Neurogastroenterol Motil (2004) **16,** 511–514 doi: 10.1111/j.1365-2982.2004.00572.x

HOT TOPIC

Real-time detection of serotonin release from enterochromaffin cells of the guinea-pig ileum

P. P. BERTRAND

Department of Physiology, University of Melbourne, Parkville, Victoria, Australia

Abstract *Serotonin (5-hydroxytryptamine; 5-HT) containing enterochromaffin (EC) cells may detect chemical or mechanical stimuli in the intestinal lumen and respond with release of 5-HT. The aim of this study was to use real-time electrochemical detection methods to detect release of 5-HT from small numbers of EC cells. In guinea-pig ileum, basal release of 5-HT from the unstimulated, unparalyzed intestine was composed of individual release events (8.4 ± 1.8 events, 0.33 ± 0.06 Hz) of different amplitudes but with similar kinetics. Local compression of the mucosa with the electrode evoked peak 5-HT release of 12.3 ± 2.8 μmol L^{-1} with a sustained release of 3.0 ± 0.7 μmol L^{-1}. Brief application of acetylcholine (ACh) or carbachol elicited a transient peak (5.7 ± 1.3 μmol L^{-1} occurring at 35 ± 18 s, n = 9) followed by cyclic release of 5-HT (9.7 ± 2.2 events, 0.40 ± 0.13 Hz, n = 6). This study shows that the release of 5-HT occurs rapidly as individual events from a small number of cells and can reach very high concentrations locally.*

Keywords *electrochemistry, enterochromaffin cells, intestine, serotonin.*

INTRODUCTION

Serotonin (5-hydroxytryptamine; 5-HT) is produced and stored in the enterochromaffin (EC) cells of the intestinal epithelium. Evoked release of this 5-HT is believed to be a critical step in the sensory transduction of chemical and mechanical information from the lumen of the gut to the intrinsic and extrinsic sensory neurons that innervate the small and large intestine.[1,2] In abnormal gastrointestinal function the process of sensory transduction may be disturbed and compounds that modulate 5-HT receptors are therapeutic.[3, 4]

The regulation of serotonin release has been studied extensively at the organ level[5] and recently using tumour-derived cell cultures[6]. In this study, techniques commonly used in the CNS and other peripheral tissues[7] have been applied to study the release of 5-HT from an intact, *in vitro* preparation. This is the first description of the real-time release of 5-HT from small numbers of intestinal EC cells. This method is suitable to study 5-HT release from a wide range of gut regions and species.

Address for correspondence
Paul P. Bertrand PhD, Department of Physiology, University of Melbourne, Parkville, Victoria 3010, Australia.
Tel: +61 3 8344 4543; fax: +61 3 8344 5818;
e-mail: p.bertrand@unimelb.edu.au
Received: 20 June 2004
Accepted for publication: 8 July 2004

MATERIAL AND METHODS

Tissue preparation

Guinea pigs were fed a standard lab diet then killed by severing the carotid arteries and spinal cord in accordance with the University of Melbourne Animal Experimentation Ethics Committee guidelines. A 3-cm long segment of distal ileum was pinned loosely to the base of a small recording chamber (volume: approximately 3 mL) and superfused with warmed (35 °C) oxygenated (95% O_2 and 5% CO_2) physiological saline (composition in mmol L^{-1}: NaCl, 117; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KCl, 4.7; $NaHCO_3$, 25; and glucose, 11).

Electrochemistry

Preparations were visualized at 20× magnification using a dissecting microscope. Serotonin oxidation was measured using single carbon fibres (7 μm) insulated with glass (200 μm of fibre exposed). They were placed above, on, or in the mucosa using a micromanipulator (MP-1, Narishige, Tokyo, Japan). Current recordings were made using a VA-10 amplifier (NPI Electronics, Tamm, Germany), digitized at 5–20 kHz (Digidata 1200A; Axon Instruments, Foster City, CA, USA), recorded on a personal computer using AXOSCOPE 8.0 (Axon Instruments) and then analyzed with ORIGIN 7.0 (MicroCal, Northampton, MA, USA).

Measurements of 5-HT oxidation commenced after 60 min or more of tissue equilibration. The carbon fibre was voltage-clamped at +400 mV where both 5-HT and the catecholamines will oxidize but melatonin which oxidises at +550 mV will not. Oxidation appears as a positive current deflection (Fig. 1A); these deflections did not appear when the electrode was held at 0 mV (eg. Fig. 1B, lower trace).

Each electrode was coated with nafion (Sigma Aldrich, St Louis, MO, USA); an anionic resin that repels ascorbic acid and metabolites of 5-HT and attracts cationic species like the catecholamines and 5-HT. Serotonin (10 μmol L^{-1}) was used to calibrate electrodes before and after recording in the mucosa. All electrodes showed a gradual and linear decrease in sensitivity over time. This effect was minimised by exchanging carbon fibre electrodes after 60 min of recording.

Data presentation and statistical analysis

Data are presented as a concentration of 5-HT calculated from oxidation currents at the tip of the electrode and the current generated by a known concentration of 5-HT. The amplitude, time to peak, 50 and 80% decay times and instantaneous frequency of release events (mean ± SEM) were calculated, with 'n' equal to the number of animals used.

RESULTS

A total of 44 preparations of guinea-pig ileum were examined for the spontaneous release of 5-HT from the mucosa. In all preparations, the oxidation current was higher with the carbon fibre touching the mucosal epithelium than just above it. In 16 of 44 preparations, when the electrode contacted the mucosa, a step-like increase in 5-HT concentration was detected (peak amplitude = 4.2 ± 1.6 μmol L^{-1}, not shown). This was maintained for as long as the electrode was in contact with the mucosa (up to an hour) and could be seen regardless of how often the electrode was placed in and out of the mucosa; these responses were not studied further.

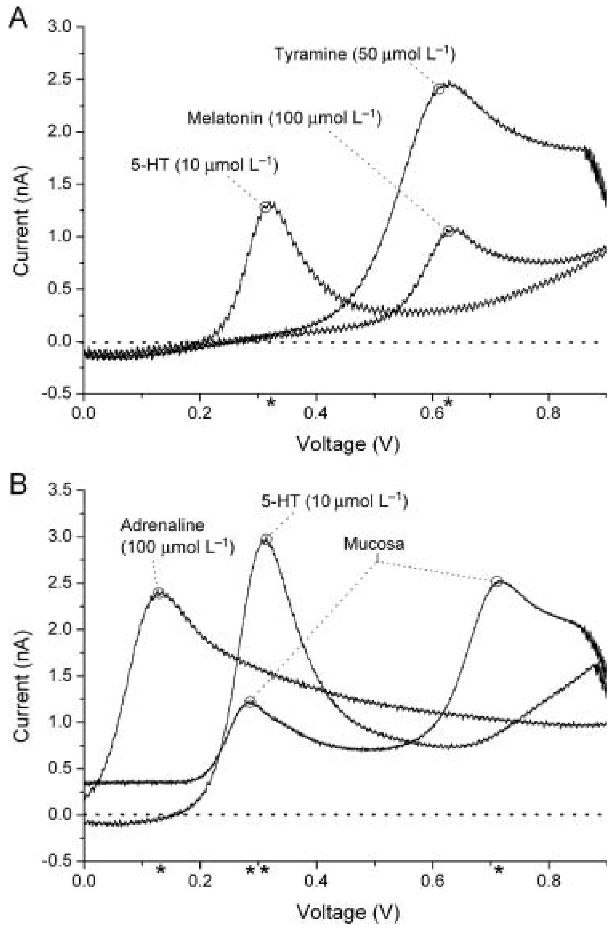

Figure 1 Separation of compounds based on oxidation potential. Oxidation currents from the same carbon fibre electrode for a variety of compounds. The voltage was ramped from −0.4 to +1 V and back down again; only the data from 0 to +1 V are shown for clarity. Current is on the y-axis and voltage is on the x-axis (with voltage increasing to the right). The scan rate was about 0.47 V s^{-1}. This protocol allows the identities of different compounds to be distinguished. (A) A family of oxidation curves for 5-HT (10 μmol L^{-1}), melatonin (100 μmol L^{-1}) and tyramine (50 μmol L^{-1}). The '*' indicates the approximate position of the peak oxidation current; for 5-HT this was 310 mV. (B) This family of oxidation curves shows 5-HT (10 μmol L^{-1}), adrenaline (100 μmol L^{-1}) and a curve taken when the electrode was place into contact with the mucosa. Note that the first peak generated from the mucosa is almost identical to that of 5-HT; the origin of the second peak is not known. On average, 5-HT oxidised at 310 ± 8 mV and the first peak from the mucosa oxidised at 309 ± 23 mV (n = 4, P > 0.5, paired, two-way t-test). Note, all traces have had the background current subtracted using a trace taken while the electrode was outside the mucosa.

In 28 of 44 preparations, contact with the mucosa produced a sharp, transient increase in the release of 5-HT (12.3 ± 2.8 μmol L^{-1}, n = 12, Fig. 2A, left inset). The peak occurred about 10 s after the estimated time

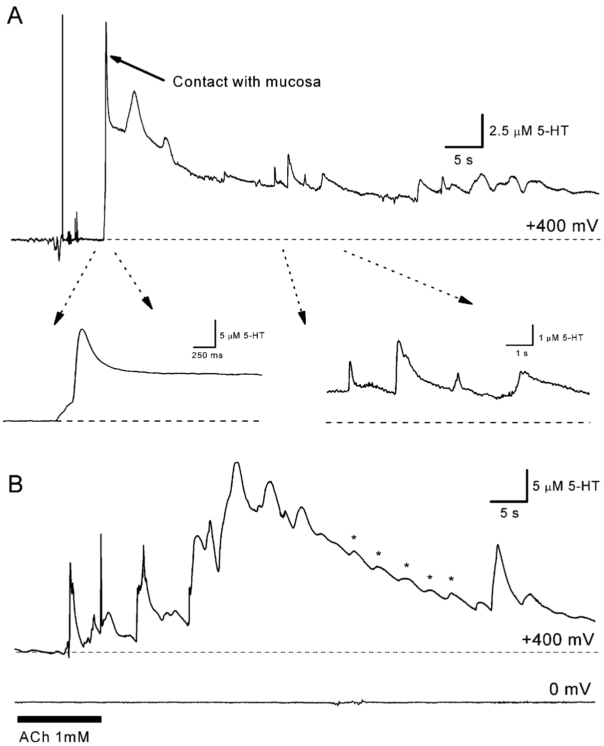

Figure 2 Detection of serotonin (5-HT) release from guinea pig mucosa. (A) The carbon fibre electrode was placed between the villi on the surface of the mucosa and basal release of 5-HT was measured. A holding potential of +400 mV was applied and an upward deflection in current was detected. Left inset: An enlargement of the transient release evoked by initial compression of the mucosa. Right inset: An enlargement of individual 5-HT release events of different amplitudes, but with similar instantaneous frequencies. (B) The carbon fibre electrode was touching the surface of the mucosa during a 30 s superfusion with acetylcholine (ACh, 1 mmol L^{-1}). Upper trace: A +300 mV holding potential, upward deflections in current were recorded that likely represent the release of 5-HT. The symbol '*' represent cyclic release events (see text). Lower trace: when the oxidizing current was removed (i.e. 0 mV holding potential) no change in current was evoked by ACh.

of contact with 50% decay at 3.4 ± 1.7 s ($n = 9$) and 80% decay at 15.2 ± 8.8 s ($n = 9$). Transient release could be evoked once every 5 min from the same section of mucosa without rundown. The release of 5-HT was apparently caused by the small amount of compression produced when the carbon fibre contacted the mucosal epithelium. In contrast, side-to-side movement of the electrode, as occurs during smooth muscle contraction, failed to elicit release ($n = 8$).

The transient release was followed by a sustained release (3.0 ± 0.7 μmol L^{-1}, $n = 13$) that was similar to that seen in the unresponsive preparations.

Table 1 Shapes of different amplitude 5-HT release events

Release event ($n = 7$)	5-HT concentration (μmol l^{-1})	Time to peak (s)	Decay 50% (s)	Decay 80% (s)
Large	2.8 ± 0.8	0.22 ± 0.09	0.64 ± 0.13	1.04 ± 0.19
Medium	1.2 ± 0.5	0.18 ± 0.04	0.56 ± 0.15	1.01 ± 0.29
Small	0.3 ± 0.1	0.31 ± 0.06	0.68 ± 0.11	1.01 ± 0.15

Three representative release events with concentrations near 0.3, 1 and 3 μmol l^{-1} were taken from seven preparations. They were analyzed for time to peak and time for 50 and 80% return to baseline. The three different amplitude events were taken from a similar point in time from each preparation. Only events with a smooth onset and decay were selected.

Superimposed upon this were individual release events (Fig. 2A, right inset). These events often stopped after a minute or two but could sometimes occur for the duration of the recording (30–60 min). Individual events occurred at an instantaneous frequency (f_{int}) of 0.34 ± 0.05 Hz ($n = 6$). There were 9.2 ± 1.6 events per compression before the events stopped or became too small to analyse. Clusters of individual release events were visually well correlated with spontaneous circular muscle contractions, but not longitudinal muscle contractions.

Individual release events with a spread of amplitudes (on average: 0.3, 1 and 3 μmol L^{-1}) were taken from each of seven preparations (Fig. 2A, right inset). Regardless of the amplitude, they all had a time to peak and 50 and 80% decay times that were statistically similar (Table 1).

Brief application of acetylcholine (ACh, 1 mmol L^{-1} to 1 mol L^{-1}) or carbachol (1 mmol L^{-1}) elicited a transient peak (5.7 ± 1.3 μmol L^{-1} at 35 ± 18 s, $n = 9$) followed by cyclic release of 5-HT (Fig. 2B). This later release was a symmetrical oscillation that occurred at an instantaneous frequency (f_{int}) of 0.40 ± 0.13 Hz ($n = 6$). On average these cycles repeated for 9.7 ± 2.2 events before stopping or becoming too small to analyse. The nicotinic agonist DMPP (1 mmol L^{-1}, $n = 5$) was only weakly active.

DISCUSSION

This is the first study to look at the real-time release of 5-HT from intact intestine. The main finding is that in the unparalyzed intestine transient, individual 5-HT release events can be reliably detected from small numbers of EC cells. These EC cells are probably then recruited to cause a more global release of 5-HT.

Detection of 5-HT

The chemical structure of 5-HT is such that it is readily oxidized at low voltages and, thus, electrochemical detection methods – where the current generated by oxidation is measured – work particularly well. Racké *et al.* have described the outflow of 5-HT from *in vitro* segments of small intestine from dog, pig, guinea pig and human (as have other groups using the same techniques).[5] In these studies, 5-HT was collected from the lumen or portal vein of 5–10 cm segments of intestine at 1–5 min intervals. The perfusate was separated using high-performance liquid chromatography and followed by electrochemical detection of 5-HT. These techniques have two main problems: they only detect release over long periods of time and the activity of many EC cells participates in the measurements.

Real-time release of 5-HT

Release of 5-HT from EC cells would be predicted to take place on the time course of 100's of milliseconds, similar to release from chromaffin cells[7] or other neuroendocrine cells (e.g. glomus cells, neuroepithelial bodies). Previous studies have sampled 5-HT concentrations once per minute or less. However, many of the receptors important for the control of 5-HT release activate and desensitize on a time course of seconds or less. Further, because 5-HT and other neurochemicals are left in contact with the tissue in between measurements, negative feedback mechanisms will be activated (e.g. autoreceptors) and reduce the amount of 5-HT measured. Determining the moment-to-moment concentrations of 5-HT is, thus, critical to understanding the role 5-HT is playing. In this study, 5-HT release was monitored in real-time, and was confirmed to take place on a very brief time scale and as the sum of many individually controlled release events.

Small numbers of EC cells participate

The number of EC cells contained within a 3 cm segment of small intestine is large; there may be 10 000 in 1 cm^2 of tissue. Enterochromaffin cells are probably not one homogeneous population, thus, sampling from a small number could potentially reveal differences in the population. It can be calculated that only about seven EC cells were very close to the exposed carbon fibre used here. To determine whether individual release events with different amplitudes resulted from differing amounts of 5-HT release, or if release was occurring at different distances from the recording electrode, the time courses of the events were analyzed. That these events had very similar kinetics suggests that for release to be detected as an individual event, it must be very close to the carbon fibre surface.

CONCLUSIONS

This study has confirmed that 5-HT release takes place rapidly and as individual release events. High-speed electrochemical techniques are, thus, suitable for re-examining 5-HT release without confounding factors from a variety of gastrointestinal tissues and in different species including human.

ACKNOWLEDGMENTS

Kind thanks to Dr Joel C. Bornstein and Ms Rebecca L. Monro for providing helpful comments on this study and special thanks to Dr James Brock for his help in setting up the technique. Supported by the NH&MRC (Australia) grant no. 299803.

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