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SPECIAL REPORT

Evidence for the involvement of P_2-purinoceptors in the cholinergic contraction of the guinea-pig ileum

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In the isolated ileum of the guinea-pig the P_2-purinoceptor antagonists PPADS and suramin: (a) strongly inhibited the cholinergic contractile effect of α,β-methylene ATP, (b) did not influence contractions evoked by exogenous acetylcholine (ACh) but, (c) moderately (by about 30%) inhibited cholinergic contractions due to electrical field stimulation (EFS), in a non-additive manner. These results suggest that an endogenous ligand that stimulates P_2-purinoceptors (possibly ATP) is involved in the contractile effect of EFS, as a positive modulator of ACh release.

Keywords: P_2-purinoceptors; intestine; PPADS; suramin; α,β-methylene ATP; acetylcholine release

Introduction The novel purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) inhibits P_2-purinoceptor-mediated responses in various tissues (Lambrecht *et al*., 1992; Ziganshin *et al*., 1993) and has proved to be a useful tool for testing the possible participation of endogenous ligands acting at these purinoceptors in nerve-mediated responses of intestinal preparations (Zagorodnyuk *et al*., 1996).

While investigating the antagonist effect and specificity of PPADS towards the indirect (acetylcholine (ACh)-mediated) contractions of the guinea-pig isolated ileum to α,β-methylene adenosine 5′-triphosphate (α,β-meATP), a P_{2X} receptor-preferring agonist (Moody & Burnstock, 1982; Sperlágh & Vizi, 1991; Kennedy & Humphrey, 1994), we observed that PPADS induces a considerable depression of the cholinergic contractions due to EFS. The present study was undertaken to test whether this depression originates from non-specific interference with smooth muscle contractility and/or transmitter release or is related to the purinoceptor blocking activity of PPADS.

Methods Segments of whole ileum from male adult albino guinea-pigs were incubated in Krebs-Henseleit solution at 37°C, oxygenated with 5% CO_2 in O_2. Longitudinal contractions were recorded isotonically, under a tension of 6 mN. The amplitude of the contractions is expressed as % of the maximal longitudinal spasm due to ACh (10^{-5} M). Electrical field stimulation (EFS; 60V, 0.1 ms pulse width, 5 Hz, trains of 5 s in the absence of atropine or 80 V, 0.1 ms, 5 Hz, trains of 20 s in the presence of atropine, 10^{-6} M) was applied through a pair of platinum wire electrodes, placed at the top and the bottom of the organ bath. Preliminary experiments have shown that contractions to EFS (5 s-trains) were invariably depressed by atropine (10^{-6} M) by over 90% ($n=12$) and those to 20 s-trains were abolished by tetrodotoxin (10^{-6} M; $n=4$). PPADS, suramin, and α,β-meATP were applied in aqueous solutions of 2×10^{-2}, 3×10^{-2} and 10^{-2} M, respectively; incubation times were 20, 40 and 2 min, respectively. Data are presented as mean ± s.e.mean. PPADS, suramin and α,β-meATP were from RBI, ACh and tetrodotoxin from Sigma.

Results α,β-meATP (10^{-5} M) caused fast contractions that reached approximately 50% of the maximal spasm due to ACh (Figure 1), but were near-maximal for α,β-meATP (data not shown). These contractions are predominantly cholinergic since they were practically abolished by 10^{-6} M atropine (95.4 ± 1.2% reduction, range 92–100%, $n=6$). The effect of α,β-meATP (10^{-5} M) faded away within 20 s, but was reproducible if each application of the drug was followed by a 20–30 min washout period. PPADS (3×10^{-5} M) strongly inhibited the contractile effect of α,β-meATP (87.4 ± 3.2% reduction, range 69.6–100%, $n=10$; Figure 1). PPADS (10^{-5} M) had a smaller inhibitory effect ($n=6$, data not shown). Suramin, another P_2-purinoceptor antagonist (see Fredholm *et al*., 1994), at 10^{-4} M also inhibited the effect of α,β-meATP (55.5 ± 3.9% reduction, range 36.8–75%, $n=11$), confirming earlier data (Kennedy & Humphrey, 1994).

Both PPADS and suramin caused an approximately 30% depression in the cholinergic contractions due to EFS (Figure 1), but left the responses to exogenous ACh ($3-10\times10^{-8}$ M) unchanged ($n=6$ for both drugs, see Figure 1 for PPADS). No further inhibition of the EFS-evoked response was produced if the concentration of PPADS was increased two fold (from 3 to 6×10^{-5} M); moreover, the partial inhibitory effect of PPADS and suramin towards the EFS-evoked contraction was non-additive: no further inhibition was produced if suramin was

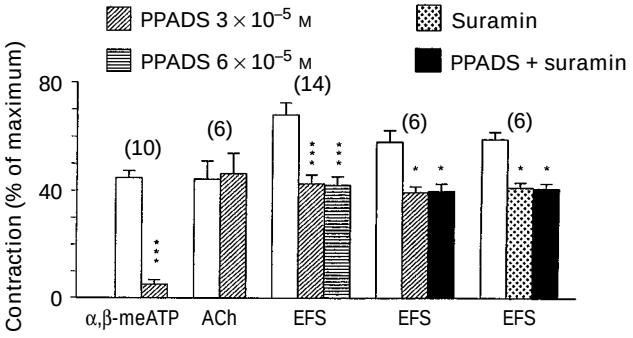

Figure 1 Contractile effects (amplitude expressed as % of the maximal longitudinal spasm to ACh, 10^{-5} M) of α,β-meATP (10^{-5} M), ACh ($3-10\times10^{-8}$ M) and EFS (5 s trains of 5 Hz). Open columns show responses before the application of PPADS ($3-6\times10^{-5}$ M) or suramin (10^{-4} M). Pretreatments are shown as shaded columns; the number of experiments is in parentheses. Asterisks indicate statistically significant differences (*$P<0.05$; ***$P<0.001$; Wilcoxon's signed-rank test was used in the case of α,β-meATP and ACh, Friedman test for several related samples for the triplets of data with EFS).

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administered to PPADS-treated ilea or *vice versa* (Figure 1). In the atropine-treated ileum, contractions due to EFS (20 s-trains) were not affected by PPADS (3×10^{-5} M); contractions reached $45.3 \pm 1.3\%$ of the maximum before and $45.0 \pm 1.5\%$ in the presence of PPADS ($n = 6$).

Discussion PPADS proved to be an efficient P_2-purinoceptor antagonist in the guinea-pig ileum. No attempt was made in the present study to establish the subtype of P_2 purinoceptor involved in the actions of α,β-meATP and the antagonists. Although PPADS has been found to exert non-specific effects in other systems in concentrations somewhat higher than that used in the present study (Vigne *et al*., 1996), in our experiments it was only the contractile effect of α,β-meATP that was strongly inhibited by PPADS, while contractions evoked by exogenous ACh and atropine-resistant contractions due to EFS were not reduced. Depression of the EFS-induced cholinergic contraction by PPADS and suramin is most probably prejunctional since these drugs did not influence the contraction to exogenous ACh. We propose that the non-additive nature of the inhibitory effect of the two antagonists speaks against a non-specific depression of the function of cholinergic neurones by PPADS or suramin. Rather, we conclude that these findings indicate the involvement of an endogenous P_2 purinoceptor ligand (possibly ATP), acting as a positive modulator of ACh release. In fact, our preliminary data show that exogenous ATP seems to activate cholinergic neurones of the gut wall, in addition to its known direct contractile effect on the smooth muscle (Moody & Burnstock, 1982; Wiklund & Gustafsson, 1988). The lack of effect of PPADS on atropine-resistant, tachykinin-mediated ileum contractions (see Barthó & Holzer, 1985) probably excludes a non-selective effect of this drug on transmitter release from myenteric neurones.

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