Neurogastroenterology & Motility

Neurogastroenterol Motil (2014) **26,** 237–246 doi: 10.1111/nmo.12254

Mucosal adenosine triphosphate mediates serotonin release from ileal but not colonic guinea pig enterochromaffin cells

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Key Messages

- Mucosal 5-HT signalling following mechanical stimulation varies in different regions of the intestinal tract.
- We studied the relationship between adenosine triphosphate (ATP), serotonin (5-HT) and mechanical stimulation from ileal and colonic mucosal tissue.
- Levels of ATP and 5-HT were monitoring using contact potential amperometry and high performance chromatography from isolated mucosal tissue from guinea pig ileum and colon.
- Ecto-ATPase inhibitor ARL67156 elevated ATP levels in the ileum and colon, but only 5-HT levels in the ileum.
- Mechanical stimulation increased levels of 5-HT in the ileum and colon, but levels returned to baseline in the presence of P2 antagonist suramin and P2Y1 inhibitor MRS2179 in the ileum.

Abstract

Background *Mechanical stimulation of the mucosal epithelium results in increased serotonin (5-HT) release from enterochromaffin (EC) cells. Little is known about how this process varies in different regions of the intestinal tract; however, purines are felt to play a role. We studied the relationship between mechanical stimulation, adenosine triphosphate (ATP), and 5-HT release from ileal and colonic mucosal tissue.* ***Methods*** *Amperometric recordings of ATP and 5-HT were carried out using an ATP biosensor and boron-doped diamond microelectrode. Levels of extracellular ATP and 5-HT were monitored using high performance liquid chromatography.* ***Key Results*** *Under basal conditions, 5-HT levels were significantly decreased in the ileum ($p < 0.001$) but not the colon in the presence of the P2 antagonist suramin (100 μM). Ecto-ATPase inhibitor ARL67156 (10 μM) elevated ATP levels in the ileum and colon (both $p < 0.001$), but only 5-HT levels in the ileum ($p < 0.001$). Exogenous ATP increased 5-HT release in the presence of tetrodotoxin in the ileum ($p < 0.001$), but had not effect in the colon. Mechanical stimulation increased levels of 5-HT in the ileum ($p < 0.001$) and colon ($p < 0.01$), but levels returned to baseline in the presence of suramin and MRS2179 in the ileum. The onset of 5-HT release was delayed following mechanical stimulation. The rise time of the ATP response was quicker than that of 5-HT during mechanical stimulation.* ***Conclusions & Inferences*** *During mechanical stimulation of the mucosal epithelium, ATP mediates 5-HT release from EC cells in the ileum, but not the colon. Mucosal 5-HT signaling following mechanical stimulation is varied in different regions of the intestinal tract.*

Keywords *adenosine triphosphate, colon, enterochromaffin cell, ileum, mechanosensory, purinergic, serotonin.*

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Received: 25 June 2013
Accepted for publication: 26 September 2013

INTRODUCTION

Mucosal signaling plays an integral role in secretomotor function and is responsible for both regulation of ion transport and motility. Within the mucosal epithelium, mechanical, and chemical stimulation of enterochro-

maffin (EC) cells drive release of serotonin (5-HT) which initiates local reflexes by activating intrinsic primary afferent neurons. This then inputs into the submucosal and myeneteric plexus to coordinate secretion and motility.[1,2] Enterochromaffin cells have been widely studied with much emphasis on understanding the various types of receptors that are present on the cell.[3,4] There have also been many studies that have also shown dynamic changes in the release of serotonin from EC cells in various conditions,[5–8] however, little is known about how mechanical stimulation of the mucosal epithelium can lead to changes in 5-HT signaling.

Various puringeric signaling molecules have been postulated to play a key role in the mechanism behind mechanically driven 5-HT release.[1,9] Adenosine triphosphate (ATP) has been widely postulated to play a role in driving mechanically evoked 5-HT release.[10,11] In human carcinoid BON cells (a widely used model of EC cells) endogenous ATP increased release of 5-HT.[12] Similar findings have also been observed in isolated pig EC cells.[13] However, endogenous application of 5-HT did not alter levels of ATP in guinea pig intestine.[12] Studies have also either directly or indirectly indicated that shaking or inducing mechanical stimulation of BON cells can also elevate ATP and 5-HT levels.[14,15] In the presence of P2 antagonist, PPADS, mechanically evoked 5-HT release was shown to be significantly reduced in BON cells, suggestive that ATP potentially played a role in mechanically driven 5-HT release.[15] From these various findings, it was postulated that mechanical stimulation of the mucosal epithelium released ATP, which activated P2 receptors, leading to elevated intracellular calcium and thus increased 5-HT and ATP release.[10] However, no study has investigated the dynamic changes in the levels of these mucosal neurochemicals, order to understand the sequence of events that occur post mechanical stimulation. The majority of these studies have been carried out using BON cells, which provide the ability to obtain mechanistic information about cellular signaling mechanisms, but require mechanical stimulation in a different facet to mucosal villi, where brush stroking and compression are often utilized. Due to the majority of studies being conducted on BON cells, little is known if ATP mediated 5-HT release is a process that governs all regions of the intestines where secretomotor function is essential.

In this manuscript, we utilized a dual sensor approach to elude the alterations in mucosal ATP and 5-HT during mechanical stimulation. Measurements were carried out in guinea pig ileum and colon tissue sections to understand if regional differences occurred.

METHODS

Tissue preparation

All animal experiments were carried out in compliance with the relevant laws (UK Home Office) and institution guidelines. Male Duncan Hartley guinea-pigs weighing 300–400 g were euthanized by CO_2 (100%) asphyxiation. The entire ileum and colon was harvested and placed in ice cold oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4 (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose). A segment of the ileum was removed 15–20 cm proximal to the ileocecal junction and a segment of colon was removed 5 cm from the anus.

Fabrication and assessment of the ATP sensor

The fabrication of the ATP sensor has been briefly described elsewhere.[16] Briefly, a 50 μm platinum wire needle-type electrode was modified for detection of ATP. The Pt needle electrode was placed in a solution of PBS buffer pH 7.2 containing 50 mM phenol, 40 mg/mL hexokinase (HEX, 250 U/mg), and 10 mg/mL glucose oxidase (GOx, 100 U/mg, both Sigma-Aldrich, St. Louis, MO, USA). The pH of the solution was maintained at pH 7.2. The microelectrode along with a 'no leak' Ag|AgCl (3 M KCl, model EE009; Cypress Systems Inc, Fresno, CA, USA) was placed in the enzyme solution for a 10 min stabilization time. The electrode was held at 0 V for 20 s, polarised to +0.9 V for 10 min for electropolymerization of the phenol film and then held at 0 V for 20 s. Following electropolymerization, the fabricated ATP biosensor was rinsed with deionized water and stored overnight at 4 °C before use.

Prior to *in vitro* recordings, the electrode was subjected to a calibration in the presence of ATP All measurements were made at +750 mV *vs* Ag|AgCl using a CHI630B potentiostat (CH Instruments, Austin, TX, USA). Sensors within 2% of the expected electrode sensitivity for ATP detection were utilized for biological measurements. All sensors were used following 2 days of storage and only for one single set of recordings.

Measurements of ATP and serotonin release

For tissue measurements, a 1 cm long segment of distal ileum or colon was then transferred to a flow bath containing oxygenated Krebs solution. The segment of ileum or colon was then cut open along the mesenteric border, lightly stretched, and pinned flat in the flow bath using stainless steel pins (50 μm diameter). The mucosal surface was face-up at the bottom of the chamber. This tissue was constantly perfused with warm (~37 °C) oxygenated Krebs' buffer. The tissue was exposed to the flowing solution for approximately 10 min prior to commencing a series of measurements. For continuous amperometric *in vitro* recordings from ileum and colon tissue; a Pt wire counter and a Ag|AgCl reference electrode were also mounted in the bath with the ATP biosensor and a boron-doped diamond (BDD) electrode, which was utilized for the detection of serotonin. Amperometric monitoring was controlled using a Biostat potentiostat (ESA Biosciences, Chelmsford, MA, USA). The ATP biosensor was poised at +750 mV *vs* Ag|AgCl, whilst for the BDD electrode was held at +650 mV *vs* Ag|AgCl. The flow bath was mounted on the stage of an inverted microscope (Model 3030; Accu-Scope, Commack, NY, USA) and perfused continuously with warm (37 °C) Krebs buffer solution at a flow rate of 2 mL/min. The

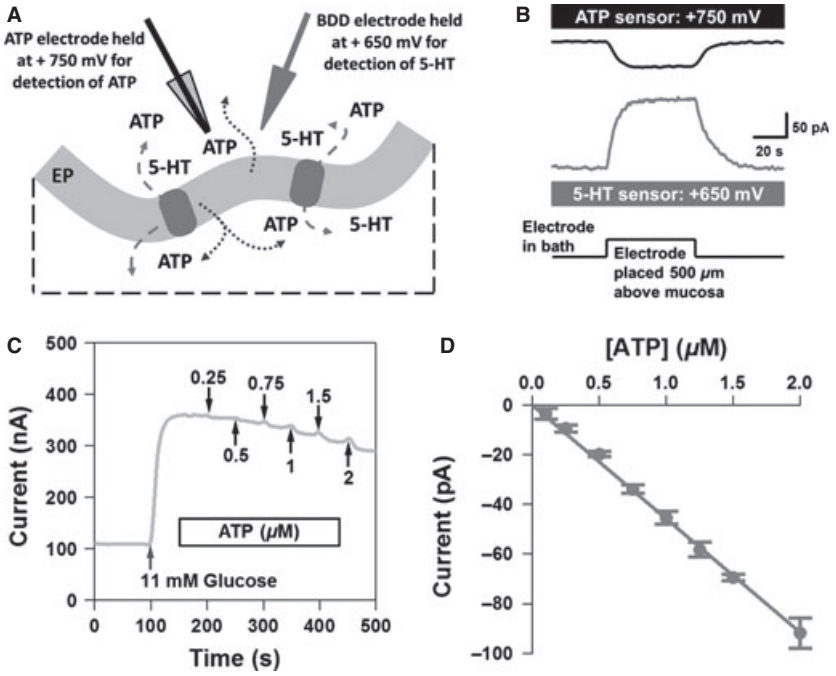

Figure 1 Amperometric detection of adenosine triphosphate (ATP) and 5-HT release using microelectrodes. (A) Experimental protocol showing the sensor and potential utilized for detection. (B) It shows the measurement protocol for recording basal levels of ATP and 5-HT. (C) It shows amperometric responses using the ATP electrode at +750 mV which is utilized to obtain the calibration plot (n = 8) shown in (D). Data shown as mean ± SD.

ATP biosensor and BDD microelectrode were mounted on micromanipulators (Model 25033; Fine Scientific Tools, Heidelberg, Germany) for reproducible placement near the mucosa (Fig. 1A).

Two types of *in vitro* protocols were carried out. To measure basal changes in 5-HT and ATP, the electrodes were placed within the bulk of the perfusing media (at least 1 cm from the tissue) to establish a stable baseline response on both electrodes. During recordings, the ATP biosensor and BDD microelectrode were carefully moved to 0.5 mm over the tissue for 60 s, before being retracted to the bulk media (Fig. 1B).

To monitor the role of mechanical stimulation on ATP and 5-HT release, the electrodes were placed at least 1 cm away from the tissue in the perfusing media to establish a baseline response. During recordings both electrodes were then carefully moved to 0.5 mm over the tissue for 60 s, before being retracted to the bulk media. During this period, 20 s into the recording, a glass capillary was utilized to stimulate the villi adjacent to the position of the electrodes. The glass capillary was utilized to distort the villi, and the force applied to the tissue was kept constant by utilizing a fixed distance moved by the micromanipulator. The mechanical stimulation was carried out for a duration of 20 s.

Chromatographic measurements

High performance liquid chromatography was utilized to monitor levels of purines and 5-HT.[17] A 1 cm^2 segment of either distal ileum or colon was transferred to a well containing warm (~37 °C) oxygenated Krebs buffer within a modified tissue well plate. This modified tissue culture plate consisted of a 16-well plate, in which the bottom of each well was lined with 5-mm thickness of Slygard 184 silicone elastomer (Dow Corning, Midland, MI, USA) to provide a surface for pinning down the tissue. The tissue sample was then cut open along the mesenteric border and pinned down flat on the modified tissue well plate. Once the tissue section was pinned down, it was washed two times using Krebs buffer to remove any waste or excised mucus during tissue transfer. Following this the tissue was then kept in 1.5 mL of warm (~37 °C) oxygenated Krebs buffer. The well plate was housed in a water bath to sustain the temperature for the duration of the experiment. After 30 min, a 250 μL aliquot of this buffer was taken and passed through a 0.2-mm syringe filter and injected for analysis. For studies of mechanical stimulation, the modified well plate was placed on a rotational stirred and investigated at various rotational speeds.

The high performance liquid chromatography (HPLC) system consisted of HP1050 autosampler, column heater kept constantly at 45 ± 0.15 °C, a quaternary pump and UV/Vis detector (Agilent, Wokingham, Berkshire, UK). A LUNA 5 mm 150 × 1.0 mm id analytical column with 4.0 × 2.0 mm, 5 mm guard column (Phenomenex, Macclesfield, UK) was utilized. The HPLC system was run with a 100 μL/min flow rate, and an injected sample volume of 5 μL was used. BAS ChromGraph software was used for control and data collection/processing. The HP1050 UV detector was set at a wavelength of 260 nm. The mobile phase was composed of 0.1 M ammonium phosphate monobasic and 10 mM tetrabutylammonium bisulfate dissolved in 1 L of deionized, distilled water, and buffered to pH 7.46 using 2 M sodium hydroxide. To prepare the mobile phase, the ammonium phosphate buffer (pH 7.46) was mixed with methanol (LCMS CHROMASOLVs for HPLC) in the ratio 95 : 5 v/v and degassed after mixing.

Data analysis

For electrochemical measurements, the difference in the current from baseline to whilst the electrode was positioned over the tissue was converted to the concentration of ATP or 5-HT released based on calibration plots obtained with the same electrode. For HPLC measurements, the peak area of the analyte was monitored and converted to concentration of the signaling molecule released. The concentration data obtained from HPLC was normalized by the surface area of the tissue. The tissue area was calculated by a pixel counting algorithm which has been widely used.[17–19]

Photographic images were taken of the modified tissue well plate in which the tissue samples were placed and the image was processed using IMAGE J software (NIH, Bethesda, MD, USA) to accurately measure the area of the pinned out isolated ileum tissue sections in pixel numbers, which was converted to cm^2. Results were presented in mean ± SD and the experimental data was statistically assessed using either a one-way or two-way ANOVA with post hoc Tukey tests.

RESULTS

Assessment of the ATP biosensor

Fig. 1C shows stable responses of ATP at various concentrations. There is a clear decrease in the current with increasing concentrations of ATP. The response time of the electrode is 11 ± 5 s. A calibration plot is shown in Fig. 1D, where a linear response is observed. The limit of detection was 9.9 ± 3.2 nM, with a sensitivity of 45.8 ± 1.22 pA/μM (n = 8). Previously, selectivity of the electrode was investigated, where no interference from adenosine mono-phosphate, adenosine di-phosphate, and serotonin were observed on the ATP biosensor.[16]

Detection of ATP and 5-HT release from mucosa

Representative traces from distal ileum and colon are shown in Fig. 2A and C. Clear responses of both ATP and 5-HT were observed when the electrodes were positioned over the mucosal surface. When the ileum was exposed to 100 μM suramin (non-selective P2 antagonist), no changes in ATP release were observed, however, there was a significant reduction in 5-HT release (Fig. 2B, $p < 0.001$, $n = 6$). When 1 μM ARL67156 (ecto-ATPase inhibitor) was perfused to the ileum, the level of both ATP and 5-HT significantly increased compared with the control response (Fig. 2B, $p < 0.001$, $n = 6$). In the distal colon, no significant differences in the ATP or 5-HT levels were observed in the presence of 100 μM suramin ($n = 6$, Fig. 2D). There was a significant increase in the level of ATP in the presence of 1 μM ARL67156 in the colon ($p < 0.001$, $n = 6$), however, a non-significant decrease in 5-HT levels were observed ($n = 6$, Fig. 2D).

Influence of exogenous ATP on 5-HT release

Recordings of 5-HT were carried out to understand the influence of perfused exogenous ATP was direct on the mucosa or via the myenteric plexus. Representative traces from ileum tissue are shown in Fig. 3A. Following the addition of 300 nM tetrodotoxin (TTX), 5-HT release in the ileum significant reduced ($p < 0.05$, $n = 6$, Fig. 3B). The 5-HT release, however, significantly increased in the ileum in the presence of 5 μM ATP and also in the presence of 300 nM TTX and 5 μM ATP ($p < 0.001$, $n = 6$, Fig. 3B). The net increase in

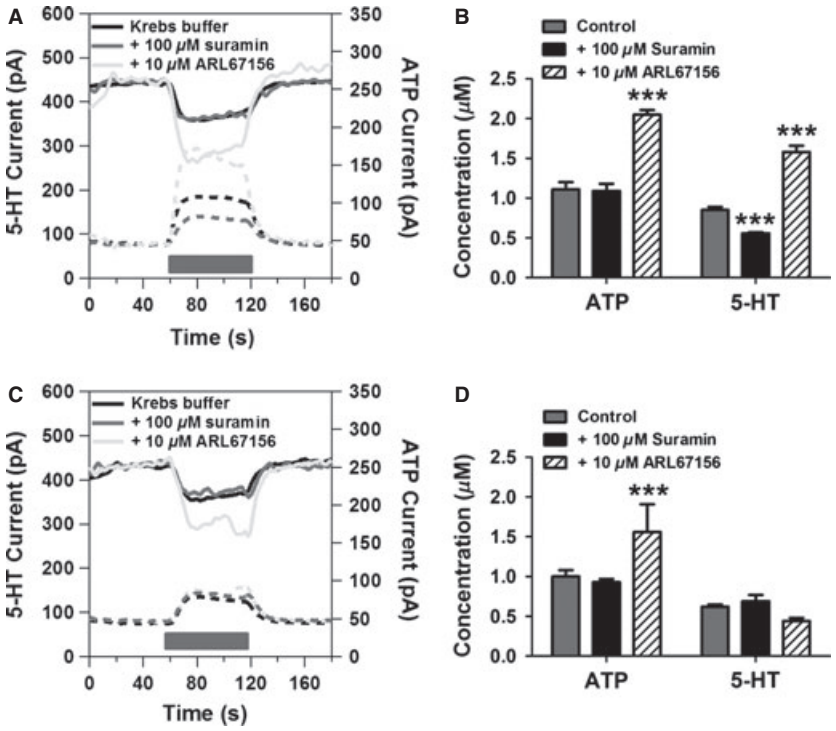

Figure 2 Basal responses of adenosine triphosphate (ATP) and 5-HT from ileal and colonic segments *in vitro*. (A) Traces from distal ileum tissue, where suramin decreased 5-HT release whilst ARL67156 increased both ATP and 5-HT release. (B) Population data from ileum traces are shown ($n = 6$). (C) Traces from distal colon tissue, where no differences in the response where obtained in the presence of suramin. ARL67156 only increased levels of ATP. (D) Population data from distal colon traces ($n = 6$). Data shown as mean ± SD, $^{***}p < 0.001$ *vs* control.

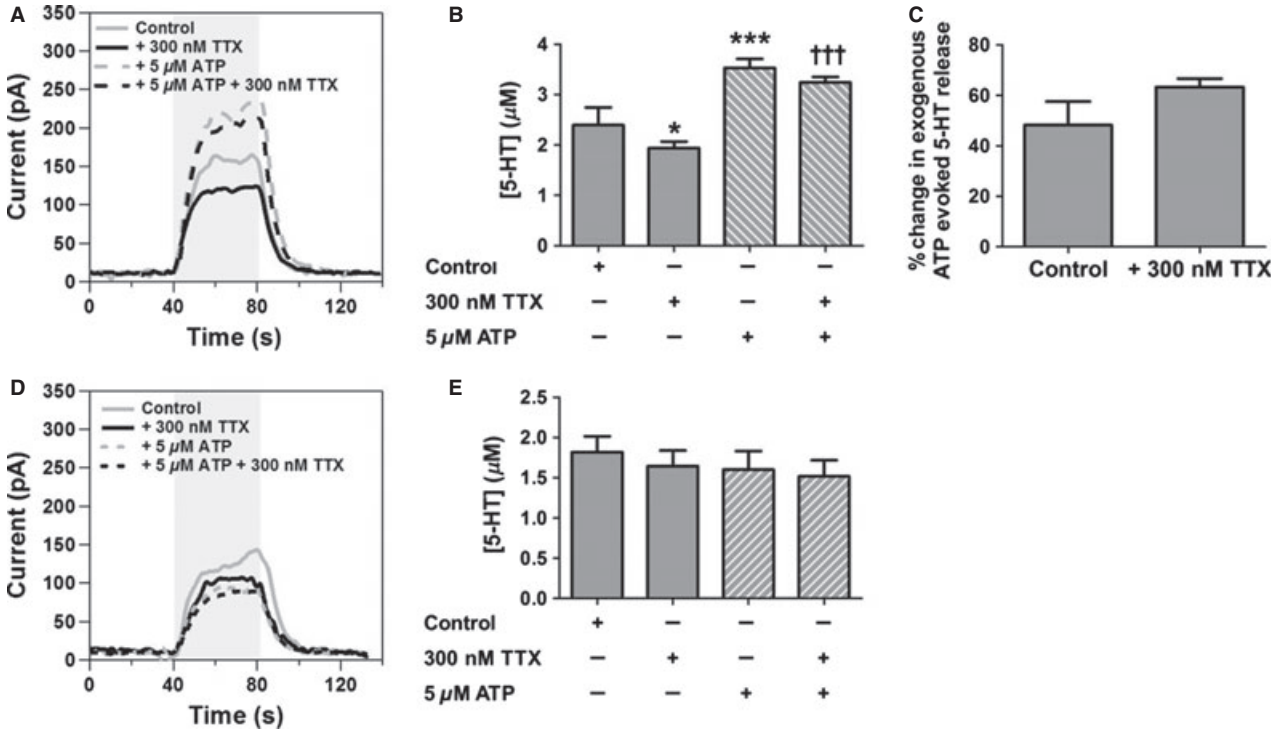

Figure 3 Exogenous adenosine triphosphate (ATP) driven 5-HT release is via a mucosal pathways. (A) 5-HT release is increased by exogenous ATP, which is not altered in the presence and absence of TTX in the distal ileum. (B) Data from ileum tissues ($n = 6$). (C) Percentage change in exogenous ATP evoked 5-HT release in the absence and presence of TTX ($n = 6$). (D) No difference in 5-HT release was observed in the presence and absence of ATP and TTX in the distal colon. (E) Data from distal colon tissues ($n = 6$). Data shown as mean ± SD, $^{*}p < 0.05$, $^{***}p < 0.001$ *vs* control, and $^{\dagger\dagger\dagger}p < 0.001$ *vs* 300 nM TTX.

exogenous ATP induced 5-HT release in the presence and absence of TTX is shown in Fig. 3C. There was no significant difference in the amount 5-HT increased in control and 300 nM TTX ($n = 6$). Representative sample traces from the colon are shown in Fig. 3D. There was no significant difference in 5-HT levels when exposed to exogenous ATP or in the presence of TTX (Fig. 3E, $n = 6$).

Influence of mechanical stimulation on ATP and 5-HT release

Chromatographic responses were carried out to monitor levels of ATP and 5-HT simultaneously. Clear separation between all key neurochemicals was observed (Fig. 4A). There was a significant increase in 5-HT levels following mechanical stimulation (50 rpm) in both ileum ($p < 0.001$, $n = 6$, Fig. 4B) and colon ($p < 0.01$, $n = 6$, Fig. 4C). However, 5-HT levels were similar to basal levels in the ileum when mechanical stimulation was carried out in the presence of 100 μM suramin ($n = 6$, Fig. 4B). In the colon, the amount of 5-HT observed during mechanical stimulation in the presence and absence of suramin was not altered ($n = 6$, Fig. 4C). Experiments in the ileum were carried out to understand the link between mechanical stimulation, ATP release and 5-HT release. Chromatographic responses were obtained at various rotational speeds. There was a significant increase in ATP levels with increased rotational speeds ($p < 0.001$, $n = 6$, Fig. 4D). There was no difference in ATP levels under control conditions and those in the presence of 100 μM suramin or 10 μM MRS2179. Levels of ATP release began to plateau around 70 rpm. There was also a significant increase in 5-HT levels with increasing rotational speeds ($p < 0.01$, $n = 6$, Fig. 4E). In the presence of 100 μM suramin and 10 μm MRS2179, there was a significant reduction in 5-HT levels at 50 and 70 rpm ($p < 0.01$, $n = 6$) rotational speeds compare with control conditions (Fig. 4E).

Electrochemical measurements were carried out on ileum tissue to verify this response. Fig. 5A shows simultaneously recordings of 5-HT and ATP during mechanical stimulation (occurring in phase 2 of the signal). The population data are shown in Fig. 5B. Compared with the response at prestimulation (basal

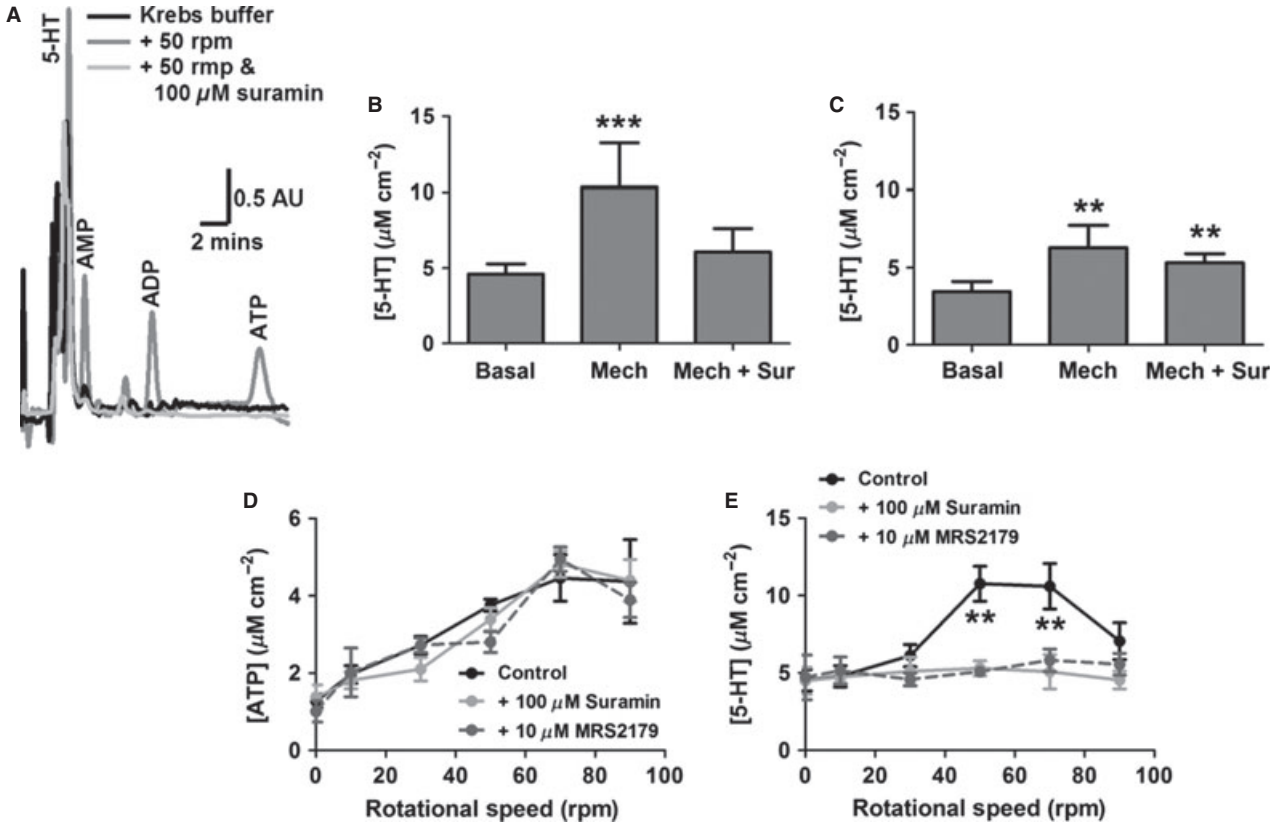

Figure 4 Alterations in extracellular adenosine triphosphate (ATP) and 5-HT levels following rotation. (A) Chromatographic responses showing changes in levels of neurochemicals following rotation in the presence of suramin in the ileum. (B) Increases in 5-HT levels were observed following mechanical rotation at 50 rpm (Mech) and return to basal levels following the additional of suramin during rotation (Mech + Sur) in the ileum ($n = 6$). (C) Increases in 5-HT levels were observed following mechanical rotation at 50 rpm (Mech) and following the additional of suramin (Mech + Sur) in the colon ($n = 6$). (D) Alterations in extracellular ATP levels following mechanical rotation ($n = 6$). (E) Alterations in extracellular 5-HT levels following mechanical rotation ($n = 6$). Data shown as mean ± SD, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs* control or basal response.

levels of both neurochemicals), there was a significant increase in 5-HT and ATP levels following mechanical stimulation ($p < 0.001$, $n = 6$, Fig. 5B). Compared with prestimulation, the post simulation levels of 5-HT were significant greater ($p < 0.001$, $n = 6$, Fig. 5B), but were not significantly different from that during mechanical stimulation. There was a significant reduction in ATP levels post stimulation when compared with that during mechanical stimulation ($p < 0.001$, $n = 6$). The levels of ATP pre- and post stimulation where not significant different ($n = 6$, Fig. 5B).

Dynamic changes in ATP and 5-HT release from mechanical stimulation

Following mechanical stimulation using a glass capillary, the dynamics of the real time ATP and 5-HT release profiles were assessed. Represented traces are shown in Fig. 6A, where the dashed line indicates the point where the mechanical stimulation was initiated. The rise time (5–95% maximal response) for ATP was 6.9 ± 0.4 s, whilst this is significantly slower for 5-HT (9.6 ± 0.6 s, $p < 0.001$, $n = 10$, Fig. 6B). The ATP release occurred simultaneous with the onset of mechanical stimulation, however, 5-HT release occurred 1.9 ± 0.2 s following the onset of mechanical stimulation ($n = 10$, Fig. 6C).

DISCUSSION

Detection of extracellular ATP and 5-HT release

This manuscript shows for the first time real-time simultaneous release of both 5-HT and ATP and thus provides a means of monitoring the dynamics of these key mucosal signaling molecules following mechanical stimulation. Extracellular release of 5-HT and ATP were also confirmed by using chromatography. Release of 5-HT using amperometry has been carried out on various animal models, where concentrations

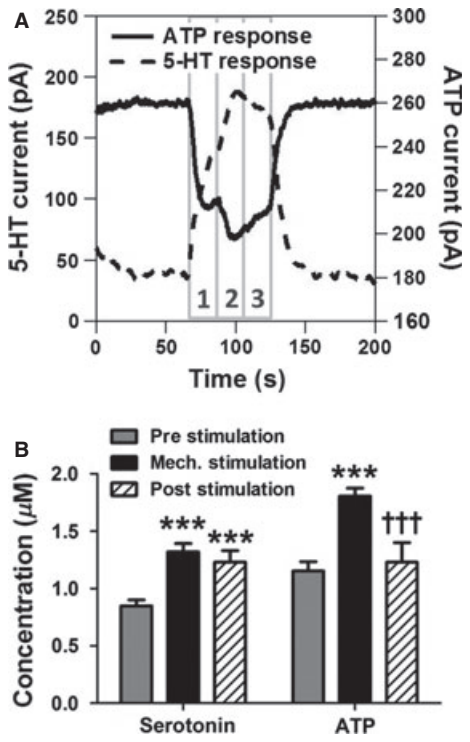

Figure 5 Amperometric responses of 5-HT and adenosine triphosphate (ATP) following mechanical stimulation using a glass capillary. (A) Simultaneous recordings of ATP and 5-HT from ileum tissue during mechanical stimulation, where 1 is the prestimulation phase, 2 is the mechanical stimulation phase, and 3 is the poststimulation phase. (B) Population from various ileum tissue segments ($n = 6$). Data shown as mean ± SD, $^{***}p < 0.001$ *vs* prestimulation and $^{\dagger\dagger\dagger}p < 0.001$ *vs* mechanical stimulation.

of 5-HT release vary.[8,20–23] Basal levels of 5-HT were ~1 μM in the ileum and decreased to ~0.7 μM in the distal colon. Other studies have shown similar trends, with 5-HT levels decreasing down the gastrointestinal tract.[24,25]

As ATP is not electro-active a biosensor was utilized for the measurement. Adenosine triphosphate biosensors have been widely used in physiological measurements.[26–28] A previous study has shown that the ATP biosensor utilized in this study was not prone to fouling and was stable for over 4 h of *in vitro* recording from intestinal tissue and the sensor was selective for ATP against other electro-active mucosal analytes like 5-HT.[16] The ATP electrode was able to provide stable responses when placed over the mucosa. The response times for both electrodes were similar when comparing the rise times of the basal responses of 5-HT and ATP. There were similar levels of ATP observed in both the ileum and distal colon. Adenosine triphosphate levels were elevated in both the ileum and colon, when the ecto-ATPase inhibitor ARL67156 was utilized, suggestive that ATP was detected by the biosensor.

Adenosine triphosphate release was shown to be linked with 5-HT release through measurements of basal levels in the ileum. When the breakdown of ATP was prevented in the ileum, 5-HT levels were elevated. This trend was not observed in the colon, suggestive that ATP does not potentially have a significant role in driving 5-HT levels. With the use of TTX, it was confirmed that this ATP induced increase in 5-HT release was a mucosal effect in the ileum and thus supported the findings of studies in BON cells, where endogenous ATP increased 5-HT levels.[11,15] Under control conditions a reduction in 5-HT levels in the presence of TTX was observed in the ileum, but not the colon. This would suggest that either primary afferent neurons have a potential feedback on the ileal EC cells or that this cell expresses similar Na^+ ion channels as neurons. However, these processes are not present in colonic EC cells, suggestive of regional specific variations in EC cell pharmacology.

When a non-specific P2 inhibitor suramin was utilized, the 5-HT response decreased in the ileum but was not affected in the colon. This suggests that the ATP mediated 5-HT release is directed through a P2 receptor. Further investigation showed that that ATP medicated 5-HT response was blocked using P2Y1 inhibitor MRS2179. Such findings are similar to those that have been proposed through mechanistic studies of BON cells. These studies have indicated that this is potentially via the P2Y receptor.[10] However, more interestingly in the colon, ATP does not mediate 5-HT release but is present in similar concentrations as that in the ileum. These results imply that there are regional differences in the role of mucosal ATP.

Mechanically driven ATP mediates 5-HT release

Initially using chromatography, 5-HT levels were elevated in the ileum following mechanical stimulation. However, this increase was suppressed in the presence of MRS2179, suggestive that mechanically stimulated 5-HT release occurred via P2Y1 receptors. When looking at the effects of various rotational speeds on the release of ATP and 5-HT, there is a significant increase in both neurochemicals in the ileum. At high rotational speeds, the level of 5-HT released decreased, which may be due to desensitization of the mucosal mechano-receptors. Importantly, suramin and MRS2179 was able to decrease mechanically driven 5-HT release and did not hinder increases in ATP release. These findings tend to show that mechanical stimulation is linked to both an increase in ATP and 5-HT release through a P2Y1 receptor in the ileum. However, in the colon, 5-HT release is elevated as anticipated, but not decreased via the non-specific P2

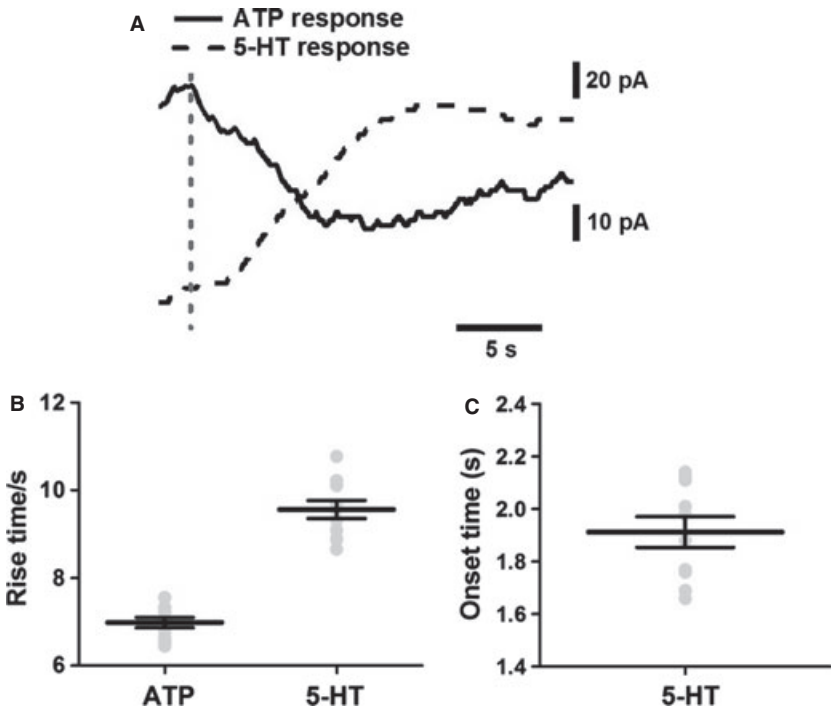

Figure 6 Analysis of neurochemical release dynamics during real-time amperometric detection of 5-HT and adenosine triphosphate (ATP) release during mechanical stimulation. (A) Representative trace showing the changes in the ATP and 5-HT signal following mechanical stimulation. The onset of mechanical stimulation is indicated by the dashed grey line. (B) Rise time of the signal measured as the time taken from 5 to 95% of the total signal amplitude (n = 10). (C) Time taken for the onset of the signal to occur is delayed only for 5-HT (n = 10). Data shown as mean ± SD, $^{***}p < 0.001$ *vs* ATP.

receptor, suggestive that ATP does not play a role in mechanically driven 5-HT release. These finding are similar to those on BON cells, where rotation also caused increases in ATP and 5-HT release.[10,15]

Interestingly, ATP was shown to be dynamically linked with increases in 5-HT release. Following mechanical stimulation with a glass capillary, increases in both ATP and 5-HT release were observed in the ileum. Post stimulation, ATP levels returned to basal, whilst 5-HT levels were sustained. This slightly varies from the BON cell studies where mechanical stimulation was felt to drive additional ATP release.[10] When studying the dynamic nature of the signal, the ATP release onset during the initiation of the stimulus, and 5-HT response was slightly delayed. The rise time of the ATP signal was greater than that of 5-HT, support that the fact that ATP mediates 5-HT release. No dynamic simultaneous measurements of ATP and 5-HT release have been carried out. These results support that ATP plays a key role that links mechanical stimulation to elevated 5-HT level in the ileum.

Mucosal ATP in colonic tissue does not influence EC cell 5-HT release

Adenosine triphosphate release in the colon was observed, however from both electrochemical and chromatographic studies, no association between ATP and 5-HT was observed. Other studies have shown as observed in this study that 5-HT release in the colon is increased by mechanical stimulation,[7,21] however, this mechanism is not via that proposed through the studies on the BON cell model. There are clear differences in the role of mucosal ATP in the ileum and the colon. Other purines such as adenosine has also been shown to influence 5-HT release from BON cells and this may be the key regulator of mechanically driven 5-HT release.[29]

Adenosine triphosphate has been associated with various functions in the mucosa, including ion transport. There have been studies linking ATP with chloride secretion[30,31] and thus may have implications in cystic fibrosis transmembrane conductance regulating.[32] Adenosine triphosphate in the mucosa of the rat bladder was shown to be altered by flavorants and odorants, thus in colonic mucosa,[33] ATP levels may be altered through chemo-sensory processes and play different roles in secretomotor function

SUMMARY AND CONCLUSIONS

Adenosine triphosphate release was observed from both guinea pig distal ileum and colon. In the distal ileum, mechanical stimulation elevated both levels of ATP and 5-HT. Analysis of the real-time signal and use of pharmacological agents suggested that following mechanical stimulation ATP release came before 5-HT

release. This indicates that ATP plays a major role in increasing 5-TH level during mechanical stimulation of the mucosal epithelium the ileum. In the distal colon, ATP did not influence 5-HT level under basal or during mechanical stimulation. These data indicate that the mechanism behind mechanically driven 5-HT release is varied in the regions of the intestinal tract and will benefit our understanding of mucosal signaling mechanisms.

ACKNOWLEDGMENTS

BAP would like to thank the University of Brighton, the Royal Society and the EPSRC for funding.

FUNDING

This research was funded by the EPSRC (EP/J000175/1), Royal Society Research grant and the University of Brighton Sabbatical grant.

DISCLOSURE

The author has no actual and potential conflict of interest.

AUTHOR CONTRIBUTION

BAP designed and performed the experiments, analyzed the data and wrote the article. The funding and experimental concept was created by BAP.

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