High Mucosal Serotonin Availability in Neonatal Guinea Pig Ileum Is Associated With Low Serotonin Transporter Expression

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**Background & Aims:** 5-Hydroxytryptamine (5-HT) is a neurotransmitter and paracrine signaling molecule in the gut. Paracrine signaling by enterochromaffin cells (EC), which release 5-HT, has not been studied in neonates. Our aim was to compare 5-HT disposition in the intestinal mucosa of neonatal and adult guinea pigs. **Methods:** 5-HT was locally measured in vitro from intestinal segments using a diamond microelectrode and continuous amperometry. The serotonin transporter (SERT) was measured using immunohistochemical and Western blot techniques. 5-HT intestinal content was measured using immunohistochemistry and high-performance liquid chromatography with electrochemical detection. **Results:** An oxidation current, reflective of local 5-HT release, was recorded with the microelectrode near the mucosal surface, and this current was larger in neonatal than in adult tissues. Mechanically stimulating the mucosa with a fine glass probe evoked an additional current in adult but not neonatal tissues. Oxidation currents were reduced by tetrodotoxin and were blocked in calcium-free solutions. Fluoxetine (1 μM) potentiated oxidation currents in adult but not neonatal tissues. SERT levels were lower in neonatal vs adult tissues. There was no difference in 5-HT content between neonates and adults but 5-hydroxyindole acetic acid/5-HT ratios were higher in adults. EC cell counts showed no difference in cell number, but EC cells were found in the crypts in neonatal and along the villi in adult tissues. **Conclusions:** SERT expression is low in neonates, and this is associated with high levels of free mucosal 5-HT and reduced metabolism. Postnatal maturation of 5-HT signaling may be important for development of neurohumoral control of intestinal motor reflexes.

Serotonin (5-hydroxytryptamine, 5-HT) is a signaling molecule released from enteric neurons1,2 and enterochromaffin (EC) cells in the mucosal layer of the gut.3,4 EC cells release 5-HT in a calcium-dependent manner and they express mechano- and chemosensitive ion channels, ligand-gated ion channels, and G-protein-coupled receptors.3,4 Activation of calcium-permeable channels or G-protein-linked receptors leads to a rise in intracellular calcium and 5-HT secretion.3,4 Regulated secretion of 5-HT by EC cells can be enhanced or inhibited by signaling molecules released from surrounding cells and by nerves supplying the mucosa.3,4 EC cells are sensory transducers that respond to mechanical or chemical stimuli applied to the mucosa causing 5-HT release.5 5-HT released from EC cells initiates motor reflexes by activating 5-HT receptors localized to the primary afferent nerve terminals.6–8 5-HT released from EC cells initiates antidromic action potentials in the intestinal primary afferent neurons,6 which then activate interneurons and motoneurons in enteric neural circuits mediating peristalsis.7,8 Clearance of 5-HT is also an important determinant of the strength and duration of excitatory signals transmitted by 5-HT. Clearance of 5-HT is accomplished through the activity of the high-affinity serotonin transporter (SERT), which is expressed by enterochromaffin cells (EC)9

The enteric nervous system (ENS) begins to mature during embryonic development when neural precursors migrate from the neural crest into the bowel wall.9 When the neural precursors reach the gut, trophic factors and extracellular matrix proteins stimulate neuronal differentiation and circuit formation.11,12 However, the ENS continues to mature in the postnatal period.13 As discussed previously, signaling between EC cells and enteric neurons is important for initiation of motor reflexes, but the status of the EC cell–ENS interaction in the early postnatal period is unknown.

To compare EC cell function in neonatal and adult intestinal tissues, it is essential to measure 5-HT concentrations very close to release sites in the intestinal mucosa. This has been accomplished using electrochemical techniques with carbon fiber microelectrodes positioned on the mucosa of guinea pig ileum maintained in vitro.14 Using this approach, it has been possible to monitor 5-HT release from a few EC cells in real time. However, electrochemical monitoring of 5-HT in vitro or in vivo is often hindered by the tendency of oxidation products to

**Abbreviations used in this paper:** EC, enterochromaffin; ENS, enteric nervous system; 5-HIAA, 5-hydroxyindole acetic acid; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; ir, immunoreactivity; SERT, serotonin transporter; TTX, tetrodotoxin.

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form an insulating film on the carbon fiber surface causing electrode fouling and signal loss.\textsuperscript{15,16} We showed recently that diamond microelectrodes are resistant to fouling, and can be used for sensitive and stable measurement of 5-HT in the intestinal mucosa in vitro.\textsuperscript{17} In the present study we used diamond microelectrodes to compare 5-HT disposition in the small intestinal mucosa of neonatal and adult guinea pigs. As there were marked differences in 5-HT handling between these groups of animals, we also used chromatographic, immunohistochemical, and Western blot techniques to probe the mechanisms underlying differences in 5-HT handling by the intestinal mucosa of neonatal and adult guinea pigs.

**Materials and Methods**

**Diamond Microelectrode Preparation**

Boron-doped diamond thin film was deposited on a sharpened 76-μm diameter Pt wire (99.99%, Aldrich Chemical, Milwaukee, WI) by microwave-assisted chemical vapor deposition (1.5 kW, 2.54 GHz, ASTeX, Woburn, MA).\textsuperscript{18,19} The diamond-coated Pt wire was affixed to a copper wire using conductive Ag epoxy and the assembly was insulated with polypropylene from a pipette tip. The insulation was applied by inserting the microelectrode into a pipette tip with about 500 μm exposed from the end and carefully heating the tapered end using the coil of micropipette puller. This softened the polypropylene and caused it to flow evenly over the diamond surface. The resulting microelectrode was conically shaped with diameter at the narrowest point of approximately 10 μm and at the widest point of approximately 80 μm. The length of the exposed electrode was 100 to 200 μm. This method reproducibly coats diamond with a thin and continuous polymer film.

**Intestinal Preparation**

All animal use protocols were approved by the Institutional Animal Use and Care Committee at Michigan State University. Neonatal guinea pigs (≤48 hours postnatal, weight 75–90 g) and young adult guinea pigs (3–4 months old, 300–400 g) were obtained from Bioport, Inc. (Lansing, MI). At this age and weight range, the animals are sexually mature (http://netvet.wustl.edu/species/guinea/guinpig.txt). Guinea pigs were lightly anesthetized via halothane inhalation, stunned, and exsanguinated by severing the major neck blood vessels. A segment of ileum was harvested 15 to 20 cm proximal to the ileocecal junction and placed in an oxygenated (95% O\textsubscript{2} and 5% CO\textsubscript{2}) Krebs’ buffer solution, pH 7.4 (composition: 117 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl\textsubscript{2}, 1.2 mmol/L MgCl\textsubscript{2}, 1.2 mmol/L NaH\textsubscript{2}PO\textsubscript{4}, 25 mmol/L NaHCO\textsubscript{3}, and 11 mmol/L glucose). A small piece (3 cm) of ileum was placed in a Sylgard-(Dow Corning, Midland, MI) lined Teflon recording chamber (6.5 cm long, 6.5 cm wide, 4 mm deep). The segment was cut open along the mesenteric attachment and was lightly stretched and pinned flat (mucosal surface up) to the chamber bottom using small stainless steel pins (Fine Science Tools, Foster City, CA). The bath was mounted on the stage of an inverted microscope (Model 3030, Accu-Scope, Sea Cliff, NY) and superfused with warm (37°C) Krebs’ solution at a flow rate of 2 mL/min. The solution temperature was controlled with an immersion heating circulator (Model 1130A, VWR Scientific, West Chester, PA), and flow was controlled with a peristaltic pump (Masterflex, Cole-Parmer, Chicago, IL). Tissues were exposed to the flowing solution for 30 minutes prior to commencing a series of measurements.

**Amperometric Measurements of 5-HT**

For continuous amperometric recording of 5-HT overflow, a 3-electrode system was used. A diamond microelectrode, a Pt wire counter electrode, and a commercial “no leak” Ag AgCl (3 M KCl, model EE009, Cypress Systems Inc, Chelmsford, MA) reference electrode were mounted in the bath to complete the electrochemical circuit. Measurements were carried out using a BioStat multichannel potentiostat (ESA Biosciences, Inc., Chelmsford, MA). The diamond microelectrode was affixed to a micromanipulator (Model 25033, Fine Scientific Tools). The tissue sample and microelectrode were placed in the center of the chamber. The electrode potential was held at 700 mV vs Ag/AgCl, which was sufficient to oxidize 5-HT at a mass transfer limited rate. The electrode was placed several centimeters away from the mucosa for several seconds before the electrode was carefully positioned about 1 mm over the tissue for 20 seconds. This established a baseline 5-HT oxidation current. A glass capillary (0.5 mm tip diameter) was used to gently touch the nearby mucosa for another 20 seconds. Movement of the glass capillary was controlled by a micromanipulator. The microelectrode was then used for another 20 seconds to record the mechanically stimulated release of 5-HT as an oxidation current. The glass capillary was then moved away from the mucosa to allow the oxidation current to return to the baseline level. Finally, the diamond microelectrode was retracted from the mucosa and the oxidation current returned to the 0 level. This procedure was repeated 5 or 6 times for each tissue section.

**High-Performance Liquid Chromatography (HPLC) Measurements of Tissue Levels of 5-HT**

Using a sharp scalpel, 1 cm\textsuperscript{2} segments of the mucosal layer were scraped from ileal tissues from neonatal and adult guinea pigs. The mucosal tissue was placed in 500 μL of ice-cold 0.1 mol/L perchloric acid. This sample was then homogenized and centrifuged at 14,000 \( \times \) g for 10 minutes prior to chromatographic analysis. The mucosa tissue samples were homogenized...
in 500 μL of ice-cold 0.1 mol/L perchloric acid and centrifuged at 14,000 g at 4°C for 10 minutes and were stored on ice prior to analysis. HPLC analysis of 5-hydroxyindole acetic acid (5-HIAA) and 5-HT was carried out on the centrifugate using a CoulArray coulometric detector (ESA Biosciences) at 400 mV. An ESA autosampler (model 542) and a 20-μL sample loop were used with a column heater maintained at 25 ± 0.15°C. An ESA HR-880 ODS analytical column (3-μm particle size, 4.6 mm i.d. × 80 mm length) along with a guard column were used for the separation. For the mobile phase (flow rate = 1.1 mL/min, ESA model 582 pump), a commercial ESA Car A phase II (ESA Biosciences) was used, which consisted of 100 mmol/L phosphate buffer and an ion pairing agent. This was mixed with 16% methanol and 8% acetonitrile. All data analysis was carried out using CoulArray for Windows (ESA Biosciences).

**Immunohistochemistry and Analysis of EC Cell Distribution**

Ileal segments were cut open along the mesenteric border and washed with cold PBS saline solution (0.01 mol/L, pH 7.2). Segments were stretched and pinned flat in a silicone elastomer-lined Petri dish, which was then filled with Zamboni’s fixative (2% [vol/vol] formaldehyde and 0.2% [vol/vol] picric acid in PBS). Tissues were fixed overnight (4°C) and then washed 3 times with DMSO at 4°C and 0.2% [vol/vol] picric acid in PBS). Tissues were washed 3 times with PBS at 10-minute intervals, followed by 3 washings in PBS at 10-minute intervals. Tissues were dehydrated in PBS/30% sucrose overnight before freezing in optimum cutting temperature mounting medium. After cryostat sectioning, sections were dried in an air-tight dessicator for 1 hour. Sections were then incubated overnight in a humidified chamber in diluted (1:200, in PBS) anti-5-HT antibody (YCS/45, rat monoclonal, Abcam, Cambridge, MA, http://www.abcam.com/index.html). Sections were then washed in PBS and incubated for 1 hour at room temperature in diluted (1:40) goat antirat IgG conjugated to fluoroscein isothiocyanate (FITC, F6258, Sigma Chemical Co., St. Louis, MO, http://www.sigmaaldrich.com). Sections were washed 3 times with PBS at 10-minute intervals and then mounted in buffered glycerol (pH = 8.6) for fluorescence microscopy. Sections were viewed using a Nikon fluorescence microscope (model TE 2000-U), and images were acquired and analyzed using MetaMorph software.

The distribution of EC cells was quantified by making measurements on 4 sections of each sample obtained from 4 adult and 4 neonatal guinea pigs. A line was drawn across the image at the crypt-villus border. The number of cells on each side of the line was counted and cell counts obtained in the 4 sections from each animal were summed to provide a single number for crypt or villus EC cells. The mean ± SEM value for the group of 4 animals was then compared using Student t test. The crypt/villus ratio of EC cell distribution for each animal was also calculated and compared.

**Western Blot Analysis**

Western blot analysis was performed on protein extracts obtained from mucosal tissues from neonatal and adult guinea pigs. Ileal segments were placed in cold (4°C) PBS (pH = 7.2) and were cut open along the mesenteric border. The mucosa was scraped from these segments using a sharp scapel and the mucosal tissues were lysed on ice in lysis buffer (10 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L EDTA, 0.2% NP40), containing a commercially available protease inhibitor cocktail (cat #P8340, Sigma-Aldrich Inc., St. Louis, MO). Lysates were centrifuged at 14,000 g at 4°C for 10 minutes to pellet nuclear proteins and any insoluble debris. The supernatant obtained from each animal was saved separately, and total protein in the supernatant was determined using a protein assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein from each sample described above were mixed with Laemmli buffer (16% mercaptoethanol [wt/vol], 6% SDS [wt/vol], 0.1% bromphenol blue [wt/vol], 30% glycerol [wt/vol], 240 mmol/L Tris, pH 6.8), separated on a 10% SDS-PAGE gel and then transferred to a nitrocellulose membrane that was maintained at 4°C overnight. Membranes were blocked with 4% milk in PBS-Tween 20 buffer for 3 hours and then incubated at 4°C overnight with the primary anti-SERT antibody (1:1500 dilution). The SERT antibody (a generous gift from Dr Randy Blakely, Vanderbilt University) was raised against the C-terminal sequence (amino acids 596 – 614, antibody #50) of the rat SERT protein. After overnight incubation, the membrane was washed with PBS and then further incubated for 1 hour at 4°C with a horseradish peroxidase-conjugated secondary antibody. The immunoreactivity was detected by a chemiluminescence kit (Pierce, Rockford, IL) and images were analyzed using Image J software. All membranes were stained with Coomassie Blue to verify equal protein loading and to quantitate total protein in each lane. The intensity of SERT bands and total protein on membranes was quantitated using Image J software (http://rsb.info.nih.gov/ij/). SERT levels in each lane were normalized to the total protein in that lane.

**Chemicals**

All chemicals and drugs were obtained from Sigma-Aldrich and used as received. All solutions were prepared with ultrapure water from a Barnstead E-Pure system (distilled, deionized, and passed over activated carbon).

**Results**

**Detection of Mucosally Released 5-HT**

Our previous work17 showed that at an electrode potential of 0.8 V, an oxidation current is measured when
a diamond microelectrode is positioned on or near the intestinal mucosal due to the overflow of 5-HT. These data are similar to those published previously in studies using carbon fiber electrodes to detect oxidation of 5-HT.14 In the present study, we used a diamond microelectrode because of its enhanced response stability and we compared 5-HT handling by the mucosa of neonatal and adult guinea pigs. We found important differences in the 5-HT signal detected in tissues from neonates and adults. When the tip of the microelectrode was inserted into the solution superfusing the intestinal tissue, no current was detected (Figure 1A). However, when the microelectrode was positioned within 1 mm of the mucosal surface, there was a marked increase in the oxidation current, and this baseline response was significantly larger in tissues from neonatal guinea pigs (Figure 1A and B). In tissues from both neonates and adults, the baseline 5-HT oxidation current was reduced significantly by the sodium channel blocker, tetrodotoxin (TTX, 0.3 µM; Figure 1B). Lowering extracellular Ca²⁺ from 2.5 to 0.25 mmol/L produced a further reduction in the baseline 5-HT oxidation current (Figure 1B).

Mechanical stimulation of the mucosa will cause release of 5-HT from EC cells. We used a fine glass capillary tube (300-µm tip diameter) to mechanically stimulate the mucosal near the end of the diamond microelectrode. Under the control of a micromanipulator, the capillary tube was gently pressed against the mucosa to deform a few villi. Mechanical stimulation evoked a significant increase in the 5-HT oxidation current over baseline levels and the stimulated response was significantly larger in neonatal compared with adult tissues (Figure 1B). The mechanically stimulated response was not altered by TTX in adult tissues but it was reduced by TTX in tissues from neonatal animals (Figure 1B). The mechanically stimulated response was blocked by the low Ca²⁺ solution in both neonatal and adult tissues (Figure 1B).

SERT is expressed in high concentrations by enterocytes in the mucosa, and is responsible for clearing 5-HT after its release from EC cells.9 It is possible that SERT expression or function is lower in the mucosa of neonates compared with adult guinea pigs. This could account for the elevated basal 5-HT level in the neonatal mucosa. Therefore, the effect of the SERT antagonist, fluoxetine (1 µmol/L), on oxidation currents measured in neonatal and adult tissues was assessed. Fluoxetine increased the baseline response and the mechanically evoked response in tissues from adult animals (Figure 2A and C). However, fluoxetine did not alter responses in neonatal tissues (Figure 2B and C).

**SERT Expression Is Reduced in Neonatal Intestine**

The pharmacologic data above suggest that SERT expression is either reduced in neonatal tissues or that SERT activity is lower in neonatal vs adult intestinal mucosa. To distinguish between these possibilities, we localized the SERT protein in frozen sections of neonatal and adult small intestine using immunohistochemical methods. SERT immunoreactivity (ir) was found in enteroctyes lining individual mucosal villi in tissues from adult animals (Figure 3A) while little or no SERT-ir was found in sections from neonates (Figure 3B). Omitting the primary antibody from the immunohistochemical
protocol removed localization of SERT in both adult and neonatal tissues (Figure 3C and D).

Although immunohistochemical techniques provide a qualitative assessment of SERT expression in the mucosa, it is difficult to make quantitative measures using this technique. Therefore, we also used Western blot techniques to compare the relative expression of SERT in the intestine of neonatal (n = 3) and adult (n = 3) guinea pigs. Figure 4A shows a Western blot revealing a band near 75 kilodaltons in extracts from the neonatal and adult intestine. This band corresponds to a prominent band at 75 kilodaltons in extracts of adult guinea pig brain (Figure 4B). The intensity of the 75-kilodalton band was similar in adult and neonatal intestinal tissues (Figure 4B). In extracts from adult and neonatal intestine, we also detected a band near 60 kilodaltons. The intensity of the 60-kilodalton band was much greater in adult samples compared with samples from neonates (Figure 4B). There were also several less intense bands of molecular weights slightly lower than 60 kilodaltons in adult and neonatal tissues.

5-HT Content Is Similar in Neonatal and Adult Intestine

The data presented above indicate that reduced SERT expression could account for the larger 5-HT oxidation currents and reduced fluoxetine sensitivity in neonatal intestine. It is also possible that the neonatal intestine contains higher levels of 5-HT and/or more EC cells. These issues were addressed in 2 ways. First, 5-HT content was measured in the intestinal mucosa from neonates and adults. 5-HT levels (Figure 5A) were similar in these 2 groups but 5-HIAA (the primary metabolite of 5-HT) levels were much higher in adult tissues (Figure 5B). Therefore, the 5-HIAA/5-HT ratio, an index of 5-HT turnover, was significantly higher in adults (Figure 5C).

Second, we used 5-HT immunohistochemistry to localize
5-HT in the EC cells of the neonatal and adult intestine (Figure 6). EC cells were easily recognized in whole wall intestinal cross-sections from adults and neonates. Cell counts revealed that the numbers of EC cells were similar in sections from neonatal and adult intestine but the cell distribution differed (Figure 7). In adult tissues, EC cells were localized predominately along the mucosal villi (Figures 6 and 7A) while in neonatal tissues, EC cells were found predominately in the crypts (Figures 6 and 7A). The difference in EC cell distribution was reflected in a difference in the crypt/villus ratio for neonatal and adult tissues (Figure 7B).

**Discussion**

**Amperometric Measurement of 5-HT Release**

5-HT release from intestinal EC cells has been studied previously either by sampling 5-HT content in overflow solutions in vitro or in blood samples obtained in vivo.\(^3\)\(^,\)\(^20\)\(^,\)\(^21\) To elicit release of detectable amounts of 5-HT, large volume samples and prolonged periods of pharmacologic or electrical stimulation were required. Although these studies have provided important data about EC cell function, overflow techniques do not allow 5-HT measurements in real time, and they have poor spatial resolution as the molecule is released from many hundreds of EC cells from extended lengths of intestine. These issues have been addressed by using amperometric methods with carbon fiber microelectrodes, which allow real-time measurement of 5-HT from the mucosa of intestinal segments maintained in vitro.\(^14\)\(^,\)\(^17\) Furthermore, these microelectrode-based techniques allow detection of 5-HT release from just a few EC cells near the electrode tip.

Although real-time and local measurements of 5-HT are feasible with carbon fibers, the duration of recordings is limited by extensive electrode fouling caused by adsorption of oxidation products and other biologic molecules present in the tissue. Electrode fouling reduces electrode sensitivity for detection of 5-HT. Diamond microelectrodes are resistant to fouling, enabling these electrodes to be used for extended periods of time for sensitive and reproducible measurement of 5-HT release from the intestine.\(^17\)

Previous work showed that currents caused by oxidation of 5-HT at the electrode tip can be discriminated from oxidation currents produced by other molecules in the mucosa.\(^14\)\(^,\)\(^17\) Using diamond microelectrodes, this can be accomplished by setting the voltage at the electrode tip to 0.8 V. When diamond microelectrodes are positioned near the mucosal surface, there is an abrupt increase in the oxidation current. The basal response was partly reduced by the neurotoxin, TTX, which indicates that 5-HT release from EC cells was partly neurogenically driven. EC cells express nicotinic and muscarinic receptors for acetylcholine, which is released from nerves supplying the mucosal layer, and acetylcholine can directly stimulate 5-HT release from EC cells.\(^22\)\(^,\)\(^23\) In our experiments, it is likely that TTX inhibited this excitatory cholinergic input. However, not all of the 5-HT release required nerve activity as a substantial fraction of the 5-HT oxidation current persisted in the presence of...
TTX, particularly in adult tissues. The TTX-resistant component was blocked by reducing extracellular Ca\(^{2+}\). This indicates that the release of 5-HT was due to Ca\(^{2+}\)-dependent exocytosis. Mechanically stimulating a few villi in adult tissues evoked an additional current that was inhibited by reduced extracellular Ca\(^{2+}\) but not by TTX. This result suggests that the mechanically evoked response was due either to direct mechanical stimulation of the EC cells or to release of substances from nonneuronal cells, which then caused Ca\(^{2+}\)-dependent exocytosis from EC cells. An important new finding from the present work is that basal and stimulated 5-HT oxidation currents in neonatal intestine greatly exceeded those measured in adult tissues.

**SERT Expression Is Reduced in Neonatal Intestine**

The elevated basal and stimulated 5-HT oxidation currents detected in neonatal compared with adult tissues could be due to 1 or more factors. The neonatal intestine could contain more EC cells with higher levels of 5-HT or 5-HT could be cleared less efficiently from the extracellular space in the neonatal intestine. As SERT is

![Western blot analysis of SERT expression in the neonatal and adult intestine.](image)

**Figure 4.** Western blot analysis of SERT expression in the neonatal and adult intestine. (A) Western blot showing multiple bands recognized by the SERT antibody in samples from 3 neonatal guinea pigs (lanes 1–3) and 3 adult guinea pigs (lanes 4–6). There is a faint band that corresponds to a prominent band near 75 kilodaltons in a protein extract from adult guinea pig brain. A prominent band near 60 kilodaltons was detected in adult extracts, but this band was present at lower levels in neonatal extracts. (B) Intensity of the 60- and 75-kilodalton bands was normalized to the total protein present in each lane. There was no difference in the intensity of the 75-kilodalton bands between adult and neonatal tissues, but the intensity of the 60-kilodalton band was significantly greater in adult compared to neonatal tissues (*P < .05). Data are mean ± SEM.

![5-HT and 5-HIAA levels in the mucosa of neonatal and adult intestine.](image)

**Figure 5.** 5-HT and 5-HIAA levels in the mucosa of neonatal and adult intestine. (A) There was no difference in 5-HT concentration in the mucosa of neonatal (n = 6) vs adult (n = 5) intestine. (B) 5-HIAA levels were lower in neonatal intestine (*P < .05). (C) The 5-HIAA/5-HT ratio was also smaller in neonatal compared to adult intestine (*P < .05). Data are mean ± SEM.
the primary mechanism for clearing 5-HT from the mucosa,⁹ the neonatal intestine may express less SERT or SERT may not function as efficiently as it does in the adult intestine. With less efficient recovery, there would be more 5-HT overflow in the neonatal intestine.

Fluoxetine is a SERT inhibitor, and if SERT is responsible for clearing 5-HT from the mucosa, it is expected that fluoxetine would enhance the 5-HT current detected near the intestinal mucosa. Consistent with this prediction we found that fluoxetine produced a marked increase in basal and stimulated oxidation currents in adult tissues. However, fluoxetine did not increase basal or stimulated currents in the intestine of neonates. These data suggest that the elevated overflow currents in neonatal tissue are due to either the absence of SERT from the neonatal intestine or that SERT is present but not functional in these tissues.

Immunohistochemical studies using an antibody raised against a portion of the rat SERT sequence⁵⁴ revealed that SERT-ir was present in enterocytes lining the mucosal villi in frozen sections of the adult intestine. Similar studies revealed that little or no SERT-ir was detectable in sections from the neonatal intestine. These data are consistent with the functional data discussed above. We sought to confirm the absence of SERT-ir from the neonatal intestine using Western blot techniques and the same rat SERT antibody. These studies revealed a complex expression of SERT-ir in protein extracts obtained from the mucosa of adult and neonatal guinea pig intestine. The SERT protein identified in rat and human brain and rat intestine has a molecular weight of approximately 75 kilodaltons. This antibody also revealed a 75-kilodalton band in protein extracts of whole guinea pig brain and in protein extracts of neonatal and adult guinea pig intestine. There was no difference in the intensity of the 75-kilodalton band between neonates and adults, suggesting that there is no difference in expression of this form of the SERT. However, a new finding presented here is that the antibody also revealed a protein band with a molecular weight of approximately 60 kilodaltons in both the neonatal and adult intestine. This 60-kilodalton band was absent from the brain but was signif-

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**Figure 6.** Immunohistochemical localization of 5-HT-ir in sections of adult and newborn intestine. (A) A cross-section of the adult intestine showing 5-HT-ir EC cells distributed in the crypt region and along the mucosal villi. (B) A cross-section of neonatal intestine showing that 5-HT-ir EC cells are localized preferentially to the crypt region.

**Figure 7.** Analysis of the distribution of EC cells in the crypts and villi of neonatal and adult guinea pigs. (A) The total number of EC cells in the mucosa did not differ between adult (n = 4) and neonatal (n = 4) guinea pigs. However, the number of EC cells in the crypt of neonatal intestine was significantly greater (* P < .05) than that in adult intestine. Alternatively, the number of EC cells in adult villi was significantly greater (* P < .05) than that in the neonatal intestine. (B) The EC cell crypt–villus ratio was significantly larger in the neonatal intestine (* P < .05). Data are mean ± SEM.
icantly stronger in protein extracts from the adult intestine. Previous work revealed that there are only modest differences in the nucleotide sequences encoding the rat and human brain and guinea pig intestine SERT protein. However, the same study showed that some antibodies recognize the rat brain SERT but do not recognize the SERT protein in the guinea pig intestine. It is possible that the SERT protein in the adult guinea pig intestine undergoes some posttranslational modification that shortens the amino acid sequence to yield the mature (functional) form of the transporter. This posttranslational processing does not occur in the neonatal intestine, and therefore the SERT protein is present but it is expressed in an immature (non-functional) form. The SERT antibody may recognize the denatured 60-kilodalton protein in Western blots, but it may not recognize the native SERT conformation in enterocytes in immunohistochemical studies. It should also be pointed out that our data are the first to describe Western blot analysis of SERT obtained from the guinea pig small intestine. Western blot studies done on protein extracts obtained from guinea pig airway smooth muscle cells maintained in culture revealed a single band with a molecular weight near 115 kilodaltons. This band might represent a dimer of individual SERT proteins (each with a molecular weight of approximately 60 kilodaltons). It is also possible that the 60-kilodalton band represents a degradation product of a larger SERT form expressed in the guinea pig intestine. Our protein isolation protocol included the use of a protease inhibitor cocktail; however, this may have been insufficient to fully block the high levels of protease activity present in the intestinal mucosa.

**5-HT Content and Differential Distribution of EC Cells in Neonatal and Adult Intestine**

There were no differences in mucosal 5-HT content in tissues obtained from neonatal and adult guinea pigs. Therefore, differences in EC cell 5-HT content cannot account for the elevated basal levels of extracellular 5-HT detected in neonatal tissues. 5-HIAA (the major degradation metabolite of 5-HT) was higher in adult tissues compared with that in neonatal tissues. The 5-HIAA/5-HT ratio is a measure of 5-HT turnover. After 5-HT is released from EC cells it is taken up by SERT expressing enterocytes where it is oxidized by monoamine oxidase to produce 5-HIAA. As functional SERT is absent from neonatal enterocytes, 5-HT cannot be transported by the enterocytes for metabolism to 5-HIAA.

We found that there were no differences in EC cell numbers in sections of the adult and neonatal intestine, and this corresponds to the similar 5-HT content measured in adult and neonatal tissues. However, in adult tissues, EC cells were localized along the length of mucosal villi while in sections from neonates, EC cells were found predominately in the crypt region. It is possible that recently differentiated EC cells in the crypt region of neonatal animals have only just begun to migrate towards the villus tip. It is also possible that EC cell movement into the villi stimulates SERT expression in enterocytes. Membrane expression and SERT activity are regulated by protein kinase C and p38–MAP–kinase-dependent pathways activated by G-protein coupled receptors, including 5-HT receptors. Enterocytes in guinea pig mucosal villi and crypts express 5-HT2A receptors and 5-HT2A receptors can activate MAP–kinase in vascular smooth muscle and renal mesangial cells. The p38–MAP–kinase-dependent pathway is particularly important for maintaining SERT membrane expression and activity. 5-HT released by EC cells as they migrate into the villi could stimulate maturation and membrane expression of SERT in the enterocytes so that as the intestine matures in the postnatal period it is prepared to buffer extracellular 5-HT released by EC cells.

**Conclusions**

The data presented here indicate that paracrine signaling by EC cells in the intestinal mucosa is not fully developed in the small intestine of neonatal guinea pigs. Specifically, functional SERT expression has not yet developed, and therefore, there is no active mechanism for clearance of 5-HT released by EC cells in the neonatal intestine. The absence of functional SERT expression causes enhanced basal and mechanically stimulated overflow of 5-HT from the mucosa. Pathophysiologic changes in SERT function or expression are also associated with increased availability of 5-HT in the intestinal mucosa. In a guinea pig model of colonic inflammation, SERT expression is reduced and 5-HT availability is increased in inflamed tissues under basal and stimulated conditions. In addition, fluoxetine increased 5-HT availability in control but not inflamed tissues. It was proposed that the increased free mucosal 5-HT in inflamed tissues might contribute to altered colonic motility known to occur in human and experimental colitis. As 5-HT released from EC cells activates motor reflexes elicited by mucosal stimulation, it might be expected that these reflexes would not be fully developed in the neonatal intestine where SERT expression is low and 5-HT availability is high. Therefore, propulsive motility might be impaired in the neonatal gut as it is in the inflamed gut. Maturation of the 5-HT signaling system in the gut might be required for postnatal development of motility patterns as the neonate transitions to oral intake of nutrients.

**References**


