Mucosal serotonin overflow is associated with colonic stretch rather than phasic contractions

Running title: Serotonin associated with colonic stretch

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Abstract

Background Many studies have shown that mucosal serotonin (5-HT) is associated with motility, however recently there have been some questions to the precise role of this transmitter. The majority of studies have focused on understanding the role of mucosal 5-HT on colonic migratory motor complexes, but very few studies have been carried out to understand how 5-HT release may be associated with other motility patterns. ***Methods*** Using distal colon segments from C57BL/6J mice, mucosal 5-HT overflow was monitored using amperometry whilst applying tension in longitudinal or circular directions to stretch the tissue. ***Key Results*** Phasic and basal 5-HT levels were not associated with the strength of phasic contractions, whilst being altered using scopolamine and L-NNA. There was a significant increase in mucosal 5-HT with longitudinal and circular muscle stretch. A greater applied force was needed to activate 5-HT release in the circular muscle. In the longitudinal muscle, 5-HT levels increased with stretch until 3 mN, after which the levels returned back to baseline. This stretch-evoked 5-HT overflow was inhibited by transient receptor potential A1 (TRPA1) agonist, 30 μM ruthenium red in both circular and longitudinal muscle preparations. The decreased 5-HT overflow after 3mN of tension was reversed using a 5-HT_4 antagonist 100 nM GR113808. ***Conclusions & Inferences*** Our findings show a relationship between colonic stretch and mucosal 5-HT overflow, whilst no relationship is observed with phasic colonic contractions. Such findings provide more insight into the role of mucosal 5-HT in influencing the pattern of colonic motility to diversify fecal propulsion.

Keywords

Serotonin, enterochromaffin cell, mechanosensitivity, TRPA1, contraction,

Key Messages

- This study shows that mucosal 5-HT is linked with colonic longitudinal and circular muscle stretch rather than phasic contractions and thus may support its role in fecal propulsion.
- This study aims to understand the association of mucosal 5-HT overflow with colonic stretch and phasic contractions
- Amperometry was utilised to monitor mucosal 5-HT overflow whilst applying tension to isolated murine distal colon
- Basal and phasic 5-HT levels did not change when the strength of phasic contractions were altered in scopolamine and L-NNA
- Basal 5-HT levels increased linearly with longitudinal colonic stretch until 3 mN, due to the activation of TRPAI ion channels. After 3 mN of applied tension, 5-HT levels decreased due to the activation of an inhibitory 5-HT_4 autoreceptor.

Introduction

Mucosal serotonin (5-HT) has been shown to play a role in gut function ever since the key initial studies by Bulbring which demonstrated that 5-HT was able to initiate propulsive motility (1). Since this study there have been a vast number of studies that have investigated the association of mucosal 5-HT with various motility patterns (2-6). Of recent there has been a high degree of controversy on the role of mucosal 5-HT, with studies debating if there is any need for mucosal 5-HT at all. Studies have highlighted that mucosal 5-HT is not essential for generating colonic migratory motor complexes (CMMCs), but 5-HT does seem to play an important role in shape and nature of this motility tone (7-11). When using TpH1$^{-/-}$ mice, where mucosal 5-HT has been depleted, CMMCs and fecal pellet propulsion still occurred, but the tone was slightly varied and the fecal pellets were much larger in diameter. This indicates that 5-HT or other mucosal signalling molecules are important to motility (12). Although a vast number of investigations have been conducted, only a few motility patterns have been investigated for their association with mucosal 5-HT and limited studies have monitored mucosal 5-HT and motility patterns simultaneously.

Electroanalytical techniques have taken prominence over the past 10 years as a suitable approach to detect real-time overflow of mucosal 5-HT and a vast number of studies have been carried out (13-16). These approaches provide a suitable platform to study the association of mucosal 5-HT release with colonic motility and a few studies have been carried out. Investigations using carbon fibre microelectrodes placed on the surface of the mucosa was utilised to show that 5-HT was linked with circular contractions in the guinea-pig and rat ileum (17, 18). These studies showed that stretch evoked reflex contractions of the circular and longitudinal muscle occurred with 5-HT release, however no mechanistic information was provided on why this correlation occurred (17).

Our study aims to investigate the relationship between mucosal 5-HT overflow and motility patterns associated with fecal propulsion. We investigated how increased colonic longitudinal and circular muscle stretch and stretch evoked phasic contractions are influenced by 5-HT overflow. Experiments were carried out using electrochemical sensors to monitor 5-HT overflow from murine colonic mucosa whilst stretch was applied at varying forces. Varying pharmacological agents were utilised to investigate how neuronal and mucosal mechanisms influenced 5-HT levels during stretch.

Materials and Methods

Animals

All procedures were carried out according to U.K. Home Office regulations and were approved by the University of Brighton Ethics Committee. Male C57BL/6J mice were obtained from Harlan UK at 8 weeks of age and housed in individual ventilated cages under barrier-reared conditions until required. Animals were maintained at 19.0 ± 1 °C, 55 % humidity and fed on a maintenance diet (RM1 (E) 801002 (Special Diet Services) chow).

Intestinal preparation

Animals aged 3 months were euthanized in CO_2, followed by cervical decapitation. A 2 cm segment of the distal colon was harvested 2 cm proximal to the anus 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) prior to experiments.

Amperometric detection of mucosal 5-HT overflow

Electrochemical measurements were made using a boron-doped diamond (BDD) microelectrode as previously described (19). A 2 cm segment of the distal colon was opened along its mesenteric border and pinned in a Sylgard®-(Dow Corning, UK) lined Teflon recording chamber and superfused with warm (37 °C) Krebs solution at a flow rate of 2 mL/min. Tissues were perfused for 30 minutes prior to commencing a series of measurements. For continuous amperometric recordings of 5-HT overflow, measurements were carried out using a BioStat™ multi-channel potentiostat (ESA Biosciences, Inc, USA). The BDD electrode was held over the tissue at +650 mV vs Ag|AgCl which was sufficient to oxidize 5-HT at a mass transfer limited rate. Using a micromanipulator, the BDD electrode was positioned several centimetres away from the mucosa for several seconds. For recordings, the distance between the tissue and electrode (T–E distance) was varied from 25 to 2000 µm and the oxidation currents were recorded at each distance.

Simultaneous detection of mucosal 5-HT overflow and colonic motility

A 2 cm segment of the distal colon was opened along its mesenteric border, placed in the recording chamber and superfused with warm (37 °C) Krebs solution at a flow rate of 2 mL/min. The tissue was either positioned to investigate circular or longitudinal muscle stretch. Half of this tissue segment was pinned down and the other half was left free and attached to a tweezer clip. The tweezer clip was attached to a force transducer using silk suture. Initially the tissue was held under resting tension. The signal from the force transducer was monitored by ADI Powerlab via a preamplifier. Traces were recorded using Chart software. For detection of 5-HT overflow, amperometric detection using a BDD electrode held at +650 mV vs Ag|AgCl was utilised as described above. The electrode was positioned in the centre of the tissue segment that was pinned down.

To understand the association of mucosal 5-HT with phasic contractions, the longitudinal muscle was placed at a tension of 1 mN and the circular muscle was placed at a tension of 3 mN whist recordings of 5-HT overflow were conducted at 10 µm T–E distance. The influence of 5-HT overflow and phasic longitudinal contractions were assessed in the presence of 1 µM scopolamine (muscarinic receptor antagonist) and 100 µM L-NNA (nitric oxide synthase blocker).

To understand how longitudinal or circular stretch influences mucosal 5-HT overflow, the muscle was held at various tensions sequentially from 0 to 6 mN for a duration of 200 s. After the tissue was held at a particular tension for 50s, amperometric recordings were carried out for 100 s at 100 µm T–E distance. Following a control measurement, the influence of and individual pharmacological compound was investigated after the tissue was allowed to rest for 30 minutes. Tissues were exposed to the following compounds: 100 µM cinnamaldehyde (CA; TRPA1 agonist), 100 nM tetrodotoxin (TTX), 30 µM ruthenium red (TRPA1 antagonist), 100 nM GR113808 (5-HT_4 antagonist) and 1 µM ondansetron (5-HT_3 antagonist).

Data Analysis

For analysis of the amperometric current response, the difference between the baseline and the current monitored at varying T–E distances were recorded and converted to the concentration of 5-HT using calibration plots. For contractility traces, the mean and standard deviations of the contractions were monitored at various applied tension. Data

was presented as mean ± standard deviation and statistical analysis was carried out using a 2-way ANOVA with Bonferroni tests using Graphpad Prism.

Results

Variations in 5-HT release over mucosal regions

Figure 1 shows how levels of mucosal 5-HT varies at different regions of the colonic mucosal surface during amperometric recordings. During these recordings, the BDD electrode was held at various T–E distances whilst moving the electrode horizontally over the tissue from zone 1 to 5 for a duration of 40 s (Figure 1A). Each zone was ~1 mm away from each other. The resultant responses at T–E distances ranging from 50 to 500 µm are shown at the various zones, where clear differences in the current was observed at the varying zones of the colonic mucosa at smaller T-E distances (Figure 1B). At larger T-E distances there was minimal variation in the levels of 5-HT observed at varying zones. In Figure 1C the mean and standard deviation from measurements at all the zones at varying T–E distances were obtained. The standard deviation increased at T–E distances closer to the tissue. 5-HT levels at the surface of the tissue based on projections would vary from 13 to 7 µM within the area monitored. This variation in 5-HT levels is only observed until a T–E distance of 150 µm, where the error is reduced.

Understanding how 5-HT overflow at various electrode distances is influenced by colonic motility

Figure 2 shows how amperometric monitoring of 5-HT is influenced by stretch-evoked spontaneous contractions. Experiments were carried out where the BDD electrode was held in a fixed position at varying T-E distances whilst phasic contractions were initiated following application of 1 mN of stretch (Figure 2A). When the T–E distance increases, less variation in the 5-HT overflow was observed for the duration of the recording. The overall responses are shown in Figure 2B, where minimal variation in the observed 5-HT overflow occurs at T–E distances greater than 100 µm. At T-E distances less than 50 µm, the 5-HT overflow trace can vary by ~ 3 µM. During the course of the recording, there was no significant difference in the amplitude of phasic contractions. In Figure 2C, the 5-HT overflow trace is shown alongside phasic contractions when the T-E distance is 10 µm. There is a clear pattern that may indicate a relationship between 5-HT overflow and phasic contractions. Therefore at this T-E distance there is the ability to study 5-HTs association

with phasic contractions. When the T-E distance is at 100 µm there is very little variation in the 5-HT overflow (Figure 2D). At this distance the 5-HT overflow is more representative of the tissue and thus can be used to understand how colonic stretch influences 5-HT overflow.

Relationship between phasic colonic longitudinal contractions and mucosal 5-HT overflow

The BDD microelectrode was held at 10 µm T-E distance at a fixed position in the centre of the tissue which was pinned down, whilst applying 1 mN of stretch to induce phasic contractions. Figure 3A-C show traces of 5-HT overflow and contractility under control conditions, in the presence of 1 µM scopolamine and 100 µM L-NNA. The standard deviation was obtained from the contractility traces for a duration of 100 s as a marker of the strength of the phasic contractions. There was a significant decrease in the strength of the phasic contractions in the presence of 1 µM scopolamine when compared to control (Figure 3D). There was a significant increase in the strength of phasic contractions in the presence of 100 µM L-NNA when compared to control (Figure 3D). The 5-HT levels were analysed by two approaches; the mean 5-HT levels were recorded for a duration of 100 s to obtain the basal 5-HT levels and the standard deviation of the 5-HT levels was obtained for 100 s to monitor the phasic 5-HT levels. Both were compared to the strength of phasic contractions under the various pharmacological treatments. Figure 3E shows how basal 5-HT levels vary with the standard deviation of phasic contractions. In the presence of either L-NNA or scopolamine, there was no change in the basal 5-HT levels even if the strength of contraction was influenced. In Figure 3F, the phasic 5-HT signal was recorded against the standard deviation of phasic contractions, where once again no differences in the 5-HT levels were observed. These results indicate that both basal and phasic 5-HT release is not associated with the strength of the phasic contractions.

Relationship between phasic colonic circular contractions and mucosal 5-HT overflow

The BDD microelectrode was held at 10 µm T-E distance at a fixed position in the centre of the tissue which was pinned down, whilst applying 3 mN of stretch to induce phasic contractions. Figure 4A-C show traces of 5-HT overflow and contractility under control conditions, in the presence of 1 µM scopolamine and 100 µM L-NNA. There was a

significant decrease in the strength of the phasic contractions in the presence of 1 μM scopolamine when compared to control (Figure 4D). There was a significant increase in the strength of phasic contractions in the presence of 100 μM L-NNA when compared to control (Figure 4D). Like that observed for longitudinal muscle phasic contractions, there was no differences in the basal or phasic levels of 5-HT in the presence of either L-NNA or scopolamine (Figure 4E&F). These results indicate that both basal and phasic 5-HT release is not associated with the strength of the phasic contractions.

Association between mucosal 5-HT overflow and stretch of colonic tissue

To monitor the association of mucosal 5-HT overflow and colonic stretch the BDD electrode was placed over the tissue in a fixed position at a T-E distance of 100 μm. During this a micromanipulator was used to apply tension on the tissue. Tension applied was increased by 1 mN every 200 s until 6 mN was applied to stretch the longitudinal or circular muscle. During this period the overflow of 5-HT was recorded for a duration of 100 s at each applied tension (Figure 5A-D). As the applied stretch increased there was an increase in the strength and frequency of phasic contractions observed (Figure 5A&B) and an increase in the variation observed in the current traces (Figure 5C&D). The mean 5-HT level for the duration of the recording was obtained from multiple experiments from isolated distal colon tissue and plotted as a function of the applied force (Figure 5E). Under resting tension, the basal 5-HT level was ~ 2μM. In preparations where longitudinal or circular stretch was applied, 5-HT levels were shown to increase with applied force. However the threshold for 5-HT release was 1 mN under longitudinal stretch as opposed to 2 mN in circular muscle stretch. In circular muscle preparations, 5-HT levels increased gradually, until levels plateaued at 5 mN of force applied. In longitudinally preparations, as increasing tension was applied the 5-HT overflow significantly increased until 3 mN of tension, where peak levels reached ~6 μM. Following this there is a decrease in the 5-HT overflow as the applied tension increased from 3 mN to 6 mN. At 6 mN the 5-HT level closely matches that observed at resting tension. For the circular muscle the trend shows a sigmoidal distribution, whilst for the longitudinal muscle the trend shows a bell shaped distribution of 5-HT release with increased applied force (Figure 5E).

Understanding the pharmacology of the colonic stretch activated 5-HT release

To understand the response of 5-HT overflow to increasing applied stretch, the influence of various pharmacological agents were carried out. In Figure 6A, there was no significant difference in the 5-HT response observed in the presence of 100 nM TTX, suggestive that mucosal 5-HT release induced by stretch of the circular muscle occurred without neurogenic regulation from enteric neurons. The response was significantly abolished at all applied tensions in the presence of 30 μM ruthenium red (Figure 6A). Under tensions less than 3 mN, there was a significant increase in the 5-HT levels observed in the presence of 100 μM cinnamaldehyde.

No significant difference in the 5-HT response observed in the presence of 100 nM TTX in the longitudinal muscle preparation, again suggestive of no enteric neurogenic input (Figure 6B). The response was significantly abolished at all applied tensions in the presence of 30 μM ruthenium red (Figure BA). Under all applied tensions except 3 mN, there was a significant increase in the 5-HT overflow in the presence of 100 μM cinnamaldehyde (Figure 6B). In Figure 6C, influence of 5-HT_3 and 5-HT_4 antagonists on 5-HT overflow in longitudinal preparations was investigated. No significant difference in the 5-HT overflow was observed until 3 mN of tension in the presence of 100 nM GR113808 and 1 μM ondansetron. At applied tensions between 4 to 6 mN there was a significant increase in the 5-HT overflow in the presence of 100 nM GR113808. The levels of 5-HT overflow in the presence of 100 nM GR113808 reached those that were observed under a tension of 3 mN in control conditions. There was no significant change in the 5-HT overflow at tensions between 4 to 6 mN in the presence of 1 μM ondansetron (Figure 6C).

Discussion

Using amperometry for monitoring 5-HT signalling during motility

Our findings show that amperometry has potential use in helping to understand the association of mucosal 5-HT with varying motility patterns, however the current response can be influenced by varying factors which can lead to false interpretation and presentation of the experimental results. Studies have clearly shown the T-E distance can have a significant variation in the observed current during recordings (20, 21). Another key influence is the heterogeneity of the mucosal surface, where EC cells are sporadically located at varying depths in the individual invaginations in the colonic columnar epithelium.

At low T-E distances, this variation can be easily observed as shown in Figure 1, where major fluctuations in the current was observed as the electrode spanned across the mucosal surface. Such variation is expected, but as shown in the Figure 1B that variation is reduced as the electrode is placed at T-E distances greater than 150 μm on colonic murine tissue. These differences in responses occur due to the extracellular profile of 5-HT in the vicinity of the tissue. At locations close to the mucosal surface, variations in the epithelium structure are more evident, but at distances further away a more homogeneous signal is observed that is most likely to be reflective of signalling from a patch of mucosal tissue.

Monitoring at a fixed location with an electrode during muscle contractions offers similar issues, however the scenario is now reversed and in this case the electrode is static and the tissue has motion. The current under these conditions will be influenced by the heterogeneity of the mucosal surface varying under the sensor during each contraction and the T-E distance may also be slightly altered as contractions create folds in the muscle that may elevate the tissue. To negate these issues we can record at varying T-E distances away from the mucosal surface, which provides the means to see both phasic and basal changes in 5-HT overflow during contractions. We have shown that at T-E distance of 10 μm, correlations of phasic 5-HT signalling with motility patterns can be made and at T-E distance of 100 μm, changes in basal 5-HT levels with motility patterns can be understood.

Various studies have utilised sensors in close proximity of the mucosal surface and noticed correlative changes in the overflow of 5-HT with motility patterns and made key associations between the two (16, 17). However in these studies there were no changes in the 5-HT signalling as the motility was influenced by pharmacological agents and it is also hard to judge if the effect observed is due to the contractions. Due to the large artifacts that can be generated on the electrode due to the sensitivity of the electrode position and distance from the mucosal surface, greater care needs to be taken during interpretation of the amperometry signals when correlating to motility.

Association of mucosal 5-HT with colonic motility patterns

Our study investigates if there is a relationship between two different motility patterns with mucosal 5-HT. When tension is applied to induce longitudinal or circular stretch, phasic contractions were observed. Our study indicates that a change in mucosal 5-HT release is associated with both longitudinal and circular muscle stretch rather than the phasic

contractions. We observed no differences in the basal or phasic 5-HT overflow response when compared to the strength of phasic contractions in the presence and absence of L-NNA and scopolamine (Figure 3&4). Our findings clearly suggest that there are no links between mucosal 5-HT and phasic contractions from the murine distal colon. When increasing tension was applied to induce longitudinal or circular stretch and basal 5-HT was monitored there was a clear increase of 5-HT overflow (Figure 5E). This finding supports that observed in the guinea-pig where stretch-evoked reflex contractions correlated with 5-HT overflow (17). Our findings show that mucosal 5-HT levels observed with increasing stretch in the presence of TTX were not different to that of control conditions, which indicates that the increased 5-HT levels are due to a mucosal signalling process and are not influenced by enteric neurogenic processes.

An interesting finding from our study is that we observed that the threshold force needed to see an increase in 5-HT overflow is greater in the circular muscle than the longitudinal muscle. Although our study does not indicate which parameter drives another, this difference in the threshold force could indicate the driver between activation of two different motility patterns and would suggest that longitudinal motility patterns would initiate prior to circular muscle patterns.

Our studies have shown that stretch-evoked 5-HT overflow can be inhibited by ruthenium red and stimulated using cinnamaldehyde. Although ruthenium red also is known to indirectly inhibit Ca^{2+} channels, the two results together support that stretch-evoked 5-HT overflow occurs through activation of TRPA1 ion channels. However as the threshold force needed to activate 5-HT in either a longitudinal or circular direction varies, there may be two different subtypes of TRP ion channels involved. Other studies have also shown using various cell lines that activation of the TRPA1 ion channels can increase release of 5-HT through elevation of intracellular Ca^{2+} (22, 23) and studies have also shown that TRPA1 agonists can induce motility (23). Our study provides direct link between TRP ion channels, 5-HT overflow and colonic stretch.

Various studies have investigated the association of mucosal 5-HT with motility patterns, where many have indicated that mucosal 5-HT may be important by not essential for the motility patterns to occur (8, 11, 12, 17). In a recent study where $TpH1^{-/-}$ mice were used to study the role of mucosal 5-HT and fecal pellet motility, larger diameter fecal pellets were observed indicating that in the absence of the mucosa greater stretch/distension is needed to sustain pellet propulsion. Our data suggests that mechanical stimulation through

stretch and potentially distension of the mucosal is associated with 5-HT, which may act and serve to reduce the threshold required for initiation of fecal pellet propulsion and thus reduce fecal pellet diameter.

Inhibition of mucosal 5-HT with increased longitudinal stretch is due to activation of inhibitory autoreceptors

Within our studies we noticed a surprising trend where there was a significant decrease in the 5-HT overflow as stretch was applied above 3 mN. This reduction in the 5-HT overflow was reversed in the presence of the 5-HT_4 antagonist GR113808. This would indicate that at a particular threshold concentration of 5-HT overflow, the 5-HT_4 inhibitory autoreceptor is activated to reduce the extracellular 5-HT level. This may occur as a feedback loop to prevent desensitization of 5-HT receptors on the primary intrinsic afferent neurons.

Various studies have shown the presence of 5-HT_4 autoreceptors on the EC cell, but depending on the animal model, species and gastrointestinal region, the role is varied from excitatory to inhibitory (24-27). Studies have also shown the influence of 5-HT receptors on other epithelial cells such as goblet cells for mucin production (26). There have been many studies that have utilised 5-HT_4 agonists to influence motility and treat constipation (26, 28) and thus there may be an interesting role on how such agents may influence pellet motility by changing the pattern of 5-HT overflow during colonic stretch.

Conclusion

Overall our study indicates that amperometry can be used at different distances over the mucosal surface to study the association of different motility patterns to 5-HT overflow. We observed that 5-HT overflow was associated with longitudinal and circular muscle stretch rather than phasic contractions in the murine distal colon. The change in 5-HT overflow during longitudinal or circular stretch is due to the activation of TRPA1 ion channels and at higher applied tension, the 5-HT overflow is significantly reduced due to the activation of 5-HT_4 autoreceptors. Our study provides new additional insight into the association of mucosal 5-HT with motility patterns.

Author contributions

BAP designed and performed the research study, analyzed the data and wrote the paper

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Figure legends

Figure 1. Monitoring 5-HT overflow over varying zones of the colonic mucosa. (A) Photograph of the mucosal tissue surface and the 5 zones where recordings were obtained for a duration of 40 s. (B) Representative trace of 5-HT overflow response obtained at varying tissue electrode distances (T-E distances) at the varying zones. (C) Mean and standard deviation response from the varying zones at different T-E, n=5 recordings.

Figure 2. Influence of phasic colonic contractions on amperometric detection of 5-HT. (A) Recording of 5-HT overflow at varying T-E distances during phasic contractions generated by applying 1 mN of longitudinal stretch. (B) Mean and standard deviation responses from 100 s of 5-HT overflow and contraction force traces at varying T-E distances. (C) 5-HT overflow responses at T-E distance of 10 µm during phasic contractions. (D) 5-HT overflow responses at T-E distance of 100 µm during phasic contractions. n=5 recordings

Figure 3. Influence of phasic colonic longitudinal contractions on 5-HT overflow. Responses of 5-HT overflow and phasic contractions under control conditions (A), 1 µM scopolamine (B) and 100 µM L-NNA (C). (D) Standard deviation of the phasic contractions monitored for 100 s in the presence of varying pharmacological treatments. (E) Changes in basal 5-HT during phasic contractions influenced by scopolamine and L-NNA and (F) Changes in phasic 5-HT during phasic contractions influenced by scopolamine and L-NNA. All measurements were carried out at T-E distance of 10 µm. n=7 animals

Figure 4. Influence of phasic circular colonic contraction on 5-HT overflow. Responses of 5-HT overflow and phasic contractions under control conditions (A), 1 µM scopolamine (B) and 100 µM L-NNA (C). (D) Standard deviation of the phasic contractions monitored for 100 s in the presence of varying pharmacological treatments. (E) Changes in basal 5-HT during phasic contractions influenced by scopolamine and L-NNA and (F) Changes in phasic 5-HT during phasic contractions influenced by scopolamine and L-NNA. All measurements were carried out at T-E distance of 10 µm. n=5 animals

Figure 5. Effect of longitudinal or circular muscle stretch on 5-HT overflow. Colonic tissue was stretched either longitudinally (A) or circularly (B) by 1 mN for approximately 200 s until 6 mN. Following 50 s, amperometric recording of 5-HT was carried out at T-E distance of 100 µm for a duration of 100 s. The resultant amperometric traces for 5-HT signalling at the varying applied force is shown in (C) for longitudinal muscle preparations and (D) for circular muscle preparations. The overall response for 5-HT overflow and applied stretch is shown in (D). Data represented as mean ± st.dev., n=4-6 animals

Figure 6. Relationship between longitudinal or circular muscle stretch and 5-HT overflow. (A) shows how 5-HT overflow at varying applied forces of circular muscle stretch is influenced by 100 µM cinnamaldehyde (CA; TRPA1 agonist), 100 nM tetrodotoxin (TTX), 30 µM ruthenium red (TPRA1 antagonist). (B) shows how 5-HT overflow at varying applied forces of longitudinal muscle stretch is influenced by 100 µM cinnamaldehyde, 100 nM tetrodotoxin, 30 µM ruthenium red. (C) Shows how 5-HT overflow at varying applied forces of longitudinal stretch is influenced by 100 nM GR113808 (5-HT_4 antagonist) and 1 µM ondansetron (5-HT_3 antagonist). Data represented as mean ± st.dev., n=4-6 animals, *p<0.05, **p<0.01, ***p<0.001 vs control (KB, krebs buffer)