Pharmacological Characterisation of NECA-Induced Fluid Secretion in Human Colon Mucosa

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Introduction

The autocoid adenosine is produced in the intestinal lumen during inflammation and is thought to be a factor in the diarrhoea-associated response in certain disease states (Sitaraman *et al.*, 2002). The adenosine receptor subtype responsible for this increase in fluid secretion has been characterized in the model colon epithelial cell line, T84, and was shown to be A_{2B} (Sitaraman *et al.*, 2002). The aim of the present study was to determine which adenosine receptor subtype is responsible for fluid secretion caused by the non-selective agonist 5'-(N-ethylcarboxamido)-adenosine (NECA) in human colonic mucosa.

Methods

Human colonic mucosa samples were obtained from 15 donors (7 female, 8 male, aged between 44 and 76) post operatively (PO) with the informed consent of the donor, and with the approval of local ethics committees. Tissues were placed in PBS at 4°C and transported immediately to the laboratory; in all cases the PO delay was less than 7 hours. The smooth muscle layers were removed and sections of mucosa mounted in Ussing chambers, containing gassed Krebs solution (95% O_2 / 5% CO_2) at 37°C, for the measurement of short circuit current (Isc, indicative of electrogenic fluid secretion). After 60 minutes equilibration, carbachol (10µM) was applied to the tissues to assess viability. After a further period of washing, adenosine receptor antagonists selective for A_1 (DPCPX, Lohse *et al.*, 1987), A_{2A} (ZM241385, Ongini *et al.*, 1999), A_{2B} (MRS1754, Ji *et al.*, 2001) and A_3 (MRS1220, Jacobson *et al.*, 1998) were applied apically at a concentration of 100nM. After a contact period of 30 minutes NECA (0.1nM – 0.1mM) was added apically by cumulative application. After addition of the final concentration of NECA, carbachol (10µM) was added as a final viability check. Statistical analysis was by one-way analysis of variance.

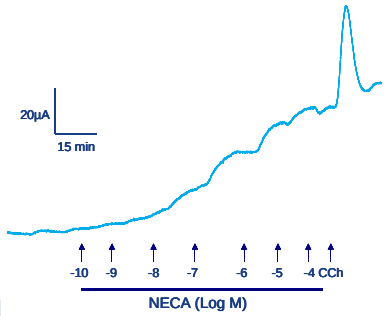

Figure 1.

Typical effect of NECA on short-circuit current (Isc) in human colonic mucosa. Trace shows concentration-dependent increases in fluid secretion.

Results

Cumulative administration of NECA in the absence of antagonist produced a concentration-dependent increase in Isc (pEC_{50} of 6.0±0.03), with a maximum response of 49.5±6.8µAcm^{-2} (n=15, Figure 1). Application of DPCPX (n=4), ZM241385 (n=6), MRS1754 (n=3), MRS1220 (n=3), or a combination of ZM241385 and MRS1754 (n=3), failed to produce a significant shift of the concentration-effect curve to NECA (Figure 2).

References

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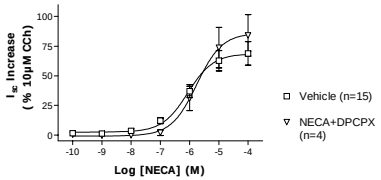
Sitaraman, S.V. *et al.* (2002). J. Biol. Chem., 6;277(36), 33188-95.

(A)



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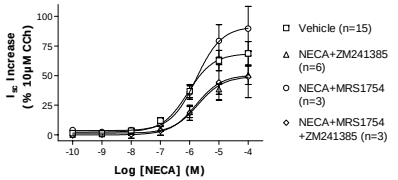

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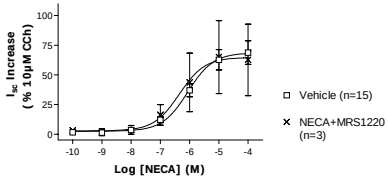

Figure 2.

Effect of selective adenosine receptor antagonists on the response of human colonic mucosa to NECA. Figures show cumulative concentration-effect curves in the absence (n=15), and presence of (A) adenosine A_1 (DPCPX, n=4), (B) A_{2A} (ZM241385, n=6), A_{2B} (MRS1754, n=3), combined A_{2A} and A_{2B} (ZM241385 plus MRS1754, n=3) and (C) A_3 (MRS1220, n=3) receptor antagonists (all 100nM). Data are expressed as the percentage of the initial carbachol challenge (CCh, 100µM).

Summary

In conclusion, contrary to published data in T84 cells, the increase in colonic mucosal fluid secretion caused by NECA *in vitro* does not appear to be mediated by any of the known adenosine receptor subtypes. The receptor responsible for this effect of NECA in human colonic mucosa has yet to be identified.

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