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ABSTRACT

Signaling by extracellular purines such as ATP and adenosine has implications for dental research on multiple levels, with the association of purinergic signaling with inflammation, mechanical strain, and pain making the system particularly relevant for the specific challenges in the oral cavity. Oral tissues express a variety of G-protein-coupled P2Y receptors for ATP and P1 receptors for adenosine in addition to ionotropic P2X receptors for ATP. When these receptors are combined with the plethora of extracellular enzymes capable of manipulating extracellular agonist levels, a complex system for regulating oral health emerges, and recent findings have begun to identify a key role for purinergic signaling in oral pathophysiology. For example, the manipulation of extracellular ATP levels by *P. gingivalis* reduces inflammasome activation and apoptosis linked to P2X₇ receptor activation. Release of ATP by periodontal ligaments may link mechanical strain to bone remodeling. Activation of P2X receptors is implicated in dental pain, and receptor antagonists represent important targets for new analgesics. Altered levels of adenosine receptors in periodontal disease also suggest a role for nucleosides in dental signaling. The intricacies of the purinergic signaling system make it well-suited for the unique concerns of dental research, and future findings will doubtless confirm this importance.

KEY WORDS: P2X₇, P2Y, inflammasome, periodontal disease, neurotransmitter, mechanosensitivity.

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Inflammation, Pain, and Pressure—Purinergic Signaling in Oral Tissues

INTRODUCTION

The purinergic signaling system is involved in a wide range of physiological processes that have an impact on dental systems, including bone remodeling (Orriss *et al.*, 2010), neuronal signaling (Burnstock *et al.*, 2011), inflammation (Ohta and Sitkovsky, 2009), epithelial transport (Novak, 2011), and stem cells (Burnstock and Ulrich, 2011). While a role for purines in oral tissues has been acknowledged for over a decade (Cook *et al.*, 1997), there has been a recent burst of new information pertaining to purinergic aspects of dental research. This review will provide a brief overview of purinergic systems before describing some of the recent findings with considerable impact on oral science.

BASIC PRINCIPLES OF PURINERGIC SIGNALING

Purinergic signaling refers to the process by which purines and pyrimidines mediate cellular responses following stimulation of specific receptors. The presence of purinergic receptors in primitive multicellular organisms (Fountain and Burnstock, 2009), combined with wide dispersal of purinergic receptors throughout the body, reflects their central role in numerous physiological and pathophysiological processes (Burnstock and Knight, 2004).

Purinergic receptors are divided into 2 main categories, termed P1 and P2 receptors (Fig. 1; Burnstock, 1978). P1 receptors respond predominantly to the nucleoside adenosine (Fredholm *et al.*, 2011). The A_{2A} and A_{2B} adenosine receptors are coupled to the G_s protein, with stimulation leading to the activation of adenylate cyclase and increased levels of cytoplasmic cAMP. The A₁ and A₃ receptors are coupled to the G_i protein, with activation leading to the inhibition of adenylate cyclase and consequently a reduction in cAMP. In addition to effects on adenylate cyclase, these receptors are differentially coupled to other G proteins and induce various downstream reactions on calcium and kinases (Klinger *et al.*, 2002).

P2 receptors are primarily activated by nucleotides and are further divided into the P2X family of ionotropic cation channels and the P2Y family of G-protein-coupled receptors (Burnstock and Kennedy, 1985; Coddou *et al.*, 2011; von Kugelgen and Harden, 2011). The 7 cloned P2X receptors, termed P2X₁₋₇, differ in their rates of inactivation and sensitivity to agonist ATP, with the P2X₇ receptor requiring the highest concentrations of ATP for activation and inactivating most slowly (North, 2002). The 8 mammalian P2Y receptors differ in their optimal agonist, with ATP, ADP, UTP, UDP, and UDP glucose preferentially activating particular receptors (Abbracchio *et al.*, 2006). P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, and P2Y₁₄ receptors are coupled to G_{q/11}, while P2Y₄, P2Y₁₂, and P2Y₁₃ are coupled to G_i and P2Y₁₁ is coupled to G_s. This variety of agonists and effectors ensures that purinergic signaling can affect an enormous number of processes in different tissues.

Figure 1. ATP is released from cells passively through pores like pannexin and connexin hemichannels, or Cl⁻ channels. Alternatively, ATP can be stored in vesicles and released via fusion with the plasma membrane. Extracellular ATP can stimulate ligand-gated P2X receptor ion channels or G-protein-coupled P2Y receptors. ADP, UTP, and UDP processed by extracellular enzymes (E) can also stimulate P2Y receptors. Additional processing by ectonucleotidases, ectonucleosidases, ectonucleotide pyrophosphatases, and ectodiphosphokinases can produce extracellular adenosine. Adenosine can stimulate G-protein-coupled A₁, A_{2A}, A_{2B}, or A₃ receptors to induce additional responses. These receptors can be present on cells neighboring those releasing the ATP for paracrine signaling, or on the same cells under autocrine signaling pathways.

The functional breadth of purinergic signaling is enhanced by the ability of most cell types to release sufficient ATP to stimulate at least some of these receptors. While ATP is used by neurons as a neurotransmitter, purines are also used for local signaling between and among many non-neuronal cell types (Burnstock, 1999; Fitz, 2007). Release is used to convey information to neighboring cells through paracrine signaling or to amplify information through autocrine networks (Lazarowski *et al.*, 2011). Every cell has a supply of cytoplasmic ATP and can release it to communicate. ATP can be released under physiological conditions through fusion of ATP-containing vesicles with the plasma membrane in classic release pathways, or, alternatively, ATP can flow down its electrochemical gradient through permeant pores or channels. The calcium-dependent fusion of vesicles containing permeant channels with the plasma membrane represents a hybrid mechanism and may explain the sometimes contradictory results (Reigada and Mitchell, 2005). Anion channels, connexin hemichannels, and pannexin hemichannels have been implicated as conduits for the passive efflux of ATP out of the cell. Multiple release mechanisms may also exist within the same cell, activated by different stimuli, with release frequently triggered by mechanical perturbations (Xia *et al.*, 2012). Under more terminal pathological conditions, cell lysis releases cellular contents including ATP into the extracellular milieu (Cook and McCleskey, 2002).

Released ATP is rapidly dephosphorylated by a series of extracellular enzymes to ADP, AMP, and adenosine (Fig. 1). The ectonucleotidase (NTPDase) family of enzymes facilitates single or double dephosphorylations, while the ectonucleotide pyrophosphatase/phosphodiesterase (EPP) family converts ATP directly into AMP and pyrophosphate (Goding *et al.*, 2003; Zimmermann, 2006). Additional extracellular enzymes such as ecto-nucleoside diphosphokinases can alter levels by interchanging terminal phosphates between purines and pyrimidines

(Lazarowski *et al.*, 1997). Adenosine is removed from extracellular space following conversion to inosine by adenosine deaminase or uptake by adenosine transporters (Thorn and Jarvis, 1996). Since ATP and adenosine can induce opposite effects on cell survival (M Zhang *et al.*, 2006), and since ectoenzymes can be located close to ATP release sites (Joseph *et al.*, 2003), the regulation of these ectoenzymes can control the effective concentration of agonists and thus the relative effect on a given receptor (Kukulski *et al.*, 2011).

PATHOPHYSIOLOGICAL ROLES OF PURINERGIC SIGNALING

The ubiquitous expression of purinergic receptors, the presence of various release mechanisms, and the plethora of enzymes capable of modifying extracellular purines and pyrimidines attest to their key role in cellular function throughout the body. Since this response involves defending the body and alerting it to potentially damaging insults, purinergic signaling has also evolved to be a central participant in pathophysiology. Of particular focus with regard to dental tissues is the role of purines in inflammation and pain (Fig. 2).

Stimulation of adenosine receptors has multiple effects on inflammatory signaling (Hasko *et al.*, 2008; Ferrero, 2011). The release of IL-10 from macrophages exposed to *E. coli* requires a functioning adenosine A_{2A} receptor (Csoka *et al.*, 2007), while cytokine release from mast cells is influenced by stimulation of the A_{2B} receptors (Auchampach *et al.*, 1997). Agonists for the A₃ adenosine receptor may be effective at reducing inflammation in rheumatoid arthritis (Silverman *et al.*, 2008) and are currently being evaluated in clinical trials. Recent attention also highlights the analgesic actions of adenosine, with both direct stimulation of P1 receptors and elevated extracellular adenosine levels following increasing ectonucleotidase activity of potential benefit (Zylka, 2011). Of particular interest is the demonstration that A₁ adenosine receptors contribute to the analgesic effect of acupuncture (Goldman *et al.*, 2010).

Extracellular ATP has long been known for its pivotal role in inflammation, and responses involve both P2X and P2Y receptors (Ferrero, 2011). Mechanosensitive release of IL-6 into the airway lumen involves ATP release and autostimulation of the P2Y₁ receptor via activation of phospholipase C and p38 MAPK (Douillet *et al.*, 2006). The response to leukotriene E4 in pulmonary cells associated with asthma is dependent upon the P2Y₁₂ receptor (Paruchuri *et al.*, 2009). Activation of the P2X₇ receptor and inflammation is widespread, with stimulation of the receptor leading to the opening of a large-diameter pore in some cases (Lister *et al.*, 2007). The P2X₇ receptor has also been linked to the inflammasome, whereby efflux of K⁺ through the channel triggers the NALP3 inflammasome, resulting in activation of caspase 1 and subsequent cleavage of pro-IL-1β into releasable mature IL-1β (Stutz *et al.*, 2009). The P2X₄ receptor has been implicated in inflammatory pain; activation of the arachidonic acid cascade following treatment with complete Freund's adjuvant was substantially reduced in P2X₄^{-/-} mice; release of PGE₂ from macrophages followed calcium influx through the P2X₄ receptor channel, p38 MAPK phosphorylation, and phospholipase A activation (Ulmann

et al., 2010). This adds to the well-documented involvement of P2X₃ (North, 2004), P2X₇ (Donnelly-Roberts and Jarvis, 2007), and P2Y receptors (Gerevich and Illes, 2004) in the detection and transmission of pain.

ATP AND GINGIVAL CELLS

A substantial role for ATP in the pathophysiology of gingival epithelial cells (GECs) has emerged over the past few years. GECs express mRNA for the P2X₂, P2X₄, P2X₅, P2X₆, and P2X₇ receptors, with the expression of the P2X₇ receptor confirmed immunohistochemically (Yilmaz *et al.*, 2008). Extracellular ATP increased rates of apoptosis, with death prevented by the P2X antagonist oxidized ATP and induced by the P2X₇R agonist BzATP. However, inclusion of *P. gingivalis* with the ATP reduced the apoptosis of GECs. An ecto-nucleoside diphosphate kinase was identified in the *P. gingivalis* genome, and a mutant strain deficient in the secreted enzyme was created. ATP hydrolysis was lower in this mutant strain, consistent with the hydrolysis of extracellular ATP by the nucleoside diphosphate kinase. In contrast to the wild type, this mutant *P. gingivalis* did not inhibit apoptosis of GECs induced by extracellular ATP. The P2X₇R is associated with the activation of a large pore permeable to molecules up to approximately 800 kDa (Surprenant *et al.*, 1996). While ATP increased uptake of the large fluorescent dye Lucifer Yellow into GECs, infection with wild-type *P. gingivalis* inhibited the ATP-evoked LY uptake. However, dye uptake was larger in cells infected with the mutant strain, and recombinant nucleoside diphosphate kinase itself inhibited dye uptake. The authors proposed that infection would normally trigger apoptosis of the GECs by stimulation of the P2X₇ receptor by ATP, but that *P. gingivalis* prevented this apoptosis by reducing extracellular ATP levels with secreted nucleoside diphosphate kinase (Yilmaz *et al.*, 2008).

P. gingivalis interferes with the inflammatory response in GECs by manipulating the activation of the P2X₇R (Yilmaz *et al.*, 2010). While infection of GECs with *P. gingivalis* enhanced expression of IL-1 β message and levels of pro-IL-1 β , secretion of IL-1 β required subsequent exposure to ATP. Caspase 1 was activated after stimulation with ATP, while knockdown of NALP3 mRNA reduced IL-1 β secretion, implicating the inflammasome. NALP3 gene expression was down-regulated after *P. gingivalis* infection. Together, these reports suggest that the

Figure 2. Proposed purinergic signaling pathways underlying the cellular responses associated with P2 receptor activation in various areas of the tooth. The involvement of purinergic receptors in cytokine expression and secretion has been examined in dental pulp cells (P2Y₆-mediated IL-6 release), in gingival fibroblasts (P2X₇-mediated IL-8 release), and in gingival epithelial cells (inflammasome activation via ATP-ligation of P2X₇R and caspase-1 cleavage of pro-IL-1 β). In addition, hemichannels have been identified as a potential conduit for ATP release from periodontal ligament cells, with stimulation of P2Y₁R leading to up-regulation of osteopontin and receptor activator of nuclear factor kappa B ligand (RANKL). Homomeric P2X₃ receptors and/or heteromeric P2X₂/P2X₃ receptors on nerve terminals in the dental pulp transmit nociceptive signals upon stimulation by ATP released from numerous neighboring sources.

P2X₇R usually plays a key role in amplifying the inflammatory signal from GECs to contain infection (Fig. 2). By hydrolyzing extracellular ATP, *P. gingivalis* reduces apoptosis and the secretion of IL-1 β to override the natural defenses of the cells.

Stimulation of the P2X₇R in gingival fibroblasts was also implicated in the up-regulation of IL-8 by the antimicrobial peptide LL-37 (Montrekechachon *et al.*, 2011). LL-37 can attract leukocytes and up-regulate immune-related genes in various cell types (Zanetti, 2004), and levels of LL-37 are perturbed in periodontitis (Puklo *et al.*, 2008). LL-37 up-regulated IL-8 through a pathway sensitive to MEK, p44/42 MAP kinase inhibitors, and P2X antagonists. Inhibition of P2X₇R with antagonist Brilliant Blue G blocked the up-regulation and secretion of IL-8 by LL-37. Whether this activation by LL-37 is mediated by an increase in ATP, or whether LL-37 augments the effect of existing ATP by partitioning into the membrane and modifying the receptor, as found elsewhere, remains to be fully resolved.

(Elssner *et al.*, 2004; Tomasinsig *et al.*, 2008). Regardless, the involvement of LL-37 may represent an important link between enhanced inflammation and purinergic signaling. The recent observation that the P2Y₁₂ antagonist clopidogrel (Plavix) reduced levels of IL-6 and TNF- α in a rat model of periodontal disease supports the pro-inflammatory role of extracellular ATP (Coimbra *et al.*, 2011).

ATP AND PERIODONTAL LIGAMENTS

In periodontal ligaments, ATP has been shown to link mechanical strain to osteopontin and the receptor activator of nuclear factor kappa-B ligand (RANKL; Fig. 2). Mechanical stress led to an up-regulation of osteopontin at mRNA and protein levels in human periodontal ligament cells (PDLs; Wongkhantee *et al.*, 2008). Osteopontin anchors osteoclasts to the surface so they can contribute to bone resorption and may be modified in periodontal disease (Sharma and Pradeep, 2006). The mechanosensitive release of osteopontin release was inhibited by the P2 receptor antagonists suramin and NF449 and specific P2Y₁R receptor antagonist MRS2179, implicating an autocrine stimulation of P2Y₁R by ATP (Wongkhantee *et al.*, 2008). The extracellular medium from stretched cells triggered an increase in osteopontin mRNA and protein expression; this medium had increased levels of ATP, and the increase in osteopontin was prevented by the soluble ecto-ATPase apyrase. The addition of ATP was sufficient to increase osteopontin expression, confirming the role of extracellular ATP in the response.

Extracellular ATP was also implicated in the stimulation of the receptor activator of nuclear factor kappa-B ligand (RANKL) in PDLs following stimulation of the P2Y₁R (Luckprom *et al.*, 2010). The ATP-induced nuclear translocation of factor NF κ B contributed to RANKL up-regulation. Since RANKL is central to osteoclast activation (Theill *et al.*, 2002), the up-regulation of both osteopontin and RANKL suggests that release of ATP following mechanical strain on PDL cells could alter the homeostasis of the periodontium by altering bone resorption.

The mechanism underlying the mechanosensitive efflux of ATP from periodontal ligaments has been proposed to involve hemichannels (Luckprom *et al.*, 2011). ATP release was prevented by the inhibitors carbenoxolone and meclofenamic acid and by connexin 43 knockdown with siRNA, although a contribution through pannexin channels was not ruled out. While an influx of carboxyfluorescein was observed after mechanical strain, consistent with the opening of a large-pore channel, this fluorescence uptake was reduced by meclofenamic acid. Heparin, thapsigargin, and 2-APB also inhibited ATP release, suggesting a role for intracellular calcium. Since the mechanosensitive release of ATP from periodontal ligaments precedes the involvement of OPN and RANKL, discussed above, inhibition of this release through the hemichannels may provide a common target.

Purines also modulate the proliferation of human periodontal ligament cells in culture (Kawase *et al.*, 2007). Prolonged incubation with at least 50 μ M of the slowly hydrolyzable ATP γ S increased caspase 3/7 levels and TUNEL staining. The corresponding reduction in viable cell number was attributed to induction of apoptosis through ATP γ S acting at P2 receptors.

Addition of hydrolyzable ATP did not activate apoptosis; the mechanism by which ATP reduced proliferation remains to be determined, but may involve modulation of cell-cycle proteins. The difference in effects between purine agonists was attributed to rapid hydrolysis of ATP; levels of the ectoATPase NTPDase1 (CD39) on PDL cells were considerable and increased with time in culture.

P2 RECEPTORS AND DENTAL PAIN

Purines make a major contribution to the transmission of pain from the oral cavity (Fig. 2). This involvement has been known for many years; in fact, the demonstration that low concentrations of ATP initiated action potentials in nociceptive neurons from dental pulp was one of the earliest reports linking purinergic signaling and the P2X₃ receptor to pain anywhere in the body (Cook *et al.*, 1997). The currents activated by ATP in these neurons had both a rapidly inactivating and more sustained component (Cook and McCleskey, 1997). Rat neurons express the P2X₃ receptor in sensory endings (Cook *et al.*, 1997). In the pulp obtained from human third molars, neurofilament-positive nerve fibers staining for the P2X₃ receptor were detected in close association with odontoblasts (Alavi *et al.*, 2001). Mechanical distortion of the odontoblasts was thought to lead to ATP release and a painful sensation after stimulation of the P2X₃ receptors on these neurons. P2X₃ receptor staining was also detected in the subodontoblastic plexus of Raschkow and in the pulp center. Expression of the P2X₃ receptor was confirmed in both myelinated and unmyelinated nerve fibers of human tissue (Sharma and Pradeep, 2006). In rats, P2X₃ receptors were detected in both pulp and substance-P-negative cells in the trigeminal ganglion, suggesting a distinct population of pain-sensing afferent fibers (Jiang and Gu, 2002). Antagonists to the homomeric P2X₃ receptor and/or the heteromeric P2X₂/P2X₃ receptors in the trigeminal subnucleus caudalis prevented sensitization following application of mustard oil to the dental pulp, suggesting involvement of the receptor in the amplification of pain signaling through secondary-level circuits (Hu *et al.*, 2002). More recently, the contribution of ATP to dental pain was supported by findings that the P2X₃ receptor agonist α,β -meATP enhanced genioglossus muscle contraction and phosphorylated ERK in the trigeminal spinal subnucleus and caudalis paratrigeminal nucleus, with both responses being inhibited by antagonist TNP-ATP (Adachi *et al.*, 2010). The enhanced expression of P2X₃ receptors on sensory neurons as compared with extracranial neurons suggests that they may provide good targets for the reduction of pain (Ambalavanar and Dessem, 2009). ATP is co-released from the trigeminal ganglia with substance P (Matsuka *et al.*, 2001), while both P2X₂ and P2X₃ receptors are expressed in trigeminal ganglion nerves (Staikopoulos *et al.*, 2007).

Purines also contribute to temporomandibular joint (TMJ) pain. The P2X₃ receptor is expressed in the TMJ (Ichikawa *et al.*, 2004), and injection of the P2X agonist α,β -meATP into the rat TMJ increased behavioral nociceptive responses, while blockage of P2X receptors with antagonist PPADS decreased the inflammatory hyperalgesia accompanying injection of carrageenan into rat TMJ (Oliveira *et al.*, 2005). P2X receptors also contribute to reflexive actions in the TMJ, since the antagonist

α,β -meATP reduced the reflex response (Watanabe *et al.*, 2010). Given the role of purinergic receptors in inflammation (Ferrero, 2011) and the importance of inflammation in the TMJ (Sessle, 2011), it is likely that purines may also contribute here, although there was no change in the expression of the P2Y₁₂ receptor from microglia in rat models of TMJ inflammation (Villa *et al.*, 2010).

While these studies suggest that the development of new P2X₃ antagonists may be of benefit in the treatment of oral pain, the receptor may be involved in the effects of traditional analgesics. For example, eugenol has been used throughout history to reduce oral pain (Markowitz *et al.*, 1992). However, eugenol reversibly inhibited ATP-induced P2X current in both capsaicin-sensitive and capsaicin-insensitive neurons, implying actions independent of Trp channels (Li *et al.*, 2008). The P2X agonist α,β -meATP induced a Ca²⁺ transient in capsaicin-insensitive neurons, which was blocked by eugenol. Eugenol was previously found by this group to antagonize voltage-gated calcium currents in dental afferent neurons (Lee *et al.*, 2005). It is conceivable that ATP released as a result of inflammatory and/or mechanical stimulation would open P2X₃ receptors, and that the corresponding influx of cations would depolarize the membrane sufficiently to activate the voltage-dependent calcium channels, although this remains to be tested directly.

PURINES, INFLAMMATORY SIGNALS, AND BONE LOSS

In addition to stimulating nociceptive receptors, the mechanosensitive release of ATP may also affect cytokine signaling from human dental pulp cells. Dental pulp cells exposed to compressive forces displayed a proportional increase in expression and release of IL-6 (Satrawaha *et al.*, 2011). The IL-6 response was both rapid and sustained, with enhanced IL-6 release maintained for at least 24 hrs. Pharmacological experiments identified the P2Y₆ receptor as mediating the pressure-induced increase in both IL-6 mRNA and release of protein. Incubation with the P2Y₆R agonist UDP, but not UTP or ATP, mimicked the response. UDP levels can be modulated by phosphate transfer by ecto-nucleoside diphosphokinase (Lazarowski *et al.*, 1997). It would thus be interesting to determine whether levels of this enzyme were altered in pulp subject to chronic pressure.

Excessive ATP acting at P2X₄ receptors may contribute to the bone loss found after periodontal surgery (Binderman *et al.*, 2007). Message levels for the receptor were increased rapidly after surgery, with the most intense antigen staining in the connective tissue of marginal gingiva and periodontal ligaments. Extracellular ATP was implicated in the bone loss, since loss was reduced by the soluble ecto-nucleotidase apyrase. Bone loss was prevented by the P2X antagonists Coomassie Brilliant Blue R and G. Coomassie Brilliant Blue has an IC₅₀ at rat P2X₇ receptors of 100 nM and at rat P2X₄ receptors of 100 μ M (Jiang *et al.*, 2000). The ability of Brilliant Blue G to reduce bone loss after periodontal surgery at 100 μ M, but not in the nanomolar range, implicates the P2X₄ receptor. The authors logically concluded that bone loss may be minimized by reducing the ability of extracellular ATP to stimulate the P2X₄ receptor. This remains a

promising avenue for treatment as specific antagonists are developed (Donnelly-Roberts *et al.*, 2008).

ADENOSINE AND SIGNALING IN DENTAL CELLS

Much less attention has thus far been paid to the actions of adenosine on P1 receptors in dental cells than to ATP on P2 receptors. However, several indications suggest this as a promising area for future development. Levels of adenosine on human gingival fibroblasts are regulated by its production by ecto-5-nucleotidase (CD73) and reduction by ecto-adenosine deaminase (Hashikawa *et al.*, 2006), suggesting that regulation of adenosine levels may contribute to the pathology. Human gingival fibroblasts have been reported to express A₁, A_{2A}, and A_{2B}, but not A₃, mRNA (Murakami *et al.*, 2001). It is worth noting that message for the A₃AR is often expressed at low levels, and the receptor has been identified in tissues where the initial detection at the molecular level was missed (X Zhang *et al.*, 2006). Stimulation of the A_{2B}AR in human gingival fibroblasts up-regulated IL-6 production (Murakami *et al.*, 2000), while stimulation of A_{2A}AR receptors increased IL-1 β -induced IL-6 and IL-8 production and the expression of hyaluronate synthase (Murakami *et al.*, 2001). Recently, it was suggested that the level of mRNA coding for adenosine receptors was altered in humans with chronic periodontitis, with levels of the A₁AR and A₃AR levels down, and levels of A_{2A}AR and A_{2B}AR increased (Sun *et al.*, 2011). Given the large number of pharmacological tools available with which to probe adenosine receptors (Gao and Jacobson, 2011), the effects of adenosine receptors are likely to be a topic of considerable interest to dental research in the future.

MANY AVENUES FOR FUTURE RESEARCH

The dental research community's recent interest in purinergic signaling is fitting, given the overlapping focus on inflammation and pain. Several key studies have demonstrated the potential importance of this signaling, and suggest that stimulation by ATP, adenosine, and related compounds will affect many additional facets of dental pathophysiology. Given the number of agonists, antagonists, and modulators under development, it is likely that, in the near future, purinergic receptors will become key pharmacological targets for the treatment of oral pathologies.

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REFERENCES

- Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, *et al.* (2006). International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from

- molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 58:281-341.
- Adachi K, Shimizu K, Hu JW, Suzuki I, Sakagami H, Koshikawa N, *et al.* (2010). Purinergic receptors are involved in tooth-pulp evoked nociceptive behavior and brainstem neuronal activity. *Mol Pain* 6:59.
- Alavi AM, Dubyak GR, Burnstock G (2001). Immunohistochemical evidence for ATP receptors in human dental pulp. *J Dent Res* 80:476-483.
- Ambalavanar R, Dessem D (2009). Emerging peripheral receptor targets for deep-tissue craniofacial pain therapies. *J Dent Res* 88:201-211.
- Auchampach JA, Jin X, Wan TC, Caughey GH, Linden J (1997). Canine mast cell adenosine receptors: cloning and expression of the A3 receptor and evidence that degranulation is mediated by the A2B receptor. *Mol Pharmacol* 52:846-860.
- Binderman I, Bahar H, Jacob-Hirsch J, Zeligson S, Amariglio N, Rechavi G, *et al.* (2007). P2X4 is up-regulated in gingival fibroblasts after periodontal surgery. *J Dent Res* 86:181-185.
- Burnstock G (1978). A basis for distinguishing two types of purinergic receptor. In: *Cell membrane receptors for drugs and hormones: a multidisciplinary approach*. Straub RW, Bolis L, editors. New York, NY: Raven Press, pp. 107-118.
- Burnstock G (1999). Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *J Anat* 194(Pt 3):335-342.
- Burnstock G, Kennedy C (1985). Is there a basis for distinguishing two types of P2-purinoreceptor? *Gen Pharmacol* 16:433-440.
- Burnstock G, Knight GE (2004). Cellular distribution and functions of P2 receptor subtypes in different systems. *Int Rev Cytol* 240:31-304.
- Burnstock G, Ulrich H (2011). Purinergic signaling in embryonic and stem cell development. *Cell Mol Life Sci* 68:1369-1394.
- Burnstock G, Krugel U, Abbracchio MP, Illes P (2011). Purinergic signaling: from normal behaviour to pathological brain function. *Prog Neurobiol* 95:229-274.
- Coddou C, Yan Z, Obsil T, Huidobro-Toro JP, Stojilkovic SS (2011). Activation and regulation of purinergic P2X receptor channels. *Pharmacol Rev* 63:641-683.
- Coimbra LS, Rossa C Jr, Guimaraes MR, Gerlach RF, Muscara MN, Spolidorio DM, *et al.* (2011). Influence of antiplatelet drugs in the pathogenesis of experimental periodontitis and periodontal repair in rats. *J Periodontol* 82:767-777.
- Cook SP, McCleskey EW (1997). Desensitization, recovery and Ca(2+)-dependent modulation of ATP-gated P2X receptors in nociceptors. *Neuropharmacology* 36:1303-1308.
- Cook SP, McCleskey EW (2002). Cell damage excites nociceptors through release of cytosolic ATP. *Pain* 95:41-47.
- Cook SP, Vulchanova L, Hargreaves KM, Elde R, McCleskey EW (1997). Distinct ATP receptors on pain-sensing and stretch-sensing neurons. *Nature* 387:505-508.
- Csoka B, Nemeth ZH, Virag L, Gergely P, Leibovich SJ, Pacher P, *et al.* (2007). A2A adenosine receptors and C/EBPbeta are crucially required for IL-10 production by macrophages exposed to *Escherichia coli*. *Blood* 110:2685-2695.
- Donnelly-Roberts DL, Jarvis MF (2007). Discovery of P2X7 receptor-selective antagonists offers new insights into P2X7 receptor function and indicates a role in chronic pain states. *Br J Pharmacol* 151:571-579.
- Donnelly-Roberts D, McGaraughty S, Shieh CC, Honore P, Jarvis MF (2008). Painful purinergic receptors. *J Pharmacol Exp Ther* 324:409-415.
- Douillet CD, Robinson WP 3rd, Milano PM, Boucher RC, Rich PB (2006). Nucleotides induce IL-6 release from human airway epithelia via P2Y2 and p38 MAPK-dependent pathways. *Am J Physiol Lung Cell Mol Physiol* 291:L734-L746.
- Elsner A, Duncan M, Gavrilin M, Wewers MD (2004). A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release. *J Immunol* 172:4987-4994.
- Ferrero ME (2011). Purinoceptors in inflammation: potential as anti-inflammatory therapeutic targets. *Front Biosci* 16:2172-2186.
- Fitz JG (2007). Regulation of cellular ATP release. *Trans Am Clin Climatol Assoc* 118:199-208.
- Fountain SJ, Burnstock G (2009). An evolutionary history of P2X receptors. *Purinergic Signal* 5:269-272.
- Fredholm BB, AP II, Jacobson KA, Linden J, Muller CE (2011). International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev* 63:1-34.
- Gao ZG, Jacobson KA (2011). Emerging adenosine receptor agonists: an update. *Expert Opin Emerg Drugs* 16:597-602.
- Gerevich Z, Illes P (2004). P2Y receptors and pain transmission. *Purinergic Signal* 1:3-10.
- Goding JW, Grobgen B, Slegers H (2003). Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. *Biochim Biophys Acta* 1638:1-19.
- Goldman N, Chen M, Fujita T, Xu Q, Peng W, Liu W, *et al.* (2010). Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nat Neurosci* 13:883-888.
- Hashikawa T, Takedachi M, Terakura M, Yamada S, Thompson LF, Shimabukuro Y, *et al.* (2006). Activation of adenosine receptor on gingival fibroblasts. *J Dent Res* 85:739-744.
- Hasko G, Linden J, Cronstein B, Pacher P (2008). Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov* 7:759-770.
- Hu B, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ (2002). P2X receptors in trigeminal subnucleus caudalis modulate central sensitization in trigeminal subnucleus oralis. *J Neurophysiol* 88:1614-1624.
- Ichikawa H, Fukunaga T, Jin HW, Fujita M, Takano-Yamamoto T, Sugimoto T (2004). VR1-, VRL-1- and P2X3 receptor-immunoreactive innervation of the rat temporomandibular joint. *Brain Res* 1008:131-136.
- Jiang J, Gu J (2002). Expression of adenosine triphosphate P2X3 receptors in rat molar pulp and trigeminal ganglia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94:622-626.
- Jiang LH, Mackenzie AB, North RA, Surprenant A (2000). Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. *Mol Pharmacol* 58:82-88.
- Joseph SM, Buchakjian MR, Dubyak GR (2003). Colocalization of ATP release sites and ecto-ATPase activity at the extracellular surface of human astrocytes. *J Biol Chem* 278:23331-23342.
- Kawase T, Okuda K, Yoshie H (2007). Extracellular ATP and ATPgammaS suppress the proliferation of human periodontal ligament cells by different mechanisms. *J Periodontol* 78:748-756.
- Klinger M, Freissmuth M, Nanoff C (2002). Adenosine receptors: G protein-mediated signalling and the role of accessory proteins. *Cell Signal* 14:99-108.
- Kukulski F, Levesque SA, Seigny J (2011). Impact of ectoenzymes on P2 and P1 receptor signaling. *Adv Pharmacol* 61:263-299.
- Lazarowski ER, Homolya L, Boucher RC, Harden TK (1997). Identification of an ecto-nucleoside diphosphokinase and its contribution to interconversion of P2 receptor agonists. *J Biol Chem* 272:20402-20407.
- Lazarowski ER, Sesma JI, Seminario-Vidal L, Kreda SM (2011). Molecular mechanisms of purine and pyrimidine nucleotide release. *Adv Pharmacol* 61:221-261.
- Lee MH, Yeon KY, Park CK, Li HY, Fang Z, Kim MS, *et al.* (2005). Eugenol inhibits calcium currents in dental afferent neurons. *J Dent Res* 84:848-851.
- Li HY, Lee BK, Kim JS, Jung SJ, Oh SB (2008). Eugenol inhibits ATP-induced P2X currents in trigeminal ganglion neurons. *Korean J Physiol Pharmacol* 12:315-321.
- Lister MF, Sharkey J, Sawatzky DA, Hodgkiss JP, Davidson DJ, Rossi AG, *et al.* (2007). The role of the purinergic P2X7 receptor in inflammation. *J Inflamm (Lond)* 4:5.
- Luckprom P, Wongkhantee S, Yongchaitrakul T, Pavasant P (2010). Adenosine triphosphate stimulates RANKL expression through P2Y1 receptor-cyclo-oxygenase-dependent pathway in human periodontal ligament cells. *J Periodontol Res* 45:404-411.
- Luckprom P, Kanjanamekanant K, Pavasant P (2011). Role of connexin43 hemichannels in mechanical stress-induced ATP release in human periodontal ligament cells. *J Periodontol Res* 46:607-615.
- Markowitz K, Moynihan M, Liu M, Kim S (1992). Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. *Oral Surg Oral Med Oral Pathol* 73:729-737.

- Matsuka Y, Neubert JK, Maidment NT, Spigelman I (2001). Concurrent release of ATP and substance P within guinea pig trigeminal ganglia in vivo. *Brain Res* 915:248-255.
- Montreekachon P, Chotjumlong P, Bolscher JG, Nazmi K, Reutrakul V, Krisanaprakornkit S (2011). Involvement of P2X(7) purinergic receptor and MEK1/2 in interleukin-8 up-regulation by LL-37 in human gingival fibroblasts. *J Periodontol Res* 46:327-337.
- Murakami S, Terakura M, Kamatani T, Hashikawa T, Saho T, Shimabukuro Y, et al. (2000). Adenosine regulates the production of interleukin-6 by human gingival fibroblasts via cyclic AMP/protein kinase A pathway. *J Periodontol Res* 35:93-101.
- Murakami S, Hashikawa T, Saho T, Takedachi M, Nozaki T, Shimabukuro Y, et al. (2001). Adenosine regulates the IL-1 beta-induced cellular functions of human gingival fibroblasts. *Int Immunol* 13:1533-1540.
- North RA (2002). Molecular physiology of P2X receptors. *Physiol Rev* 82:1013-1067.
- North RA (2004). P2X3 receptors and peripheral pain mechanisms. *J Physiol* 554(Pt 2):301-308.
- Novak I (2011). Purinergic signalling in epithelial ion transport: regulation of secretion and absorption. *Acta Physiol (Oxf)* 202:501-522.
- Ohta A, Sitkovsky M (2009). The adenosinergic immunomodulatory drugs. *Curr Opin Pharmacol* 9:501-506.
- Oliveira MC, Parada CA, Veiga MC, Rodrigues LR, Barros SP, Tambeli CH (2005). Evidence for the involvement of endogenous ATP and P2X receptors in TMJ pain. *Eur J Pain* 9:87-93.
- Orriss IR, Burnstock G, Arnett TR (2010). Purinergic signalling and bone remodelling. *Curr Opin Pharmacol* 10:322-330.
- Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, et al. (2009). Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. *J Exp Med* 206:2543-2555.
- Puklo M, Guentsch A, Hiemstra PS, Eick S, Potempa J (2008). Analysis of neutrophil-derived antimicrobial peptides in gingival crevicular fluid suggests importance of cathelicidin LL-37 in the innate immune response against periodontogenic bacteria. *Oral Microbiol Immunol* 23:328-335.
- Reigada D, Mitchell CH (2005). Release of ATP from RPE cells involves both CFTR and vesicular transport. *Am J Physiol Cell Physiol* 288:C132-C140.
- Satrawaha S, Wongkhantee S, Pavasant P, Sumrejkanchanakij P (2011). Pressure induces interleukin-6 expression via the P2Y6 receptor in human dental pulp cells. *Arch Oral Biol* 56:1230-1237.
- Sessle BJ (2011). Peripheral and central mechanisms of orofacial inflammatory pain. *Int Rev Neurobiol* 97:179-206.
- Sharma CG, Pradeep AR (2006). Gingival crevicular fluid osteopontin levels in periodontal health and disease. *J Periodontol* 77:1674-1680.
- Silverman MH, Strand V, Markovits D, Nahir M, Reitblat T, Molad Y, et al. (2008). Clinical evidence for utilization of the A3 adenosine receptor as a target to treat rheumatoid arthritis: data from a phase II clinical trial. *J Rheumatol* 35:41-48.
- Staikopoulos V, Sessle BJ, Furness JB, Jennings EA (2007). Localization of P2X2 and P2X3 receptors in rat trigeminal ganglion neurons. *Neuroscience* 144:208-216.
- Stutz A, Golenbock DT, Latz E (2009). Inflammasomes: too big to miss. *J Clin Invest* 119:3502-3511.
- Sun CX, Wall NR, Angelov N, Ririe C, Chen JW, Boskovic DS, et al. (2011). Changes in mRNA expression of adenosine receptors in human chronic periodontitis. *Chin J Dent Res* 14:113-120.
- Surprenant A, Rassendren F, Kawashima E, North RA, Buell G (1996). The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* 272:735-738.
- Theill LE, Boyle WJ, Penninger JM (2002). RANK-L and RANK: T cells, bone loss, and mammalian evolution. *Annu Rev Immunol* 20:795-823.
- Thorn JA, Jarvis SM (1996). Adenosine transporters. *Gen Pharmacol* 27:613-620.
- Tomasinsig L, Pizzirani C, Skerlavaj B, Pellegatti P, Gulinelli S, Tossi A, et al. (2008). The human cathelicidin LL-37 modulates the activities of the P2X7 receptor in a structure-dependent manner. *J Biol Chem* 283:30471-30481.
- Ulmann L, Hirbec H, Rassendren F (2010). P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. *EMBO J* 29:2290-2300.
- Villa G, Ceruti S, Zanardelli M, Magni G, Jasmin L, Ohara PT, et al. (2010). Temporomandibular joint inflammation activates glial and immune cells in both the trigeminal ganglia and in the spinal trigeminal nucleus. *Mol Pain* 6:89.
- von Kugelgen I, Harden TK (2011). Molecular pharmacology, physiology, and structure of the P2Y receptors. *Adv Pharmacol* 61:373-415.
- Watanabe T, Tsuboi Y, Sessle BJ, Iwata K, Hu JW (2010). P2X and NMDA receptor involvement in temporomandibular joint-evoked reflex activity in rat jaw muscles. *Brain Res* 1346:83-91.
- Wongkhantee S, Yongchaitrakul T, Pavasant P (2008). Mechanical stress induces osteopontin via ATP/P2Y1 in periodontal cells. *J Dent Res* 87:564-568.
- Xia J, Lim JC, Lu W, Beckel JM, Macarak EJ, Laties AM, et al. (2012). Neurons respond directly to mechanical deformation with pannexin-mediated ATP release and autostimulation of P2X7 receptors. *J Physiol* 590(Pt 10):2285-2304.
- Yilmaz O, Yao L, Maeda K, Rose TM, Lewis EL, Duman M, et al. (2008). ATP scavenging by the intracellular pathogen *Porphyromonas gingivalis* inhibits P2X7-mediated host-cell apoptosis. *Cell Microbiol* 10:863-875.
- Yilmaz O, Sater AA, Yao L, Koutouzis T, Pettengill M, Ojcius DM (2010). ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by *Porphyromonas gingivalis*. *Cell Microbiol* 12:188-198.
- Zanetti M (2004). Cathelicidins, multifunctional peptides of the innate immunity. *J Leukoc Biol* 75:39-48.
- Zhang M, Budak MT, Lu W, Khurana TS, Zhang X, Laties AM, et al. (2006). Identification of the A3 adenosine receptor in rat retinal ganglion cells. *Mol Vis* 12:937-948.
- Zhang X, Zhang M, Laties AM, Mitchell CH (2006). Balance of purines may determine life or death of retinal ganglion cells as A3 adenosine receptors prevent loss following P2X7 receptor stimulation. *J Neurochem* 98:566-575.
- Zimmermann H (2006). Ectonucleotidases in the nervous system. *Novartis Found Symp* 276:113-128.
- Zylka MJ (2011). Pain-relieving prospects for adenosine receptors and ectonucleotidases. *Trends Mol Med* 17:188-196.