Chapter

Functions of Purinergic Receptors

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Abstract

Purinergic receptors, also known as purinoceptors, are a family of plasma membrane molecules found in many mammalian tissues. Purinergic receptors are transmembrane receptors consisting of two main categories. P1 receptors are stimulated by adenosine. Those that respond to extracellular nucleotides (ATP, ADP, UTP and UDP) are P2 receptors. The P2X receptors are ligand-gated ion channels. The P1 and P2Y receptors are bound to the G protein. Both of these metabotropic receptors are distinguished by taking into account their reactivity to specific activators. P1 and P2Y receptors are widely distributed in the brain, heart, kidneys and adipose tissue.

Keywords: purinergic receptors, P1, P2, ATP, UTP

1. Introduction

1.1 The possible physiological sources of nucleotides and nucleosides

ATP and other described nucleotides treat both metabotropic (P2Y) and ionotrophic (P2X) receptors. P2X receptors subunits (P2X1–P2X7) form ligand-gated cation channels, like homomultimers or heteromultimers. P2X3 subunits contribute to the ion permeability pathway by joining the fields of each subunit. P2X3R has the lowest recorded relative Ca^{2+} permeability of the family. P2X7, in addition to cation channels, is associated to contain large cytolytic pores; that are found in macrophages and brain microglial cells. P2Y receptors can activate or inhibit adenylate cyclase according to the subtype and consequently the type of coupled G protein. Adenylate cyclase and especially for the Ca^{2+} channel inhibition appears. P2Y receptors form as subset of G-protein-linked receptors; most mate to phospholipase C via the G protein, but inhibition of adenylate cyclase and N-type Ca^{2+} channels and activation of K^{+} channels also occurs. The expressed P2Y receptors are generally pharmacologically distinguished by the rank order of the agonists; some prefer pyrimidine to purine. Several P2Y receptors have a very common tissue distribution [1]. Molecular structures of ATP and BzATP are shown in **Figure 1**.

Adenine nucleotides inhibit isoproterenol and forskolin-induced cyclic AMP accumulation in C6-2B rat glioma cells. This inhibition occurs in the presence of a phosphodiesterase inhibitor. Adenine nucleotides did not cause effects in measurements of the direct phosphodiesterase activity in intact cells. Pretreatment of C6-2B glioma cells with pertussis toxin blocked the inhibitory effects of P2Y-purinergic receptor agonists. A number of ATP and ADP analogs produced a rank potency order (2-methylthioadenosine 5′-triphosphate> or = 2-methylthioadenosine 5′-diphosphate > adenosine 5′-O-(2-thiodiphosphate) > 2-chloro-adenosine ADP = adenosine 5′-O-(3-thio- triphosphate) > ATP > UTP) expected from a P2Y-purinergic receptor activation; and the P2X-purinergic receptor agonists, alpha,

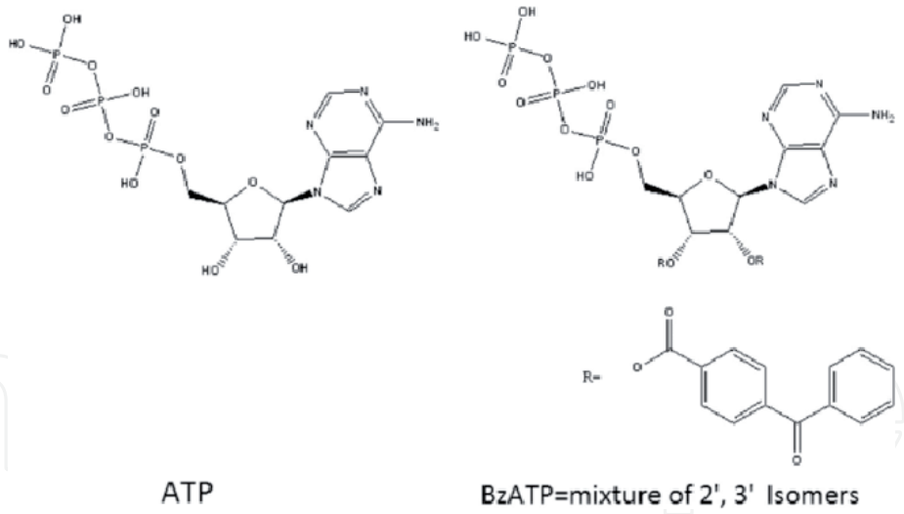

Figure 1.
Molecular structures of ATP and BzATP [2].

beta-methyleneadenosine 5′-triphosphate and beta, gamma-methylene-adenosine 5′-triphosphate had no effect. Phospholipase C activity occurs in response to P2-purinergic receptor activation in many target tissues, therefore the effects of P2Y receptor agonists on inositol phosphate accumulation were measured in C6-2B cells. No evidence was found for the regulation of P2Y-purinergic receptor mediated by inositol lipid metabolism under conditions where the activation of muscarinic cholinergic receptor or AIF4 increased the inositol phosphate accumulation. These results indicate that a P2-purinergic receptor subtype with different signaling characteristics is present on the C6-2B rat glioma cells. Although this receptor expresses the general pharmacological properties of a phospholipase C-linked P2Y-purinergic receptor, it may represent a unique receptor subtype because it inhibits adenylyl cyclase [3].

Purines were thought to be limited to the intracellular compartment in which they were used for energy processing, nucleic acid synthesis and a large number of biochemical reactions. Karl Lohmann isolated ATP, which is the key intracellular energy currency, in 1929 [4]. However, adenosine and adenosine triphosphate (i) are abundant biochemical components of the tumor microenvironment, (ii) are strong modulators of immune cell responses and cytokine release, and (iii) are key players in host-tumor interaction. In addition, both nucleotides directly affect tumor cell growth. Adenosine is a potent immunosuppressant (mainly effective at A2A receptors) and a cell growth modulator (mainly effective at A3 receptors). ATP is a proinflammatory agent (effective in P2Y1, P2Y2, P2Y4, P2Y6 and P2Y12 and at P2X4 and P2X7 receptors), an immunosuppressant (effective in P2Y11) and a growth promoter (effective in P2Y1, P2Y2 and P2X7). This complex signaling network produces a number of inhibitory and stimulating responses that affect immune cell function, tumor growth and metastatic spread.

Purinergic receptors, represented by many families, are the most abundant receptors in living organisms, possibly occurring in the early stages of evolution. Purinergic signaling in peripheral and central nervous systems is a rapidly expanding field. Examination of these receptors makes it possible to develop therapeutic strategies for these disorders with novel mechanisms of action, including purulent, pathogenic conditions, including pain, trauma, ischemia, epilepsy, migraine, psychiatric disorders and drug dependence [5].

In micromolar/nanomolar concentrations, extracellular adenosine triphosphate (ATP) has been shown to produce significant functional changes in a wide variety of normal and transformed cell types. Although ATP can be specifically released

from the cytosol of damaged cells, it is also packaged in some exocytotic vesicles/granules containing conventional neurotransmitters and hormones. Various biological responses to ATP are mediated by various P2-purinergic cell surface receptors activated upon binding of ATP and other nucleotides. Recent physiological, biochemical and pharmacological studies have shown that there are multiple types of ATP receptor subtypes. These include: (1) G-protein-bound ATP receptors that induce inositol phospholipid hydrolysis, Ca^{2+} mobilization and activation of protein kinase C; (2) ATP receptors that directly activate non-selective cation channels in plasma membranes of various cell types and (3) ATP receptors capable of producing cytotoxic or activation responses in T lymphocytes and other immune effector cells by rapid induction of surface membrane pores permeable to ions and endogenous metabolites (with molecular weights until 900 Da). In addition to these functional criteria, these default ATP receptor subtypes can be pharmacologically distinguished by characteristic potency for various structurally modified ATP analogs [6].

Intracellular nucleotides play a fundamental and ubiquitous role in energy metabolism, nucleic acid synthesis and enzyme regulation. It is widely understood that extracellular nucleotides and nucleosides carry out important biological actions in many tissues and cells [7]. GLUTs (facilitate transport of glucose into the cells), SGLTs (facilitate the re-absorption of glucose back into circulation) and KATP (ATP-sensitive potassium channels) metabolic sensors play an important role in glucose homeostasis and metabolism in the body and in many specific organs (e.g., intestine, pancreas, heart, skeletal muscle and brain) [8].

KATP bind metabolic signals to cell excitability and play an important role in many tissues, including regulation of insulin secretion, control of vascular tone and protection of neurons and muscles from ischemia. KATP channels are octameric complexes consisting of four sulfonylurea receptors (SUR.x) and four inwardly rectifying potassium channels (Kir6.x). They are regulated by intracellular ATP and ADP. While ATP inhibits channel activity, ADP antagonizes the inhibitory effect of ATP in the presence of Mg^{2+} and stimulates the channel activity. These gate properties are essential for this channel to detect metabolic changes in cells. Thus, in pancreatic β cells, the [ATP]/[ADP] ratio increases in response to blood glucose levels augment, leading to closure of the KATP channel, membrane depolarization, activation of voltage-gated Ca^{2+} channels and insulin release. However, when the blood glucose levels are low, the [ATP]/[ADP] ratio decreases, KATP channels are opened and insulin secretion decreases [9].

The catastrophic channels in the pancreas are activated as follows; in the event of β-cell glucose level is increased, the intracellular ratio of ATP to ADP also increases, leading to closure of the KATP channel, depolarization of cells and insulin release [10].

The P-sensitive K′(K + [ATP]) stream is thought to be regulated by GTP-binding proteins (G proteins), but the pathways combining the receptor, G protein and channel are not identified. The regulation of tolbutamide-sensitive K′[ATP] current in neonatal rat ventricular myocytes is determined. Activated ATP-sensitive K + (K + [ATP]) channels are present in cells when intracellular ATP levels decrease. Intracellular ATP levels are found when intracellular ATP levels are reduced in the cell, intracellular skeletal muscle, brain and pancreas. Little is known about the function of K + [ATP] channels in heart cells, although their important role in controlling insulin secretion from pancreatic P-cells has been well established. When pharmacologically activated, these channels greatly reduce the duration of action potential and have been proposed to be responsible for the shortening of action potential in metabolically dangerous ischemic muscles. However, the ATP concentration in the metabolically blocked caste remains above the level, which prevents the channels in

the excised membrane patches. A possible explanation for this discrepancy is that the ATP sensitivity of the channels can be modulated by intracellular mechanisms [11].

ATP-responsive K^+ channels, called KATP channels, provide a link between cellular metabolism and membrane electrical activity in various tissues. Channel isoforms are targets for compounds that stimulate and inhibit their activity resulting in membrane hyperpolarization and depolarization, respectively. Vascular smooth muscle and stimulating insulin secretion loosening are examples for these situations above [12]. Adenosine agonists and the openers of the ATP-sensitive potassium (KATP) channel were reported to limit infarct size (IS) [13]. ATP-sensitive potassium (KATP) channels are well defined in the heart, skeleton and smooth muscle, pancreatic cells, pituitary, central and peripheral nervous system both electrophysiologically and pharmacologically. The activities and hence the various cellular functions are controlled by the cellular metabolism. In general, the changes in ATP (causing channel closure) and in MgADP (channel activating) are believed to have dual metabolism to channel activity 6. It is described that the cellular localization of two mRNA transcripts that is expected to generate ATP-sensitive K^+ channels in the murine brain. There is evidence that the KATP channel in pancreatic cells is composed of a Kir6.2 and a complex of SUR1 subunits. KATP channels with similar characteristics (type I) have been described in various neurons, including those with brain cortex, basic nigra, caudate, and hippocampus [14].

Almost all tumor cells and all immune cells express plasma membrane receptors for extracellular nucleosides (adenosine) and nucleotides (ATP, ADP, UTP, UDP and sugar UDP). The tumor microenvironment is characterized by an unusually high concentration of ATP and adenosine. Adenosine is an important determinant of the immunosuppressive tumor environment. Serial hydrolysis of extracellular ATP catalyzed by CD39 and CD73 is the main pathway for adenosine formation in the tumor interstitium. Extracellular ATP and adenosine mold are both host and tumor responses. Depending on the activated specific receptor, extracellular purines mediate host-side immunosuppression or immunostimulation and tumor-side growth stimulation or cytotoxicity. Recent developments in this area provide the key to deciphering this complex scenario, using the potential benefits of therapy. Preclinical data indicate that targeting the adenosine producing pathway or adenosinergic receptors attenuates immunosuppression and strongly inhibits tumor growth. On the other hand, the growth of experimental tumors is strongly inhibited by targeting the receptor of P2X7 ATP selective cancer and immune cells. The role of extracellular purines (purinergic signaling) acts in host-tumor interaction and highlights new treatment options from recent advances. There is now a consensus that ATP and adenine are the main components of tumor microenvironment (TME), in which TME affects tumor growth, immune cell functions and tumor-host interaction in different ways. In view of the widespread observation that many malignant tumors overexpress several P1R or P2R subtypes, a simple approach would require targeting these receptors with selective receptors to suppress tumor receptor growth. On the same line of interference, the enzymes involved in the metabolism of extracellular nucleotides and nucleosides (CD39, CD73 and adenosine deaminase) are viewed. Although the effectiveness of several simple preclinical models has been proven, this simple approach is clearly very pure. P1Rs, P2Rs and ATP/adenosine-disrupting enzymes are expressed together with host immunostimulatory and stromal cells, which have very important functions for host integrated complex formation around the tumor. Careful selection of the candidate purinergic receptor in combination with modulators of extracellular adenosinergic pathways may allow inhibition of tumor cell growth and concomitantly increase the anti-tumor host response. This anti-cancer agent will provide an additional powerful weapon for combinative treatments [15].

Nucleotides and their receptors are emerging as potential actors in host-tumor interaction, such as new and important inflammatory and immune modulators. A large number of P2 and P1 receptors expressed by tumor and inflammatory cells exhibiting different ligand affinities for P2 and P1 receptor subtypes are modulated by local factors obtained from ectonucleotides and ADA on the nucleotide and adenosine concentration. In vivo data supports in vitro evidence that the reduction of the intratumour adenosine concentration and the targeting of the P2X7 receptor have a potent antitumor effect. Therefore, investigating the purinergic signaling in cancer opens promising perspectives for the development of innovative therapeutics [16].

Purinergic signaling has been focused on the tumor-associated immune response; nucleotides and nucleosides have strong direct effects on the tumor cells themselves. Stimulation of P2Y receptors (P2Y1 and P2Y2) promotes growth, therefore, depending on the expressed P2Y receptor subtypes, the accumulation of ATP in the tumor microenvironment is likely to promote tumor growth. In addition to P2Y receptors, P2X7 plays a role in tumor growth. It is a long-standing observation that most malignant tumors over-express P2X7 [17]. It is known that this receptor mediates a strong cytotoxic response [18]. Therefore, why a tumor should over-express a "suicide" receptor is determined. However, cytotoxicity is most commonly triggered by pharmacological (i.e., near millimolar) ATP doses. In contrast, the activation of P2X7 by the endogenously released ATP produces a trophic, growth-promoting effect [19].

Nucleotides and nucleosides in airway surface fluid regulate mucociliary clearance (MCC) activities, the primary natural defense mechanism that removes foreign particles and pathogens from airway surfaces. These effects in the airways are mainly mediated by two purinergic receptor subtypes, the Gq-coupled ATP/UTP-sensing P2Y2 receptor and the Gs-conjugated A2b adenosine receptor. Activation of the A2b receptor results in cyclic AMP-dependent activation of the Cın1 channel of the cystic fibrosis transmembrane regulator (CFTR) and stimulation of the ciliary pulse frequency. Agonist activation of the P2Y2 receptor promotes the inhibition of CFTR-dependent and CFTR-independent Cl secretion, ciliary beating and mucin secretion as well as Na^+ absorption [20].

The phenomenon about the process reveals the participation of a biological cascade. In this context, adenosine agonists and ATP-sensitive K^+ channel (KATP) openers mimic some protective effects of the preconditioning process. Furthermore, these effects are reversed by adenosine antagonists and KATP blockers; this suggests that the release of adenosine and activation of KATP channels through adenosine Al receptors may constitute an early step in ischemic cerebral preconditioning [21].

Activation of the adenosine receptor, protein kinase C (PKC) and ATP-sensitive potassium (KATP) channel is known to trigger preconditioning. The data provides direct evidence that the KATP channel, rather than the adenosine receptor, is the effector downstream of PKC in initiating PKC-mediated preconditioning. Both the adenosine receptor and the KATP channel are required to promote the actual protective effect during continuous hypoxia [22]. The possibility of joining the biological stage of protective event involvement of adenosine A1 receptors and KATP channels, associated with cross-tolerance between KA-induced epileptic tolerance or KA-induced epilepsy and global ischemia, is evaluated [23].

2. Classification of the P1 and P2 receptors

The antinociceptive effects of adenosine via preconceptional P1 (A1) purinoceptors in the spinal cord and the pain-enhancing effects of adenosine with P1 (A2)

purinoceptors in the environment have shown great interest in the development of P1 agonists and antagonists and P2X antagonists as potential analgesic drugs [24].

Purinergic receptors are divided into adenosine receptors (P1) and nucleotide receptors (P2). The receptors for nucleotides are also divided into two main groups: ligand-gated ion channels (P2X) and G-protein-associated receptors (P2Y). Upon vascular injury, platelets are collected at the site of injury to form a hemostatic plug. Abnormal activation of platelets leads to thrombosis, resulting in greater paralysis and a risk of myocardial infarction. The importance of ADP in hemostasis and thrombosis greatly underscores the significance of understanding the function of these receptors that would enable development of potent and safe antithrombotic drugs. This makes ADP receptors antagonize or interfere. Purinergic receptors are shown in **Figure 2**.

The molecular mechanisms of platelet activation caused by ADP are now evident. The separation and interactions of a unique P2T receptor concept into three components (i.e., P2Y1, P2Y12 and P2X1 receptors) helped to explain the intracellular and physiological effects of ADP on platelets. The interaction of signaling events under P2Y1 and P2Y12 receptors is a new physiological response and may be a general mechanism of α1bβ3 integrin activation by all physiological agonists. It is also shown to be a mechanism of activation for another platelet integrin αVβ3 [25].

Results from this observation include a fourth P2 receptor subtype on platelets and P2Y12 receptor binding to other G proteins. The precise signaling mechanisms and pathways mediated by these three P2 receptor subtypes will provide a better understanding of the molecular mechanisms of ADP-mediated physiological responses in platelets and, in general, agonist-induced platelet activation [26].

Two types of purinergic receptors were distinguished: P1 purines are the most sensitive to adenosine and AMP, competitively blocked by methylxanthines; P2 purinoceptors are most sensitive to ATP and ADP, and quinidine is blocked (although not competitive) by 2-substituted imidazoline, 2,2′-pyridylisatogen and apamine, and their use leads to the production of prostaglandins. Adenosine reduces the heart rate and contraction force of the atrium and the ventricles of most species. It disrupts conduction in the atrioventricular node and has a particularly strong effect on the sinoatrial pacemaker. These effects are blocked by methylxanthines and strengthened by dipyridamole. ATP is also a contractility potent inhibitor of the heart of most species but initially has a positive inotropic effect on the frog's heart, followed by inhibition (possibly due to adenosine after rapid disintegration of ATP). Adenyl compounds allow the expansion of coronary vessels. ATP and ADP are the most potent, and adenosine and AMP are partially about 25%, whereas inosine, adenine and hypoxanthine are almost inactive. Adenosine acts on the presynaptic P1 purinoceptors, reducing the release of noradrenaline and acetylcholine from the adrenergic and cholinergic nerve terminals. These effects are blocked by methylxanthines. Adenosine probably induces

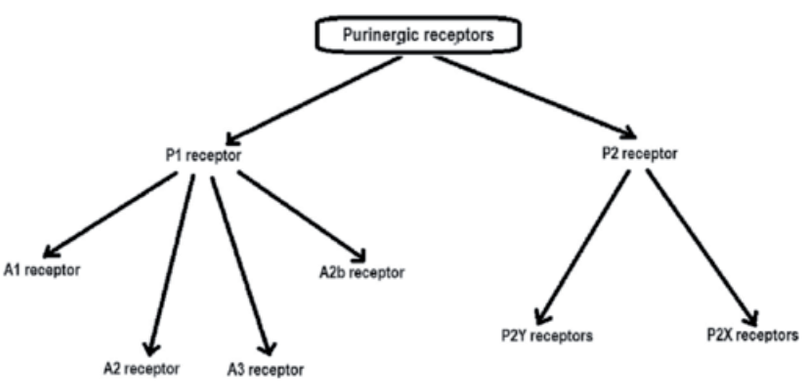

Figure 2.
Purinergic receptors [2].

adenylate cyclase in the three main regions of the heart (i.e., heart muscle, vascular smooth muscle and nerve terminals), causing changes in cAMP accumulation and a reduction in Ca^{2+} flux. The mechanism of action of ATP is unknown. There is strong evidence for a physiological role for adenyl compounds in the regulation of coronary blood flow. The concentration of adenosine, ischemia-induced ATPs acting on the P1 purinorecceptors, acting on vasoconstriction of vasodilatation from cardiac hyperactivity, may be increased by the release of intranural purinergic nerves and acting on P2 purinoseptors. Some purinergic nerves affect sinoatrial pacemaker activity, and adenosine may be a physiological presynaptic modulator of cardiothoracic activity of both adrenergic and cholinergic nerves [27]. ATP and other purine nucleotides and nucleosides have strong regulatory (similar to neurotransmitter) effects attributed to interaction with a specific plasma membrane receptor 1. To date, receptor mechanisms underlying purinergic activation have been poorly characterized. One problem was the variability of excited effects in different tissues [28]. It was suggested that most of the effects could be explained if two different receptors, called P1 and P2, have different properties for agonists and antagonists. The receptor mechanisms in the parotid gland have been extensively studied. ATP causes a significant increase in membrane conductivity, radioactive Rb flow and amylase secretion. The effects of ATP are similar to those induced by acetylcholine (ACh) and α-adrenergic agonists but are still present when cholinergic and adrenergic blocking agents are used. The latency and return potential of the effects induced by ATP can be compared to those of autonomic agonists. General structures of different classes of P2X7 antagonists are shown in **Figures 3** and **4**.

Since the sequence of action of the nucleotide sequence was ATP > ADP > AMP, adenosine had no effect, and the response could be blocked by quinidine, but not by theophylline [29]. Activation of P2Y1 and P2Y12 receptors by secreted ADP induced by agonists such as thrombin, thromboxane and collagen is the main mechanism of platelet activation. P2X1 receptors also contribute to the change of platelet shape and enhance calcium mobilization. Cloning of the P2Y12 receptor and knockout in

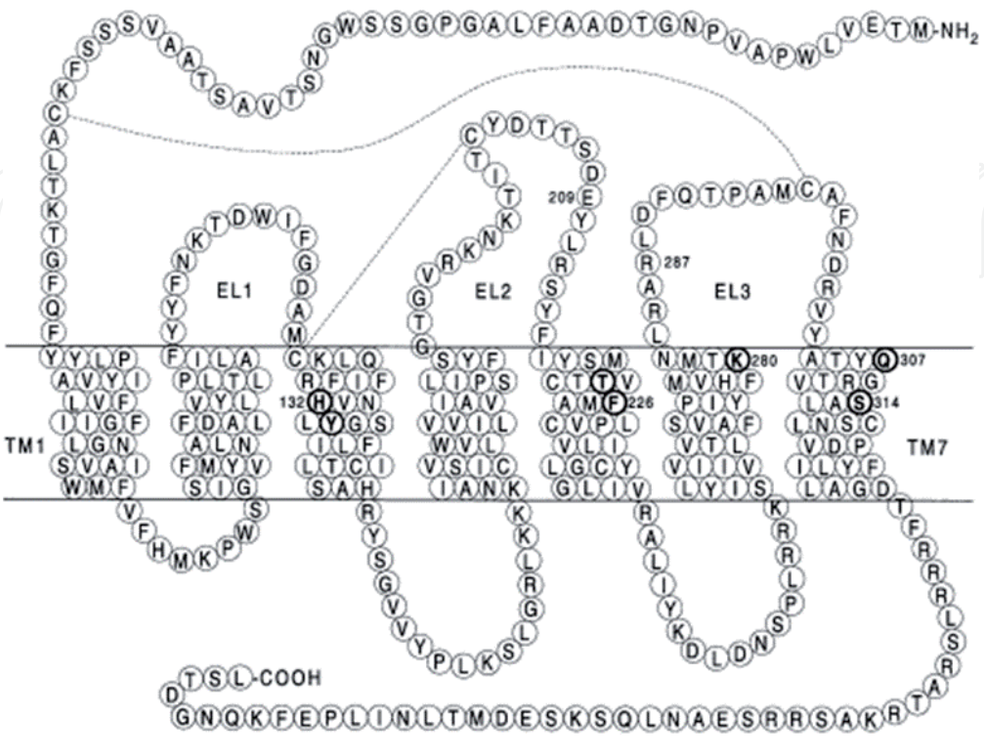

Figure 3.
General structures of different classes of P2X7 antagonists [2].

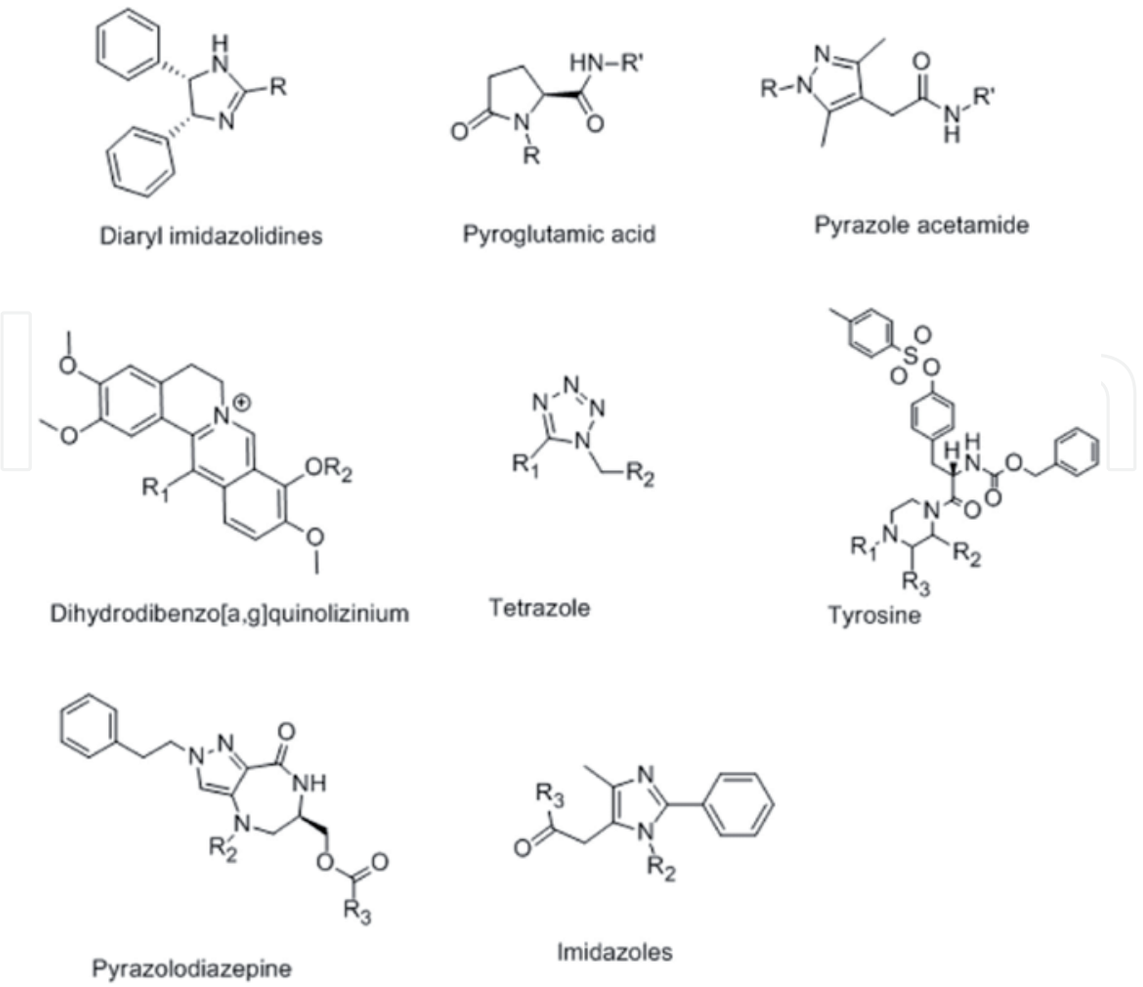

Figure 4.
General structures of different classes of P2X7 antagonists [2].

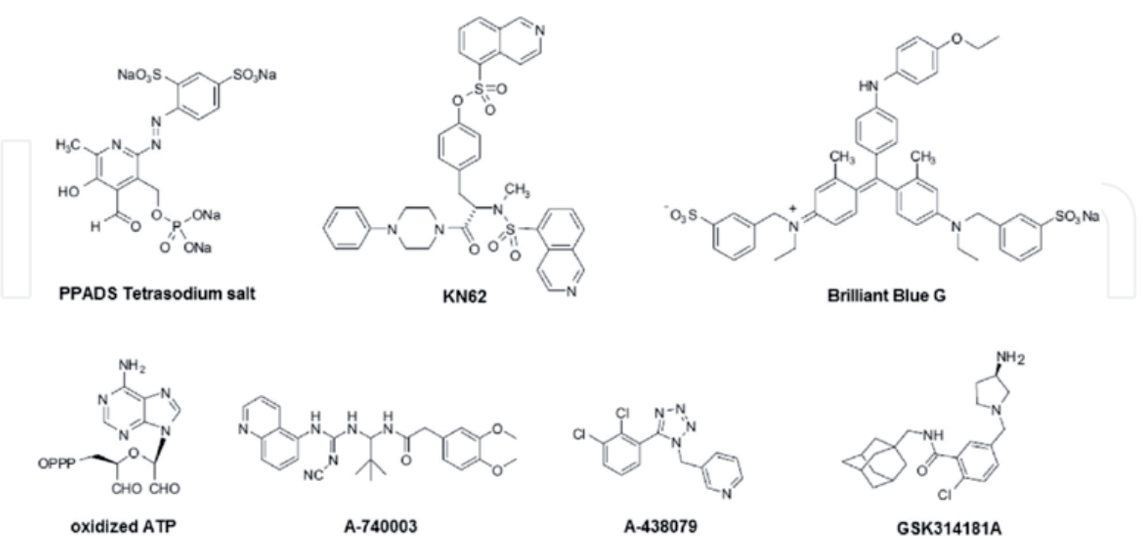

Figure 5.
Chemical structures of literature P2X7 receptor antagonists [2].

subsequent mice promises a better understanding of downstream signaling events. Interestingly, mouse platelets missing from the P2Y1 receptor may enter partial deposition with high ADP concentrations [30]. Chemical structures of literature P2X7 receptor antagonists are shown in **Figure 5**.

3. Functions of the purinergic receptors in metabolism

3.1 Functions of the purinergic receptors in the cardiac system

Multiple P2 receptor subtypes are located in the microglia pain paths as both initiator and modulator. Activation of homomeric P2X3 receptors probably contributes to some aspects of acute nociception and acute inflammatory pain. In contrast, activation of heteromeric P2X23 receptors appears to modulate the duration of nociceptive sensitivity associated with nerve injury or chronic inflammation. In addition, P2X4, P2X7 and P2Y12 receptors can serve to protect nociceptive sensitivity through sensitization of other nociceptive receptors such as TRPV1 channels or complex neural-glial cell interactions under conditions of continuous nociceptive activation on microglia. P2X3, P2X4, P2X7 and P2Y12 receptor antagonists are used for neuropathic pain. The study is being investigated for orally biologically available P2X3, P2X23, P2X4, P2X7 and P2Y12 receptor antagonists with molecules able to cross the blood-brain barrier and not be degraded in vivo during the pain treatment. In particular, the P2X7 receptor has become the main target for inflammatory neuropathic pain and a number of selective P2X7 receptor antagonists have been developed. There is also a review describing the recent advances in the development of adenosine receptor ligands as anti-inflammatory drugs [31]. Other therapeutic approaches, including the development of agents that control the expression of receptors and the selective inhibition of known ectonucleotides, as well as the development of agents that prevent the degradation of ATP, are considered. It is also expected the mechanisms underlying the transport of ATP to be understood, although it is clear that many different cell types secrete physiologically to ATP in response to mechanical deterioration, hypoxia and various agents. Hopefully, when this becomes clearer, agents will be developed that will increase or enhance the release of ATP, another useful pathway that stands out as a therapeutic strategy. In this context A134757, a novel adenosine kinase (AK) inhibitor, alleviated tactile allodynia by means of spinal action sites in peripheral nerve injury rats and added increased evidence that EC inhibitors may be useful analgesic agents [32]. Furthermore, human macrophages express purinic receptors with the rIFN-an modulated P2Z subtype [33].

Purinergic receptor expression in human dendritic cells is associated with the pharmacological and biochemical evidence that immature and mature cells express P2Y and P2X subtypes associated with intracellular Ca^{2+}, membrane depolarization and secretion of inflammatory cytokines. ATP-activated Ca^{2+} mobilization is biphasic, which is rapidly released from intracellular stores and releases a second delayed flow in the plasma membrane. Prolonged exposure to ATP was toxic to dendritic cells (swollen, lost from typical dendrites, phase lents were exhibited, separated from substrate, and eventually died). These changes strongly elicited the expression of the cytotoxic receptor P2X7 as confirmed by the ability of dendritic cells to become permeable to membrane impermeable dyes such as Lucifer's yellow or ethidium bromide. P2X7 receptor ligand 2 isolation, 3 z-(4-benzoylbenzoyl) -ATP was a better agonist, followed by an increase in Ca^{2+} and ATP-induced plasma membrane depolarization. ATP oxidized and the P2X7 antagonist KN-62, a covalent blocker of P2X receptors, inhibited both permeabilization and ATP-induced Ca^{2+} changes. The following purinoceptors were expressed by immature and mature dendritic cells: P2Y1, P2Y2, P2Y5 and P2Y11 and P2X1, P2X4 and P2X7. Finally, stimulation of matured cells with LPS by ATP induced IL-1 and TNF-α release. Therefore, these receptors can provide a new way of modulating dendritic cell function [34]. Glutamate is a well-known excitotoxic agent that can lead neurons and astrocytes to death when it is present as the primary mediator of stimulatory neurotransmission in the central nervous system and in extracellular milieu [35].

The ability to release this transmitter demonstrates the direct involvement of astrocytes in glutamatergic neuronal transmission and in the excitotoxic effect of glutamate [36]. Although this last problem has been proven, a large number of evidence suggests that astrocyte-derived glutamate has complex effects on neurons that play a modulator role in synaptic transmission. In the hippocampus, it modulates the excitability of the interneurons and strengthens the inhibitory transmission; it also acts on the stimulating axon terminals of the CA1 region to increase the probability of spontaneous glutamate release [37]. At the same time, astrocytic glutamate has a direct effect on hippocampal pyramidal neurons by activating extra-synaptic NMDARs and triggering episodic inward currents (SICs) characterized by slow kinetics. Interestingly, this NMDAR response may occur simultaneously in many CA1 neurons, and this increases the likelihood of synergistic neuronal activity [38].

The results show that activation of different purinergic receptors in the hippocampus are mediated by two types of glutamate release, each of which induces a different response in CA1 pyramidal neurons. In the first release, P2X7R is not included. This release of glutamate mediated for astrocytes evokes transient NMDAR-mediated responses (SICs) in CA1 pyramidal neurons, which represent a sign of astrocyte-neuron communication. In the second type of release, a receptor similar to P2X7 may be included. This phenomenon is enhanced under nonphysiological conditions, increasing the likelihood of contributing to the excitotoxic effect of glutamate in the brain.

The presence of a non-cholinergic, non-adrenergic component in the vertebrate autonomic nervous system is now well established. Evidences that ATP is a donor released from some of these nerves include

a. synthesis and storage of ATP

b. release of ATP from nerves when stimulated

c. externally applied ATP mimicking the action of the nerve-secreting donor

d. the presence of enzymes that inactivate ATP

e. exogenously administered ATP and drugs that produce similar blocking or enhancing effects on response to nerve stimulation.

A basis for distinguishing two types of purinergic receptors has been proposed based on four criteria: relative forces of agonists, competitive antagonists, changes in cAMP levels and stimulation of prostaglandin synthesis. P1 purinoceptors are therefore most susceptible to adenosine and are blocked in competition with methylxanthines, and their use leads to changes in cAMP accumulation; P2 purinoceptors are most sensitive to ATP, but, although not competitive, quinidine is blocked by 2-substituted imidazolines, 2,2′-pyridylisatogen and apamine, and their use leads to prostaglandin production. P2 purinoceptors mediate the response of smooth muscle to ATP released from purinergic nerves, while P1 purinogens mediate the presynaptic effects of adenosine on adrenergic, cholinergic and purinergic nerve terminals [39].

The discovery of a P2X purinoceptor (a ligand-gated ion channel triggered by ATP) that is selectively expressed by small-diameter sensory neurons has led to the investigation of ATP sources involved in the initiation of different types of nociception and pain types including sympathetic nerves, endothelial cells and tumor cells. To a lesser extent, ATP stimulates the sensory nerve endings in the skin, causes severe pain, and causes a significant increase in discharge from sensory neurons.

The molecular structure of the PZX3 purinepeptor is associated with the nociceptor and is associated with the search for the PZX3 purinoceptor to identify selective antagonists (Xenopus oocytes and/or transfected cells can be expressed herein).

When defined, such antagonists can be tested in vitro models of different types of pain. It is clear that PZX3 purine receptors are not the only receptors involved in pain, so a synergy with receptors for other pain-modulating agents (such as bradykinin, histamine and S-hydroxytryptamine) should be investigated. ATP acts on receptors on sensory nerve terminals; it has been reported that ATP acts on the dorsal horn neurons in the spinal cord after release from a subpopulation of small primary afferent nerves in the pain pathways.

There are multiple P2 receptor-mediated mechanisms in which ATP can alter nociceptive sensitivity following tissue damage. Evidence from various experimental strategies, including genetic degradation studies and the development of selective antagonists, modulates pain in the activation of P2X receptor subtypes, including P2X3, P2X2 / 3, P2X4 and P2X7 and P2Y (eg P2Y2) receptors. For example, administration of A-317491, a selective P2X3 antagonist, has been shown to effectively block both hyperalgesia and allodynia in different pathological painful animal models. Antisense oligonucleotides administered intrathecally to target P2X4 receptors reduce tactile allodynia following nerve damage. Selective antagonists for the P2X7 receptor also reduce sensitivity in animal models of inflammatory and neuropathic pain; This provides evidence that purinergic glial nerve interactions are important modulators of harmful sensory neurotransmission. In addition, activation of P2Y2 receptors leads to the planning of polmodal transient receptor potential-1 receptors. Thus, ATP acts either directly on neurons (P2X3, P2X2/3 and P2Y receptors) or directly on multiple purinergic receptors that are indirectly affected by neural-glial cell interactions (P2X4 and P2X7 receptors). The development of selective antagonists for some of these P2 receptors has greatly helped to investigate the nociceptive role of ATP. This perspective highlights some of the recent advances to identify selective P2 receptor ligands that enhance the investigation of ATP-related pain sensitivity modulation [40].

3.2 Function of the purinergic receptors in the retina

P2X receptors are ligand-gated ion channels which are activated by adenosine triphosphate and expressed in a wide variety of tissues. The expression of various types of purinergic P2X receptors is shown in defined retinal ganglion cells (RGCs) of the adult rat retinas. The single-cell reverse transcription polymerase chain reaction (SC-RT-PCR) resulted in a positive amplification signal for all P2X receptor subunit mRNAs studied (P2X3X5, P2X7). Immunohistochemistry with antibodies specific to the P2X3,4 receptor subunit showed the label of neurons in the ganglion cell layer and internal nuclear layer. The data suggest that extracellular ATP is directly effective on RGCs through several types of P2X receptors and may provide neuromodulatory effects in the retinal information processing [41]. For example, in the retina, glutamate release from acetamides modulates the spike activity in ganglion cell that is most likely driven by mild stimulation with a presynaptic action [42].

3.3. Function of the purinergic receptors in the heart

Both the adenosine receptor and the ATP-responsive K (KATP) channel mediate the intact heart-protective effect of ischemic preconditioning. The data [43] provides direct evidence that the myositis KATP channel is effective downstream of the adenosine Al receptor in mediating direct preconditioning of cardiac myocytes.

A study by Liang and Gross [44] showed that the functional opioid receptors were found in chick cardiac ventricular myocytes. The activation of the receptors by the nonselective opioid receptor agonist morphine can result in a PC-like effect. The protective effect of morphine in myocytes was probably mediated by the activation of the K1 (KATP) channel, which is sensitive to mitochondrial origin, ATP. However, the identity of the specific subtype of the said opioid receptor and the signaling pathway for mediating cardioprotective effect to the mitochondrial KATP channel from the receptor is unknown [45].

3.4 Function of the purinergic receptors in cancer

Reports documenting the activity of convergent ATP and its metabolites on cancer growth demonstrate a clear issue of how we can benefit from purifying cancer from purinergic signaling. Schematically, two paths are possible for the host and/or the tumor side to interfere. The available evidence in several experimental tumor models clearly demonstrates that decreasing adenosine concentration inhibits tumor progression and prevents metastasis [46]. The concentration of adenosine in the tumor interstitium can be reduced by downregulation of CD39 and/or CD73 or by upregulation of CD26. Alternatively, ADA may be targeted to the tumor as a PEG-ADA conjugate [47]. Also, on the host side, the beneficial effect of ATP release is demonstrated by the ability to activate the P2X7/inflammatory axis including the immune cells [48]. Thus, a pharmacological strategy may be based on the administration of CD39 inhibitors with double beneficial effect to maintain adequate levels of ATP for immunostimulation and to prevent adenosine accumulation.

When the use of purinergic receptors as a novel treatment for non-melanoma skin cancers is investigated; purinergic receptors binding adenosine-5′-triphosphate are expressed in human cutaneous keratinocytes. Previous studies in rat and human epidermis have proposed functional roles for purinergic receptors in the regulation of proliferation, differentiation and apoptosis. Immunohistochemical analysis of frozen sections in human basal cell carcinomas and squamous cell carcinomas for P2X5, P2X7, P2Y1, P2Y2 and P2Y4 receptors was performed with detailed analysis of the archive material of tumor subtypes in paraffin sections. Functional studies were performed using the human cutaneous squamous cell carcinoma cell line (A431), where purinergic receptor subtype agonists were applied to cells and changes in cell number were measured by a colorimetric assay. P2X5 and P2Y2 receptors have been extensively expressed in basal cell carcinomas and squamous cell carcinomas. P2X7 receptors were expressed in the necrotic center of nodular basal cell carcinomas and in apoptotic cells in superficial multifocal and infiltrative basal cell carcinomas and squamous cell carcinomas. P2Y1 receptors were expressed only in tumors surrounding the stroma. P2Y4 receptors were found in basal cell carcinomas but not in squamous cell carcinomas. P2X5 receptors appear to be associated with differentiation. P2X7 receptor agonist benzoylbenzoyl-adenosine 5 accordingly-triphosphate and high concentrations of adenosine 5 (-tophosphate (1000) 5000 concentM) lead to a significant decrease in A431 cell number ($p < 0.001$), while P2Y2 receptor agonist uridine 5 triphosphate significantly induced proliferation (p. <0.001). It is shown that non-melanoma skin cancers express functional purinergic receptors and significantly reduce in vitro cell numbers of P2X7 receptor agonists [49].

3.5 Function of the purinergic receptors in the fetal epidermis

It is expressed the expression of P2X5, P2X7, P2Y1 and P2Y2 receptor subtypes in the 8–11-week human fetal epidermis associated with proliferative proliferation markers (proliferative cell nuclear antigen (PCNA) and Ki-67), keratinocyte differentiation

(cytokeratin K10 and involucrin) and apoptosis marker (TdT-mediated dUTP-biotin nick end labeling (TUNEL) and anti-caspase-3). Immunohistochemistry showed that each of the four receptors was expressed in spatially distinct regions of the developing epidermis: the P2Y1 receptors were found in the basal layer, the P2X5 receptors were found mainly in the basal and intermediate layers, and both P2Y2 and P2X7 receptors were in the periderm. Colocalization assays have proposed different functional roles for these receptors. In fetal keratinocytes positive for PCY and Ki-67, P2Y1 receptors were found, indicating a role in proliferation. Double-labeled P2X5 receptors with differentiated fetal keratinocytes, which are positive for cytokeratin K10, show that they play a role in differentiation. It was expressed in periderm cells, positive for P2X7 receptors collozed with anti-caspase-3 antibody and also positive for TUNEL, suggesting a role in periderm cell apoptosis. P2Y2 receptors have been found only in periderm cells and may play a role in the release of chloride and fluid into the amniotic fluid [50].

3.6 Function of the purinergic receptors in the kidney

Autocrine and paracrine signals in the kidney nephron have been a widely used hypothesis for decades. The lumen of the nephron is an ideal autocrine and paracrine signal microenvironment. Any agonist released from the glomerulus or released in the proximal tubule or other proximal segments is then retained in the nephron lumen and is present to interact with the lumen receptors. Similar signals in the renal interstitium are also possible and possible. In fact, for many autocrine and paracrine agonists, the receptors have been characterized on the lumen membrane and serosal membrane of many nephron segments. An important autocrine and paracrine agonist family in the kidneys are purinergic agonists. In addition to extracellular ATP, metabolites (ADP, 5′-AMP and adenosine) are released by renal epithelial cells. These compounds are also freely filtered in the glomerulus and are in the final urine. ATP and adenosine receptors are also expressed on the lumen and serosal side of many nephron segments. This review discusses purinergic signaling by nucleotide agonists from ATP release to ATP receptors to extracellular ATP mediated effects in renal epithelial function. These themes are the areas in which our

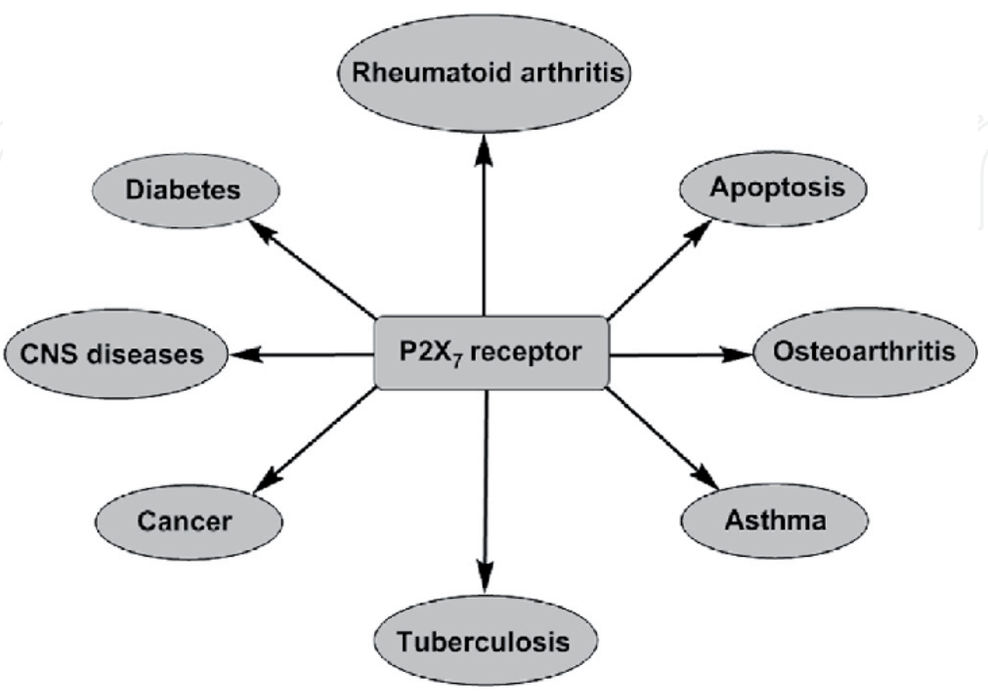

Figure 6.
P2X7 receptor and diseases [2].

laboratory focuses on normal and diseased epithelial cells in normal and polycystic kidneys and other tissues. The physiological roles of extracellular purinergic signals in the kidney and other tissues began to emerge [51]. $P2X_7$ receptor and diseases are shown in **Figure 6**.

4. Conclusions

Purinergic receptors are a family of newly characterized plasma membrane molecules in the field of signaling. As a result of many scientific studies, these receptors have been associated with many cellular functions including vascular reactivity, apoptosis and cytokine secretion; learning and memory, locomotor and feeding behavior and proliferation and migration of sleep neural stem cells. Activation of these receptors is partly due to the release of ATP (or UTP) from the cells, usually in the determination of cellular damage. Further studies are needed to better define the functions of purinergic receptors and to better understand the effect of extracellular micro-environment on their functions.

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