European Journal of Pharmacology 740 (2014) 442–454

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

α,β-meATP mimics the effects of the purinergic neurotransmitter in the human and rat colon

Míriam Martínez-Cutillas [a], Víctor Gil [a], Diana Gallego [b], Noemí Mañé [a], Pere Clavé [b,c], María Teresa Martín [a,b], Marcel Jiménez [a,b,*]

[a] Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain
[b] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, (CIBERehd), Instituto de Salud Carlos III, Barcelona, Spain
[c] Fundació de Gastroenterologia Dr Vilardell and Department of Surgery, Hospital de Mataró, Mataró, Barcelona, Spain

ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form
13 June 2014
Accepted 17 June 2014
Available online 10 July 2014

Keywords:
$P2Y_1$ receptors
Purines
Junction potential
Smooth muscle
Colonic contractility

ABSTRACT

The purine receptor involved in inhibitory responses in the gastrointestinal tract has been recently identified. $P2Y_1$ receptor activation mediates the fast component of the inhibitory junction potential (IJPf) and the non-nitrergic relaxation. The aim of the present work has been to investigate which purinergic agonist better mimics endogenous responses. We used different agonist and antagonist of P2 receptors. Contractility and microelectrode experiments were used to compare the effects of exogenously added purines and electrical field stimulation (EFS)-induced nerve mediated effects in rat and human colonic strips. In rat colon, the IJPf and EFS-induced inhibition of contractions were concentration-dependently inhibited by the $P2Y_1$ antagonist MRS2500 but not by iso-PPADS or NF023 (P2X antagonists) up to 1 μM. In samples from human colon, EFS-induced inhibition of contractions was inhibited by either MRS2500 or apamin (1 μM) but not by iso-PPADS. In both species, α,β-meATP, a stable analog of ATP, caused inhibition of spontaneous contractions. α,β-meATP effect was concentration-dependent (EC_{50}: 2.7 μM rat, 4.4 μM human) and was antagonized by either MRS2500 or apamin but unaffected by P2X antagonists. ATP, ADP, β-NAD and ADP-ribose inhibited spontaneous contractions but did not show the same sensitivity profile to purine receptor antagonists as EFS-induced inhibition of contractions. The effect of α,β-meATP is due to $P2Y_1$ receptor activation leading the opening of sKca channels. Accordingly, α,β-meATP mimics the endogenous purinergic mediator. In contrast, exogenously added putative neurotransmitters do not exactly mimic the endogenous mediator. Quick degradation by ecto-nuclease or different distribution of receptors (junctionally vs extrajunctionally) might explain these results.

1. Introduction

The purinergic neurotransmitter(s) responsible for non-adrenergic, non-cholinergic (NANC) inhibitory responses in the gastrointestinal (GI) tract has still not been identified. Several purines including adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate sodium salt (ADP), β-nicotinamide adenine dinucleotide hydrate (β-NAD) or even adenosine 5′-diphosphoribose sodium salt (ADP-ribose) are nowadays possible candidates for this role (Burnstock et al., 1970; Mutafova-Yambolieva et al., 2007; Durnin et al., 2012). Recently, the receptor responsible for purinergic neuromuscular transmission has been identified. $P2Y_1$ receptor antagonists such as MRS2179, MRS2279 and MRS2500 have been valuable pharmacological tools to demonstrate the crucial role of $P2Y_1$ receptors in purinergic neuromuscular transmission (Gallego et al., 2006; Wang et al., 2007; Gallego et al., 2008a, 2008b; Grasa et al., 2009). MRS2500 is considered the most potent $P2Y_1$

Abbreviations: IJPf, fast inhibitory junction potential; EFS, electrical field stimulation; NANC, non-adrenergic non-cholinergic; GI, gastrointestinal; NO, nitric oxide; L-NNA, Nω-nitro-L-arginine; α,β-meATP, α, β- methylene adenosine 5′-triphosphate lithium salt; ADPβS, adenosine 5′-[β-thio]diphosphate trilitium salt; β-NAD, β-nicotinamide adenine dinucleotide hydrate; ADP, adenosine 5′-diphosphate sodium salt; ATP, adenosine 5′-triphosphate; ADP-ribose, adenosine 5′-diphosphoribose sodium salt; UTP, uridine-5′-triphosphate; UDP-glucose, uridine diphosphate glucose; ODQ, oxadiazolo [4,3-α]quinoxalin-1-one; MRS2500, (1R,2S,4S,5S-4-[2-Iodo-6-(methylamino)-9H-purin-9-yl]-2-(phosphonooxy)bicyclo[3.1.0]hexane-1-methanol dihydrogen phosphate ester tetraammonium salt; NF023, 8,8'-[carbonyl*bis*(imino-3,1-phenylenecarbonylimino)]*bis*-1,3,5-naphthalene-trisulphonic acid hexasodium salt; iso-PPADS, pyridoxalphosphate-6-azophenyl-2',5'-disulfonic acid tetrasodium salt; NF157, 8,8'-[Carbonyl*bis*[imino-3,1-phenylenecarbonylimino(4-fluoro-3,1-phenylene)carbonylimino]]*bis*-1,3,5-naphthalene-trisulfonic acid hexasodium salt; ARL67156, ARL67156 trisodium salt hydrate; TTX, tetrodotoxin; sKca, small conductance calcium-activated potassium channels; AUC, area under curve

* Corresponding author at: Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain. Tel.: +34 93 581 1566; fax: +34 93 581 2006.
E-mail address: marcel.jimenez@uab.es (M. Jiménez).

http://dx.doi.org/10.1016/j.ejphar.2014.06.048
0014-2999/

antagonist (Kim et al., 2003; Cattaneo et al., 2004) and has proven to be inactive on other purine receptors such as P2X (Bradley et al., 2011; Doyle et al., 2014), $P2Y_{12}$ (Hechler et al., 2006) and $P2Y_{13}$ (Gao et al., 2010). Studies in knocked-out mice have confirmed $P2Y_1$ receptor relevance on purinergic junction potentials and smooth muscle relaxation in the colon (Gallego et al., 2012; Hwang et al., 2012), stomach and cecum (Gil et al., 2013). A consensus about the involvement of the $P2Y_1$ receptor in smooth muscle relaxation in the whole GI tract now exists (King, 2012; Gil et al., 2013; Goyal et al., 2013). The identification of $P2Y_1$ receptors has been crucial in understanding the process of co-transmission between purines and NO and in establishing pharmacological criteria for identifying potential agonists able to mimic endogenous responses (Gallego et al., 2006; Mutafova-Yambolieva et al., 2007; Gallego et al., 2011; Durnin et al., 2012; Gil et al., 2013).

α,β-methylene adenosine 5′-triphosphate lithium salt (α,β-meATP) is an unselective P2X receptor agonist (Alexander et al., 1999). The effect of α,β-meATP on smooth muscle excitability differs depending on the species and area of the GI tract. Consistent with an effect on P2X receptors, α,β-meATP produces smooth muscle depolarization and contraction. An excitatory junction potential due to activation of $P2X_1$ receptors has been reported in the guinea-pig tenia caeci (Zhang and Paterson, 2005). α,β-meATP induces non-selective cation inward currents triggering smooth muscle depolarization and contraction in the canine colon (Lee et al., 2005). However, in other tissues, α,β-meATP causes smooth muscle hyperpolarization and/or relaxation (Zagorodnyuk et al., 1996; Storr et al., 2000; Ishiguchi et al., 2000; Giaroni et al., 2002; De Man et al., 2003; Van Crombruggen et al., 2007; King and Townsend-Nicholson, 2008). The receptor involved in α,β-meATP effect is not clear and high concentrations of P2X antagonists such as PPADS are often used for its identification. It is important to investigate if high concentrations of P2X antagonists can inhibit $P2Y_1$-mediated responses. Little is known about α,β-meATP effect in human smooth muscle excitability. In the jejunum, α,β-meATP causes hyperpolarization and partial IJP desensitization (Xue et al., 1999) and in the human colon, the relaxation induced by α,β-meATP is blocked by high concentrations of MRS2179 (Auli et al., 2008), suggesting a possible involvement of $P2Y_1$ receptors.

Accordingly, the aim of this paper is to establish a pharmacological methodology to characterize the receptor involved in endogenous purinergic responses and to investigate which of the exogenously added purines better mimics the neurotransmitter. This can be used in future studies where $P2Y_1$ agonists might be useful to treat purinergic motor disorders (Strong et al., 2010; Roberts et al., 2012).

2. Materials and methods

2.1. Solutions and drugs

The composition of the Krebs solution was (in mM): glucose, 10.1; NaCl, 115.5; $NaHCO_3$, 21.9; KCl, 4.6; NaH_2PO_4, 1.1; $CaCl_2$, 2.5 and $MgSO_4$, 1.2 (pH 7.3–7.4). The Krebs solution (37 ± 1 °C) was bubbled with carbogen (95% O_2 and 5% CO_2). "Non-adrenergic non-cholinergic" (NANC) conditions were obtained by adding phentolamine, propranolol and atropine (1 μM) to the Krebs solution to block α- and β-adrenoceptors and muscarinic receptors.

The following drugs were used: nifedipine, apamin, Nω-nitro-L-arginine (L-NNA), α, β- methyleneadenosine 5′-triphosphate lithium salt (α,β-meATP), adenosine 5′-[β-thio]diphosphate trilitium salt (ADPβS), β-nicotinamide adenine dinucleotide hydrate (β-NAD), adenosine 5′-diphosphate sodium salt (ADP), adenosine 5′-triphosphate (ATP), Adenosine 5′-diphosphoribose sodium salt (ADP-ribose), uridine-5′-triphosphate (UTP), uridine diphosphate glucose (UDP-glucose), phentolamine, oxadiazolo[4,3-α]quinoxalin-1-one (ODQ), nifedipine, atropine sulfate (Sigma Chemicals, St. Louis, USA), propranolol, (1R,2S,4S,5S)-4-[2-Iodo-6-(methylamino)-9H-purin-9-yl]-2-(phosphonooxy)bicyclo[3.1.0]hexane-1-methanol dihydrogen phosphate ester tetraammonium salt (MRS2500), 8,8′-[carbonyl *bis*(imino-3,1-phenylenecarbonylimino)] *bis*-1,3,5-naphthalene-trisulphonic acid hexasodium salt (NF023), Pyridoxalphosphate-6-azophenyl-2′,5′-disulfonic acid tetrasodium salt (iso-PPADS), 8,8′-[Carbonyl *bis*[imino-3,1-phenylenecarbonylimino(4-fluoro-3,1-phenylene)carbonylimino]]*bis*-1,3,5-naphthalenetrisulfonic acid hexasodium salt (NF157), ω-conotoxin GVIA, ARL 67156 trisodium salt hydrate (ARL67156) (Tocris, Bristol, UK), tetrodotoxin (TTX) (Latoxan, Valence, France). Stock solutions were made by dissolving drugs in distilled water except for nifedipine and ODQ which were dissolved in 96% ethanol and L-NNA which was dissolved in Krebs solution by sonication.

2.2. Rat tissue preparation

Male Sprague-Dawley rats (300–350 g; 8–10 weeks old) were kept at a constant room temperature (19–21 °C) and humidity (60%), with a lighting cycle of 12 h light/12 h dark and ad libitum access to water and food. Animals were stunned by a sharp blow to the head before being decapitated and bled. The mid colon was quickly placed in carbogenated Krebs solution. The mesenteric fat was removed and the colon was opened along the mesenteric border and pinned to a Sylgard base with the mucosa facing upwards. The mid colon was distinguished according to the longitudinal orientation of the folds of the mucosa (total length about 5 cm in the center of the colon) taking into account the anatomical criteria previously described (Alberti et al., 2005). Mucosa and submucosa layers were carefully removed and circular muscle strips were cut into strips 1 cm long and 0.3 cm wide. This procedure was approved by the Ethics Committee of the Universitat Autònoma de Barcelona.

2.3. Human tissue preparation

Tissue specimens of human sigmoid colon ($n=64$) were obtained from patients (34 females and 30 males, average age 68) during colon resections of neoplasm. The person that performed the experiments and analyzed the tracings was initially not aware of this information and accordingly, gender and age were not considered as variables of this study. Colon segments from macroscopically-normal marginal regions were collected and transported to the laboratory in cold saline buffer. The tissue was placed in Krebs solution on a dissection dish and the mucosa layer was gently removed. Circular muscle strips (10 × 4 mm) were cut. The patients provided informed consent. Ethics committee of the Hospital of Mataró (Barcelona, Spain) approved the experimental procedure.

2.4. Intracellular microelectrode recording

Muscle strips were pinned to the base of a Sylgard coated chamber, circular muscle side up, and continuously perfused with Krebs solution. Strips were allowed to equilibrate for approximately 1 h before recording. Circular smooth muscle cells were impaled with sharp glass microelectrodes filled with 3 M KCl (30–60 MΩ). Membrane potential was measured using standard electrometer Duo773 (WPI Inc., Sarasota, FL, USA). Tracings were displayed on an oscilloscope 4026 (Racal-Dana Ltd., Windsor, UK) and simultaneously digitalized (100 Hz) using PowerLab 4/30 system and Chart 5 software for Windows (all from ADInstruments, Castle Hill, NSW, Australia). Nifedipine (1 μM) was used to