ELSEVIER

European Journal of Pharmacology 375 (1999) 203–210

ejp

www.elsevier.nl/locate/ejphar

Review

Connections between P_2 purinoceptors and capsaicin-sensitive afferents in the intestine and other tissues

Loránd Barthó [a,*], László Lénárd Jr. [a], Zsófia Lázár [a], Carlo A. Maggi [b]

[a] *Department of Pharmacology and Pharmacotherapy, University Medical School of Pécs, Szigeti u. 12. H-7643 Pécs, Hungary*
[b] *Research Division, A. Menarini Pharmaceuticals, Firenze, Italy*

Accepted 30 April 1999

Abstract

Relations between P_2 purinoceptors and capsaicin-sensitive sensory neurons include an excitatory action of P_2 purinoceptor agonists on spinal afferent neurons, as well as release of ATP from afferents at their central and peripheral endings, and a possible participation of ATP in nociception and/or in 'local efferent' responses mediated by sensory nerves at the periphery. The present paper briefly summarizes available evidence on these interrelations. Ample evidence shows that ATP and other P_2 purinoceptor agonists can activate primary afferent neurons, through $P2X_3$ receptors and probably other purinoceptors as well, but evidence for an involvement of P_2 purinoceptors in nociception or in 'local efferent' responses due to activation of primary afferents is, at best, circumstantial. The possibility is also dealt with that P_2 purinoceptor activation may cause small intestinal contraction with the mediation of capsaicin-sensitive sensory neurons and that the motor response to capsaicin in this tissue may involve the release of a P_2 purinoceptor stimulant from sensory nerves. Our data show that cholinergic contractions of the guinea-pig ileum in response to the P_2 purinoceptor agonist α,β-methylene ATP (α,β-meATP) are blocked by atropine, but not by in vitro capsaicin pretreatment (which completely blocks the contractile action of capsaicin). Cholinergic ileum contractions due to capsaicin (2 μM) are insensitive to suramin (a P_2 purinoceptor antagonist; 100 μM). In the presence of antagonists acting at tachykinin NK_1 and NK_2 receptors, however, suramin (100 μM) causes a significant inhibition of the capsaicin-evoked contraction. These data indicate that capsaicin-sensitive nerves are not involved in the excitatory effect of α,β-methylene ATP on myenteric neurons. On the other hand, ATP is probably involved in the 'non-tachykininergic' component of the capsaicin-induced excitatory response of the small intestine. ATP may originate from sensory neurons and probably acts as activator of myenteric nerves.

Keywords: Capsaicin; P_2 purinoceptor; ATP; Sensory neuron; Myenteric plexus

1. Introduction

In the past 30 years conclusive evidence has accumulated that purine nucleotides, in addition to their important intracellular functions, also play a role as extracellular neurotransmitters and/or modulators (see Burnstock, 1972 for the first systematic formulation of the hypothesis). They act on P_1 and P_2 purinoceptors, P_1 receptors being most important in mediating the modulatory effects of adenosine (usually negative), while P_2 purinoceptors mediate the actions of ATP and related substances. Of the two main types of P_2 purinoceptors (seven of each have been recognised so far) P_{2X} receptors are ligand-gated ion channels, while P_{2Y} receptors are linked to G-proteins. ATP probably functions as fast neurotransmitter and co-transmitter in neuro-neuronal communication, as well as in transmission between nerves and vascular and non-vascular smooth muscle and possibly other cell types as well. It can convey both excitatory and inhibitory messages. Examples of co-transmission include noradrenaline with ATP in sympathetic nerves supplying vessels, the vas deferens and intestinal circular muscle (Venkova and Krier, 1993), acetylcholine with ATP in parasympathetic nerves of the urinary bladder and others. ATP collaborates with vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase activating polypeptide (PACAP), nitric oxide (NO) or tachykinins, respectively, in relaxing the ileal or colonic circular muscle or the taenia caeci or contracting the bile duct of the guinea-pig (Crist et al., 1992; Zagorodnyuk et al., 1996; Barthó et al., 1998; Patacchini et al., 1998). Various aspects of purinergic transmission have been re-

* Corresponding author. Tel.: +36-72-324122; Fax: +36-72-211761; E-mail: lbartho@apacs.pote.hu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00253-8

viewed by Burnstock (1972, 1990, 1997), Vizi (1979); Sawynok and Sweeney (1989); Illés and Norenberg (1993); Fredholm et al. (1994); Humphrey et al. (1995); North and Barnard (1997) and others. A most interesting aspect of purinergic transmission is P_2 purinoceptor-sensory neuron relations.

2. Connections between P_2 purinoceptors and capsaicin-sensitive afferents: a brief overview

P_2 purinoceptors and capsaicin-sensitive primary afferent neurons may be interrelated in two possible ways (see Kennedy and Leff, 1995; Burnstock, 1996; Burnstock and Wood, 1996). First, exogenous and endogenous purinoceptor agonists may stimulate peripheral and/or central endings and somata of sensory nerves that are equipped with P_2 purinoceptors. This leads to afferent impulse generation, as well as release (at both ends of afferent neurons) of sensory neurotransmitters. Sources of endogenous ATP might be, among others, degrading cells (such as tumor cells), and sympathetic neurons. Second, capsaicin-sensitive sensory neurons contain and release endogenous purinoceptor agonists that may contribute to both nociceptive neurotransmission and 'local efferent' responses (see Szolcsányi, 1984; Holzer, 1988 for reviews; Geppetti and Holzer, 1996 for monograph) brought about by the release of biologically active substances from these neurons in the skin, mucous membranes, and visceral organs, including the gastrointestinal tract (Barthó and Holzer, 1985; Holzer and Barthó, 1996).

2.1. P_2 purinoceptors on primary afferents

A number of pharmacological studies indicate that ATP and related substances are able to activate sensory neurons. These substances cause pain when applied to the blister base in humans (Bleehen and Keele, 1977). Subplantar injection of α,β-meATP and, less potently, ATP induces nociceptive behavioural responses in the rat (Bland-Ward and Humphrey, 1997), with an involvement of P_{2X}-like receptors. ATP also shows excitation in the tail-spinal cord preparation (Trezise and Humphrey, 1996) and activates articular nerve afferents in rats (Dowd et al., 1998); pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), a P_2 purinoceptor antagonist (Lambrecht et al., 1992) abolishes the action of ATP and α,β-meATP. Adjuvant-induced arthritis enhances or induces spontaneous activity in part of the fibres tested, but no evidence has been found (by using PPADS as antagonist) for an involvement of endogenous ATP in the arthritis-induced sensitization (Dowd et al., 1998). The number of the appropriate units found (showing both spontaneous activity and sensitivity to exogenous ATP) was, however, rather low in this study; so the concept of ATP involvement in afferent sensitization probably needs further testing in various experimental conditions.

Electrophysiological experiments with intracellular recording show that ATP depolarizes sensory structures like dorsal root ganglion cells (Jahr and Jessell, 1983; Krishtal et al., 1983; Bean, 1990; Bean et al., 1990; Bouvier et al., 1991; Li et al., 1997), vagal sensory (nodose) ganglion cells (Krishtal et al., 1983, 1988; Li et al., 1993; Khakh et al., 1995; Robertson et al., 1996) and cells of the trigeminal ganglion (Krishtal et al., 1983). A depolarizing effect of ATP on the isolated vagus nerve (Trezise et al., 1994) may also reflect an effect on sensory fibres, which constitute the majority of vagal fibres. In most studies, a non-selective cation channel is responsible for the excitation. Although the characteristics of the receptors involved are not uniform, they all can be categorized as P_2 purinoceptors. RNAs for all six already cloned P_{2X} receptor subunits are expressed in dorsal root, nodose and trigeminal ganglia (Collo et al., 1996; Vulchanova et al., 1997). An intriguing development in the field is that, in the rat, the presence of the mRNA for the $P2X_3$ subunit is restricted to small-diameter afferents of the dorsal roots and nodose ganglia (Chen et al., 1995; Lewis et al., 1995). In the dorsal root ganglion, the expression of the $P2X_3$ mRNA is strongly decreased by neonatal capsaicin desensitization, indicating that capsaicin-sensitive sensory neurons are encountered (Chen et al., 1995). Lewis et al. (1995) could identify the genetic message for the $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_4$ subunits of the P_{2X} receptor in the rat dorsal root ganglion. Co-expression of cation channels consisting of combinations of these subtypes in transfected human embryonic kidney (HEK293) cells showed that a heteromultimer of $P2X_2$ and $P2X_3$ subunits is readily formed and most closely resembles characteristics (in terms of agonist potencies, desensitization and antagonist effects) of natural P_2 purinoceptors in rat dorsal root ganglia.

2.2. ATP release from afferents

Spinal afferent neurons are known to contain and release ATP, as shown by the classical works of Holton and Holton (1954), Holton (1959) and it was thought at a time that ATP mediates antidromic vasodilatation (for which calcitonin gene-related peptide (CGRP) is a more likely candidate these days). The release of ATP was confirmed by later work (Sweeney et al., 1989). Also, the abundant distribution of P_2 purinoceptors in the spinal dorsal horn (Bo and Burnstock, 1994; Brake et al., 1994; Valera et al., 1994; Collo et al., 1996; Soto et al., 1996; Burnstock, 1997), as well as a potent excitatory action of ATP (but not AMP and adenosine) on dorsal horn neurons (Jahr and Jessell, 1983; Fyffe and Perl, 1984; Salter and Henry, 1985) or neurons of the trigeminal sensory nucleus (Salt and Hill, 1983) is suggestive of ATP release from primary afferents. The finding that sensory transmitters such as substance P and CGRP facilitate the effect of ATP on the $P2X_2$ receptor expressed in *Xenopus* oocytes (Wildman et

al., 1997) opens up possible new ways of transmitter interaction in afferent transmission. It should be noted, however, that more data indicate the possible involvement of purinergic transmission in processing of non-noxious than of noxious stimuli (among others Fyffe and Perl, 1984). ATP, released by K^+ from rat dorsal horn synaptosomes may partly originate from terminals of large-, but not capsaicin-sensitive small-diameter fibres (Sawynok et al., 1993). Yet, an antinociceptive effect of the P_2 purinoceptor antagonists suramin (Ho et al., 1992; Driessen et al., 1994), Evans-blue, trypan blue, reactive blue 2, but not PPADS has been reported (Driessen et al., 1994).

There is little direct evidence for the involvement of ATP in the 'local efferent' responses due to activation of peripheral terminals of primary afferents. An intriguing possibility is that ATP might cause an endothelial release of NO by a process not involving the NO synthase (Kakuyama et al., 1998) and thereby mediate NANC relaxation of the mesenteric vascular bed in response to electrical stimulation. In this study, the vasorelaxation due to electrical field stimulation was inhibited by the P_{2Y} purinoceptor antagonist basilene blue and further reduced by a CGRP receptor antagonist. The response studied was, however, only partly capsaicin-sensitive.

Clearly, in spite of the promising data available, the exact relations between nociceptive afferents and P_2 purinoceptors are far from understood; especially more potent and selective antagonists are needed before purinoceptor involvement in physiological/pathophysiological processes can be assessed. This area is also of great potential pharmacological interest, in that it offers the possibility of developing novel analgesics, anti-inflammatory agents, or drugs for more specific purposes, such as cystitis-associated bladder dysmotility (see Burnstock, 1997).

3. P_2 purinoceptors and the small intestine

There is a huge number of enteric neurons with differing morphological, neurochemical and functional characteristics (Furness and Costa, 1980; Costa and Brookes, 1994; Wood, 1994). Pioneering observations indicating that adenine nucleotides may be neurotransmitters and/or neuromodulators in the digestive tract have been reviewed by Burnstock (1972). Different parts of the digestive tracts of various species possess many kinds of purinergic receptors. The P_{2Y} purinoceptor may predominate on the smooth muscle cells (see among others Lambrecht, 1996). Neuronal purinoceptors may be of the P_{2X} type or may poorly fit into the current classification (see below); they can be located on excitatory and inhibitory motoneurons and probably on interneurons as well. It follows from this that purinoceptor antagonists can only be critically used for identifying the biological functions (e.g., the role played in motility) by endogenous ATP and related substances. Also, ATP can excite more than one purinoceptor subtype and its breakdown products make the picture even more complicated. Effectiveness of the antagonist used against exogenous ATP (not only against more receptor specific ATP analogues), as well as specificity of action of the antagonist(s) need to be proven before conclusions concerning the roles played by ATP are drawn; such data obtained at one type of preparations cannot be automatically transferred to other types of gastrointestinal tissue.

In the guinea-pig ileum ATP and related substances have multiple actions and there are some contradictions in the data available. ATP causes contraction (Kamikawa et al., 1977); no detailed pharmacological analysis has been performed in this study, but it was noted that indomethacin reduced the response. The authors themselves, however, noted the limits of the specificity of the action of indomethacin (10–20 μM). It is worth noting that in a specificity study in the guinea-pig ileum, comparing the inhibitory effect of indomethacin on the arachidonic acid-evoked contractions with the depression of the cholinergic 'twitch' contraction to field stimulation 1–3 μM of indomethacin was found to be the safe and effective concentration range (Barthó, 1978).

Multiple effects of ATP, ADP, β,γ-methylene ATP and α,β-meATP in the guinea-pig ileum have been described (Moody and Burnstock, 1982). All these agents caused the longitudinal muscle to contract, however, the effect of ATP was resistant to atropine or tetrodotoxin, while that of α,β-meATP was apparently mediated by cholinergic motoneurons of the myenteric plexus (see also Kennedy and Humphrey, 1994; Matsuo et al., 1997). At the same time, an inhibition of the twitch response to electrical field stimulation was found with ATP, β,γ-methylene ATP and adenosine, an effect inhibited by the P_1-purinoceptor antagonist theophylline. It was concluded that ATP acted through its breakdown products on this receptor. α,β-meATP slightly inhibited the twitch at lower but potentiated it at higher concentrations. Wiklund and Gustafsson (1986, 1988a,b) and Wiklund et al. (1985) confirmed the contractile action of adenine nucleotides (through a P_2-like receptor on the smooth muscle) in the guinea-pig ileum, as well as the marked inhibition of the electrically-evoked twitch response by ATP and ADP. The contractile action of α,β-derivatives such as α,β-meATP was also confirmed but, at variance to what has been reported earlier (Moody and Burnstock, 1982) it was found resistant to tetrodotoxin and hyoscine, but dependent on prostaglandin production as shown by a complete blockade by indomethacin (3 μM) (Wiklund and Gustafsson, 1988b). The inhibitory action of ATP and ADP (to a lesser extent also α,β-meATP) on the electrically-evoked cholinergic contractions seems to be a P_1-purinoceptor mediated effect (blocked by 8-*p*-sulphophenyltheophylline); evidence is presented that ATP and ADP act by themselves on these receptors, not via their degradation to AMP and adenosine. From these studies it seems probable that the

purinoceptor(s) involved is not easy to categorize. Analyzing the effect of a series of agonists (including, among others, ATP, α,β-meATP, 2-methylthio ATP and β,γ-methylene ATP) and some antagonists (suramin and reactive blue 2 or cibacron blue, the latter for P_{2Y} receptors) led Kennedy and Humphrey (1994) to conclude that receptors on cholinergic nerves show some characteristics of the P_{2X} purinoceptor while those on the longitudinal muscle more closely resemble P_{2Y} purinoceptors (even if they are not inhibited by reactive blue 2), but a final categorization needs further work. Matsuo et al. (1997) found that the contractile effect of α,β-meATP was antagonized by suramin, but not by PPADS. This latter finding with PPADS is at variance with our own data (Barthó et al., 1997).

Neurochemical experiments have shown both enhancement (Sperlágh and Vizi, 1991) and inhibition of the stimulated acetylcholine release by α,β-meATP in the guinea-pig ileum (Katsuragi et al., 1993). The latter effect was, however, blocked by theophylline, and ATP (released from the target tissue) was proposed as mediator (see also Matsuo et al., 1997).

Specific antagonists make it possible to study the involvement of endogenous ligands of P_2 purinoceptors (ATP being the main candidate) in motor responses evoked by various stimuli. Such an involvement in the electrically-evoked cholinergic contraction of the guinea-pig ileum longitudinal muscle was proposed by Barthó et al. (1997). In this study, it was confirmed that α,β-meATP (10 μM) causes cholinergic contraction. This response, but not that to exogenous acetylcholine, is strongly inhibited by PPADS (30 μM) or suramin (100 μM). Both P_2 purinoceptor antagonists cause an approximately 30% inhibition of electrically-evoked cholinergic contractions, which seems to be a 'ceiling' effect, since the inhibition will not increase if suramin is added to PPADS or vice versa, or if the concentration of PPADS is doubled. PPADS also antagonizes the complex motor effects of ATP in the longitudinal muscle of the guinea-pig ileum (L. Barthó, under preparation). Although the extent of the proposed modulation in the above study is not too large, it might represent a pharmacological correlate of P_2 purinoceptor-mediated fast depolarization evoked by preganglionic electrical stimulation demonstrated in the guinea-pig myenteric plexus by electrophysiological studies (Galligan and Bertrand, 1994; Zhou and Galligan, 1996; LePard et al., 1997). Pharmacological analysis of membrane responses to exogenous nucleotides (Barajas-Lopez et al., 1993, 1996; Kimball et al., 1996; Zhou and Galligan, 1996) on guinea-pig myenteric neurons seems to indicate that the fast depolarization and intracellular free Ca^{2+} accumulation, evoked by these agonists is mediated through more than one type of receptor (P_1-like, P_{2X}-like and P_{2Y}-like) with partly unusual properties. It seems that in electrophysiological studies PPADS is probably a more specific antagonist than suramin against the excitatory action of ATP on the P_{2X}-like receptor (cf. Humphrey et al., 1995). Thus, ATP could function as an alternative to acetylcholine in myenteric neuro-neuronal transmission. Yet, our data (in preparation) show that the 'hexamethonium-resistant peristaltic reflex' (Barthó et al., 1987a, 1989; Holzer et al., 1998) is not inhibited by suramin or PPADS.

P_1 purinoceptors mediate negative modulation in the intestine. Exogenous and endogenous stimulants of P_1 purinoceptors inhibit the release of acetylcholine (Vizi, 1979) and of substance P from the myenteric plexus (Barthó et al., 1985; Christofi et al., 1990; Moneta et al., 1997).

4. Capsaicin-sensitive innervation of the small intestine

The sensory stimulant drug capsaicin causes a nerve-mediated contraction in the guinea-pig ileum (Barthó and Szolcsányi, 1978). There is ample evidence that this drug stimulates endings of extrinsic (spinal) afferent nerves in the gut wall which in turn activate myenteric neurons by releasing chemical mediators (Barthó and Szolcsányi, 1978; Szolcsányi and Barthó, 1978; Barthó et al., 1982). There is evidence (by the use of substance P tachyphylaxis) for the involvement of endogenous tachykinins in the activating effect of capsaicin on myenteric neurons (Barthó et al., 1982; Chahl, 1982; see Barthó and Holzer, 1985; Holzer and Barthó, 1996 for reviews). The exact nature of the transmitters that are released from sensory nerve endings and activate myenteric neurons is, however, not clear, all the more because the expectation that neuronal tachykinin NK_3 receptors may be responsible for the excitation of myenteric neurons has not been supported by recent findings (Patacchini et al., 1995). A combined inhibition of tachykinin NK_1 and NK_3 receptors by the antagonists [O-Pro^9, (Spiro-γ-lactam)Leu^{10}, Trp^{11}]physalaemin (1–11) (GR 82 334; Hagan et al., 1991) and (*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl) propyl) -4-phenylpiperidine-4-yl)-*N*-methylacetamide (SR 142 801; Emonds-Alt et al., 1995), however, caused a significant inhibition in the amplitude of the capsaicin-induced contraction (Barthó et al., 1999). We assume that, similarly to the tachykinin NK_3 receptors (Laufer et al., 1985; Croci et al., 1995; Giuliani and Maggi, 1995; Holzer and Holzer-Petsche, 1997a,b) also the tachykinin NK_1 receptors involved are localized on myenteric neurons (cf. Portbury et al., 1996), since the contractile effect of capsaicin is abolished by tetrodotoxin (Barthó and Szolcsányi, 1978; Barthó et al., 1982, 1999), whereas the release of neuropeptides and probably other biologically active substances by capsaicin is resistant to tetrodotoxin (see Barthó and Holzer, 1985); the opening of the non-specific cation channel associated with the vanilloid receptor (Caterina et al., 1997) is independent of tetrodotoxin-sensitive Na^+ channels. The activation of myenteric neurons by afferent nerve endings seems not to involve cholecystokinin-like peptides or CGRP (Barthó et al., 1987b, 1993), two stimu-

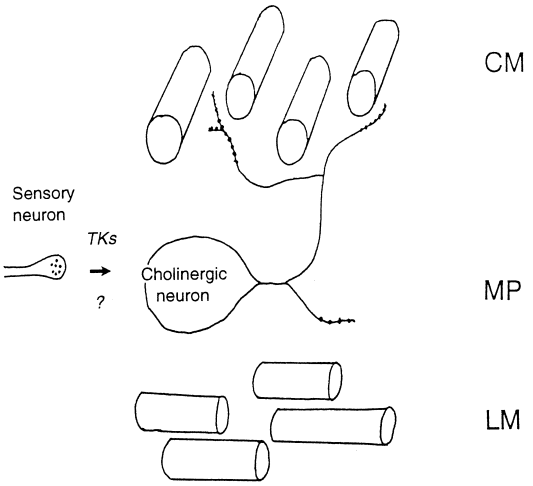

Fig. 1. Activation of a myenteric cholinergic motoneuron by a nearby capsaicin-sensitive sensory neuron. There is strong evidence for the participation of tachykinins (TKs), but part of the transmitters involved is unknown. CM: circular muscle, MP: myenteric plexus, LM: longitudinal muscle.

lants of myenteric neurons that may appear in primary afferents. Although there may be morphological basis for acetylcholine being released from primary afferents themselves (Sann et al., 1995), the blocking action of tetrodotoxin and the lack of inhibitory effect of hexamethonium, respectively, do not favour the assumption that acetylcholine coming from sensory nerves contributes to the excitation of the smooth muscle directly or to the activation of myenteric neurons.Consequently, part of the neurotransmitters utilized by sensory neurons is still unknown (Fig. 1). Thus, if ATP is released in sufficient quantities from primary afferents, an activation of P_2 purinoceptors might be involved in the activation of myenteric ganglion cells in the mechanism of the capsaicin-evoked motor response.

5. Testing of capsaicin-sensitive afferent-P_2 purinoceptor interactions in the guinea-pig ileum

As mentioned above, both capsaicin and α,β-meATP cause acetylcholine-mediated longitudinal contractions in the guinea-pig ileum (see Barthó and Holzer, 1985; Holzer and Barthó, 1996; Barthó et al., 1997). The effect of α,β-meATP is inhibited by suramin or PPADS. In conventional organ bath experiments (see Barthó et al., 1997; Barthó et al., 1999 for methodological details) we tested the possibility that (1) α,β-meATP may stimulate sensory nerves that in turn activate myenteric cholinergic neurons; (2) ATP, released from primary afferents may participate in the contractile effect of capsaicin.

Ad (1). A functional blockade of capsaicin-sensitive neurons in the gut wall can be achieved by an in vitro pretreatment with capsaicin (10 μM for 15 min). This blockade is characterized by an irresponsiveness of the intestine to the contractile effect of capsaicin and of activation of capsaicin-sensitive nerves by mesenteric nerve stimulation (Barthó and Szolcsányi, 1978; Szolcsányi and Barthó, 1978). In the present experiments, such a pretreatment (followed by a 45-min washout period) failed to significantly affect the contractile action of α,β-meATP (3 or 10 μM) (Table 1). These contractions due to α,β-meATP (3 and 10 μM) were fully blocked by atropine (1 μM) (Table 1).

Ad (2). Suramin (100 μM) had no effect on the contractile action of capsaicin (2 μM) in the absence of tachykinin antagonists ($n = 8$, data not shown). However, in the presence of the tachykinin NK_1 receptor antagonist GR 82 334 (3 μM) and the tachykinin NK_3 receptor antagonist SR 142 801 (0.1 μM) *plus* suramin (100 μM) the excitatory motor response to capsaicin (2 μM) was significantly smaller than that in the presence of the tachykinin receptor antagonists only (Table 1).

These data speak against a sensory stimulant action of α,β-meATP being involved in its activating effect on myenteric neurons. It is probable that the purinoceptors involved are located on the myenteric neurons themselves. Conversely, ATP release from primary afferent neurons cannot account for the activation of myenteric cholinergic neurons in the course of the excitatory action of capsaicin if tachykinin receptors are not blocked. The inhibitory action of suramin in the presence of tachykinin receptor antagonists, however, point to the participation of P_2 purinoceptors in the 'non-tachykininergic' component of the contractile effect of capsaicin in the ileum, probably as activators of myenteric neurons.

Table 1

Pretreatment	α,β-meATP 3 μM	α,β-meATP 10 μM	*n*
–	42.3 ± 2.2	51.6 ± 1.7	(11)
Capsaicin (10 μM)	40.3 ± 2.7	52.3 ± 3.5	(11)
–	40.1 ± 5.2	52.8 ± 3.4	(6)
Atropine (1μM)	2.3 ± 0.3[a]	2.3 ± 0.6[a]	(6)
	Capsaicin (2 μM)		
GR 82 334 (3 μM) + SR 142 801 (0.1 μM)	44.6 ± 2.2		(9)
Suramin (100 μM) + GR 82 334 (3 μM) + SR 142 801 (0.1 μM)	34.4 ± 3.0[a]		(9)

Contractions of the guinea-pig ileum expressed as percent of the maximal longitudinal spasm due to acetylcholine (10 μM). Influence of various drugs on responses to α,β-meATP and capsaicin. Significant differences, compared to the respective control group, ([a]) $P < 0.05$ (Wilcoxon's signed rank test).

6. Concluding remarks

Primary afferents are equipped with P_2 purinoceptors that may be involved in nociception under physiological and pathological conditions, but evidence for this is largely circumstantial. To exactly determine this involvement (a problem of great potential importance not only for basic science but also for clinical pain management) more potent and specific P_2 purinoceptor antagonists are probably needed than those we have today. In the guinea-pig ileum, P_2 purinoceptors on primary afferents are not involved in the contractile effect of α,β-methylene ATP. Also, little direct evidence is available for an involvement of ATP, acting on P_2 purinoceptors, in 'local efferent' responses brought about by a release of biologically active substances from capsaicin-sensitive afferents upon activation. In the intestine, we present evidence for a mediating role of ATP in the 'non-tachykininergic' component of the excitatory effect of capsaicin.

Acknowledgements

This work was supported by the A. Menarini Foundation, the Hungarian Ministry of Public Health (ETT T-375/1996), Ministry of Education (FKFP 1287/1997) and the National Research Foundation (OTKA T-013045, T-020277, and T026463).

References

Barajas-Lopez, C., Barrientos, M., Espinoza-Luna, R., 1993. Suramin increases the efficacy of ATP to activate an inward current in myenteric neurons from guinea-pig ileum. Eur. J. Pharmacol. 250, 141–145.

Barajas-Lopez, C., Huizinga, J.D., Collins, S.M., Gerzanich, V., Espinoza-Luna, R., Peres, A.L., 1996. P_{2X} purinoceptors of myenteric neurones from the guinea-pig ileum and their unusual pharmacological properties. Br. J. Pharmacol. 119, 1541–1548.

Barthó, L., 1978. Adrenergic responses of the guinea-pig ileum: inhibition by prostaglandins and potentiation by non-steroid anti-inflammatory drugs (NSAIDs). Arch. Int. Pharmacodyn. Ther. 235, 238–247.

Barthó, L., Szolcsányi, J., 1978. The site of action of capsaicin on the guinea-pig isolated ileum. Naunyn-Schmiedeberg's Arch. Pharmacol. 305, 75–81.

Barthó, L., Holzer, P., 1985. Search for a physiological role of substance P in gastrointestinal motility. Neuroscience 16, 1–32.

Barthó, L., Holzer, P., Lembeck, F., Szolcsányi, J., 1982. Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to substance P. J. Physiol. (London) 332, 157–167.

Barthó, L., Pethô, G., Rónai, Z., 1985. Theophylline-sensitive modulation of non-cholinergic neutrotransmission in the guinea-pig ileum. Br. J. Pharmacol. 86, 315–317.

Barthó, L., Holzer, P., Lembeck, F., 1987a. Is ganglionic transmission through nicotinic receptors essential for the peristaltic reflex in the guinea-pig ileum?. Neuropharmacology 26, 1663–1666.

Barthó, L., Holzer, P., Lembeck, F., Lippe, I.Th., Setnikar, I., 1987b. Evaluation of a new and potent cholecystokinin antagonist on motor responses of the guinea-pig intestine. Br. J. Pharmacol. 90, 753–761.

Barthó, L., Holzer, P., Leander, S., Lembeck, F., 1989. Evidence for an involvement of substance P, but not cholecystokinin-like peptides, in hexamethonium-resistant intestinal peristalsis. Neuroscience 28, 211–217.

Barthó, L., Kóczán, G., Maggi, C.A., 1993. Studies on the mechanism of the contractile action of rat calcitonin gene-related peptide and of capsaicin on the guinea-pig ileum: effect of hCGRP(8-37) and CGRP tachyphylaxis. Neuropeptides 25, 325–329.

Barthó, L., Lénárd, L. Jr., Maggi, C.A., 1997. Evidence for the involvement of P_2 purinoceptors in the cholinergic contraction of the guinea-pig ileum. Br. J. Pharmacol. 121, 1507–1508.

Barthó, L., Lénárd, L. Jr., Szigeti, R., 1998. Nitric oxide and ATP co-mediate the NANC relaxant response in the guinea-pig taenia caeci. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 496–499.

Barthó, L., Lénárd, L. Jr., Patacchini, R., Halmai, V., Wilhelm, M., Holzer, P., Maggi, C.A., 1999. Tachykinin receptors are involved in the 'local efferent' motor response to capsaicin in the guinea-pig small intestine and oesophagus. Neuroscience 90, 221–228.

Bean, B.P., 1990. ATP-activated channels in rat and bullfrog sensory neurons: concentration-dependence and kinetics. J. Neurosci. 10, 1–10.

Bean, B.P., Williams, C.A., Ceelen, P.W., 1990. ATP-activated channels in rat and bullfrog sensory neurons: current–voltage relation and single-channel behavior. J. Neurosci. 10, 11–19.

Bland-Ward, P.A., Humphrey, P.P.A., 1997. Acute nociception mediated by hindpaw P2X receptor activation. Br. J. Pharmacol. 122, 365–371.

Bleehen, T., Keele, C.A., 1977. Observations on the algogenic actions of adenosine compounds on the human blister base preparation. Pain 3, 367–377.

Bo, X., Burnstock, G., 1994. Distribution of [^{3}H]α,β-methylene ATP binding sites in rat brain and spinal cord. NeuroReport 5, 1601–1604.

Bouvier, M.N., Evans, M.L., Benham, C.D., 1991. Calcium influx induced by stimulation of ATP receptors on neurones cultured from rat dorsal root ganglia. Eur. J. Neurosci. 3, 285–291.

Brake, A.J., Wagenbach, M.J., Julius, D., 1994. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. Nature 371, 519–523.

Burnstock, G., 1972. Purinergic nerves. Pharmacol. Rev. 24, 509–581.

Burnstock, G., 1990. Overview: purinergic mechanisms. Ann. New York Acad. Sci. 603, 1–18.

Burnstock, G., 1996. A unifying purinergic hypothesis for the initiation of pain. Lancet 347, 1604–1605.

Burnstock, G., 1997. The past, present and future of purine nucleotides as signalling molecules. Neuropharmacology 36, 1127–1139.

Burnstock, G., Wood, J.N., 1996. Purinergic receptors: their role in nociception and primary afferent neurotransmission. Curr. Opin. Neurobiol. 6, 526–532.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature (London) 389, 816–824.

Chahl, L.A., 1982. Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 212–215.

Chen, C.C., Akopian, A.N., Sivilotti, L., Colquhoun, D., Burnstock, G., Wood, J., 1995. A P_{2X} purinoceptor expressed by a subset of sensory neurons. Nature (London) 377, 428–431.

Christofi, F.L., McDonald, T.J., Cook, M.A., 1990. Adenosine receptors are coupled to release of tachykinin(s) from enteric nerve endings. J. Pharmacol. Exp. Ther. 253, 290–295.

Collo, G., North, R.A., Kawashima, E., Merlo-Pich, E., Neidhart, S., Surprenant, A., Buell, G., 1996. Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. J. Neurosci. 16, 2495–2507.

Costa, M., Brookes, S.J., 1994. The enteric nervous system. Am. J. Gastroenterol. 89, S129–S137.

Crist, J.R., He, X.D., Goyal, R.K., 1992. Both ATP and the peptide VIP are inhibitory neurotransmitters in guinea-pig ileum circular muscle. J. Physiol. (London) 447, 119–131.

Croci, T., Landi, M., Emonds-Alt, X., LeFur, G., Manara, L., 1995. Neuronal NK-3 receptors in the guinea-pig ileum and taenia coli: in vitro characterization by their first non-peptide antagonist, SR 142 801. Life Sci. 57, 361–366.

Dowd, E., McQueen, D.S., Chessell, I.P., Humphrey, P.P.A., 1998. P_{2X} receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint. Br. J. Pharmacol. 125, 341–346.

Driessen, B., Reimann, W., Selve, N., Friderichs, E., Bültmann, R., 1994. Antinociceptive effect of intrathecally administered P_2 purinoceptor antagonists in rats. Brain Res. 666, 182–188.

Emonds-Alt, X., Bichon, D., Ducoux, J.P., Heaulme, M., Miloux, B., Poncelet, M., Proietto, V., Van Broeck, D., Vilain, P., Neliat, G., Soubrié, P., Le Fur, G., Breliere, J.C., 1995. SR 142801, the first potent non-peptide antagonist of the tachykinin NK_3 receptor. Life Sci. 56, PL27–PL32.

Fredholm, B.B., Abbracchio, M.P., Burnstock, G., Daly, J.W., Harden, T.K., Jacobson, K.A., Leff, P., Williams, M., 1994. Nomenclature and classification of purinoceptors. Pharmacol. Rev. 46, 143–156.

Furness, J.B., Costa, M., 1980. Types of nerve in the enteric nervous system. Neuroscience 5, 1–20.

Fyffe, R.E.W., Perl, E.R., 1984. Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin?. Proc. Natl. Acad. Sci. U.S.A. 81, 6890–6893.

Galligan, J.J., Bertrand, P.P., 1994. ATP mediates fast synaptic potentials in enteric neurons. J. Neurosci. 14, 7563–7571.

Geppetti, P., Holzer, P. (Eds.), 1996. Neurogenic Inflammation. CRC Press, Boca Raton.

Giuliani, S., Maggi, C.A., 1995. Effect of SR 142801 on nitric oxide-dependent and independent responses to tachykinin NK_3 receptor agonists in isolated guinea-pig colon. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 512–519.

Hagan, R.M., Ireland, S.M., Bailey, F., McBride, C., Jordan, C.A., Ward, P., 1991. A spirolactam conformationally-constrained analogue of physalaemin which is a peptidase resistant, selective tachykinin NK_1 receptor agonist. Br. J. Pharmacol. 102, 168P.

Ho, B.T., Huo, Y.Y., Newman, R.A., Levin, V.A., 1992. Analgesic activity of anticancer agent suramin. Anti-Cancer Drugs 3, 91–94.

Holton, P., 1959. The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. J. Physiol. (London) 145, 494–504.

Holton, F.A., Holton, P., 1954. The capillary dilator substances in dry powders of spinal roots; a possible role of adenosine triphosphate in chemical transmission from nerve endings. J. Physiol. (London) 126, 124–140.

Holzer, P., 1988. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768.

Holzer, P., Barthó, L., 1996. Sensory neurons in the intestine. In: Geppetti, P., Holzer, P. (Eds.), Neurogenic Inflammation. CRC Press, New York, pp. 153–167.

Holzer, P., Holzer-Petsche, U., 1997a. Tachykinins in the gut: Part I. Expression, release and motor function. Pharmacol. Ther. 73, 173–217.

Holzer, P., Holzer-Petsche, U., 1997b. Tachykinins in the gut: Part II. Roles in neuronal excitation, secretion and inflammation. Pharmacol. Ther. 73, 219–263.

Holzer, P., Lippe, I.Th., Heinemann, A., Barthó, L., 1998. Tachykinin NK_1 and NK_2 receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine in vitro. Neuropharmacology 37, 131–138.

Humphrey, P.P., Buell, G., Kennedy, I., Khakh, B.S., Michel, A.D., Surprenant, A., Trezise, D.J., 1995. New insights on P_{2X} purinoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 585–596.

Illés, P., Norenberg, W., 1993. Neuronal ATP receptors and their mechanism of action. Trends Pharmacol. Sci. 14, 50–54.

Jahr, C.E., Jessell, T.M., 1983. ATP excites a subpopulation of rat dorsal horn neurones. Nature (London) 304, 730–733.

Kakuyama, M., Vallance, P., Ahluwalia, A., 1998. Endothelium-dependent sensory NANC vasodilatation: involvement of ATP, CGRP and a possible NO store. Br. J. Pharmacol. 123, 310–316.

Kamikawa, Y., Serizawa, K., Shimo, Y., 1977. Some possibilities for prostaglandin mediation in the contractile response to ATP of the guinea-pig digestive tract. Eur. J. Pharmacol. 45, 199–203.

Katsuragi, T., Shirakabe, K., Soejima, O., Tokunaga, T., Matsuo, K., Sato, C., Furukawa, T., 1993. Possible transsynaptic cholinergic neuromodulation by ATP released from ileal longitudinal muscles of guinea-pigs. Life Sci. 53, 911–918.

Kennedy, I., Humphrey, P.P.A., 1994. Evidence for the presence of two types of P_2 purinoceptor in the guinea-pig ileal longitudinal smooth muscle preparation. Eur. J. Pharmacol. 261, 273–280.

Kennedy, C., Leff, P., 1995. Painful connection for ATP. Nature 377, 385–386.

Khakh, B.S., Humphrey, P.P.A., Surprenant, A., 1995. Electrophysiological properties of P_{2X}-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac neurones. J. Physiol. (London) 484, 385–395.

Kimball, B.C., Yule, D.I., Mulholland, M.W., 1996. Extracellular ATP mediates Ca^{2+} signaling in cultured myenteric neurons via a PLC-dependent mechanism. Am. J. Physiol. 270, G587–G593.

Krishtal, O.A., Marchenko, S.M., Pidoplichko, V.I., 1983. Receptor for ATP in the membrane of mammalian sensory neurons. Neurosci. Lett. 35, 41–45.

Krishtal, O.A., Marchenko, S.M., Obukhov, A.G., Volkova, T.M., 1988. Receptors for ATP in rat sensory neurones: the structure-function relationship for ligands. Br. J. Pharmacol. 95, 1057–1062.

Lambrecht, G., 1996. Design and pharmacology of selective P_2 purinoceptor antagonists. J. Auton. Pharmacol. 16, 341–344.

Lambrecht, G., Friebe, T., Grimm, U., Windscheif, U., Bungardt, E., Hildebrandt, C., Bäumert, H.G., Spatz-Kümbel, G., Mutschler, E., 1992. PPADS, a novel functionally selective antagonist of P_2-purinoceptor mediated responses. Eur. J. Pharmacol. 217, 217–219.

Laufer, R., Wormser, U., Friedman, Z.Y., Gilon, C., Chorev, M., Selinger, Z., 1985. Neurokinin B is a preferred agonist for a neuronal substance P-receptor and its action is antagonized by enkephalin. Proc. Natl. Acad. Sci. U.S.A. 82, 7444–7448.

LePard, K.J., Messori, E., Galligan, J.J., 1997. Purinergic fast excitatory post-synaptic potentials in myenteric neurons of guinea-pig: distribution and pharmacology. Gastroenterology 113, 1522–1534.

Lewis, C., Neidhart, S., Holy, C., North, R.A., Buell, G., Surprenant, A., 1995. Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. Nature (London) 377, 432–435.

Li, C., Peoples, R.W., Li, Z., Weight, F.F., 1993. Zn^{2+} potentiates the excitatory action of ATP on mammalian neurons. Proc. Natl. Acad. Sci. U.S.A. 90, 8264–8267.

Li, C., Peoples, R.W., Weight, F.F., 1997. Enhancement of ATP-activated current by protons in dorsal root ganglion neurons. Pflügers Arch. - Eur. J. Physiol. 433, 446–454.

Matsuo, K., Katsuragi, T., Fujiki, S., Sato, C., Furukawa, T., 1997. ATP release and contraction mediated by different P_2-receptor subtypes in guinea-pig ileal smooth muscle. Br. J. Pharmacol. 121, 1744–1748.

Moneta, N.A., McDonald, T.J., Cook, M.A., 1997. Endogenous adenosine inhibits evoked substance P release from perifused networks of myenteric ganglia. Am. J. Physiol. 272, G38–45.

Moody, C.J., Burnstock, G., 1982. Evidence for the presence of P_1-purinoceptors on cholinergic terminals in the guinea-pig ileum. Eur. J. Pharmacol. 77, 1–9.

North, R.A., Barnard, E.A., 1997. Nucleotide receptors. Curr. Opin. Neurobiol. 7, 346–357.

Patacchini, R., Barthó, L., Holzer, P., Maggi, C.A., 1995. Activity of SR 142,801 at peripheral tachykinin receptors. Eur. J. Pharmacol. 278, 17–25.

Patacchini, R., DeGiorgio, R., Barthó, L., Corinaldesi, R., Maggi, C.A., 1998. Evidence that tachykinins are the main NANC excitatory transmitters in the guinea-pig common bile duct. Br. J. Pharmacol. 124, 1703–1711.

Portbury, A.L., Furness, J.B., Southwell, B.R., Wong, H., Walsh, J.H., Bunnett, N.W., 1996. Distribution of neurokinin-2 receptors in the guinea-pig gastrointestinal tract. Cell Tissue Res. 286, 281–292.

Robertson, S.J., Rae, M.G., Rowan, E.R., Kennedy, C., 1996. Characterization of a P_{2X}-purinoceptor in cultured neurons of the rat dorsal root ganglia. Br. J. Pharmacol. 118, 951–956.

Salt, T.E., Hill, R.G., 1983. Excitation of single sensory neurones in the rat caudal trigeminal nucleus by iontophoretically applied adenosine 5′-triphosphate. Neurosci. Lett. 35, 53–57.

Salter, M.W., Henry, J.L., 1985. Effects of adenosine-5′-monophosphate and adenosine 5′-triphosphate on functionally identified units in the cat spinal dorsal horn. Evidence for a differential effect of adenosine 5′-triphosphate nociceptive vs. non-nociceptive units. Neuroscience 15, 815–825.

Sann, H., McCarthy, P.W., Mäder, M., Schemann, M., 1995. Choline acetyltransferase-like immunoreactivity in small diameter neurones of the rat dorsal root ganglion. Neurosci. Lett. 198, 17–20.

Sawynok, J., Sweeney, M.I., 1989. The role of purines in nociception. Neuroscience 32, 557–569.

Sawynok, J., Downie, J.W., Reid, A.R., Cahill, C.M., White, T.D., 1993. ATP release from dorsal spinal cord synaptosomes: characterization and neuronal origin. Brain Res. 610, 32–38.

Soto, F., Garcia-Guzman, M., Gomez-Hernandez, J.M., Hollmann, M., Karschin, C., Stühmer, W., 1996. $P2X_4$: an ATP-activated ionotropic receptor cloned from rat brain. Proc. Natl. Acad. Sci. U.S.A. 93, 3684–3688.

Sperlágh, B., Vizi, E.S., 1991. Effect of presynaptic P_2 receptor stimulation on transmitter release. J. Neurochem. 56, 1466–1470.

Sweeney, M.I., White, T.D., Sawynok, J., 1989. Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord. J. Pharmacol. Exp. Ther. 248, 447–454.

Szolcsányi, J., 1984. Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In: Chahl, L.A., Szolcsányi, J., Lembeck, F. (Eds.), Antidromic Vasodilalation and Neurogenic Inflammation. Akadémiai Kiadó, Budapest, pp. 27–55.

Szolcsányi, J., Barthó, L., 1978. New type of nerve-mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin. Naunyn-Schmiedeberg's Arch. Pharmacol. 305, 83–90.

Trezise, D.J., Humphrey, P.P.A., 1996. Activation of peripheral sensory neurones in the neonatal rat tail by ATP. Br. J. Pharmacol. 117, 103P.

Trezise, D.J., Kennedy, I., Humphrey, P.P., 1994. The use of antagonists to characterize the receptors mediating depolarization of the rat isolated vagus nerve by α,β-methylene adenosine 5′-triphosphate. Br. J. Pharmacol. 112, 282–288.

Valera, S., Hussy, N., Evans, R.J., Adami, N., North, R.A., Surprenant, A., Buell, G., 1994. A new class of ligand-gated ion channel defined by P_{2X} rreceptor for extracellular ATP. Nature (London) 371, 516–519.

Venkova, K., Krier, J., 1993. Stimulation of lumbar sympathetic nerves evokes contractions of the cat colon circular muscle mediated by ATP and noradrenaline. Br. J. Pharmacol. 110, 1260–1270.

Vizi, E.S., 1979. Presynaptic modulation of neurochemical transmission. Prog. Neurobiol. 12, 181–290.

Vulchanova, L., Riedl, M.S., Shuster, S.J., Buell, G., Surprenant, A., North, R.A., Elde, R., 1997. Immunohistochemical study of the $P2X_2$ and $P2X_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. Neuropharmacology 36, 1229–1242.

Wiklund, N.P., Gustafsson, L.E., 1986. Neuromodulation by adenine nucleotides, as indicated by experiments with inhibitors of nucleotide inactivation. Acta Physiol. Scand. 126, 217–223.

Wiklund, N.P., Gustafsson, L.E., 1988a. Indications for P_2 purinoceptor subtypes in guinea-pig smooth muscle. Eur. J. Pharmacol. 148, 361–370.

Wiklund, N.P., Gustafsson, L.E., 1988b. Agonist and antagonist characterization of the P_2-purinoceptors in the guinea-pig ileum. Acta Physiol. Scand. 132, 15–21.

Wiklund, N.P., Gustafsson, L.E., Lundin, J., 1985. Pre- and postjunctional modulation of cholinergic neuroeffector transmission by adenine nucleotides. Acta Physiol. Scand. 125, 681–691.

Wildman, S.S., King, B.F., Burnstock, G., 1997. Potentiation of ATP-responses at a recombinant $P2X_2$ receptor by neurotransmitters and related substances. Br. J. Pharmacol. 120, 221–224.

Wood, J.D., 1994. Physiology of the enteric nervous system. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract, 3rd edn., Raven Press, New York, pp. 423–482.

Zagorodnyuk, V., Santicioli, P., Maggi, C.A., Giachetti, A., 1996. The possible role of ATP and PACAP as mediators of apamin-sensitive NANC inhibitory junction potentials in circular muscle of guinea-pig colon. Br. J. Pharmacol. 119, 779–786.

Zhou, X., Galligan, J.J., 1996. P_{2X} purinoceptors in cultured myenteric neurons of guinea-pig small intestine. J. Physiol. (London) 496, 719–729.