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Inhibition of cyclooxygenase-2 and EP_1 receptor antagonism reduces human colonic longitudinal muscle contractility in vitro

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ABSTRACT

We investigated the contribution of cyclo-oxygenase enzyme inhibition and prostamide agonism on human colonic contractility in vitro. The effects of the non-specific COX inhibitor diclofenac were compared against selective COX-2 inhibition via nimesulide, the prostanoid EP_1 receptor antagonist SC19220 or the prostaglandin prodrug/prostamide receptor agonist bimatoprost, on potency of contraction to acetylcholine in human colonic circular and longitudinal muscle strips. Pre-treatment with either nimesulide (10^{-5} M) or diclofenac (10^{-6} M) caused a significant decrease in the potency of acetylcholine-evoked longitudinal muscle contraction, but did not inhibit acetylcholine-evoked circular muscle contraction. Pre-treatment with the EP_1 receptor antagonist SC19220 (10^{-5} M) similarly decreased cholinergic potency in longitudinal muscle, without influence on circular muscle contraction. The prostamide agonist bimatoprost (10^{-6} M) increased basal circular and longitudinal muscle tone, but did not alter cholinergic potency in either muscle layer. In conclusion, colonic longitudinal muscle contraction is augmented by COX-2 activity, most likely via PGE_2 acting at EP_1 receptors. While colonic contraction is tonically modulated by bimatoprost, it does not share the same functional properties attributed to other endogenous COX-2 metabolites on colonic contractile function.

1. Introduction

Prostaglandins form a diverse class of eicosanoids with varying effects on gastrointestinal function, influencing motility, secretion and blood flow [1]. In the gut, the production of prostaglandins occurs through the action of both recognized cyclo-oxygenase isozymes COX-1 and COX-2 [2]. Studies demonstrating that COX inhibition alters colonic contractility support a role for endogenous prostaglandins in the tonic control of gut motility [3,4]. COX-1 is produced constitutively, is expressed within the muscularis propria and has been shown to modulate neurogenic contractility [3]. COX-2 is also expressed in most layers of the healthy human colon, including the muscularis propria [5,6]. While inducible COX-2 expression is augmented during experimental and clinical colitis [7,8], evidence suggests it may play an important constitutive regulatory role in excitatory transmission to the healthy colon, where it modulates cholinergic contraction [4,7] and small bowel motility [9]. These findings have allowed us to reappraise the role of COX-2 in gastrointestinal function as one operating not solely from states associated with upregulated expression, such as in inflammation, but as a part of the control of normal gut function. In addition to prostanoid production, COX-2 is also involved in the production of a novel class of bioactive prostaglandin ethanolamides (prostamides) from endocannabinoids [10]. Evidence suggests that the prostamides have distinct actions conferred via separate receptors from conventional prostaglandins [11]. This is typified by the selectivity of purported prostamide receptor-selective ligands such as bimatoprost [12,13]. The biological roles of such COX-2 derived molecules in the gut have not yet been elucidated.

In the present study, we investigated the functional effects of inhibition of cyclo-oxygenase enzymes COX-1 and COX-2 and prostaglandin EP_1 receptor antagonism on changes in human colonic longitudinal and circular muscle contractility. In addition, we compared colonic contractility with the purported prostamide receptor-selective/prostaglandin prodrug agonist bimatoprost [14], to discern whether any pattern of influence on contractility was one that might be associable with COX-derived mediators.

2. Materials and methods

2.1. Tissue collection and preparation

Experiments were performed on colonic tissue taken from patients referred to The Royal Adelaide Hospital. All studies were approved and performed in accordance with the guidelines of The

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Human Research Ethics Committee of The Royal Adelaide Hospital in accordance with the Helsinki Declaration.

Specimens of human colon were prepared following colectomy from 25 patients with intestinal carcinoma (mean age 71.0 ± 2.8 years, range 58–87 years). The samples collected were obtained from macroscopically normal regions within the colon not involved with malignancy and were not affected by colitis, fibrosis or inflammation as assessed using independent standard histological techniques. Specimens of full thickness tissue were placed in ice-cold (4 °C) carbogenated (95% O_2, 5% CO_2) Kreb's solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.6, $MgSO_4$ 1.2, NaH_2PO_4 1.3, glucose, 11, $CaCl_2$ 2.5. Samples were transferred to the research laboratory, typically within 20 min of resection.

After removal of the mucosa and submucosa, tissue was divided under sharp dissection in the longitudinal orientation to give two uniform bands of circular and longitudinal muscle, of dimensions 3 mm × 15 mm, together with the attached myenteric plexus. Isolated muscle strips were mounted in Kreb's-filled organ baths at 37 °C and continuously bubbled with carbogen. One end of the sutured tissue was fastened to a glass tissue support, whilst the other end was attached to an isometric force transducer (FT03, Grass Instruments, Quincy MA). The tissue was placed under an initial tension of 0.8 g, which we have found allows optimal development of contractile responses overall in longitudinal muscle. Tissue strips were then left to equilibrate for 60 min, with replacement of Kreb's solution every 15 min. Once equilibrated, tissue strips were exposed to acetylcholine (10^{-4} M) in order to establish responsiveness of the tissue. Preparations which responded poorly to acetylcholine at this stage were discarded from further studies.

2.2. Muscle strip contractility studies

In order to specifically examine excitatory contractile responses, the nitric oxide synthase inhibitor N_ω-Nitro-L-arginine (L-NNA; 10^{-4} M) [15] was added as a pre-treatment to longitudinal muscle preparations, in order to dampen inhibitory neuronal nitrergic activation. A contact time of at least 20 min was allowed before performing further treatments. As neuronal nicotinic receptor activation is linked predominantly to nitrergic innervation in the human sigmoid colon [16], L-NNA pre-treatment provides for a solely myogenic basis for contraction in response to exogenous acetylcholine.

Muscle strip responses to increasing concentrations of exogenous acetylcholine were then measured, with final bath concentrations of 10^{-8} to 10^{-3} M acetylcholine achieved using a cumulative protocol. Maximum contraction was recorded and the strips washed out with fresh Kreb's solution, left to return to baseline tension and L-NNA (10^{-4} M) reapplied.

Either the non-specific COX enzyme inhibitor diclofenac (10^{-6} M) or COX-2 specific inhibitor nimesulide (10^{-5} M) was then administered to investigate the effects of endogenous cyclo-oxygenase metabolites on acetylcholine-evoked colonic contraction, at concentrations effective in producing enzyme blockade [17,18]. In additional experiments, the effects of either a prostanoid EP_1 receptor antagonist SC19220 (10^{-5} M) or prostamide-selective receptor agonist, bimatoprost (10^{-6} M) on acetylcholine-evoked colonic muscle strip contractions were tested. These concentrations were chosen as the threshold for biological activity in colon tissue [19,20]. SC19220 or bimatoprost were incubated in colonic muscle strips for at least 10 min prior to acetylcholine administration.

2.3. Drugs

SC19220 (2-Acetylhydrazide 10(11H)-carboxylic acid 8-Chlorodibenz[b,f][1,4]oxazepine-10(11H)-carboxylic acid), acetylcholine chloride, sodium diclofenac, nimesulide (N-(4-Nitro-2-phenoxyphenyl) methanesulfonamide and N_ω-Nitro-L-arginine (L-NNA) were obtained from Sigma–Aldrich (Sydney, Australia). Bimatoprost (7-[3,5-dihydroxy-2-(3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-*N*-ethyl-hept-5-enamide) was diluted in saline to the required concentration from the commercial formulation (Lumigan®, Allergan Inc., Australia). Acetylcholine was dissolved in saline (0.9%, w v^{-1}) and L-NNA in deionised water. Diclofenac and nimesulide were dissolved in ethanol. Further drug dilutions were made in deionised water prior to being used. Incubation of tissue with the equivalent organ bath concentration of solvent alone (0.02%, v v^{-1} maximum) has been previously shown to not affect colonic contractility [21].

2.4. Data analysis and statistical procedures

Isometric muscle responses were amplified and digitized via an analogue/digital interface (Quad Bridge and PowerLab 4/20; AD Instruments, Sydney) prior to being acquired onto a personal computer. All tension measurements were acquired and managed onto computer hard drive using Chart™ software (version 4.0.4, AD Instruments).

Contractility in response to acetylcholine was measured as a percentage of the maximal response evoked to potassium chloride (KCl; 0.12 M). Separate time control preparations for muscle strips were performed which included vehicle administration, in order to eliminate any tissue fatigue/desensitisation. All data was analysed using the graphical and statistical analysis program Prism 4.03 (GraphPad, San Diego, CA). pD_2 values for acetylcholine-evoked contraction, alone and in the presence of the various drug interventions, were expressed as the negative logarithm of the EC_{50} values as calculated by Prism against the acetylcholine concentration–response curves. EC_{50} (agonist concentration eliciting 50% maximum response) values were calculated via sigmoidal non-linear regression of acetylcholine concentration–response data using Prism. pD_2 values were then used in repeated measures one-way ANOVA with Bonferonni's post hoc testing to enable statistical comparison between control and antagonist/inhibitor intervention treatment groups. Statistical significance was set at a P value < 0.05 for all analyses.

3. Results

3.1. Functional organ bath responses

Acetylcholine (ACh: 10^{-8} to 10^{-3} M) elicited concentration-dependent contraction in circular and longitudinal muscle strips. Incubation with nimesulide (10^{-5} M) significantly altered acetylcholine-evoked contraction in longitudinal muscle strips (Fig. 1b), such that there was a significant decrease in potency. In contrast, nimesulide was without effect on circular muscle cholinergic contractile potency (Fig. 1a).

The presence of diclofenac (10^{-6} M) elicited a similar profile of reduction in potency in response to acetylcholine compared with nimesulide in colonic longitudinal muscle strips. That is, in longitudinal muscle diclofenac (10^{-6} M) evoked a reduction in cholinergic contractile potency (Fig. 2b), while it was without effect on circular muscle contractility (Fig. 2a).

Pre-treatment with the EP_1 receptor antagonist SC19220 (10^{-5} M) caused a rightward shift in the acetylcholine concentration–response curve such that it evoked a significant decrease in acetylcholine potency in longitudinal muscle (Fig. 3b). SC19220 was without significant effect on the acetylcholine-evoked contraction in circular muscle (Fig. 3a).

Bimatoprost (10^{-6} M) significantly increased baseline tension in longitudinal muscle preparations (0.150 ± 0.03 g, P < 0.001) and