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Research Overview

Expanding Field of Purinergic Signaling

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT This article attempts to paint a broad picture of the extraordinary explosive recent developments in the purinergic signaling field. After a brief historical review and update of purinoceptor subtypes, the focus is on the physiological roles of purines and pyrimidines. These are considered both in terms of short-term signaling in neurotransmission, secretion, and vasodilatation and in long-term (trophic) signaling in development, regeneration, proliferation, and cell death. Examples of trophic signaling include cartilage development in limb buds, glial cell proliferation, development of skeletal muscle, changes in receptor expression in smooth-muscle phenotypes, maturation of testicular spermatids, and bone remodeling. Plasticity of purinoceptor expression in pathological conditions is described, including the increase in the purinergic component of parasympathetic nervous control of the human bladder in interstitial cystitis and outflow obstruction and in sympathetic cotransmitter control of blood vessels in hypertensive rats, the appearance of $P2X_7$ receptors in the glomeruli of the kidney from diabetic and transgenic hypertensive animal models, and up-regulation of $P2X_1$ and $P2Y_2$ receptor mRNA in hearts of rats with congestive heart failure. The role of $P2X_3$ receptors in nociception is considered, and a new hypothesis about purinergic mechanosensory transduction in the gut is explored. A personal view of some of the areas ripe for future development concludes this article, including a discussion of different strategies that could lead to the development of purinergic therapeutic agents. Drug Dev. Res. 52:1–10, 2001.

Key words: P2X receptors; P2Y receptors; neurotransmission; secretion; trophic signaling

INTRODUCTION

The purinergic signaling field was born more than 30 years ago. It had a very troubled postnatal period and now, at last, it seems to be blossoming into puberty with the promise of many good times to come as it matures. This article starts with a brief historical review of the field and an update of purinoceptor subtypes and then focuses on the physiological and pathophysiological roles of purines and pyrimidines, stressing some of the new and exciting avenues for future developments.

EARLY HISTORY

As is well known by now, the field started with the seminal article by Drury and Szent-Györgyi in 1929, in which they showed potent extracellular actions of purine nucleotides and nucleosides on the heart. In a 1959 landmark article, Pamela Holton showed release of ATP during antidromic stimulation of sensory nerves to the rabbit ear artery in sufficient amounts to produce changes in vascular tone. Then, in 1970 in Melbourne, we found evidence that ATP is a neurotransmitter in nonadrenergic, noncholinergic (NANC) nerves supplying the gut [Burnstock et al., 1970], and in 1972, I put forward the purinergic neurotransmission hypothesis [Burnstock, 1972].

PURINOCEPTOR SUBTYPES

Implicit in the purinergic neurotransmission hypothesis was the presence of postjunctional receptors for ATP [Burnstock, 1976a], and a basis for distinguishing P1 (adenosine) from P2 (ATP/ADP) receptors was pro-

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posed [Burnstock, 1978] that helped resolve some of the earlier ambiguous reports. These were complicated by the breakdown of ATP to adenosine by ectoenzymes, so that some of the actions of ATP were directly on P2 receptors while others were due to indirect action via P1 receptors. It was not, however, until 7 years later that Burnstock and Kennedy [1985] proposed a basis for distinguishing two types of P2 purinoceptor, P2X and P2Y, based largely on pharmacological criteria. Soon after, Gordon [1986] proposed two further P2 subtypes, namely, P2T on platelets and P2Z on macrophages. Later, it was recognized that pyrimidines as well as purines are potent extracellular messengers, and they were named P2U receptors [O'Connor et al., 1991].

Williams remarked that "this random walk through the alphabet was not really satisfactory." It was fortunate that in the early 1990s studies of transduction mechanisms [Dubyak, 1991] and cloning of both P2Y [Lustig et al., 1993; Webb et al., 1993] and P2X [Brake et al., 1994; Valera et al., 1994] receptors was reported. This led Abbracchio and Burnstock [1994] to put forward a new nomenclature system, which is now widely accepted. They proposed that there were two families of P2 purinoceptors—P2X ionotropic ligand-gated ion channel receptors and P2Y metabotropic G protein–coupled receptors. This framework allowed for a logical expansion as new receptors were identified. There are currently seven subtypes of P2X receptors and six subtypes of P2Y receptors that are clearly recognized [Ralevic and Burnstock, 1998]. In addition, there have been recent suggestions for a $P2X_8$ receptor [Bo et al., 2000] and several new P2Y subtypes [King et al., 2001]. Earlier, four subtypes of P2 receptors were cloned, namely, A_1, A_{2A}, A_{2B}, and A_3 [Ralevic and Burnstock, 1998].

P2X Receptor Family

P2X receptors are characterized by two transmembrane domains, short intracellular N- and C-termini and an extensive extracellular loop with conservation of 10 cysteines. It has become apparent that the pharmacology of the recombinant P2X receptor subtypes expressed in oocytes or other cell types is often different from the pharmacology of P2Y-mediated responses in naturally occurring sites. There are several contributing factors to explain these differences. First, it is now recognized that three P2X units (or possibly four) form the ionic pore and that heteromultimers as well as homomultimers are involved. For example, heteromultimers are clearly established for $P2X_{2/3}$ [Lewis et al., 1995; Radford et al., 1997], $P2X_{4/6}$ [Lê et al., 1998], $P2X_{1/5}$ [Torres et al., 1998; Haines et al., 1999], and $P2X_{2/6}$ [King et al., 2000]. $P2X_7$ does not form heteromultimers, and $P2X_6$ will not form a functional homomultimer [Torres et al., 1999; North and Surprenant, 2000]. Second, spliced variants of P2X receptor subtypes might play a part. For example, a spliced variant of $P2X_4$ receptor, while it is nonfunctional on its own, can potentiate the actions of ATP through the full-length $P2X_4$ receptors [e.g., Townsend-Nicholson et al., 1999]. Third, the presence of powerful ectoenzymes in tissues that rapidly break down purines and pyrimidines is not a factor when examining recombinant receptors [Zimmermann, 1996].

Early studies of the distribution of P2X receptor subtypes based on Northern blot and in situ hybridization studies [Collo et al., 1996] have been extended substantially, after antibodies to these receptors became available, by immunohistochemical localization at both light [Vulchanova et al., 1996; Bradbury et al., 1998; Chan et al., 1998a; Xiang et al., 1998a,b, 1999; Bo et al., 1999; Gröschel-Stewart et al., 1999a,b; Bardini et al., 2000; Brouns et al., 2000; Lee et al., 2000a,b] and electron microscopic levels [Llewellyn-Smith and Burnstock, 1998; Loesch and Burnstock, 1998, 2000; Loesch et al., 1999]. For example, while it was originally thought that smooth muscle contained only $P2X_1$ receptors, there is now evidence for the presence of $P2X_2$, $P2X_4$, and probably $P2X_5$ receptors as well as both homomultimers and heteromultimers [see, for example, Nori et al., 1998; Hansen et al., 1999; Lewis and Evans, 2000]. $P2X_1$ receptors, which, in earlier studies, were not considered to be present in the brain, now have been found at postjunctional sites in synapses in the cerebellum [Loesch and Burnstock, 1998]. In current studies in my laboratory, Lele Jiang has found alternating molecules of the $P2X_1$ receptor and connexin 43 in discs in the heart, perhaps involved in gap junction regulation. The $P2X_1$ receptor is characterized by rapid desensitization and potent actions of α,β-methylene ATP (α,β-meATP), and there are now very potent selective antagonists for this receptor, such as trinitrophenyl ATP [Lewis et al., 1998] and diinosine pentaphosphate [King et al., 1999].

The $P2X_2$ receptor is widespread in the central nervous system and has been found at both pre- and postsynaptic sites in the hypothalamus [Loesch et al., 1999]. A feature of the $P2X_2$ receptor is lack of fast desensitization and extreme sensitivity to acidity and Zn^{2+} [King et al., 1996; Wildman et al., 1998, 1999a,b]. $P2X_3$ receptors are interesting in that they are predominantly localized in sensory nerves, particularly the small nociceptive neurons in the dorsal root ganglia and trigeminal and nodose ganglia [Bradbury et al., 1998]. The central projections are located in inner lamina II of the dorsal horn of the spinal cord [Llewellyn-Smith and Burnstock, 1998], and peripheral extensions have been noted in the skin, tongue, and tooth pulp [Bo et al., 1999; Gröschel-Stewart et al., 1999a; Burnstock, 2000; Alavi et al., 2001]. There is recent evidence for $P2X_2$ and $P2X_3$ labeling of endothelial cells of microvessels in brain, thymus, thyroid, and gut

and also in epithelial cells in the thyroid [Gröschel-Stewart et al., 1999b; Glass and Burnstock, 2001; Glass et al., 2000; Loesch and Burnstock, 2000].

$P2X_4$ and $P2X_6$ receptors are prominent in the central nervous system and are unique among P2X subtypes in that responses to purines mediated by these receptors are potentiated by suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid, and reactive blue 2, agents commonly used as P2 antagonists [Bo et al., 1995; Séguéla et al., 1996; Ralevic and Burnstock, 1998; Townsend-Nicholson et al., 1999]. $P2X_5$ receptors have been shown to be associated with proliferating and differentiating epithelial cells in the skin and hair follicles [Gröschel-Stewart et al., 1999a], in the bladder and ureter [Lee et al., 2000b], and in the vagina [Bardini et al., 2000]. This receptor subtype is also prominent in a number of cell types in embryonic development [Meyer et al., 1999a; Bar-Isaac et al., 2000].

$P2X_7$ receptors are unique in that, as well as a cation pore, a large, 4-nm pore can be formed, which appears to be linked with apoptosis, perhaps associated with the elongated C-terminus of this receptor [Surprenant et al., 1996]. It is particularly interesting that in recent studies in our laboratory, the $P2X_7$ receptor has been found to be internalized in cells that, under pathological conditions such as ischemia and cancer, become externalized, leading to apoptosis. Fluorescent green protein coupled to $P2X_7$ receptors will provide a valuable technique for observing the movement of receptors in living cells [see, for example, Dutton et al., 2000].

P2Y Receptor Family

P2Y receptors, in common with other G protein–coupled receptors, have seven transmembrane domains, an extracellular N-, and intracellular C-terminus. The conservation between the different subtypes is greatest in the transmembrane domains, the C-terminus showing the greatest diversity. $P2Y_1$ receptors, which were first cloned from chick brain, where they are ATP-selective, now appear to be ADP-selective in mammals and humans. In particular, 2-methylthioADP is a potent agonist [Hechler et al., 1998], and MRS 2179 is a potent antagonist [Boyer et al., 1998]; MRS 2269 and MRS 2286 have been identified as selective antagonists [Brown et al., 2000]. At $P2Y_2$ and $P2Y_4$ receptors in the rat, ATP and UTP are equipotent, but the two receptors can be distinguished with antagonists, that is, suramin blocks $P2Y_2$ and Reactive blue 2 blocks $P2Y_4$ [Bogdanov et al., 1998; King et al., 1998]. The $P2Y_3$ receptor is regarded as an orthologue of $P2Y_6$ by many researchers in the field, while $P2Y_5$, $P2Y_9$, and $P2Y_{10}$ appear to be orphan receptors with no evidence of a functional role.

$P2Y_6$ is UDP-selective, while $P2Y_7$ turned out to be a leukotriene receptor [Yokomizo et al., 1997]. $P2Y_8$ is a receptor cloned from frog embryos, where all the nucleotides are equipotent [Bogdanov et al., 1997], but no mammalian homologue has been identified to date, apart from a recent report of $P2Y_8$ mRNA in undifferentiated HL60 cells [Adrian et al., 2000]. $P2Y_{11}$ is unusual in that there are two transduction pathways, adenylate cyclase as well as inositol trisphosphate, which is the second messenger system used by the majority of the P2Y receptors. The $P2Y_T$ receptor found on platelets was not cloned until recently and seems likely to represent one of a subgroup of P2Y receptors for which transduction is entirely through adenylate cyclase. A receptor on C6 glioma cells and possibly a receptor in the midbrain, selective for a diadenosine polyphosphate, also may operate through adenylate cyclase. An interesting question has arisen by analogy with other G protein–coupled receptors as to whether dimers can form between the P2Y subtypes.

PHYSIOLOGY

Non-neuronal Short-Term Signaling

A major step forward was the proposal in 1976 that more than one transmitter can be released from nerve terminals (the cotransmitter hypothesis) [Burnstock, 1976b]. This is now widely accepted, and the focus is on defining the "chemical coding" of various nerve types, that is, to describe the combination of neurotransmitters in these nerves and their projections to various sites, such as smooth muscle, secretory cells, or other nerves. In the autonomic nervous system, the coding of prejunctional fibers also is under investigation.

There is now supporting evidence that ATP is a cotransmitter in many nerve types, probably reflecting the primitive nature of purinergic signaling [Burnstock, 1996, 1999a]. Thus, there is now evidence for ATP as a cotransmitter with noradrenaline and neuropeptide Y in sympathetic nerves, for ATP with acetylcholine and vasoactive intestinal peptide in some parasympathetic nerves, for ATP with nitric oxide and vasoactive intestinal peptide in enteric NANC inhibitory nerves, and for ATP with calcitonin gene–related peptide and substance P in sensory-motor nerves. There is also evidence for ATP with γ-aminobutyric acid in retinal nerves and for ATP with glutamate or with dopamine in nerves in the brain. In sympathetically innervated tissues, such as vas deferens or blood vessels, ATP produces fast responses mediated by P2X receptors and followed by a slower component mediated by G protein–coupled α-adrenoceptors; neuropeptide Y usually acts as a pre- or postjunctional modulator of the release and/or action of noradrenaline and ATP. Similarly, for parasympathetic nerves supplying the urinary bladder, ATP provokes a fast, short-lasting twitch response via P2X receptors, whereas the slower component is mediated by G pro-

tein–coupled muscarinic receptors. In the gut, ATP released from NANC inhibitory nerves produces the fastest response, nitric oxide gives a less rapid response, and vasoactive intestinal peptide produces slow tonic relaxations. In all cases of cotransmission, there are considerable differences in the proportion of the cotransmitters in nerves supplying different regions of the gut or vasculature and between species. The plasticity of expression of different cotransmitters in development and in different pathological conditions is discussed later in this article.

Some influential articles were published in *Nature* in 1992, which provided the first clear evidence for nerve-nerve purinergic synaptic transmission. One of these articles showed that excitatory postsynaptic potentials in the celiac ganglion were reversibly antagonized by suramin, a P2X antagonist [Evans et al., 1992], which was reported concurrently in an independent study [Silinsky et al., 1992]. Similar experiments were carried out in the medial habenula in the brain, showing reversible block of excitatory postsynaptic potentials by suramin [Edwards et al., 1992]. Since then, there have been many articles describing either the distribution of various P2 receptor subtypes in the brain and spinal cord or electrophysiological studies of the effects of purines in brain slices, isolated nerves, and glial cells [Gibb and Halliday, 1996; Abbracchio, 1997; Robertson, 1998; Burnstock, 1999b]. Synaptic transmission also has been found in the myenteric plexus [Zhou and Galligan, 1996; LePard et al., 1997; Spencer et al., 2000] and in various sensory and sympathetic and pelvic ganglia [Zhong et al., 1998, 2000a,b; Dunn et al., 2000].

Non-neuronal Signaling

It would be an error to think of purinergic signaling only in relation to excitable tissues, because there are now many examples of purinoceptor-mediate responses in non-neuronal and non-muscular cell types. Such examples include endothelial cells, which express $P2Y_1$, $P2Y_2$, and probably $P2Y_4$ receptors that, when occupied, release nitric oxide leading to vasodilatation. Moreover, there has been the more recent discovery of P2X receptors in endothelial cells, probably leading to the formation or regulation of gap and tight junctions involved in permeability [Loesch and Burnstock, 2000]; P2Y receptors in pancreatic β-cells involved in insulin secretion [Loubatières-Mariani and Chapal, 1988] and $P2Y_2$ receptors in hepatocytes [Schöfl et al., 1999]; $P2Y_T$, $P2X_1$, and $P2Y_1$ receptors in platelets [Kunapuli and Daniel, 1998]; and $P2Y_2$ on myelinating Schwann cells and $P2Y_1$ receptors on non-myelinating Schwann cells [Mayer et al., 1998]. P2 receptors also are involved in signaling to endocrine cells, leading to hormone secretion [Chen et al., 1995b; Lee et al., 1996; Tomić et al., 1996; Törnquist et al., 1996; Sperlágh et al., 1999; Glass et al., 2000a; Vainio and Törnquist, 2000].

Long-Term (Trophic) Signaling

In addition to the examples of short-term signaling described here, there are now many examples of purinergic signaling concerned with long-term events, such as development and regeneration, proliferation, and cell death [Abbracchio, 1996; Neary et al., 1996; Abbracchio and Burnstock, 1998]. For example, α,β-meATP produces proliferation of glial cells, whereas adenosine inhibits proliferation. A $P2Y_8$ receptor was cloned from the frog embryo, which appears to be involved in the development of the neural plate [Bogdanov et al., 1997]. $P2Y_1$ receptors seem to have a role in cartilage development in limb buds and in development of the mesonephros [Meyer et al., 1999b]. $P2X_5$ and $P2X_6$ receptors have been implicated in the development of chick skeletal muscle [Meyer et al., 1999a]. In recent studies of purinoceptor expression in the mouse myotubes, we have shown progressive expression of $P2X_5$ (from E14 to E18), $P2X_6$ (from E16 to E18), and $P2X_2$ (from E18 to postnatal day 7) [Bar-Isaac et al., 2000].

In a study of purinoceptor signaling in smooth-muscle phenotypes from the aorta, $P2X_1$ was prominent in the contractile phenotype. P2Y subtypes were also present, but in the synthetic phenotype grown in culture, the $P2X_1$ receptor was not detectable, while $P2Y_1$ and $P2Y_2$ receptors were substantially upgraded [Erlinge et al., 1998]. $P2X_2$, $P2X_3$, $P2X_5$, and $P2X_7$ receptors have been shown to be expressed in spermatids in distinct developmental stages in the seminiferous epithelium of the rat testis [Glass et al., 2000b]. This may be an interesting new target in the development of contraceptives. There are several reports implicating P2X and P2Y receptors in osteoclasts and osteoblasts involved in bone remodeling [Morrison et al., 1998; Wiebe et al., 1999; Dixon and Sims, 2000; Hoebertz et al., 2000].

PLASTICITY OF PURINOCEPTOR EXPRESSION AND PATHOPHYSIOLOGY

Plasticity in Disease

It is by now well established that the autonomic nervous system shows marked plasticity. The expression of cotransmitters and receptors shows dramatic changes in

Fig. 1. Schematic of a novel hypothesis about purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on $P2X_3$ receptors on low-threshold subepithelial intrinsic sensory nerve fibers (labeled with calbindin), contributing to peristaltic reflexes. ATP released during extreme distension also acts on $P2X_3$ receptors on high-threshold extrinsic sensory nerve fibers (labeled with isolectin B4, IB4) that send messages via the dorsal root ganglia (DRG) to pain centers in the central nervous system.

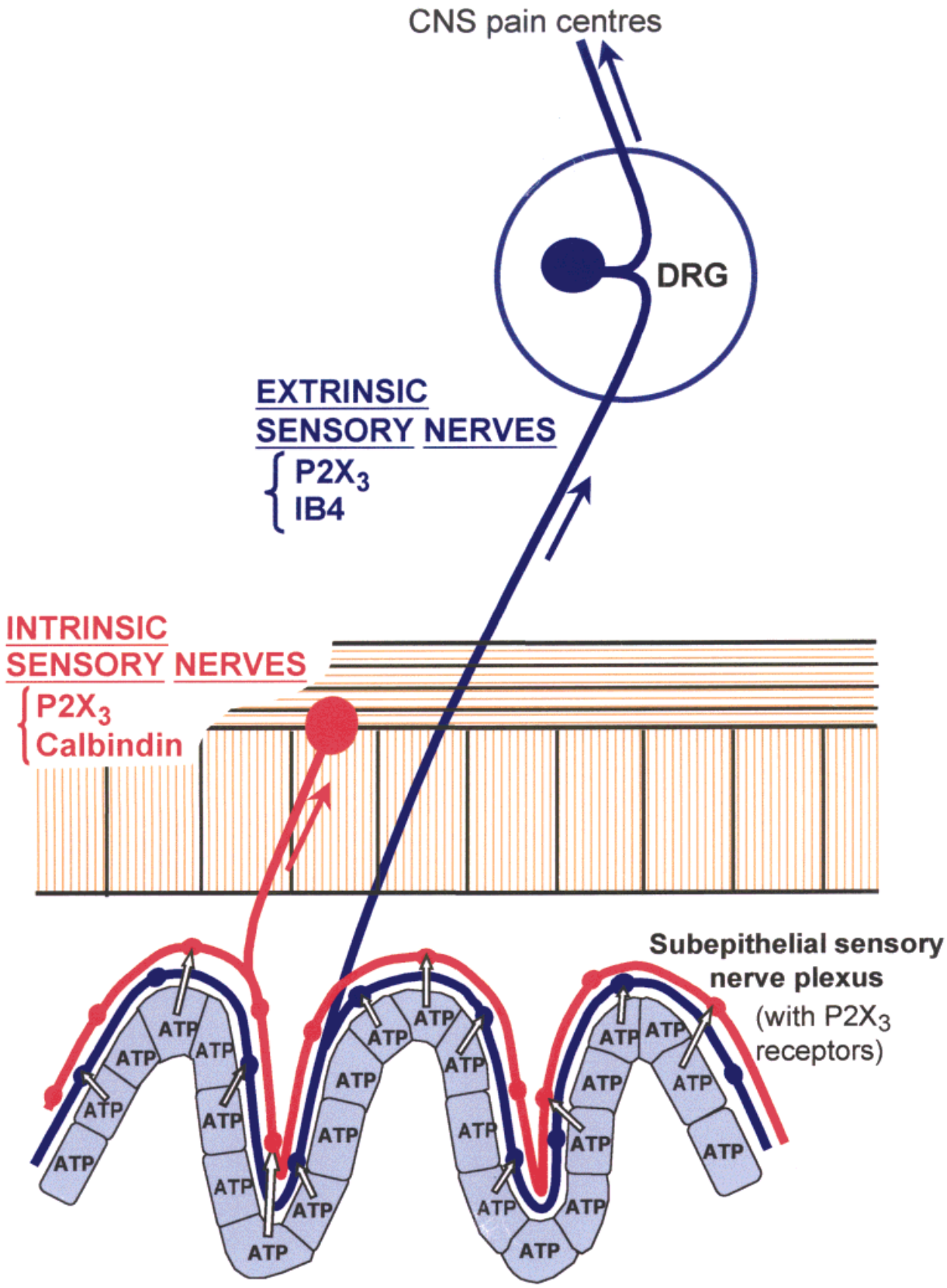
CNS pain centres
DRG
EXTRINSIC
SENSORY NERVES
P2X3
IB4
INTRINSIC
SENSORY NERVES
P2X3
Calbindin
Subepithelial sensory
nerve plexus
(with P2X3
receptors)
ATP

Figure 1.

development and aging, in nerves that remain after trauma or surgery, and in disease conditions [Burnstock, 1981, 1986, 1990, 1991; Milner and Burnstock, 1994; Abbracchio and Burnstock, 1998]. A few examples follow.

It is well known that although the purinergic component of parasympathetic transmission of the urinary bladder is between 40% and 70% in experimental laboratory animals, it is very small compared with muscarinic transmission in human bladder, despite the fact that P2X receptors are clearly present [Burnstock, 2001]. However, there are now a number of examples where the purinergic component in the human bladder is increased up to 40% in pathophysiological conditions, such as interstitial cystitis [Palea et al., 1993], outflow obstruction [Smith and Chapple, 1994; Bayliss et al., 1999], and possibly also neurogenic bladder [Wammack et al., 1995].

Another example where ATP plays a significantly greater cotransmitter role is in sympathetic nerves supplying hypertensive blood vessels [Vidal et al., 1986; Bulloch and McGrath, 1992; Brock and Van Helden, 1995]. In the healthy kidney, $P2X_1$ receptors are prominent in pre-glomerular arterioles and larger vessels, but P2X receptor expression is never seen in the glomerulus [Chan et al., 1998a]. However, recent studies in our group have shown prominent expression of $P2X_7$ receptors in the glomerulus of damaged kidneys from both transgenic hypertensive mice and streptozotocin-induced diabetic rats [Chan et al., 1998b]. Up-regulation of $P2X_1$ and $P2Y_2$ receptor mRNA in hearts of rats with congestive heart failure has been reported [Hou et al., 1999].

$P2X_3$ Receptors and Nociception

There have been various reports over the years concerning ATP on sensory nerves. In 1995, the $P2X_3$ receptor was cloned [Chen et al., 1995a; Lewis et al., 1995] and was shown to be expressed predominantly on nociceptive neurons in sensory ganglia [Burnstock, 2000]. Sensory nerve terminals in the tongue are strongly immunopositive for the $P2X_3$ receptor [Bo et al., 1999], and we have developed a tongue sensory-nerve preparation to examine the pathways of purinergic sensory signaling [Rong et al., 2000]. ATP and α,β-meATP applied to the tongue were shown to activate sensory afferent fibers preferentially in the lingual nerve but not the taste fibers in the chorda tympani.

A new hypothesis for purinergic mechanosensory transduction in visceral organs involved in the initiation of pain has been proposed [Burnstock, 1999c]. It is suggested that distension of tubes (such as the ureter, salivary ducts, and gut) and sacs (such as urinary bladder and gallbladder) leads to the release of ATP from the lining epithelial cells. ATP diffuses to the subepithelial sensory nerve plexus to stimulate $P2X_3$ and/or $P2X_{2/3}$ receptors, which mediate messages to pain centers in the central nervous system. It has been established that ATP is released from the epithelial cells in the distended bladder [Ferguson et al., 1997] and ureter [Knight et al., 1999]. $P2X_3$ receptors also have been identified in subepithelial nerves in the ureter [Lee et al., 2000b] and in the bladder [Cockayne et al., 2000]. Recording in a $P2X_3$ knockout mouse, we have shown that the micturition reflex is impaired and that responses of sensory fibers to $P2X_3$ agonists are gone, suggesting that $P2X_3$ receptors on sensory nerves in the bladder have a physiological as well as a nociceptive role [Cockayne et al., 2000]. Similarly, $P2X_3$ receptors on projections of neurons from the nodose ganglia supplying neuroepithelial bodies in the lining of the lung [Brouns et al., 2000] may also mediate pathophysiological events, perhaps involved in protective responses to noxious gases.

I conclude this review by proposing another hypothesis about sensory nerves in the gastrointestinal tract. It is known that the sensory nerves in the gut arising from dorsal root ganglia are labeled with $P2X_3$ receptors (and isolectin B4), but we now have been able to show that the intrinsic sensory neurons in both myenteric and submucous plexuses, which are labeled with calbindin, also show positive immunoreactivity for $P2X_3$. It is proposed that during moderate distension, low-threshold intrinsic enteric sensory fibers are activated via $P2X_3$ receptors by ATP released from mucosal epithelial cells, leading to reflexes concerned with propulsion of material down the gut. In contrast, it is proposed that with substantial distension, which often is associated with pain, higher-threshold extrinsic sensory fibers are activated by ATP released from the mucosal epithelia, which pass messages through the dorsal root ganglia to pain centers in the central nervous system (Fig. 1). We are carrying out experiments to test this hypothesis.

FUTURE DEVELOPMENTS

Some of the areas of research that seem likely to predominate in the next few years include studies of knockout mice for receptor subtypes, in addition to the $P2X_1$, $P2X_2$, $P2X_3$, $P2X_7$, $P2Y_1$, or $P2Y_2$ knockout mice that have been reported to date [Léon et al., 1999; Cesare et al., 2000; Cockayne et al., 2000; Homolya et al., 2000; Mulryan et al., 2000]. It is likely that there will be many studies of purinergic signaling in the brain, focusing, it is hoped, on physiological and behavioral roles, in addition to applying purines and pyrimidines to neurons and glial cells in brain slices and in culture. An expansion of studies to the long-term trophic roles of purines and pyrimidines in a variety of systems is highly desirable, in particular, in embryonic development and wound healing. There is likely to be substantial interest in the therapeutic development of purinergic agents for a variety of diseases. The therapeutic strategies are likely to extend beyond the development of selective agonists and antago-

nists for different P2 receptor subtypes to the development of agents that control the expression of P2 receptors, of inhibitors of extracellular ATP breakdown, and of ATP transport enhancers and inhibitors. The interactions of purinergic signaling with other established signaling systems also will be an important way forward.

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