Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides

Roland Seifert and Günter Schultz

Uridine and uracil nucleotides are involved in the regulation of various cell functions. Here, Roland Seifert and Günter Schultz *review the evidence that, rather than by binding to purinoceptors, pyrimidine nucleotides exert their effects by binding to distinct pyrimidinoceptors, which are coupled to pertussis toxin-sensitive G proteins in human phagocytes. However, many questions remain to be answered: no antagonists for these pyrimidinoceptors are available, and binding studies have not been carried out; the receptor proteins and subtypes have not been characterized; and little is known about the G proteins and effector systems involved, or the regulation of storage and release of pyrimidine nucleotides.*

Extracellular adenosine and adenine nucleotides play an important role in the regulation of many cell functions[1–3]. Adenosine binds to adenosine A_1 or adenosine A_2 receptors, leading to inhibition or activation of adenylyl cyclase and other effector systems regulated by guanine nucleotide binding proteins (G proteins)[1]. Extracellular adenine nucleotides bind to P_2 purinoceptors which are subdivided into P_{2x} and P_{2y} purinoceptors according to the potency of purinergic agonists to activate cell functions[2,3]. In addition, cell type-specific purinoceptors have been described in mast cells and platelets[2].

Occupation of purinoceptors with agonists results in the activation of a variety of effector systems such as phospholipase C, Ca^{2+} channels, superoxide- ($O_2^{\cdot-}$)-forming NADPH oxidase of HL-60 leukemic cells and an inhibition of adenylyl cyclase[4–11]. In the case of inhibition of adenylyl cyclase in rat hepatocytes and activation of phospholipase C and NADPH oxidase in HL-60 cells, purinoceptors have been shown to couple functionally to pertussis toxin-sensitive G proteins[4,8,10,11].

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Pyrimidinergic regulation of cell function

It is known that extracellular uridine and uracil nucleotides are also effective activators of cell functions (Table I). However, relatively little attention has been paid to these observations. UTP is as effective as ATP in inducing relaxation of guinea-pig trachea[12]. In addition, extracellular UTP results in dilatation of intra- and extracranial arteries[13–15]. By contrast, uracil nucleotides and uridine also effectively induce vasoconstriction and an increase in the systemic blood pressure[13,14,16–22]. These opposite effects of uracil nucleotides may be due to the fact that both vascular smooth muscle cells and endothelial cells are activated by UTP. Thus, UTP may induce endothelium-dependent relaxation of blood vessels, presumably via the production of prostacyclin[7,15,23]. It has been suggested that the contraction of intracranial arteries by UTP plays an important role in the pathogenesis of the vasospasm following cerebral injury, as platelets and brain tissue are rich sources of uracil nucleotides[18,21,24,25].

The effects of extracellular uracil nucleotides are not restricted to the vasculature. UTP induces various metabolic changes in perfused rat liver, such as stimulation of the release of glucose, K^+ and Ca^{2+}, and inhibition of O_2 uptake[22]. In addition, UTP results in the mobilization of intracellular Ca^{2+} from non-mitochondrial stores and Ca^{2+} influx from the extracellular space in Madin–Darby canine kidney cells, Ehrlich ascites tumor cells, J774 macrophages and human neutrophils[9,26–28]. In platelets and in neutrophils, uracil nucleotides induce aggregation[11,29,30]. UTP activates $O_2^{\cdot-}$ formation in HL-60 cells differentiated with dibutyryl cAMP[10]. In human neutrophils, UTP potentiates $O_2^{\cdot-}$ formation and exocytosis of β-glucuronidase stimulated by formyl peptides[9–11]. As pretreatment with pertussis toxin inhibits UTP-induced $O_2^{\cdot-}$ formation in HL-60 cells and neutrophil aggregation, it is likely that the effects of UTP are mediated via G proteins[10,11]. UTP also enhances retinoic acid-induced myeloid differentiation of HL-60 cells[31]. Furthermore, UTP and CTP have recently been reported to activate phospholipase C in cultured rat anterior pituitary cells[32].

Differences between purinergic and pyrimidinergic regulation

As there is no apparent stereochemical similarity between adenine and uracil nucleotides, the question arises whether the effects of the uracil nucleotides are mediated via purinoceptors or via separate pyrimidinoceptors (Fig. 1). Forsberg *et al.*[7] suggested that uracil nucleotides may bind to a subgroup of purinoceptors, whereas Martin *et al.*[15] suggested that certain purinoceptors also recognize pyrimidine bases. These interpretations, however, are not very satisfactory, as only the stereospecificity of nucleotide receptors for purine bases would justify the term 'purinoceptors'.

Indeed, there are several reports of dissociations between the effects of extracellular adenine and uracil nucleotides, suggesting the existence of specific pyrimidinoceptors (Table II).

- There are substantial differences in ATP- and UTP-induced contractions of intracranial and extracranial arteries with respect to desensitization, potency order of nucleotides, effects of various pharmacological agents and the release of 5-HT (Refs 16, 19 and 20).
- In perfused rat liver, there are

TABLE I. Activation of cell functions by extracellular uridine and by uracil nucleotides

Cell type/tissue	Effects	Ref.
Trachea (guinea-pig)	Relaxation (UTP)	12
Arteries (various species)	Dilatation (UTP, UDP)	13–15
Arteries (various species)	Contraction (UTP, UDP, UMP, uridine, CTP)	13,14,16–21
Portal vein (rat)	Contraction (UTP, UDP, UMP)	16,22
Intact organism (various species)	Increase of blood pressure (UDP, UMP, uridine, UDP-glucose)	16
Endothelium (pig, cattle)	Formation of prostacyclin and inositolphosphates (UTP)	7,23
Liver (rat)	Inhibition of O_2 uptake, stimulation of glucose output, K^+ uptake and release of K^+ and Ca^{2+} (UTP, UDP)	22
Madin–Darby kidney cells (dog)	Ca^{2+} influx, intracellular Ca^{2+} mobilization (UTP)	26
J774 macrophages (mouse)	Ca^{2+} influx, intracellular Ca^{2+} mobilization (UTP)	27
Ehrlich ascites tumor cells (mouse)	Ca^{2+} influx, intracellular Ca^{2+} mobilization (UTP)	28
HL-60 cells (human)	$O_2^{\cdot -}$ formation, enhancement of differentiation (UTP)	10,31
Neutrophils (human, rat)	Potentiation of exocytosis and $O_2^{\cdot -}$ formation, aggregation, Ca^{2+} influx (UTP)	9,11,30
Platelets (human)	Aggregation (UDP)	29
Pituitary cells (rat)	Formation of inositol phosphates (UTP, CTP)	32

Most effective or potent pyrimidinergic agonists are given in parentheses.

dissociations between the effects of ATP and UTP on several metabolic parameters[22].

- In HL-60 cells, the effects of ATP but not of UTP on NADPH oxidase or phospholipase C are partially resistant to inhibition by pertussin toxin, suggesting that purino- and pyrimidinoceptors couple to different populations of G proteins[8,10]. In addition, ATP-induced activation of $O_2^{\cdot -}$ formation in HL-60 cells is less sensitive to inhibition by activators of adenylyl cyclase than is the activation induced by UTP[10].
- In neutrophils and J774 macrophages, uracil nucleotides are more effective activators of certain cell functions than the corresponding adenine nucleotides[11,27]. In J774 macrophages, ATP but not UTP induces a generalized increase in plasma membrane permeability[27]. Activation of human neutrophils by purine nucleotides shows less pronounced base specificity than the activation induced by pyrimidine nucleotides[11]. In addition, the effectiveness order of adenine nucleotides and the corresponding uracil nucleotides to activate neutrophils is quite different[11].

Stereoselectivity of pyrimidinergic cell activation

One classical property of plasma membrane receptors is their ability to discriminate stereoselectively various structurally related compounds. Figure 1 illustrates the structure–activity relationship for pyrimidinergic activation of NADPH oxidase in HL-60 cells. The effects of pyrimidine nucleotides are stereospecific with respect to the length and structure of the phosphate chain, to the substitution of the ribose moiety and to the base. In most cell types examined so far, UTP is more effective than UDP, UMP and uridine, and TTP and CTP are only relatively weak agonists. UTP induces dilation and contraction of blood vessels, whereas uridine exclusively induces contraction.

These data raise the question whether, like adenosine and adenine nucleotide receptors, uridine receptors are a class of receptor different from uracil nucleotide receptors. In addition, pyrimidinergic activation shows differences in the nucleotide specificity between different cell types, suggesting heterogeneity among pyrimidinoceptors. However, pyrimidinergic activation of cell functions must be studied in much more detail, using a broad variety of pyrimidine nucleotides, before these questions can be answered definitely. These tasks may be facilitated by the use of phosphorothioate analogues of uracil nucleotides; this technique has recently been used in the study of pyrimidinoceptors of human neutrophils[11].

Characterization of pyrimidinoceptors

So far, no antagonists for pyrimidinoceptors are available. In addition, pyrimidinoceptors have not been characterized by binding studies, and the receptor proteins have not been identified.

At platelet purinoceptors the phosphorothioate analogues of GTP and GDP, guanosine 5′-*O*-(3-thiotriphosphate) and guanosine 5′-*O*-(2-thiodiphosphate), are competitive antagonists of ADP[33]. In the case of neutrophil pyrimidinoceptors, the corresponding phosphorothioate analogues of UDP and UTP are agonists[11]. Arylazidoaminopropionyl ATP and adenosine 5′-[αβ-methylene]triphosphate are antagonists at certain purinoceptors[34]. By analogy, the corresponding uracil nucleotides may be antagonists at pyrimidinoceptors.

The characterization of pyrimidinoceptors by receptor binding studies will be a difficult task, as pyrimidine nucleotides apparently have rather low affinity for their receptors. In addition, extracellular nucleotides including uracil nucleotides may rapidly be degraded by ectonucleotidases[2], and recent evidence suggests that extracellular nucleoside triphosphates are substrates for protein kinases catalysing the phosphorylation of several cellular proteins[35]. At least for neutrophil nucleotide receptors, however, it is not likely that transphosphorylation reactions catalysed by nucleoside diphosphate kinase are involved in regulation of cellular functions by extracellular purine and pyrimidine nucleotides[11].

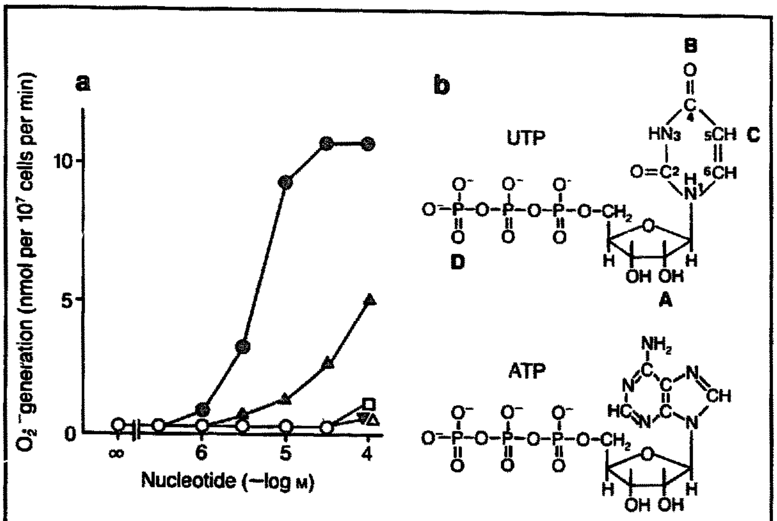

Fig. 1. Structure–activity relationship of pyrimidinergic activation of NADPH oxidase in HL-60 cells. **a:** *Concentration–response function for activation of $O_2^{\cdot -}$ formation in dibutyryl cAMP-differentiated HL-60 cells.* ●, *UTP;* ▲, *dUTP;* ▼, *UDP;* □, *CTP;* △, *TTP.* **b:** *Structures of UTP derivatives and ATP. UTP was the most effective and most potent of the pyrimidine nucleotides tested (IC_{50} 5 μM). Deoxy-UTP (deoxyribose instead of ribose,* **A***) is less potent than UTP. CTP (amino group instead of keto group,* **B***, in pyrimidine ring position 4), TTP (methyl instead of hydrogen,* **C***, in pyrimidine ring position 5) and UDP (lacks the terminal phosphate group,* **D***) are very poor activators of NADPH oxidase. Pyrimidinoceptors in HL-60 cells therefore stereoselectively recognize the base, the ribose moiety and the polyphosphate chain of pyrimidine nucleotides.* (See Ref. 10 for experimental details.)

Uridine 5′-[α-^{35}S-thio]triphosphate and uridine 5′-[β-^{35}S-thio]-diphosphate may be useful ligands for binding studies at certain pyrimidinoceptors, by analogy with the use of adenosine 5′-[α-^{35}S-thio]triphosphate and adenosine 5′-[^{35}S-thio]diphosphate for the characterization of purinoceptors[36,37]. Another possible approach would be to use stereospecific labelling of plasma membrane proteins to identify and to isolate the receptor proteins. Interestingly, Tauber *et al.*[38] observed that labelled uridine and UTP covalently bind to specific plasma membrane proteins in rat liver. It remains to be determined, however, whether covalent binding of uridine and uracil nucleotides to proteins is causally linked to pyrimidinergic activation of cell functions, as activation of NADPH oxidase in HL-60 cells by UTP is a reversible process[10].

Desensitization of pyrimidinoceptors and receptor synergism

Desensitization of pyrimidinoceptors has been shown for several cell types. In myeloid cells the mechanisms underlying desensitization of pyrimidinoceptors may be similar to those of formyl peptide receptors: cytochalasin B potentiates the stimulatory effects of both formyl peptides and UTP on $O_2^{\cdot -}$ formation[10,39], probably by preventing receptor sequestration and by enhancing the expression of plasma membrane receptors. In human neutrophils and HL-60 cells cross-desensitization between purinoceptors and pyrimidinoceptors occurs[10,11]. These

TABLE II. Differences between the effects of extracellular purine and pyrimidine nucleotides on cell functions

Parameter	Purine nucleotides	Pyrimidine nucleotides	Ref.
Rabbit ear artery, contraction			19
Pretreatment with [α,β-CH_2]ATP	desensitization	potentiation/partial desensitization	
Rabbit basilar artery, contraction			20
Treatment with phentolamine or reactive blue B	no effect	enhancement	
Pretreatment with ATP[γS]	desensitization	no effect	
Pretreatment with UTP	no effect	desensitization	
Rat femoral vasculature, contraction or dilatation			14
Antagonism by methysergide or phentolamine, 5-HT release	yes	no	
Perfused rat liver, various metabolic changes			22
O_2 consumption	increase	decrease	
Glucose output after withdrawal	no	yes	
Initial K^+ uptake	transient	prolonged	
K^+ release after withdrawal	no	yes	
Ca^{2+} release	more effective	less effective	
HL-60 cells, $O_2^{\cdot -}$ formation			10
Pertussis toxin sensitivity	partial	complete	
Sensitivity to inhibition by cAMP-increasing agents	more resistant	more sensitive	
Human neutrophils, potentiation of $O_2^{\cdot -}$ formation			11
Base specificity	ITP > ATP = GTP	UTP > CTP, TTP inactive	
Effectiveness order	ATP[γS] > ATP > ADP > (RP)-ATP[βS], ADP[βS] inactive	UTP[γS] > UTP = UDP[βS] = (RP)-UTP[βS], UDP inactive	
J774 macrophages, Ca^{2+} influx			27
Generalized increase in plasma membrane permeability (> 100 μM)	yes	no	

[α,β-CH_2]ATP, adenosine 5′-[α,β-methylene]triphosphate; **ATP[γS]**, adenosine 5′-*O*-[3-thiotriphosphate]; **ADP[βS]**, adenosine 5′-*O*-[2-thiodiphosphate]; **(RP)-ATP[βS]**, RP-diastereomer of adenosine 5′-*O*-[2-thiotriphosphate]; **UTP[γS]**, uridine 5′-*O*-[3-thiotriphosphate]; **UDP[βS]**, uridine 5′-*O*-[2-thiodiphosphate]; **(RP)-UTP[βS]**, (RP)-diastereomer of uridine 5′-*O*-[2-thiotriphosphate]

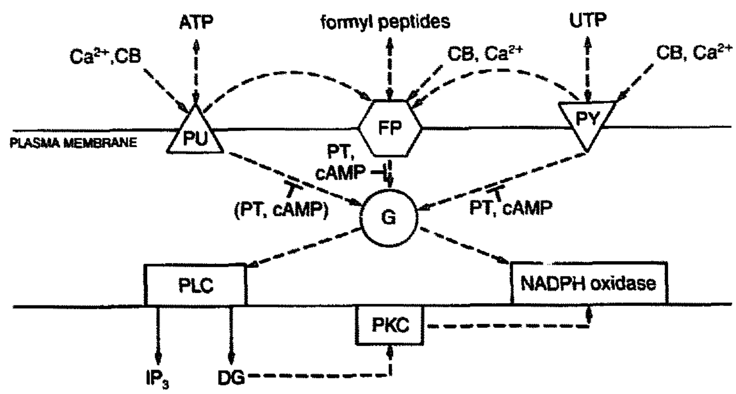

Fig. 2. Mechanisms involved in pyrimidinergic activation of NADPH oxidase in human myeloid cells. **CB**, *cytochalasin B;* **DG**, *diacylglycerol;* **G**, *G protein;* **FP**, *formyl peptide receptor;* **IP_3**, *inositol 1,4,5-trisphosphate;* **PKC**, *protein kinase C;* **PLC**, *phospholipase C;* **PT**, *pertussis toxin;* **PU**, *purinoceptor;* **PY**, *pyrimidinoceptor. There are several functional similarities in the effects of formyl peptides and purine and pyrimidine nucleotides on activation of NADPH oxidase: they require extracellular Ca^{2+}, are potentiated by cytochalasin B, are reversible, are subject to homologous desensitization and are inhibited by an increase in the intracellular concentration of cAMP (Refs 9–11). In addition, their effects are inhibited by pertussis toxin which ADP-ribosylates G proteins, thereby functionally uncoupling receptors from their effector systems[8,10,11]. Activation of NADPH oxidase by receptor agonists is achieved either directly via G proteins or indirectly through protein kinase C and Ca^{2+}-dependent processes[9–11]. Evidence for the existence of pyrimidinoceptors distinct from purinoceptors in these cells comes from the findings that the effects of UTP are more susceptible to inhibition by pertussis toxin and an increase in cAMP than those of ATP and that the effectiveness order of adenine nucleotides and the corresponding uracil nucleotides to activate cell functions is quite different[10,11]. Furthermore, ATP and UTP potentiate the effects of formyl peptides[9–11]. This synergism may be due to alterations in the number and/or affinity state of formyl peptide receptors, additive activation of G proteins and/or generation of intracellular signal molecules.*

results do not necessarily argue against the existence of different types of nucleotide receptor, but rather may support the concept that the two classes of receptor are closely related functionally.

Different classes of intercellular signal molecule interact synergistically to activate human neutrophils[11]. This is also the case for the interaction of pyrimidinoceptors and receptors for formyl peptides, platelet activating factor and leukotriene B_4 to activate $O_2^{\cdot-}$ formation, exocytosis and aggregation in human myeloid cells[9–11]. The mechanisms underlying synergistic interaction of these receptors may be complex. They may involve increases in the affinity of receptors, additive or synergistic activation of different pools of G proteins and amplified generation of intracellular signal molecules, such as diacylglycerol, Ca^{2+} and arachidonic acid. Studying the interactions of extracellular pyrimidine nucleotides with other intercellular signal molecules in non-myeloid cell types should help elucidate the role of pyrimidines in regulating cell function.

Functional coupling to G proteins and effector systems

The characterization of the coupling of pyrimidinoceptors to G proteins is another important task. NADPH oxidase is coupled to pyrimidinoceptors via pertussis toxin-sensitive G proteins[10]. By analogy with purinergic activation, pyrimidinergic activation of phospholipase C in HL-60 cells is also likely to be pertussis toxin sensitive[8]. However, it is not known whether, as is the case for NADPH oxidase[10], purinergic and pyrimidinergic activation of phospholipase C in these cells show differential pertussis toxin sensitivity.

In pertussis toxin-insensitive signal transduction systems, the interaction of pyrimidinoceptors with G proteins will be more difficult to demonstrate; a possible approach would be to test the sensitivity of agonist binding to guanine nucleotides. The functional similarities between purinoceptors and pyrimidinoceptors make adenylyl cyclase, phospholipase C and Ca^{2+} and K^+ channels likely candidates as pyrimidinergic effector systems.

Storage and release of pyrimidine nucleotides

Only limited information is available concerning the storage and regulation of release of uracil nucleotides. Uracil nucleotides are stored in granules of platelets and may be released from these cells upon stimulation[24]. Uracil nucleotides are also present at concentrations of up to 0.7 μmole g^{-1} fresh weight in liver, kidney and brain[25]. By analogy with adenine nucleotides, uracil nucleotides may be released from cells under a variety of pathological conditions such as trauma, hypoxia and inflammation[2]. Because uracil nucleotides activate cell functions in the concentration range 1 μM to 1 mM, pyrimidinergic regulation is likely to take place *in vivo*. The question of whether there is a more specific and controlled release of UTP into the extracellular space from intracellular stores of neurons, chromaffin or mast cells or from the cytosol deserves further investigation.

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The mechanism by which extracellular pyrimidine nucleotides regulate cell functions shows many properties characteristic of receptor-mediated processes. These include stereospecificity for agonists, reversibility and desensitization of activation, involvement of G proteins, the generation of intracellular signal molecules and activation of cellular effector systems. There is substantial indirect evidence for the existence of pyrimidinoceptors distinct from purinoceptors.

Information so far available suggests that many cell types possess pyrimidinoceptors and that these receptors are heterogeneous. Figure 2 summarizes the mechanisms involved in pyrimidinergic activation of human myeloid cells. HL-60 cells and human neutrophils are useful model systems to study the pyrimidinergic regulation of cell functions. However, the physiological relevance of pyrimidinergic regulation of cellular functions is as yet poorly understood, owing to the lack of information on how the release of pyrimidine nucleotides is controlled.

The development of potent and selective agonists and antagonists for pyrimidinoceptors is essential to characterize these receptors, and may provide a novel approach to intervene in various pathological states such as inflammatory processes and vascular diseases.

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Pharmacological modulators of DNA-interactive antitumor drugs

John S. Lazo and Robert R. Bahnson

The poor therapeutic index and limited efficacy of current cancer chemotherapeutic agents represent an important pharmacological problem. Athough there has been a significant increase in our understanding of the mechanisms by which anticancer drugs kill mammalian cells, identification of new, effective anticancer agents during the last decade has been exceeding slow. Thus, attention has focused on understanding the causes of drug resistance and on either sensitizing tumor cells to existing anticancer agents using what could be called 'chemoenhancers', or protecting non-malignant tissues against serious untoward effects using 'chemoprotectors'. John Lazo and Robert Bahnson *review recent strategies attempting to modulate the activity of antineoplastic drugs.*

When demand exceeds supply, people look for ways to increase the usefulness of the existing supply. So it currently is in cancer research: there are many malignancies that do not respond to chemotherapy and too few exciting novel drugs to test. This, combined with an elevated understanding of the molecular basis of resistance to anticancer drugs, has kindled interest in identifying and developing pharmacological modulators of existing cancer chemotherapeutic agents to improve their therapeutic indices. Two classes of modulator are being examined: chemoenhancers, which would sensitize tumor cells; and chemoprotectors, which would selectively protect non-malignant tissue.

To be a successful chemoenhancer or chemoprotector, an agent must exhibit little toxicity itself; many such agents have no anticancer activity at all and some have other useful pharmacological properties. The clinically successful combination of methotrexate with leucovorin (folinate) provides an important precedent for the chemoprotective approach; cisplatin, currently one of the most popular anticancer drugs,

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