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Purinergic modulation of pacemaker Ca^{2+} activity in interstitial cells of Cajal

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Abstract

Purinoceptors are widely distributed throughout the body, and are thought to have important contributions to numerous functions. In this study, we characterised the contribution of purinoceptors to the mechanisms underlying spontaneous rhythmicity of the gastro-intestinal tracts. Using cell cluster preparations (100–200 μm diameter) obtained from murine ileum, we measured spontaneous intracellular Ca^{2+}($[Ca^{2+}]_i$) oscillations in the presence of nifedipine, as an index of pacemaker $[Ca^{2+}]_i$ activity in interstitial cells of Cajal (ICCs, c-Kit-immunopositive cells), the pacemaker cells for gastrointestinal motility. This small preparation also contained smooth muscle and enteric neurones. Using various purinoceptor agonists and an antagonist, we characterised both TTX-sensitive and insensitive modulations of pacemaker $[Ca^{2+}]_i$ activity in ICCs. Continuous application of either ATP, ATPγS, suramin or α,β-methylene ATP (α,β-meATP) suppressed pacemaker $[Ca^{2+}]_i$ activity. The inhibitory effect of α,β-meATP was completely abolished by a prior application of TTX. On the other hand, even in the presence of TTX, continuous application of 2-methylthio ATP (2-MeSATP) at concentrations greater than 30 μM caused a prompt rise followed by a slow decline of the baseline $[Ca^{2+}]_i$, and pacemaker $[Ca^{2+}]_i$ oscillations were gradually suppressed during the decline. Neither UTP nor α,β-meATP at high concentrations (30–100 μM) produced a similar $[Ca^{2+}]_i$ response. These results suggest that the TTX-resistant, direct purinergic modulation of pacemaker $[Ca^{2+}]_i$ activity in ICCs is mediated via P2X purinoceptors distinct from those involved in TTX-sensitive modulation. The slow decline may be attributed to desensitisation of these purinoceptors. The possible involvement of other purinoceptors is also discussed.

Keywords: Purinoceptors; Calcium oscillation; c-Kit-immunopositive cells; Interstitial cells of Cajal; Tetrodotoxin

1. Introduction

Gastrointestinal motility is driven by spontaneous electrical activity, termed slow waves. The pacemaker-like cells underlying this spontaneous activity have been identified using the receptor tyrosine kinase, c-Kit, and are referred to as interstitial cells of Cajal (ICCs) due to their histological features (Maeda et al., 1992; Ward et al., 1994; Tokutomi et al., 1995; Huizinga et al., 1995). Pacemaker activity in the gastrointestinal tracts seems to display several unique properties, such as low voltage sensitivity and high temperature dependency in frequency (Tomita, 1981). There is now accumulating evidence that intracellular Ca^{2+} ($[Ca^{2+}]_i$) oscillations in ICCs act as the primary mechanism to produce the pacemaker

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activity (Suzuki et al., 2000; Yamazawa and Iino, 2002; Torihashi et al., 2002; van Helden and Imtiaz, 2003; Takaki, 2003; Aoyama et al., 2004; Ishikawa et al., 2004; Liu et al., 2004). However, despite increasing insights into the mechanisms underlying gastrointestinal pacemaker activity, it remains unclear how these mechanisms are modulated by neurotransmitter systems.

Purinoceptors are widely distributed throughout the body, and are thought to have important contributions to numerous functions. Ionotropic (P2X) and G-protein-coupled metabotoropic purinoceptors (P2Y) are known to exist in smooth muscle cells and autonomic neurones (Giaroni et al., 2002; Poole et al., 2002). In the gut, ATP has been shown to be released from nerve varicosities in the myenteric plexus (Al Humayyd and White, 1985). Also, ATP is possibly released from smooth muscle during spontaneous contractions (Katsuragi et al., 1996) and from enteric glia cells (Jessen and Burnstock, 1982). Importantly, it has recently been shown that ICCs express purinoceptors by immunostaining (Burnstock and Lavin, 2002). Therefore, purinergic signalling may play an important role in modulating pacemaker activity in the gut.

We have recently developed a cell cluster preparation from mouse ileum. This preparation enables us to relatively easily stain ICCs with fluorescent Ca^{2+} indicators, and contains essential members to investigate mechanisms underlying spontaneous rhythmicity: smooth muscle, c-Kit-immunopositive interstitial cells (equivalent to ICCs) and enteric neurones. In the present study, using this cell cluster preparation, we examined effects of several nucleotides on the pacemaker $[Ca^{2+}]_i$ oscillations in ICCs, and found TTX-sensitive and insensitive modulations.

2. Materials and methods

2.1. Cell cluster preparation

The preparation of cell clusters in the present study has been described previously (Nakayama and Torihashi, 2002; Torihashi et al., 2002). The mice used in the present study were treated ethically according to the Guidelines for the Care and Use of Animals approved by the Physiological Society of Japan. BALB/c mice (10–20 days after birth) were killed by cervical dislocation. The smooth muscle layers (both circular and longitudinal) including the myenteric plexus were carefully dissected from ileum (5 cm in length, from 1 cm below the pyloric ring to the caecum), and incubated in Ca^{2+}-free Hanks' solution containing collagenase (1 mg/ml, Wako Chemical, Osaka, Japan), trypsin inhibitor (2 mg/ml, type I-S, Sigma, St Louis, MO, USA), ATP (0.3 mg/ml, Seikagakukogyo, Tokyo, Japan), and bovine serum albumin (2 mg/ml, fraction V, Sigma) for 40 min at 37 °C. The muscle preparation was then triturated with fire-blunted glass pipettes. The resultant cell clusters were plated onto a lab-made culture dish (a silicone ring approx. 20 mm in diameter on a cover glass of 25 mm in diameter and 0.12–0.17 mm thick, coated with pig collagen (Nitta Gelatin, Osaka, Japan)), and kept in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Rockville, MD, USA) supplemented with 10% foetal bovine serum (Sigma) and antibiotics (streptomycin 30 μg/ml and penicillin 30 units/ml; Life Technologies) at 37 °C. After 2–3 days of incubation, the cultured cell clusters were used for Ca^{2+} imaging.

Previous immunohistochemical examinations revealed that cell cluster preparations were mainly composed of smooth muscle cells stained with anti-γ-enteric actin antibody (1:200; ICN, Costa Mesa, CA, USA). The presence of enteric neurones and ICCs were confirmed by staining with anti-PGP9.5 antibody (1:8000; Ultracone, England) and ACK2 (ACK2, 10 μl/ml) (Nakayama and Torihashi, 2002; Torihashi et al., 2002). The percentage of ICCs contained was at most 3–5% (Liu et al., 2004). The diameter of cell clusters used in Ca^{2+} imaging was 100–200 μm. According to Kunze and Furness (1999) (2500 nerve cells per mm gut), each cell cluster preparation was expected to contain a sizable number of enteric neurones. However, it was also considered that the enzymatic and mechanical treatments possibly impaired innervation to ICCs in cell clusters, leading to variations in the TTX-sensitive purinergic modulations of pacemaker $[Ca^{2+}]_i$ activity caused via purinoceptors.

2.2. Ca^{2+} imaging and evaluation of mechanical activity

The cultured cell cluster preparations were incubated in 'normal' solution containing approximately 8 μM Fluo3-AM (acetoxymethly ester of Fluo-3: Dojindo, Kumamoto, Japan) and detergents (0.02% Pluronic F-127, Dojindo; 0.02% cremophor EL, Sigma) for 3–4 h at room temperature. A CCD camera system (Argus HiSCA, Hamamatsu Photonics, Hamamatsu, Japan) was used to continuously monitor digital images of Fluo-3 emission light. The cell clusters were illuminated at 488 nm, and emission light of 515–565 nm was detected. Digital images (0.963 μm/pixel) were normally collected at approximately 300 ms intervals. Changes in fluorescence emission intensity (F) were expressed as F_t/F_0, where F_0 is the basal fluorescence intensity obtained at the start of the experiment. During Ca^{2+} imaging, the temperature of the recording chamber was kept at 35 °C using a micro-warm plate system (MP10DM, Kitazato Supply, Fujinomiya, Japan). When the amplitude of $[Ca^{2+}]_i$ transients fell below 10% of the control value or within the noise level, the oscillation was judged to have ceased.