells, placenta, T cells, thymus	ADP>UTP>>ATP	RB2, PPADS, suramin
$P2Y_{11}$	Spleen, intestine, granulocytes	BzATP>ATPγS>ATP	Suramin, RB2
$P2Y_{12}$	Platelets	ADP	Clopidogrel, ATP

TABLE 3. Possible Use of Substances Modulating P2 Receptors in Clinical Practice

Possible therapeutic effects	Agonists		Antagonists	
	P2X	P2Y	P2X	P2Y
Analgetic effect [15,18,20]			+	
Antiinflammatory effect [8,39,65]			+	+(?)
Antiepileptic effect [22]			+	
Antitumor effect [54,55]	+			
Hypoglycemic effect [34,61]		+		
Antiaggregant effect [9,40,46]				+
Antiarrhythmic effect [38,60]			+(?)	+(?)
Hypotensive effect [5,6,7]		+		
Spasmolytic effect [12,51,52,58]		+	+	
Male contraception [28,47]			+	
Stimulation of birth activity [64]	+			
Suppression of bone resorption [27]			+	
Decrease in intraocular pressure [53]	+(?)			

increases the severity of pain and inflammation [8,23]. P2 receptors are present on cells involved in inflammatory and immune responses (*e.g.*, neutrophils, lymphocytes, and mast cells) [25,31]. P2 receptor antagonists inhibit the development of inflammatory processes [39,65]. These data indicate that P2 receptor antagonists hold promise as antiinflammatory drugs with a new mechanism of action.

The atropine-resistant contractile response of the urinary bladder in mammals is mediated by P2 receptors [17,51,58]. Probably, the purinergic component plays a particular role under pathological conditions. For example, patients with chronic interstitial cystitis and enuresis are characterized by a pathologically increased sensitivity to ATP [50]. Selective P2X antagonists hold much promise in this respect.

In recent years we studied the clinical significance of P2 receptors in human tissues. At the late gestational period contractile activity of human uterus is mediated by P2 receptors. This reaction is not observed in nonpregnant uterus [64]. The data suggest that P2 receptors are expressed in human myometrium during pregnancy. Physiologically, these changes promote uterine contractions in labor. Therefore, preparations exciting P2 receptors can be used as stimulators of labor and contractile activity of the uterus.

These data indicate that studies of P2 receptors are of considerable fundamental and clinical importance. In the immediate future selective agonists and antagonists of P2 receptors effective *in vitro* and *in vivo* will be created. This approach to the synthesis of medicinal preparations holds much promise.

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