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Commentary

Uracil nucleotides: From metabolic intermediates to neuroprotection and neuroinflammation

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ABSTRACT

Uracil nucleotides (i.e., UTP and UDP) have been known for years as fundamental intermediates in the de novo synthesis of the other pyrimidine nucleotides, which altogether represent key building blocks for nucleic acid synthesis. In addition, their sugar conjugates (i.e., UDP-glucose and UDP-galactose) enter in several biochemical routes, for example leading to glycogen biosynthesis, and protein and lipid glycosylation, which in turn contribute to the synthesis of essential components of the cellular plasma membrane. More recently, the existence of a ''pyrimidinergic transmission'' has arisen from the discovery that several purinergic G protein-coupled P2Y receptors can be activated also or exclusively by uracil nucleotides and sugar conjugates. The number of these receptors is continuously growing over years with the discovery that previously ''orphan'' G protein-coupled receptors are actually responding to this class of molecules. Therefore, new unforeseen effects mediated by uracil derivatives have emerged, in particular in the nervous system, and previously unexplored avenues for the pharmacological manipulation of this system are currently under investigation. In this commentary we shall try to put together our current knowledge on the biochemical and receptor-mediated effects of uracil nucleotide derivatives with a specific focus on the nervous system in order to depict a clearer view of the importance of the pyrimidinergic system in both physiological and pathological conditions.

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Abbreviations: APP, amyloid precursor protein; BBB, blood–brain barrier; BDNF, brain-derived growth factor; CGRP, calcitonin-gene related peptide; CNS, central nervous system; CNT2, concentrative nucleoside transporter 2; CREB, cAMP-responsive element binding protein; CTP, cytidine triphosphate; CysLTs, cysteinyl-leukotrienes; DRG, dorsal root ganglia; EGF, epidermal growth factor; ER, endoplasmic reticulum; FGF2, fibroblast growth factor 2; GalT-1, UDP-galactose:ceramide glycosyltransferase; GFAP, glial fibrillary acidic protein; Glu1P, glucose-1-phosphate; GS, glycogen-synthase; KA, kainic acid; LPS, lypopolysaccharide; MCAo, middle cerebral artery occlusion; NANA, *N*-acetyl-neuraminic acid; NDPK, ecto-nucleoside diphosphokinase; NGF, nerve growth factor; NPCs, neural stem/precursor cells; PDGF, platelet derived growth factor; PP_i, inorganic phosphate; RGD, arginine-glycine-aspartic acid; ROS, reactive oxygen species; TG, trigeminal ganglia; TNFα, tumor necrosis factor alpha; TRPV1, vanilloid receptor 1; TSP-1, trombospondin 1; UDP-Gal, UDP-galactose; UDP-GlcA, UDP-glucuronic acid; UDP-Glc, UDP-glucose; Urd, uridine; Ura, uracil; VEGF-2, vascular endothelial growth factor 2.

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1. Introduction

In the early 1970s Prof. Burnstock anticipated the existence of the so-called ''purinergic transmission'' by raising the hypothesis that ATP could be co-stored and co-released together with ''classical'' neurotransmitters at nerve endings to act selectively on specific membrane receptors [1]. Despite the initial, and in some cases prolonged, skepticisms of a part of the scientific community, after more than 30 years solid evidence has progressively accumulated clearly demonstrating that adenine nucleotides and nucleosides play fundamental roles in all tissues and organs (upon both physiological and pathological conditions), and do not simply behave as energy donors, metabolic cofactors, or nucleic acid components [2].

More recently, also uracil nucleotides have gained the center stage thanks to the discovery of their ability to modulate cellular and tissue pathophysiology through the activation of specific membrane receptors, suggesting that also a ''pyrimidinergic transmission'' exists. Through the deorphanization of various G protein-coupled receptors, the number of uracil nucleotide-activated receptors is continuously growing. Nevertheless, several important issues still need to be clarified, such as the mechanisms of uracil nucleotide storage and release and the physiological roles played by the various receptor subtypes in the different tissues and organs. In this commentary we shall focus on the currently known and predictable significance of pyrimidinergic transmission in the nervous system.

2. Intracellular sources of uracil nucleotides and sugar conjugates

Intracellular pyrimidine nucleotides derive from two major biochemical routes: the de novo biosynthesis and the salvage pathway. The former is the most important source of pyrimidine nucleobases in the liver and in actively proliferating tissues, such as epithelia and cancer cells [3]. In the brain, the high biosynthetic activity of neonatal tissue is rapidly declining so that the salvage pathway becomes increasingly important throughout the lifespan [4].

2.1. *De novo pyrimidine biosynthesis*

An oversimplified scheme of the extremely complex pyrimidine metabolic pathway is shown in Fig. 1. Through the sequential activity of various multidomain polypeptides encompassing different enzymatic activities (for review, see Ref. [4]) leading to the formation of orotate and orotidine-5′-P as intermediates, UMP is synthesized which is in turn phosphorylated to UDP and UTP. The latter nucleotides represent the central molecules in the synthesis of the whole pyrimidine pool, since (i) both can be reduced to corresponding deoxyribonucleotides, that are subsequently dephosphorylated to dUMP, methylated through the so-called ''methyl cycle'', and finally phosphorylated to thymidine deoxynucleotide derivatives (Fig. 1), and (ii) UTP can be aminated to cytidine triphosphate (CTP), that represents not

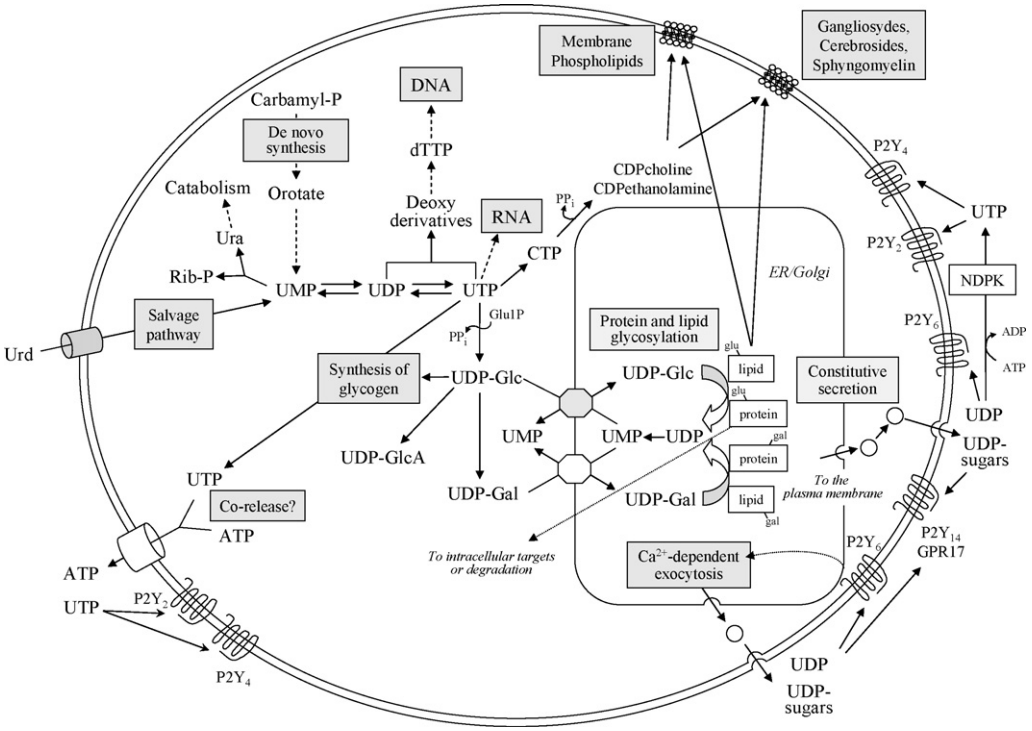

Fig. 1 – Schematic representation of the metabolic pathways leading to uracil nucleotides biosynthesis and of their intra- and extra-cellular utilization. The biosynthetic routes and the final outcomes of the biochemical pathways involving uracil nucleotides are enclosed in gray boxes. See text for details. For the sake of clarity, only uracil nucleotide-activated P2Y receptors are shown among the P2 receptor family. *Abbreviations*: Ura, uracil; Urd, uridine; UDP-Glc, UDP-glucose; UDP-GlucA, UDP-glucuronic acid; UDP-Gal, UDP-galactose; NDPK, ecto-nucleoside diphosphokinase; Glu1P, glucose-1-phosphate; PP_i, inorganic phosphate; Rib-P, ribose-1-phosphate.

only a key molecule in the biosynthesis of nucleic acids but also a fundamental metabolic intermediate (see below; Fig. 1). Expression and function of the enzymes involved in pyrimidine biosynthesis are strictly regulated both at the transcriptional and post-transcriptional level, leading to the expansion of the intracellular nucleotide pool during DNA synthesis in the S-phase of the cell cycle. This increase can be further enlarged to three- to four-fold following malignant transformation [4].

2.2. The pyrimidine salvage pathway

The salvage pathway represents the less energy sparing way for a cell to restore its intracellular nucleotide pools. While purine nucleobases (i.e., adenine and guanine) can be directly recycled to corresponding nucleotides, in the case of pyrimidine only nucleosides (i.e., uridine, cytidine, and thymidine) can be phosphorylated by specific cytosolic or mitochondrial enzymes to the mono-, di- or tri-phosphate derivatives. As previously mentioned, this salvage route is extremely important in the central nervous system (CNS) where, despite the very low rate of de novo biosynthesis, high amount of pyrimidine nucleotides are needed to be utilized in several essential biosynthetic pathways (see below). Thus, for the adult brain the recycle of nucleosides taken up from the blood becomes the primary source of uracil nucleotides. In humans, uridine represents the majority of the circulating pyrimidine pool, with its plasma concentrations maintained at about 3–5 μM [5]. In rats, cytidine blood concentration is about threefold higher than uridine [6], but uridine is more efficiently transported across the blood–brain barrier (BBB) due to the action of highly effective Na^+-dependent concentrative nucleoside transporters (named CNT2), which also transport purines [7]. This leads to a sevenfold difference in uridine concentration over cytidine in rat brain extracellular fluid [8]. The observation that the dietary supply of uridine can increase in parallel its blood and brain concentrations of about twofold [6] opens interesting opportunities for the development of uridine as therapeutic agent for various brain pathologies (see Section 6).

2.3. Synthesis of sugar conjugates

UTP derived from either the de novo synthesis or the salvage pathway can be conjugated to glucose-1-phosphate or to acetyl-glucosamine-1-phosphate to give raise to UDP-glucose or UDP-*N*-acetyl-glucosamine, respectively (Fig. 1) [9]. UDP-*N*-acetyl-glucosamine is a substrate for sialic acid and UDP-*N*-acetyl-galactosamine biosynthesis [9]. UDP-glucose can be further metabolized: (i) by UDP-glucose dehydrogenase which oxidates it to UDP-glucuronic acid which in turn conjugates to xeno- and endo-biotics in the liver allowing their detoxification [10], and (ii) by galactose-1-phosphate uridyl transferase which exchanges glucose with galactose to form UDP-galactose which enters in the synthesis of glycoprotein, glycolipids, and extracellular matrix component (see also below). UDP-glucose can also be directly epimerized to UDP-galactose by UDP-galactose-4-epimerase, and this pathway becomes particularly important in specific physiological conditions, such as lactose synthesis during breast-feeding.

Sugar conjugates synthesized in the cytosol have to be delivered to the endoplasmic reticulum (ER) and to the Golgi apparatus where they serve as substrates for glycosylation reactions (see Section 3.1) [11]. Concentration of sugar conjugates in their target organelle (up to 20 times with respect to cytosol) is mediated by various nucleotide sugar/UMP antiporters located in the ER and Golgi membrane (Fig. 1) [12].

3. Biochemical roles of uracil nucleotides

Besides representing one of the building blocks of RNA, uracil nucleotides possess a central role in a huge variety of biochemical pathways controlling physiological events such as cell survival, proliferation, and differentiation, as well as pathological behaviors, such as tumorigenesis.

3.1. Protein glycosylation reactions

The addition of simple or complex sugar moieties to a protein occurs co-translationally exclusively in the lumen of the Golgi apparatus [13]. The predominant sugars found in glycoproteins are galactose, mannose, fucose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine and *N*-acetyl-neuroaminic acid (NANA). Glucose can be abundantly found in nascent glycoproteins, but it is almost completely removed following protein maturation. Sugars are covalently bound to either an asparagine (N-glycoproteins) or to serine, threonine, and hydroxylysine residues (O-glycoproteins) [14]. Carbohydrate modifications of proteins play fundamental roles in various cellular processes. For instance, the presence of glycoproteins on the outer layer of the plasma membrane allows oligosaccharides to be exposed in the extracellular milieu (where they contribute to cell adhesion and cell-to-cell communication), and the addition of a carbohydrate tag targets newly synthesized lysosomal enzymes to their site of action [15]. More recently it has emerged that the coupling to carbohydrate moieties is necessary to check and promote the correct folding and maturation of newly synthesized proteins [14]. Moreover, misfolded or aberrant proteins are targeted to disruption by the further addition of a glucose moiety, leading to protein secretion in the cytoplasm, ubiquitination and lysosomal degradation [14].

As already mentioned in Section 2.3, sugars utilized for glycoprotein synthesis need to be activated by binding to nucleotides, mainly UDP (and to a lesser extent GDP for mannose, and CDP for NANA). *O*-Glycosylation occurs through the direct addition of nucleotide-bound sugar to the protein chain thanks to the activity of specific glycosyl-transferase enzymes, whereas *N*-glycosylation needs the intervention of a lipid intermediate called dolichol phosphate which receives the sugar residues from nucleotide–sugar complexes and translocates them to the target protein [14]. Newly formed glycoproteins are then addressed to their final destination, or to degradation, via binding to various chaperone proteins and receptors located within the ER [16]. Another important class of glycosylated compound is represented by glycosaminoglycans, composed by a proteic core synthesized in the ER to which complex carbohydrate moieties delivered by uracil

sugar conjugates are added in the Golgi, and that participate to the biosynthesis of extracellular matrix in nearly all tissues [17]. Defects in the glycosylation and galactosylation pathways lead to dramatic cognitive deficits and learning impairment (see Section 6).

3.2. *Biosynthesis of phospholipids and glycosphyngolipids*

Cell membranes are mainly made of phospholipids, and the maintenance of their relative amount is fundamental to preserve membrane integrity and to determine their optimal fluidity. Biosynthesis of membrane phosphatidylcholine, the most abundant phospholipid in the brain, and of phosphatidylethanolamine proceeds through the so-called Kennedy pathway [6]. The amine moiety (i.e., choline or ethanolamine) needs to be activated via coupling to CDP prior to its addition to diacylglycerol, leading to the production of CDP-choline or CDP-ethanolamine and PP_i. As shown in Sections 2.1 and 2.2, CTP intracellular pool can derive either from the re-phosphorylation of cytidine or from the metabolism of uridine (Fig. 1). Due to its more efficient transportation from blood to brain with respect to cytidine, circulating uridine represents the major source of CTP for the brain tissue [6], and, as a consequence, UTP intracellular levels directly control the cellular availability of newly synthesized phospholipids (see below).

Not only proteins, but also lipids (such as ceramides) can be glycosylated and galactosylated as well within the ER lumen [13]. Galactosylation of ceramides is mediated by the UDP-galactose:ceramide galactosyltransferase (GalT-1) enzyme, that is exclusively expressed in highly specialized cells, such as myelinating cells, spermatogonia and some epithelial cells. The UDP-galactose transporter physically interacts with the GalT-1 enzyme, thus allowing a continuous supply of substrate for the galactosylation reaction [13].

Addiction to ceramide of various sugar and sialic acid residues or of choline (released from CDP-choline) results in gangliosides and sphingomyelin synthesis, respectively [17]. In the CNS, these complex molecules are important components of the plasma membrane outer layer and of the myelin sheath surrounding axons.

3.3. *Synthesis of glycogen*

Glycogen is a polysaccharide and represents the major glucose storage form in the body. It is mainly synthesized by the liver and muscles, but it can be also produced by the brain, uterus and other cell types. The elongation of the glycogen molecule proceeds through sequential steps, each leading to the attachment of one glucose subunit donated by UDP-glucose to the growing polysaccharide chain, with the concomitant release of UDP [18]. The enzyme controlling glycogen production is glycogen-synthase (GS), whose activity and protein level are strictly modulated by intracellular UDP-glucose concentration, which in turn depends upon glucose and UTP availability. In fact, a drop in intracellular UTP concentrations results in a much lower rate of glycogen synthesis, and can be rescued by addition of uridine to the culture medium [18]. Moreover, glucose starvation causes a rapid depletion of intracellular UDP-glucose, which in turn inhibits GS activity and decreases GS mRNA and protein levels [19].

4. Release of uracil nucleotides

In the early 1990s, the demonstration of a regulated extracellular ATP release [20] strongly supported the hypothesis on the existence of a ''purinergic transmission'' raised by Burnstock [1], and contributed to foster the interest in this research field. Since then, various G protein-coupled receptors subtypes responding to extracellular adenine nucleotides were cloned, some of which responded equipotently or even exclusively to uracil nucleotides [21] (see Section 5). Moreover, together with the wide distribution of these receptors, various tissue and cellular responses were described after application of uracil nucleotides (see Section 5), strongly suggesting that like their adenine counterparts they might also act as extracellular signalling molecules. For many years, however, no evidence of a regulated release of uracil nucleotides from cells was provided. The first demonstration of shear stress-induced uracil nucleotide release from endothelial cells came from Saiag et al. [22]. An accurate measurement of the extracellular uracil nucleotides concentration was provided few years later in a series of seminal works made by Lazarowski's and Harden's groups, together with the demonstration of uracil nucleotides release from both resting and stressed cells [23,24].

More recently with the discovery that a previously ''orphan'' G protein-coupled receptor (formerly known as GPR105) is selectively activated by nucleotide-sugars conjugates, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid and UDP-*N*-acetyl-glucosamine [25], also these latter uracil derivatives emerged as potential extracellular signalling molecules. The receptor was then included in the P2Y nucleotide receptor family with the name of $P2Y_{14}$ (see Section 5.2) [26], and immediately afterwards Lazarowski's group provided compelling evidence of sugar nucleotides release from resting, mechanically stimulated [27], and from thrombin-exposed astrocytoma cells [12].

Secretory cells, such as platelets, show a regulated exocytotic uracil nucleotides release [28], but at variance from adenine nucleotides such a mechanism has not been definitively demonstrated in neurons, and no clues of UTP storage in synaptic vesicles has been provided yet. Nonetheless, the observation that UDP-sugar conjugates are transported and highly concentrated in the ER and Golgi, and that the glycosylation reactions produce UDP as a secondary product (see Section 3.1) has allowed to raise the intriguing hypothesis that uracil derivatives might be released from cells as additional cargo molecules during delivery of glycoproteins and other glycoconjugates to the plasma membrane (Fig. 1) [12]. Adenine nucleotides are present in the lumen of the Golgi as well, and might be co-released with uracil derivatives [12].

To date several mechanisms have been proposed for the extracellular release of uracil nucleotides and sugar derivatives in non-secretory cells upon either physiological or pathological conditions:

(a) Basal UTP release has been observed in primary astrocytes, and astrocytoma cell lines, and it has been hypothesized to occur in parallel with ATP through a yet-to-be identified common membrane transporter (Fig. 1) [12]. Furthermore,

UDP, UDP-glucose and other nucleotides might also be basally released as a consequence of the physiological delivery of newly synthesized protein or glycolipids to the plasma membrane (see above). UDP can be then extracellularly phosphorylated to UTP by an ecto-nucleoside diphosphokinase (NDPK; Fig. 1) exchanging a phosphate residue between UDP and ATP, with the contemporary production of ADP [23,24,28]. This raises the challenging hypothesis that an autocrine/paracrine basal activation of P2Y uracil-sensitive receptors might contribute to maintain cell physiology (see Section 6).

It is worth noting that the concentrations of both UTP (up to 70 nM) [23] and UDP-glucose (from about 10 to 22 nM) [27] detected in the bulk extracellular culture medium are probably underestimating the real levels that can be attained in the close proximity of the plasma membrane, and consequently the effects of uracil derivatives on P2Y receptors also upon basal conditions.

(b) Stressful conditions have been shown to increase the release of uracil derivatives through non-lytic mechanisms. For instance, mechanical shear stress induces uracil nucleotides and UDP-sugars release from primary astrocytes as well as from glioma cells [27,28]. Indeed, increased UDP-glucose concentrations have been detected in the extracellular medium of 1321N1 cells stimulated with thrombin, whose brain concentrations are known to increase upon stressful conditions (see also below) [12]. Furthermore, the application of a 50% hypotonic shock as a mechanical stress to A549 airway epithelial cells resulted in a biphasical Ca^{2+}-dependent adenine and uracil nucleotide release [29,30]. Moreover, the contribution of P2Y receptors to nucleotide release has been also demonstrated, thus allowing to hypothesize the existence of an autocrine/paracrine loop activated by nucleotides just released from the cell [29]. This autoamplifying circuit might occur not only in pulmonary epithelial cells, but also in other cell types, therefore suggesting the existence of a regulated secretory pathway that could contribute to increase the extracellular availability of uracil nucleotides upon pathological or stressful emergency situations (Fig. 1).

(c) Cell lysis such as necrotic death causes the extracellular release of the whole cytoplasmic and organelle content including nucleotides (which come also from the degradation of nucleic acids), whose concentration therefore dramatically increases in the close proximity of the damaged tissue. During brain ischemia the sustained release of adenine nucleotides has been well documented [31] whereas to date no evidence of uracil nucleotides release has been provided, although its existence can be postulated. The major limitation to the evaluation of uracil nucleotides concentration in the extracellular milieu during ischemic episodes was the lack of a reliable quantification assay. With the development of such an assay [28] (see also above), the research groups of Lazarowski and Erlinge have been recently able to demonstrate a sustained release of uracil nucleotides during cardiac ischemia in pig [32] and during myocardial infarction in man [33], thus opening the way for the demonstration of UTP, UDP, and UDP-sugars release also in brain ischemia.

5. Old and new receptors activated by uracil nucleotides

5.1. $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors

$P2Y_2$ (equally activated by ATP and UTP), $P2Y_4$ (with UTP more potent than ATP), and $P2Y_6$ (activated by UDP) receptors are widely expressed by different cellular subtypes both in the peripheral and central nervous system [21]. In the CNS, $P2Y_2$ receptors have been localized on pyramidal neurons in the hippocampus and prefrontal cortex, on supraoptic magnocellular neurosecretory neurons in the hypothalamus and on neurons in the dorsal horn of the spinal cord [34]. $P2Y_2$ receptors have been also found in oligodendrocytes, and Schwann cells [21], and all the uracil-responding P2Y receptors have been found expressed by rat cortical astrocytes [35], as well as by N9 microglial cells [36].

In addition, $P2Y_4$ and $P2Y_6$ receptor subtypes have been identified in the cerebellum and hippocampus at the mRNA, but not at the protein level [34]. More recently, a functional $P2Y_6$ receptor has been characterized in rat cultured microglial cells [37], and in neural precursor cells mRNA for $P2Y_4$ and $P2Y_6$ receptors were described [38] (see Section 5.1.2).

In the periphery, trigeminal ganglia express all the uracil nucleotides receptors with $P2Y_2$ and $P2Y_4$ receptors functionally coupled to the modulation of $[Ca^{2+}]_i$ in both neurons and satellite glial cells, whereas $P2Y_6$ receptor was functional only in the latter [39]. A subpopulation of dorsal root ganglia neurons express high levels of $P2Y_2$ receptor [40], which has been also found expressed in several astrocytoma and neuroblastoma cell lines [21].

5.1.1. *Reactive astrogliosis*

Astrocytes have the ability to respond to traumatic and inflammatory CNS insults through major and rapid modifications of their morphology and cell structure and with increased proliferation, a process globally known as ''reactive astrogliosis''. It occurs in several acute and chronic pathologies such as trauma, stroke and Alzheimer's disease, and it is characterized by cell body hypertrophy and hyperplasia, by an increased expression of the typical astrocytic hallmark (named glial fibrillary acidic protein; GFAP), as well as by cytoskeletal rearrangement involving extracellular matrix, and enhanced cell migration and proliferation [41]. Reactive astrocytes produce and release inflammatory mediators, such as cytokines and chemokines, and various growth factors. In the last 15 years, the overall positive or negative outcome of reactive astrogliosis has been debated in the scientific community. In fact, several lines of evidence demonstrate that migrating astrocytes constitute a barrier (the so-called ''glial scar'') isolating healthy tissue from the surrounding damaged areas [41], as it happens in stroke where reactive astrocytes migrate to the border of the injured area slowing down the spreading of the necrotic ischemic core. It has been also very recently hypothesized that at least a subpopulation of reactive astrocytes might de-differentiate upon pathological conditions to a stem cell state and reacquire their multipotency, in an attempt to counteract cell death [42]. These cells could then be instructed to generate new neurons to substitute the lost neuronal population, or new oligoden-

drocytes in the case of demyelinating disorders. On the other hand, excessive or chronic accumulation of astrocytes can also produce deleterious effects thereby preventing neuronal regeneration within the damaged areas [41]. The in-depth understanding of the various neurotransmitter and cytokine systems controlling these two opposite sides of the astrocytic reaction might therefore help finding appropriate pharmacological approaches to exploit the beneficial effects of reactive astrogliosis by in parallel limiting the deleterious ones.

Stimulation of primary rat astrocytes with UTP has been demonstrated to promote cell migration, which could represent a typical early feature of astrogliosis [43]. This effect was obtained with ATP as well, whereas UDP was not active, suggesting an involvement of the $P2Y_2$ receptor. In particular, UTP induced a time dependent up-regulation of α_v, β_3 and β_5 integrins, membrane receptors which are able to bind to extracellular matrix proteins containing an arginine-glycine-aspartic acid (RGD) motif and whose overexpression during pathological events triggers astrocytic migration [43]. $P2Y_2$ receptor was shown to directly interact with $\alpha_v\beta_3/\beta_5$ integrins complexes via an RGD motif found in its first extracellular loop [44]. Moreover, astrocyte migration was completely abolished by silencing the $P2Y_2$ receptor, strongly supporting its central role in controlling astrocytic reaction to harmful events [43]. These findings have been further supported by the observation that the interaction between $P2Y_2$ receptor and α_v integrin is at the basis for the signalling through G_{12} and G_o, leading to cell migration in human astrocytoma 1321N1 cells expressing the recombinant $P2Y_2$ receptor (1321N1-$P2Y_2$ cells) [43].

UTP-stimulated 1321N1-$P2Y_2$ cells have been utilized as a particularly useful system to understand the role of the $P2Y_2$ receptor in neuroprotection. In fact, UTP positively regulates the anti-apoptotic genes bcl-2 and bcl-xl through the phosphorylation of the transcription factor CREB, whereas the pro-apoptotic gene bax was down regulated [45]. Moreover, microarray analysis showed an enhanced expression of genes for neurotrophins, neuropeptides and growth factors, therefore suggesting an important role for $P2Y_2$ receptor in promoting nerve tissue regeneration through the positive involvement of surrounding astrocytes. To further confirm this hypothesis, conditioned medium from 1321N1-$P2Y_2$ cells treated with 100 μM UTP for 24 h significantly enhanced neurite outgrowth of PC-12 cells comparable to the effects obtained with NGF treatment [45]. It is also worth noting that a Src-dependent transactivation of EGF, PDGF and VEGF-2 receptors has been described to occur in 1321N1-$P2Y_2$ [46], therefore directly linking this UTP-activated purinoceptor to the trophic and pro-survival effects that are typically mediated by growth factors. In the same cellular model, UTP leads to the α-secretase dependent cleavage of the amyloid precursor protein (APP) with the release of the non-amyloidogenic product sAPPα [47], described to have neurotrophic and neuroprotective activities. Taken together, these observations play in favour of an overall neuroprotective activity played by the astrocytic $P2Y_2$ receptor subtype.

Not only the $P2Y_2$, but also the $P2Y_4$ receptor subtype has been implicated in the modulation of astrocytic function following pathological events. In fact, extracellular UTP stimulated a robust overexpression of thrombospondin 1 (TSP-1), through the activation of $P2Y_4$ receptor in an in vitro model of traumatic CNS injury [48]. TSP-1 belongs to the family of thrombospondins, multidomain glycoproteins involved in cell-to-cell and cell-to-matrix interactions, which are secreted by astrocytes during nervous system development and allow astrocytes to interact with neurons behaving as recognition molecules. TSP-1 released by astrocytes after brain injury promotes dendritic growth and axonal sprouting, and enhances synaptogenesis. The formation of new synapses is an extremely important process, which allows lesioned neurons to survive and regenerate through the glial scar. These results could support the concept that mediators released during the early phase of reactive astrogliosis might be able to stabilize neurons and their synapses in areas surrounding the injury [48], and one of the factors controlling this event is likely to be UTP.

A strong expression of both $P2Y_2$ and $P2Y_4$ receptors subtypes has been also described on the end feet of astrocytes surrounding brain microvessels and capillaries [49]. This suggests that UTP released from endothelial cells composing the blood–brain barrier in response to various physiological or pathological situations might contribute to deliver the message to brain astrocytes, which may participate to the brain reaction to peripheral stimuli [50].

5.1.2. *Cell proliferation and differentiation*

Uracil nucleotides could also have unexplored potential in brain pathologies because of their effects on cell proliferation and differentiation. UTP significantly enhanced $P2Y_4$ receptor expression in human neuroblastoma SH-SY5Y cells, known to respond with neuronal differentiation and development of long-term potentiation to increased intracellular levels of cAMP [51]. This ligand-activated up-regulation of $P2Y_4$ receptor is correlated with cell differentiation, promoting neurite outgrowth and elongation [52]. Furthermore, SH-SY5Y cells transiently overexpressing $P2Y_4$ receptor and exposed to UTP showed an increased induction of cell death in parallel to cell differentiation [52]. The mechanism at the basis of these events is unknown, but it can be hypothesized that a trigger of differentiation (e.g., UTP) can generate contradictory signals within a tumor cell population, which might eventually lead to cell growth inhibition, and induction of cell death. This could have important clinical application for the pharmacological treatment of neuroblastoma.

In 1321N1 astrocytoma cells stably transfected with the $P2Y_6$ receptor, UDP was found protective against tumor necrosis factor-alpha (TNFα)-induced apoptosis [53]. Interestingly, in the same experimental model UDP was not able to counteract cell death induced by oxidative stress or chemical ischemia [53], therefore suggesting the existence of a highly selective mechanism of action. On the contrary, in cultured hippocampal neurons UDP has been found to elicit a strong mitochondrial depolarization in the absence of Ca^{2+} responses and to stimulate the generation of radical oxygen species (ROS) whereas UTP has no effect [54]. These observations suggest that the $P2Y_6$ receptor might exert opposite effects in cellular damage, senescence, and post-ischemic neuroinflammation probably depending upon the toxic stimulus involved.

In the very last years, a great interest has raised on the study of embryonic and adult neural stem/precursor cells (NPCs), which represent a wonderful evidence of brain plasticity. In fact, they can proliferate, migrate to the site of injury and differentiate into neurons or glia. Therefore they are potentially suitable for gene therapy in brain tumors, transplantation in acute or chronic neurodegenerative diseases, or, in the case of adult endogenous stem cells, pharmacological manipulation to be recruited to the site of injury. In vitro experiments on human mesencephalic NPCs (hmNPCs) demonstrated that extracellular UTP augmented cell proliferation in presence of the mitogens EGF and FGF2 [38]. In addition, UTP and UDP increased dopaminergic differentiation when added to growth factor-free medium, as indicated by the appearance of the dopaminergic marker tyrosine hydroxylase. The effects of UTP on both proliferation and differentiation were abrogated by the P2Y receptor antagonists PPADS and suramin [38]. Moreover, RT-PCR and microarray analysis revealed the presence of the $P2Y_1$, $P2Y_4$, $P2Y_6$ and $P2X_4$ receptor subtypes, indicating that the effects mediated by UTP and UDP could be due to the activation of both $P2Y_4$ and $P2Y_6$ receptors. ATP abolished both proliferation and differentiation, in apparent contrast with the fact that it was described as a proliferative agent in various systems, including astrocytes and neural stem cells [55,56]. Nevertheless, since UTP can be degraded to UDP, the proliferative effect could be mediated only by the latter nucleotide, acting on the $P2Y_6$ receptors. For the same reason, the anti-proliferative effect of adenine nucleotides could be mediated by adenosine, a metabolite of ATP, active at P1 receptors [38].

Importantly, due to the selective loss of dopaminergic neurons in the substantia nigra as the leading cause of Parkinson's disease, the finding that UDP can both induce stem cell proliferation and dopaminergic differentiation could represent a starting point for the development of therapeutic approaches exploiting NPCs transplantation to the damaged areas or stimulation of endogenous neurogenesis through $P2Y_{4/6}$ receptors activation.

5.1.3. *Modulation of microglia activation*

Microglia, the brain immune cells, play an important role in the phagocytosis of dying neurons and cellular debris during infections or brain injuries. After neuronal damage, microglial cells migrate to the affected site, where they can release a variety of cytokines and neurotrophic factors [57]. ATP not only stimulates the release of various biologically active substances from microglia but it also regulates cell motility through the $P2Y_{12}$ receptor subtype and other $G_{i/o}$-coupled P2Y receptors [57,58]. UDP-treated rat microglial cells rapidly changed their morphology through actin reorganization leading to a strong phagocytosis of zymosan particles in the medium [37]. UDP-induced phagocytosis was significantly reduced by MRS2578, a selective antagonist at the $P2Y_6$ receptor subtype and was nearly abolished by the treatment with $P2Y_6$ antisense oligonucleotides. The effect was also inhibited by thapsigargin and staurosporin indicating the recruitment of a pathway mediated by PLC-linked increases of $[Ca^{2+}]_i$ and PKC activation [37].

The expression and function of microglial $P2Y_6$ receptors in vivo was demonstrated by intraperitoneal administration of kainic acid (KA) to rats. Western blotting and immunocytochemical analysis showed an increased number of poorly ramified activated microglial cells with up-regulated $P2Y_6$ receptors [37]. The co-localization with the neuronal marker NeuN suggested the active phagocytosis of damaged or dead neurons by microglial cells, thus demonstrating that they migrate and proliferate in response to a KA-induced damage. Furthermore, a time course measurement of extracellular uracil nucleotides showed a 2–3-fold early increase in UTP concentrations after KA injection with respect to untreated animals. UTP was subsequently degraded to UDP, the endogenous ligand of $P2Y_6$ receptor, which might in turn have acted as a chemotactic signal [37].

The definitive proof of a key role played by the $P2Y_6$ receptor subtype in the modulation of microglial phagocytosis came from the injection of fluorescent microspheres in the hippocampal CA3 regions after KA administration. The selective knocking down of the $P2Y_6$ receptor by antisense oligonucleotide strategy significantly inhibited the number of phagocytosed particles, clearly demonstrating that $P2Y_6$-mediated signals are important for microglial phagocytosis even in vivo [37].

5.1.4. *Modulation of pain signalling*

Pain signals from the periphery are integrated and conveyed to CNS at the level of sensory neurons located in dorsal root ganglia (DRG) near to the spinal cord, whereas painful sensations form the head and facial districts are mainly elaborated by the trigeminal ganglion (TG) [59]. Besides neurons, also glial cells (named satellite glial cells), which anatomically surround sensory neurons, are found in all ganglia [39]. In recent years, evidence has emerged indicating that also these cells can actively participate to pain transmission by releasing and/or sensing chemical mediators (e.g., CGRP, bradykinin, etc.), which in turn influence and modulate neuronal firing [59].

At the site of injury, tissue damage associated to inflammation or ischemia produces an array of substances, which are able to act on nociceptor terminals to elicit or exacerbate pain transmission. The P2 receptor agonist ATP is one of these mediators, and its most important receptors involved in this process are the $P2X_2$ and $P2X_3$ ionotropic receptor subtypes, selectively expressed by a subpopulation of DRG neurons [60]. However, recent observations have demonstrated also the involvement of P2Y receptors in pain transmission. Among them, the $P2Y_1$ and $P2Y_2$ receptor subtypes are highly expressed by DRG and TG sensory neurons [61], whereas $P2Y_4$ receptor is mostly expressed by a subpopulation of large diameter neurons, which seem to be involved in the transmission of tactile allodynia [61]. mRNA encoding for the $P2Y_6$ receptor subtype has been reported in DRG as a whole, but it has not been localized on sensory neurons [40], suggesting that it might be expressed by satellite glial cells (see also below).

Most nociceptors in both rodents and humans are found on small neurons with unmyelinated axons, which represent the 60–70% of neurons in DRG. In small neurons, both ATP and UTP act as potent nociceptive signals: $P2Y_2$ receptor evoked a delayed train of action potentials that persisted many seconds after the removal of the stimulus [40]. Moreover, UTP-

activated $P2Y_2$ receptors induced CREB phosphorylation in a subset of rat sensory neurons, leading to an increase of calcium influx. Phosphorylated CREB is known to promote the expression and release of a variety of neuropeptides, such as CGRP, substance P and BDNF, which are up-regulated in sensory neurons in response to injury or inflammation [62].

Capsaicin is one of the most potent molecules known to activate nociceptors, such as TRPV1. Interestingly, most of the capsaicin-sensitive fibers are also UTP-sensitive cutaneous and mechanoceptor fibers, suggesting a positive association between capsaicin and UTP [63]. To further confirm this hypothesis, UTP-induced thermal hyperalgesia in vivo is abolished in mice lacking TRPV1, whereas it is preserved in $P2Y_1$-deficient mice [64], and patch clamp analyses using mouse DRG neurons indicated the involvement of $P2Y_{2/4}$ receptor subtypes. Furthermore, both ATP and UTP potentiated the capsaicin-evoked currents in cultured rat DRG neurons, where in the presence of extracellular nucleotides the threshold temperature for the activation of TRPV1 was reduced from 42 to 35 °C, thus causing pain sensation even at body temperature [64]. In addition most UTP-sensitive fibers also respond to the P2X receptor agonist αβ-methyleneATP, thereby assessing a crosstalk between adenine- and uridine-sensitive P2 purinoceptors [63]. PPADS and RB2 inhibited the stimulatory effects of nucleotides, whereas suramin, which blocks $P2Y_2$ but not $P2Y_4$ receptors, abolished the effect of UTP, and the $P2Y_6$ receptor agonist UDP has no effect on pain transmission [63]. Taken together these results strongly play in favour of a pro-nociceptive role for the $P2Y_2$ receptor subtype expressed by DRG neurons.

Surprisingly, behavioral studies demonstrated that intrathecally administered UTP and UDP to normal rats elevated the mechanical nociceptive threshold in the paw-pressure test and prolonged the thermal nociceptive latency in the tail-flick test [65]. Furthermore, in neuropathic pain model, intrathecal administration of UTP suppresses the tactile allodynia induced by ligation of the sciatic nerve [65]. These findings suggest that the activation of spinal $P2Y_{2/4/6}$ receptors could reduce neuropathic pain, in contrast to P2X receptors and to data obtained in in vitro models (see above). A possible explanation of the analgesic effects of uracil nucleotides is that UTP-activated P2Y receptors increase $[Ca^{2+}]_i$ in dorsal horn astrocytes, and this may modulate the allodynic information of neighboring nociceptive neurons [65]. In contrast, intracerebroventricular administration of UTP had no anti-algogenic effect, indicating that the analgesic effects are obtained only with the stimulation of spinal P2Y receptors [66].

At present, no functional in vivo studies have been performed on the role of uracil nucleotide-activated receptors in pain signalling in TG. In our research group, we have demonstrated that mouse TG express all the cloned P2Y receptors at the mRNA level. Single cell calcium imaging revealed the presence of functional UTP-activated $P2Y_2$/$P2Y_4$ receptors on both neurons and glia (together with ADP-activated $P2Y_{1,12,13}$ receptors), whereas UDP-sensitive $P2Y_6$ receptors were only found functional on a small percentage of satellite glial cells [39]. Interestingly, exposure of cultures to the pro-inflammatory and algogenic stimulus bradykinin, which down-regulated neuronal $P2X_3$ receptor functions, significantly increased P2Y-receptor-mediated responses on satellite glial cells in parallel. In particular, the percentage of UDP-responding cells underwent a 2.5-fold increase, suggesting the possible development of an attempt to decrease pain transmission after exposure to a noxious stimulus [39].

5.2. *$P2Y_{14}$: a receptor for sugar nucleotides*

The previously orphan G protein-coupled receptor GPR105 is the first receptor identified as being selectively activated by the uracil sugar nucleotides UDP-glucose and UDP-galactose [25]. After its deorphanization, it was therefore included in the growing family of P2Y nucleotide receptors and re-named as $P2Y_{14}$ [26]. The amazing discovery that also UDP-sugar conjugates can activate a cell membrane receptor has further confirmed that also molecules that were believed to behave as mere metabolic intermediates (see Section 3) can instead contribute to signal transduction, and has therefore opened up new avenues in the fields of neurotransmission and cell-to-cell communication.

The $P2Y_{14}$ receptor subtype has been initially found widely expressed in peripheral tissues (e.g., spleen, kidney, liver and lung) and immune cells (e.g., neutrophils and lymphocytes) [21,25]. Immunohistochemical analysis of post mortem human brain sections revealed the ubiquitous glial expression of $P2Y_{14}$ receptor mainly localized in the white matter and more sparsely in the gray matter. In particular, a specific immunoreactivity was found in cerebral cortex, hippocampus, cerebellum, thalamus, basal ganglia and brainstem. The co-localization with the marker GFAP confirmed the astroglial expression of the receptor, whereas the presence of the receptor in a small population of GFAP-negative cells has not been fully investigated [67].

Well before its deorphanization, the $P2Y_{14}$ rat ortholog, called VTR 15-20, has been found expressed by both microglia and astrocytes [68]. After treatment with the pro-phagocytotic agent zymosan, expression of VTR 15-20 was significantly up regulated in microglial (5.4-fold) and astrocytic cultures (2.7-fold) compared to control values. Similar results were observed in cells treated with the immunogenic agent LPS although to a lesser extent [68]. These data were further confirmed in vitro in N9 microglial cells, where an overnight exposure to LPS increased the functional response of the $P2Y_{14}$ receptor subtype [36].

Intraperitoneal administration of LPS to rats induced a significant increase in the mRNA expression of $P2Y_{14}$ receptor in cerebral cortex, hippocampus, and hypothalamus, whereas no significant up-regulation was observed in spleen. The effect induced by immunological challenge is probably a result of the activation or proliferation of astrocytes (e.g., during reactive astrogliosis) as well as microglial cells [67].

Conversely, in vivo injection of KA, which was described to induce microglial activation (see also above), resulted in a significant down-regulation of VTR 15-20 in hippocampus and hypothalamus, but not in cortex. This may be due to a differential effect of KA or to a diverse regulation of the VTR 15-20 transcript depending upon the brain area [68].

Under physiological conditions, a basal activation of the $P2Y_{14}$ receptor subtype might be guaranteed by the constitutive release of UDP-sugar conjugates from ER and Golgi (see Section 2.3), although its physiological significance is still

unknown. Its overactivation might instead occur during cellular stress or lysis with the consequent leakage of intracellular UDP-glucose and UDP-galactose content (see Section 4). Thus, upon stressful conditions the functions of the $P2Y_{14}$ receptor subtype are likely to be linked to neuroimmune responses to infection, inflammation or even mechanical and traumatic insults [67].

5.3. *GPR17: a new dual receptor for uracil nucleotides and cysteinyl-leukotrienes*

Clues about the possible existence of an additional UDP-sugar sensitive G protein-coupled receptor came from studies performed in U373 MG astrocytoma cells, where exposure to UDP-glucose reduced forskolin-stimulated cAMP accumulation in a concentration dependent manner, despite the lack of the transcript encoding for the $P2Y_{14}$ receptor subtype [69].

These observations confirmed the hypothesis on which our research group has been working for the last 5 years, and finally we have recently cloned and characterized the orphan receptor named GPR17, demonstrating that its endogenous ligands are the uracil nucleotides UDP, UDP-glucose and UDP-galactose [70]. Interestingly, GPR17 is also activated by cysteinyl-leukotrienes (CysLTs), a family of pro-inflammatory molecules derived from arachidonic acid metabolism. A functional crosstalk between nucleotides and CysLTs in mediating inflammatory responses to cerebral hypoxia and trauma was previously postulated [71], but GPR17 represents the first fully characterized example of G protein-coupled receptor showing a dual pharmacology [70] since in vitro GPR17 is antagonized by both P2Y (i.e., MRS2179 and Cangrelor) and CysLT antagonists (i.e., Montelukast and Pranlukast).

GPR17 is highly expressed in brain, heart and kidney, highly vascularized tissues that may undergo ischemic damage. Upon physiological conditions, GPR17 is expressed by neuronal cells in various brain areas, but not by astrocytes [70]. Since it is well known that during stroke neurons and astrocytes in the infarct area undergo apoptotic and necrotic cell death, leading to a massive release of pro-inflammatory molecules and nucleotides [31], to get insights into the possible effects mediated by GPR17 in brain damage, we took advantage of an in vivo model of brain ischemia (the middle cerebral artery occlusion, MCAo, in rat) [70].

An early overexpression of GPR17 by dying neurons within the ischemic core was observed after MCAo induction (Lecca et al., unpublished data), and both the pharmacological inhibition of GPR17 (with the purinergic antagonist Cangrelor or the CysLT antagonist Montelukast) as well as its knockdown by means of specific antisense oligonucleotides markedly prevented damage progression [70]. Thus our results clearly suggest a key role played by GPR17 in the development of brain damage and in the advancement of its overall outcome.

To date, we still do not have definitive answers on the role played by GPR17 in brain function upon physiological conditions. Moreover, several hypotheses might be raised on the roles played by GPR17 during the progression of brain damage following MCAo. For instance, it can be hypothesized that the increased concentrations of UDP and UDP-sugar nucleotides in the extracellular milieu (which have been already observed during heart ischemia; see above and Section 6) may be a signal of membranes breakdown with GPR17 acting as a sensor of cellular damage. Its early up-regulation after cerebral ischemia may thus represent an attempt to counteract brain damage with a neuroprotective strategy. On the other hand, CysLTs are universally known as inflammatory mediators and therefore GPR17 overexpression could finally exacerbate post-ischemic inflammation and contribute to brain damage.

The existence of complex interactions between uracil nucleotide and CysLT systems during inflammatory events is further confirmed by receptor studies in vitro, where the CysLT1 receptor (specifically responding to LTD_4) is also influenced by extracellular UDP concentrations [72]. This was not due to a competition between UDP and CysLT since nucleotides were not able to compete with the receptor-binding site labelled with LTD_4 [72]. UDP was instead found to desensitize the LTD_4-induced $[Ca^{2+}]_i$ response in a concentration-dependent manner, whereas LTD_4 had no effect on P2Y receptor responses, suggesting a unilateral nucleotide-mediated regulation of the CysLT1 receptor [72]. It is therefore possible that nucleotide-induced desensitization of the CysLT1 receptor represents a feedback mechanism used by the cells to protect themselves from the increase of inflammatory mediators, which are typical of several acute and chronic pathological processes.

6. Possible pathophysiological roles of extracellular uracil nucleotides in the nervous system

The overall analysis of the literature data available on the biochemical and receptor-mediated effects of uracil nucleotides in the nervous system plays in favour of their global protective activity (Fig. 2). This was already suggested by early evidence demonstrating that uridine might preserve brain functions by maintaining brain metabolism during severe hypoglycemia and ischemia [73,74]. Also the pro-algogenic activity of neuronal $P2Y_2$ receptor in DRG can be interpreted as a positive strategy to sense tissue damage and to enable a protective reaction. Conversely, UTP or its metabolite UDP can modulate nociceptive spinal transmission via astroglial $P2Y_{2/4/6}$ receptors, exerting an analgesic effect. These data support the hypothesis that uracil nucleotides analogues may represent a novel class of analgesics (Fig. 2).

As reviewed in Section 3, intracellular uracil nucleotides directly control the cellular availability of glycosylated membrane components, such as phospholipids, sphyngolipids, and ceramides. Their optimal synthesis plays a fundamental role in the development of the nervous system; for instance, deletion of the glucosylceramide synthase enzyme in mouse brain leads to severe neural defects and death soon after birth [75]. Along this line, it has been recently demonstrated that a chronic dietary administration of uridine can significantly improve hippocampal-dependent memory in rats reared under impoverished conditions [76], and increase acetylcholine synthesis and release in the striatum of aged rats [77]. These effects have been mainly explained with the ability of uridine to increase the synthesis of the various

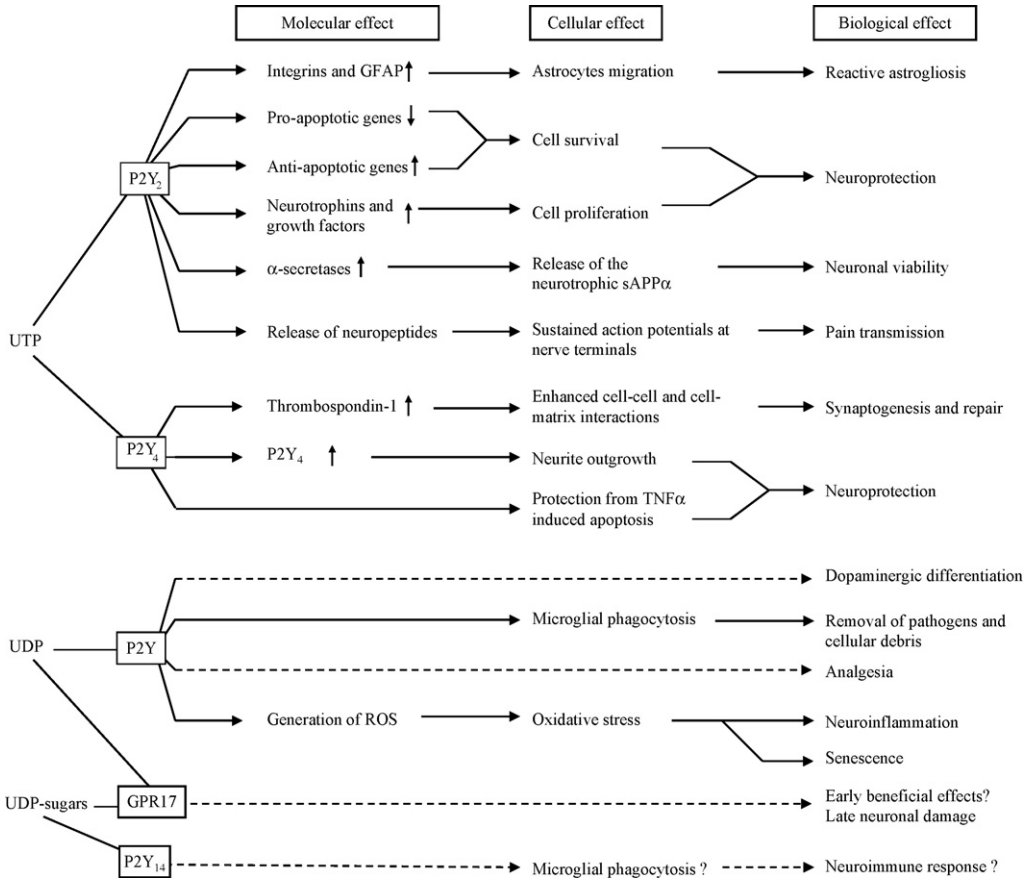

Fig. 2 – Uracil nucleotides-mediated effects in the nervous system. Through the activation of specific P2Y receptor subtypes, uracil nucleotides can induce a variety of molecular and cellular effects leading to neuroprotection, neuroinflammation, modulation of pain transmission, cell survival or proliferation. Some of these events, extensively described in the text, are here summarized. Dashed lines indicate that the molecular or cellular events leading to the biological effect are yet-to-be identified.

membrane lipid components, which in turn ameliorate neuronal functions. However, a significant contribution to these beneficial events of an improved activation of uracil nucleotides-sensitive P2Y receptor can be envisaged, due to the known ability of the $P2Y_2$ and $P2Y_4$ receptor subtypes to stimulate growth factor synthesis, to foster synaptogenesis, and to promote neurite outgrowth [45,48] (see Sections 5.1.1 and 5.1.2 and Fig. 2).

Moreover, a growing number of congenital disorders of glycosylation has been identified in humans in the last few years [17]. They are mostly characterized by severe neurological clinical symptoms whose degree increases in parallel with the loss of glycosylation reactions, leading to embryonic lethality when glycosylations are virtually absent [17]. One of the most common disorders of glycosylation is represented by galactosemia, which is caused by deficiency or mutations in the genes encoding for the enzymes involved in galactose metabolism [17,78]. The result of such an impairment is the toxic cellular accumulation of galactose and the lack of glycosylated proteins, leading to severe neurological, motor, and endocrinological defects [78].

Definitive data on the mechanisms of galactose toxicity have not been provided yet, and also in the case of congenital defects of glycosylation the most harmful event seems to be represented by the lack of fundamental membrane components. However, also uracil nucleotides are released as additional cargo molecules during the physiological delivery of glycosylated proteins and lipids from the ER to the plasma membrane (see Section 4), and it can be speculated that in the absence of appropriate glycosylation reactions also the ER content (and the consequent release) of UDP and UDP-sugars is dramatically reduced. Consequently, it can be also hypothesized that the lack of a constitutive neuroprotective or neurotrophic activation of uracil-nucleotide sensitive P2Y receptors is contributing to the development of the clinical symptoms of such pathologies, thus confirming the possible beneficial effects of endogenous uracil nucleotides. These important issues surely warrant further investigations.

In this scenario, UTP-stimulated reactive astrogliosis through the activation of the $P2Y_2$ (and to a lesser extent, $P2Y_4$) receptor subtype can be identified as an attempt to block tissue damage spreading by fostering migration of reactive astrocytes, formation of a glial scar, the expression of anti-apoptotic genes and growth factors, and neuron viability and synaptogenesis. (see Section 5.1). The $P2Y_2$ receptor might thus represent a novel target for the development of therapies

aimed at minimizing the deleterious effects of chronic astrogliosis associated with brain injury or diseases [43].

The contribution of UDP-sugar conjugates to neuroinflammation and reactive astrogliosis still needs to be fully characterized, but it can be envisaged based on the up-regulation of the $P2Y_{14}$ receptor upon immunological challenge, and on the massive release of UDP-glucose that can be induced by challenging astrocytes with thrombin [12]. Since leakage of thrombin from the blood into the brain parenchyma has been shown to occur under a number of acute and chronic brain pathological conditions following the disruption of the BBB (e.g., stroke, ischemia, and multiple sclerosis) [12], the consequent release of UDP-glucose and other sugar conjugates might then contribute to the development of the glial reaction to injury.

The double-edged nature of the inflammatory and glial reaction in brain pathologies has been recently pointed out (see also Section 5.1.1). To our current knowledge, it seems that an initial acute inflammatory reaction to harmful stimuli might prove beneficial for tissue recovery. However, when prolonged over time it changes to a deleterious event thus contributing to the progression of neurodegeneration [41]. This could be also the case for the newly identified uracil-responding GPR17 receptor that plays a key role in promoting brain damage following cerebral ischemia (see Section 5.3) [70].

No definitive proofs of the role played by uracil nucleotides during brain ischemia have been provided yet. However, several clear demonstrations of their protective role against cell death induced in cardiomyocytes in vitro by chemical or hypoxic damage [79,80] or by ATP and TNFα [81] and in the ischemic heart in vivo [82] and ex vivo [83] are now emerging. UTP-mediated protective effects on cardiac cells and tissue seem to be mainly mediated by the activation of the $P2Y_2$ receptor subtype. Interestingly, UTP is able to preserve mitochondrial function by inducing a transient membrane depolarization which results protective against the subsequent chemical or hypoxic stress, a sort of ''ischemic preconditioning'' [80]. Since a $P2Y_2$-like receptor has been identified on the outer mitochondrial membrane [84], it can be hypothesized the mitochondrial uracil-sensitive receptors are involved in the maintenance of cellular energetic homeostasis. Due to the high similarity between cardiac and brain ischemia, these new data suggest that such a protective effect mediated by a least some of the UTP- or UDP-activated receptors can be envisaged upon ischemic or hypoxic events also in the CNS.

7. Conclusions

To date available data suggest that uracil nucleotides exert physiologically relevant actions through their metabolic actions and the basal activation of membrane receptors, so that alterations in these activities proved extremely harmful. Upon acute injury, the increased release of uracil nucleotides accompanied by receptor up-regulation might represent the first attempt to counteract damage and to deliver a danger signal to the surrounding tissue. On the other hand, upon prolonged and chronic pathological conditions the dysregulation of these protective activities might contribute to spread the damage resulting in deleterious effects. It is conceivable that the pharmacological manipulation of this system will therefore allow fostering its beneficial effects thereby reducing the noxious ones.

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