Uptake of Serotonin at the Apical and Basolateral Membranes of Human Intestinal Epithelial (Caco-2) Cells Occurs through the Neuronal Serotonin Transporter (SERT)

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Received January 28, 2003; accepted April 4, 2003

ABSTRACT
Serotonin plays important physiological functions at the intestinal level. However, nothing is known concerning its inactivation mechanisms in the human intestine. So, the aim of this work was to characterize the uptake of serotonin at the apical and basolateral membranes of human intestinal epithelial (Caco-2) cells. Uptake of [3H]serotonin at the apical membrane of Caco-2 cells was specific and Na\(^+\)-, Cl\(^-\)-, and potential-dependent. It was concentration dependently inhibited by several monoamines (with the following rank order of potency: serotonin \(\geq\) dopamine \(\geq\) noradrenaline) and tricyclic and nontricyclic antidepressants (with the following rank order of potency: fluoxetine \(>\) desipramine \(>\) cocaine \(>\) GBR 12909). In contrast, it was not affected by corticosterone (0.01–100 \(\mu M\)) and was only partially inhibited by decynium-22 (0.001–10 \(\mu M\)). Transepithelial apparent permeability \(P_{\text{app}}\) to [3H]serotonin in the apical-to-basolateral direction was reduced by desipramine (0.4 \(\mu M\)) and fluoxetine (0.02 \(\mu M\)), and it was not \(\text{Na}^+\)-dependent nor affected by corticosterone (100 \(\mu M\)). Uptake of [3H]serotonin at the basolateral membrane of Caco-2 cells was \(\text{Na}^+\)-dependent and reduced by desipramine (0.4 \(\mu M\)) and fluoxetine (0.02 \(\mu M\)), and it was not affected by corticosterone (100 \(\mu M\)). The \(P_{\text{app}}\) to [3H]serotonin in the basolateral-to-apical direction was reduced by desipramine (0.4 \(\mu M\)) and fluoxetine (0.02 \(\mu M\)), and it was not affected by \(\text{Na}^+\) omission or by corticosterone (100 \(\mu M\)). Reverse transcriptase-polymerase chain reaction indicates that mRNA of the neuronal serotonin transporter (SERT) is present in Caco-2 cells and in human small intestine. In conclusion, these results suggest that human intestinal epithelial Caco-2 cells functionally express SERT, both at their apical and basolateral cell membranes.

The largest store of serotonin in the body is found in the gastrointestinal tract, corresponding to over 95% of the body’s serotonin (Erspamer, 1966). Most of the gastrointestinal serotonin is contained in enterochromaffin (EC) cells of the mucosal epithelium, within which serotonin is synthesized from L-tryptophan and stored in secretory granules. Serotonin is also present in serotonergic neurons of the enteric nervous system. Several functions of enteric serotonin have been identified in the past years. First, serotonin acts as the neurotransmitter of a subset of myenteric interneurons (Wade et al., 1994). Second, serotonin contained in EC cells, being released in response to chemical or mechanical stimuli, affects gastrointestinal motility (it initiates peristaltic reflexes by acting on intrinsic sensory neurons and musculature) and intestinal electrolyte transport (it initiates secretory reflexes by acting on intrinsic sensory neurons and mucosal cells) (Engel et al., 1984; Imada-Shirakata et al., 1997). Moreover, extrinsic sensory neurons activated by serotonin initiate sensations from the bowel, which may include nausea, bloating, and pain (for review, see Gershon, 1999). Additionally, serotonin present in EC cells has been shown to regulate the rate of proliferation of neighboring epithelial cells (Tutton and Barkla, 1987), and to inhibit the intestinal absorption of sugars (Arruebo et al., 1989) and L-leucine (Salvador et al., 1997).

If serotonin plays a role as a mucosal signaling molecule, a mean of inactivating intramuscular serotonin would be predicted. The action of transmitters is characteristically limited temporally and spatially; in the absence of an adequate inactivating mechanism, receptors for serotonin would likely desensitize. The enzymes that catabolize serotonin (at the intestinal level, monoamine oxidase and glucuronyl transferase; Blashko and Levine, 1966) are located intracellularly.

ABBREVIATIONS: EC, enterochromaffin; SERT, serotonin transporter; OCT, organic cation transporter; TEER, transepithelial resistance; \(P_{\text{app}}\), apparent permeability; RT-PCR, reverse transcriptase-polymerase chain reaction; RT, reverse transcriptase; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NAT, noradrenaline transporter; DAT, dopamine transporter; GBR 12909, 1-(2-bis(4-fluorophenyl)-methoxy)-ethyl-4-3-phenyl-propyl)piperazine.
So, serotonin must be internalized by the cells that possess these enzymes before being enzymatically inactivated (Wade et al., 1996; Chen et al., 1998).

A neuronal serotonin transporter (SERT) has been cloned in rats (Blakely et al., 1991; Hoffman et al., 1991), mice (Chang et al., 1996), bovine (Mortensen et al., 1999), and humans (Ramamoorthy et al., 1993). This transporter is sodium- and chloride-dependent and is specifically inhibited by serotonin-selective reuptake inhibitors (e.g., fluoxetine). However, there are no serotonergic neurons in the gastrointestinal mucosa (Furness and Costa, 1982) and no mucosal nerves with the ability to take up serotonin have been found (Gershon and Sherman, 1982). Interestingly, mucosal epithelial cells possessing SERT might take up serotonin to activate it. Indeed, rat and guinea pig mucosal epithelial cells have been found to express an mRNA encoding SERT, display SERT immunoreactivity, and specifically take up serotonin (Takayanagi et al., 1995; Wade et al., 1996; Chen et al., 1998).

Because nothing is known concerning the mechanism(s) of inactivation of serotonin in the human intestine, the purpose of this study was to characterize the uptake of serotonin by human intestinal epithelial (Caco-2) cells. Caco-2 cells are an epithelial cell line derived from a human colon adenocarcinoma, which mimics the human intestinal absorptive epithelium (for review, see Delie and Rubas, 1997). Because rat and guinea pig intestinal epithelial cells possess SERT (see above), we investigated the presence of mRNA encoding SERT in Caco-2 cells, and the functional expression of SERT in these cells. Moreover, because nothing is known concerning the subcellular localization of SERT in intestinal epithelial cells, we characterized the uptake of serotonin at both the basolateral and apical membranes of Caco-2 cells and the transepithelial transport of serotonin across Caco-2 monolayers in the apical-to-basolateral and basolateral-to-apical directions. Finally, because Caco-2 cells and the intestinal mucosa express a number of Na+-independent transporters for organic cations, belonging to the organic cation transporter (OCT) family (OCT1, OCT2 and EMT, also known as OCT3) (Bleasby et al., 2000; Chen et al., 2001; Martel et al., 2001; Hayer-Zillgen et al., 2002), the putative participation of these transporters in the uptake and transepithelial transport of serotonin in Caco-2 cells was also assessed.

Materials and Methods

Cell Culture. The Caco-2 cell line was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and was used between passage 10 and 67. Cells were maintained in a humidified atmosphere of 5% CO2, 95% air and were grown in minimum essential medium (Sigma-Aldrich, St. Louis, MO) supplemented with 20% fetal calf serum, 25 mM HEPES, 100 units ml−1 penicillin, 100 μg ml−1 streptomycin, and 0.25 μg ml−1 amphotericin B (all from Sigma-Aldrich). Culture medium was changed every 2 to 3 days and the culture was split every 7 days. For subculturing, the cells were removed enzymatically (0.25% trypsin-EDTA, 5 min, 37°C), split 1:3, and subcultured in plastic culture dishes (21 cm2; 60 mm; Corning Glassworks, Corning, NY). For uptake studies, Caco-2 cells were seeded on 24-well plastic cell culture clusters (2 cm2; 16 mm; Corning Glassworks), and the experiments were performed 7 to 9 days after the initial seeding. For permeability studies, cells were grown on semipermeable polycarbonate filters (1-cm2 surface area, 0.4-μm pore size) suitable for mounting in Ussing chambers (Snapwell; Corning Glassworks), thereby allowing study of vectorial transepithelial transport, i.e., basal-to-apical and apical-to-basal transport.

Permeability studies were performed 12 to 15 days after the initial seeding. In both uptake and permeability studies, the cell medium was free of fetal calf serum for 24 h before the experiments.

Uptake Studies. The uptake experiments were performed with Caco-2 cells incubated in Hanks' medium with the following composition: 137 mM NaCl, 5 mM KCl, 0.8 mM MgSO4, 1.0 mM MgCl2, 0.33 mM Na2HPO4, 0.44 mM KH2PO4, 0.25 mM CaCl2, 0.15 mM Tris-HCl, and 1.0 mM sodium butyrate, pH 7.4. The buffer also contained 1 mM ascorbic acid and 100 μM pargyline, to prevent oxidation and metabolism of serotonin by monoamine oxidase, respectively. Initially, the growth medium was aspirated and the cells were washed with Hank's medium at 37°C; then the cell monolayers were preincubated for 20 min in Hanks' medium at 37°C. Uptake was initiated by the addition of 0.3 ml of medium at 37°C containing 200 nM [3H]serotonin. At the end of the incubation period, incubation was stopped by placing the cells on ice and rinsing the cells with 0.5 ml of ice-cold Hank's medium. The cells were then solubilized with 0.3 ml of 0.1% (v/v) Triton X-100 (in 5 mM Tris-HCl, pH 7.4) and placed at 37°C overnight. Radioactivity in the cells was measured by liquid scintillation counting.

Effect of Drugs. Drugs to be tested were present during both the preincubation and incubation periods.

Effect of Ionic Composition of the External Medium. To study the influence of extracellular Na+ and Cl− on the uptake of [3H]serotonin, cells were preincubated and incubated in NaCl-free Hanks' medium, NaCl being isotonically replaced by LiCl and NaF, respectively. To study the influence of the membrane potential on the uptake of [3H]serotonin, cells were preincubated and incubated in NaCl-free Hanks' medium, NaCl being isotonically replaced by KCl.

Permeability Studies. Confluent and differentiated Caco-2 cells were used for permeability studies. Cell monolayer integrity and confluence was assessed by measurement of transepithelial resistance (TER) before the beginning of each experiment, by using an epithelial voltohmmeter fitted with planar electrodes (EVOM; World Precision Instruments, Stevenage, UK). Experiments were conducted only in those cell monolayers that showed a TER > 150 Ω, after correction for the resistance obtained in a Snapwell without cells. TER was also determined after each experiment to determine the effect of test substances on monolayer integrity (see Results).

Cells were removed from the growth medium, washed twice with Krebs' medium (containing 125 mM NaCl, 4.85 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 0.4 mM K2HPO4, 1.6 mM KH2PO4, 1.2 mM CaCl2, 10 mM glucose, 1 mM ascorbic acid, and 0.1 mM pargyline, pH 7.4), placed in Ussing chambers (1.131-cm2 surface area; World Precision Instruments) and bathed on mucosal and serosal sides by 5 ml of Krebs' medium, at 37°C, and continuously oxygenated. After a 20-min equilibration period, [3H]serotonin was added to either the mucosal (apical) or serosal (basolateral) chamber to give a final concentration of 100 nM. Samples (0.5 ml) were removed from the acceptor chamber every 10 min, for 90 min, and replaced with fresh medium. Samples (50 μl) were also taken from the donor chamber at the beginning and end of each experiment to monitor donor chamber [3H]serotonin concentration during the experiment. Samples were analyzed by liquid scintillation counting and results are expressed as apparent permeability (Papp) (see below).

As a further test of the integrity of the cell monolayers, transport of the paracellular marker phenol red across the monolayer was determined in each experiment. Phenol red (10 μM) was added to the donor chamber at the beginning of the permeability study, and the amount of phenol red in the acceptor chamber was determined after 90 min. Phenol red was determined spectrophotometrically (at 560 nm) after addition of 10% (v/v) 0.1 N NaOH to the samples. Transepithelial transport of phenol red was always less than 5%/h of the initial concentration.

Effect of Drugs. Drugs to be tested were present in the donor chamber medium during the 20-min equilibration period and the 90-min duration of the permeability study.

Effect of Ionic Composition of the External Medium. To test the influence of extracellular Na+ and NaCl of the Krebs' medium...
present in the donor chamber was isotonically replaced by LiCl during the 20-min equilibration period and the 90-min duration of the permeability study.

Protein Determination. The protein content of Caco-2 cells was determined as described by Bradford (1976), with bovine serum albumin as standard.

RT-PCR. Total RNA from human small intestine (Ambion, Austin, TX) and Caco-2 cells was used. Total RNA was extracted from Caco-2 cells by the method of Chomczynski and Sacchi (1987). Caco-2 cell cultures with 10 days of culture were used and for 24 h before the RNA extraction, the cell medium was made free of fetal bovine serum. Unless otherwise stated, all reagents used were molecular biology reagents from Sigma-Aldrich.

A total of 20 μg of total RNA was incubated at 37°C for 30 min with 150 units of RNase-free DNase I (Sigma-Aldrich) in 100 μl of 5 M MgCl₂, 50 mM triethanolamine-HCl, pH 7.5, to degrade any residual DNA. The RNA was extracted with phenol-chloroform, precipitated with ethanol and dissolved in water. For cDNA synthesis, 5 μg of the RNA thus prepared was incubated at 45°C for 1 h in a total volume of 20 μl with 200 units of Superscript II reverse transcriptase (RT; Invitrogen, Carlsbad, CA), in 10 μl of a mixture containing 0.375 mM dNTP per dNTP, 3 mM MgCl₂, 75 mM KCl, 50 mM Tris-HCl, pH 8.3 (25°C), 10 mM dithiothreitol, and 40 units of RNase inhibitor (RNaseOUT; Invitrogen). For paired negative controls, RT was omitted. After heat inactivation of the reaction mixture (10 min at 95°C) and addition of 5 μl of 0.5 mg/ml DNase-free RNase A (Sigma-Aldrich) in 10 mM Tris-HCl, pH 8.0, and 50% (v/v) glycerol, the cDNA was incubated at 37°C for 30 min, to degrade unreacted mRNA. Using 4 μl of this cDNA, PCR was performed. The PCR reaction mixture (50 μl) contained 0.5 μM per primer, 0.2 mM per dNTP, 2.5 mM MgCl₂, and 2 units of TaqDNA polymerase in the buffer provided (Invitrogen). The following primers, for human SERT and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were used: 5′-CAT CTG GAA AGG CGT CAA G-3′ (forward primer SERT), 5′-CGA AAC GAA GCT GTG CAT G-3′ (reverse primer SERT), 5′-ACT GGC GTC TTC ACC ACC AT-3′ (forward primer GAPDH), 5′-TCC ACC ACC CTG TTG CTG TA-3′ (reverse primer GAPDH). Thermocycling consisted of 1 cycle at 94°C for 2 min (for denaturing) followed by 30 cycles of 30 s at 95°C, 30 s at 55°C, and 90 s at 72°C (for amplification), and final elongation at 72°C for 7 min. The predicted sizes of the PCR products were (in base pairs) 319 (SERT) and 682 (GAPDH). Ten microliters of each individual PCR reaction was then run on a 1.6% agarose gel and visualized with an ultraviolet transilluminator (Vilber Lourmat, Marne La Vallée, France) using ethidium bromide staining, a COHU charge-coupled device camera, and the appropriate filters for UV light.

Calculations and Statistics. For the analysis of the time course of [3H]serotonin accumulation, the parameters of eq. 1 were fitted to the experimental data by a nonlinear regression analysis, using a computer-assisted method (Motulsky et al., 1994).

\[
A(t) = \frac{k_{in} k_{out}}{k_{in} + k_{out}} (1 - e^{-k_{in} t})
\]  

(1)

A(t) represents the accumulation of [3H]serotonin at time t, k_{in} and k_{out} the rate constants for inward and outward transport, respectively, and t the incubation time. A_{max} is defined as the accumulation at steady state (t \to \infty). k_{in} is given in microliters per milligram of protein per minute and k_{out} in minutes.

For the calculation of IC_{50} values, the parameters of the Hill equation for multisite inhibition were fitted to the experimental data by a nonlinear regression analysis, using a computer-assisted method (Motulsky et al., 1994).

\[
P_{app} = \frac{dQ}{dt} \cdot 1/(A \cdot C_1)
\]  

(2)

where dQ/dt (moles per second) is the transport rate, C_1 (moles per cubic centimeter) is the initial concentration in the donor chamber, and A (square centimeters) is the surface area.

Arithmetic means are given with S.E.M. and geometric means are given with 95% confidence limits. Statistical significance of the difference between various groups was evaluated by one-way analysis of variance followed by Dunnett’s test. For comparison between two groups, Student’s t test was used. Differences were considered to be significant when P < 0.05.

Materials. [3H]-5-Hydroxytryptamine (5-[1,2-3H(N)]-hydroxytryptamine creatinine sulfate ([3H]serotonin); specific activity 23.7 Ci mmol⁻¹) (New England Nuclear, Dreieich, Germany); Triton X-100 (Merek, Darmstadt, Germany); cocaine hydrochloride (Uquipa, Sacavém, Portugal); desipramine hydrochloride (Ciba-Geigy, Basel, Switzerland); corticosterone, decynium-22 (1,1'-diethyl-2,2'-cyanine iodide), dopamine hydrochloride, fluoxetine hydrochloride, GBR 12909, HEPES, 5-hydroxytryptamine creatinine sulfate, pargyline hydrochloride, phenol red (phenoisulfophthalein) sodium salt, (−)-noradrenaline hydrochloride, Tris (tris(hydroxymethyl)-aminomethane, trypsin-EDTA solution (Sigma-Aldrich).

When the drugs to be tested were dissolved in ethanol, the final concentration of the solvent in the buffer was 1%. Controls for these drugs were run in the presence of the solvent.

Results

Studies on the Apical Uptake of [3H]Serotonin by Caco-2 Cells

Time Course of [3H]Serotonin Apical Uptake. In the first series of experiments, Caco-2 cells were incubated at 37°C with 200 nM [3H]serotonin for various periods of time (Fig. 1). Analysis of the time course of accumulation revealed a k_{in} of 9.9 ± 1.5 μl mg protein⁻¹ min⁻¹, a k_{out} of 0.018 ± 0.005 min⁻¹, and an A_{max} of 110.5 ± 13.2 pmol mg protein⁻¹ (n = 51). In other words, an amount of Caco-2 cells corresponding to 1 mg of cell protein cleared 9.9 μl of incubation medium of [3H]serotonin per minute, and simultaneously 2% of intracellular [3H]serotonin left the cells per minute. In the presence of 1 mM serotonin, both the k_{in} and the A_{max} were significantly reduced (to 3.0 ± 0.9 μl mg protein⁻¹ min⁻¹ and 3.5 ± 0.2 pmol protein⁻¹, respectively). On the contrary, the k_{out} was significantly increased (to 0.176 ± 0.056 min⁻¹). As shown in Fig. 1, uptake of [3H]serotonin was linear with time for up to 6 min of incubation. So, in all the subsequent experiments, cells were incubated with [3H]serotonin for 6 min, to determine initial rates of uptake.

Substrate Specificity of the Apical Uptake of [3H]Serotonin. To characterize the substrate specificity of the apical inward transport mechanism for [3H]serotonin in Caco-2 cells, the effect of several monoamines on [3H]serotonin up-

![Fig. 1. Time course of [3H]serotonin apical uptake by Caco-2 cells. Cells were incubated at 37°C with 200 nM [3H]serotonin in the absence (control; n = 5–9) or presence of 1 mM 5-hydroxytryptamine (serotonin 1 mM; n = 3). Exponential functions were fitted to the experimental data. Shown are means ± S.E.M.](image-url)
take was evaluated (Fig. 2). As can be observed, [3H]serotonin uptake was concentration dependently inhibited by the three monoamines tested. Analysis of the IC50 values of these compounds shows that the ranking order of potency was serotonin ≫ dopamine ≫ noradrenaline (Table 1). Because the inhibitory potency of these compounds reflects their affinity for the transporter, the affinity of the transporter responsible for [3H]serotonin uptake by Caco-2 cells decreases in the order serotonin ≫ dopamine ≫ noradrenaline.

**Inhibitor Specificity of the Apical Uptake of [3H]Serotonin.** To characterize the inhibition profile of the transporter responsible for the apical uptake of [3H]serotonin, the effect of inhibitors of several distinct transport mechanisms for monoamines was tested.

In the first series of experiments, the effects of fluoxetine, desipramine, cocaine, and GBR 12909 were studied (Fig. 3). These compounds are known inhibitors of the neuronal SERT, the neuronal noradrenaline transporter (NAT), and the neuronal dopamine transporter (DAT). However, they differ in their potencies toward these distinct transporters. As shown in Fig. 3, all of these drugs potently and concentration dependently reduced [3H]serotonin apical uptake. Their IC50 values were calculated and are presented in Table 1. The inhibitory ranking order of potency of the compounds was fluoxetine > desipramine > cocaine > GBR 12909.

The putative modification of [3H]serotonin apical uptake by decynium-22 and corticosterone, which are known inhibitors of Na+ independent transporters of organic cations belonging to the OCT family (OCT1, OCT2, and EMT), was also studied (Fig. 4). Corticosterone had no significant effect at all concentrations tested (0.01–100 μM). Decynium-22, however, concentration dependently reduced uptake of [3H]serotonin. At the highest concentration tested (10 μM), decynium-22 reduced uptake of [3H]serotonin to 53 ± 2% of control (n = 4).

**Effect of Ionic Replacement on the Apical Uptake of [3H]Serotonin.** To test the dependence of the apical uptake of [3H]serotonin on extracellular Na+ and Cl−, we measured

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**TABLE 1**

Inhibition by various compounds of the apical uptake of [3H]serotonin by Caco-2 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
<th>95% CI</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>0.0155</td>
<td>(0.007–0.032)</td>
<td>4–6</td>
</tr>
<tr>
<td>Desipramine</td>
<td>0.363</td>
<td>(0.206–0.641)</td>
<td>4</td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.643</td>
<td>(0.252–1.59)</td>
<td>4</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>1.41</td>
<td>(0.96–2.09)</td>
<td>4</td>
</tr>
<tr>
<td>5HT</td>
<td>0.998</td>
<td>(0.605–1.65)</td>
<td>4</td>
</tr>
<tr>
<td>Dopamine</td>
<td>247.9</td>
<td>(130.5–470.8)</td>
<td>4</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>398.9</td>
<td>(141.7–1123)</td>
<td>4</td>
</tr>
</tbody>
</table>
the uptake in the absence of NaCl, which was substituted with either LiCl or NaF. As can be seen in Fig. 5, substitution of Na⁺ with Li⁺ and substitution of Cl⁻ with F⁻ caused dramatic decreases in the uptake of [³H]serotonin. So, it is concluded that [³H]serotonin uptake is dependent on both extracellular Na⁺ and Cl⁻.

To determine whether uptake of [³H]serotonin is dependent on the membrane potential, NaCl was substituted with KCl. This substitution caused not only a marked inhibition of the uptake in relation to control cells but also in relation to uptake in cells in which NaCl was replaced by LiCl (Fig. 5), supporting the conclusion that [³H]serotonin uptake is potential-dependent.

Studies on the Permeability of [³H]Serotonin across Caco-2 Monolayers

Time Course of Transepithelial Permeability to [³H]Serotonin. Permeability to [³H]serotonin (100 nM) across Caco-2 monolayers was examined in both the apical-to-basolateral and basolateral-to-apical directions, and is depicted in Fig. 6. As can be seen, the flux was essentially linear for up to 90 min, and the $P_{app}$ was similar in the apical-to-basolateral (13.8 ± 0.75 cm/s × 10⁻⁶; n = 4) and basolateral-to-apical (15.4 ± 1.28 cm/s × 10⁻⁶; n = 6) directions.

Effect of Drugs and Ionic Replacement on [³H]Serotonin Transepithelial Permeability. Desipramine (0.4 μM) and fluoxetine (0.02 μM) caused a significant reduction in the $P_{app}$ to [³H]serotonin (100 nM) in both the apical-to-basolateral and basolateral-to-apical directions (Fig. 7). These two compounds also caused a significant reduction in the amount of [³H]serotonin present in the cells at the end of the 90-min permeability study, both in the apical-to-basolateral and basolateral-to-apical permeability experiments (Fig. 7). Under control conditions, the cellular content of [³H]serotonin at the end of the experiment was 24.5 ± 2.2 and 40.5 ± 3.4 pmol/Snapwell (n = 4–6), in the apical-to-basolateral and basolateral-to-apical permeability studies, respectively. So, desipramine and fluoxetine seem to inhibit both the apical and basolateral cellular uptake of [³H]serotonin and the apical-to-basolateral and basolateral-to-apical transepithelial transport of this amine in Caco-2 cells.

Fig. 6. Bidirectional permeability to [³H]serotonin across confluent monolayers of Caco-2 cells. The permeability to [³H]serotonin (100 nM) was examined in the apical-to-basolateral (AP-BL) and basolateral-to-apical (BL-AP) directions. Experiments were performed in Ussing chambers at 37°C. Each value represents arithmetic mean ± S.E.M. of four to six experiments.

The influence of extracellular Na⁺ on the cellular uptake and transepithelial transport of [³H]serotonin was next examined. Substitution of NaCl in the donor chamber by LiCl caused a marked reduction in the cellular content of [³H]serotonin at the end of the 90-min permeability study, in both the apical-to-basolateral and basolateral-to-apical permeability experiments (Fig. 8). However, this treatment did not cause a change in the $P_{app}$ to [³H]serotonin across cell monolayers (Fig. 7).

Corticosterone (100 μM) did not affect either the apical or basolateral cellular uptake of [³H]serotonin or the apical-to-basolateral and basolateral-to-apical $P_{app}$ to [³H]serotonin across Caco-2 monolayers (Figs. 7 and 8).

None of the drugs tested (desipramine, fluoxetine, and corticosterone) nor Na⁺ omission produced any significant change in TEER, measured at the end of the experiment (data not shown).

Expression of SERT in Caco-2 Cells and Human Small Intestine. RT-PCR analysis of SERT expression in Caco-2 cells and human small intestine was performed (Fig. 9). RT-PCR resulted in amplification products of the expected size (319 base pairs) in both Caco-2 and human small intestine samples. So, both Caco-2 cells and the human small intestine express the mRNA of SERT.

Discussion

The aim of this work was to characterize the mechanism(s) involved in the uptake of serotonin by human intestinal epithelial cells. The experiments were performed using an established epithelial cell line derived from a human colon adenocarcinoma (Caco-2 cells). Caco-2 cells are an enterocyte-like cell line proved as an appropriate model system for intestinal epithelial permeability studies (Delie and Rubas, 1997).

Recent studies have shown that rat and guinea pig intestinal epithelial cells express an mRNA encoding SERT, display SERT immunoreactivity, and specifically take up serotonin (Takayanagi et al., 1995; Wade et al., 1996; Chen et al., 1998). However, nothing was known concerning serotonin inactivation (including the putative participation of SERT) in...
the human intestine. By using RT-PCR, we found that both Caco-2 cells and the human small intestine express mRNA encoding SERT. So, we decided to investigate the putative participation of SERT in the uptake of serotonin by Caco-2 cells. Moreover, because the exact membrane location of SERT (i.e., basolateral, apical or both) was not determined in these former studies, we aimed also at determining the membrane localization of this transporter.

In relation to the uptake of serotonin at the apical membrane of Caco-2 cells, we can conclude, from the results obtained, that it is most probably mediated by SERT. First, in the apical uptake experiments, we found several similarities between the characteristics of the uptake of \(^{3}H\)serotonin by Caco-2 cells and SERT-mediated transport. These similarities between the characteristics of the uptake of \(^{3}H\)serotonin across confluent monolayers of Caco-2 cells. The \(P_{app}\) to \(^{3}H\)serotonin (100 nM) was examined in the apical-to-basolateral (AP-BL; \(n = 3\)–5) and basolateral-to-apical (BL-AP; \(n = 4\)–6) directions. Experiments were performed in Ussing chambers at 37°C. Each value represents arithmetic mean ± S.E.M. * significantly different from control \((P < 0.05)\).

Fig. 7. Effect of desipramine (0.4 μM), fluoxetine (0.02 μM), sodium omission (0 mM NaCl), and corticosterone (100 μM) on the bidirectional permeability to \(^{3}H\)serotonin across confluent monolayers of Caco-2 cells. The \(P_{app}\) to \(^{3}H\)serotonin (100 nM) was examined in the apical-to-basolateral (AP-BL; \(n = 3\)–5) and basolateral-to-apical (BL-AP; \(n = 4\)–6) directions. Experiments were performed in Ussing chambers at 37°C. Each value represents arithmetic mean ± S.E.M. * significantly different from control \((P < 0.05)\).

include: 1) dependence on external Na\(^+\) and Cl\(^-\); 2) dependence on cell membrane potential; 3) the ranking order of the inhibitory potencies of monoamines; and 4) the ranking order of the inhibitory potencies of tricyclic and nontricyclic antidepressants.

In the present study, the ranking order of inhibitory potencies of the three monoamines tested was serotonin \(\geq\) dopamine \(\geq\) noradrenaline. These three amines are known substrates of SERT, and this ranking order is in perfect agreement with the ranking order of substrate affinity of SERT (serotonin \(\geq\) dopamine \(\geq\) noradrenaline) (Eshleman et al., 1999; Miller et al., 2001). Moreover, it is clearly distinct from the ranking order of substrate affinity of two other neuronal monoamine transporters, the NAT, and the DAT (for both of them: dopamine \(\geq\) noradrenaline \(\geq\) serotonin; for review, Graefe and Bönisch, 1988). Of worth noticing is the fact that the \(K_m\) value of serotonin relative to SERT-mediated transport (0.3–0.6 μM; Blakely et al., 1991; Miller et al., 2001) is very similar to its IC\(_{50}\) value (0.998 μM) obtained in the present study (which should be very approximate to its \(k_l\) value, because we used a substrate concentration below the \(K_m\) value).

In relation to the inhibitors tested, their ranking order of potency was found to be fluoxetine \(\geq\) desipramine \(\geq\) cocaine \(\geq\) GBR 12909. This ranking order of potency is also characteristic of SERT-mediated transport (e.g., Blakely et al., 1991). In contrast, NAT and DAT have clearly distinct inhibitory profiles. Whereas NAT is most potently inhibited by the tricyclic antidepressants desipramine and cocaine, DAT is most potently inhibited by GBR 12909 (Miller et al., 2001).

Second, in the transepithelial permeability studies, the...
cellular uptake was clearly dependent on extracellular Na⁺ and inhibited by desipramine and fluoxetine, and the trans-epithelial permeability (as assessed by \( P_{\text{app}} \)) to [³H]serotonin in the apical-to-basolateral direction was inhibited by desipramine and fluoxetine. The concentration of desipramine (0.4 μM) and fluoxetine (0.02 μM) used in these experiments corresponds to \( 1 \times \text{IC}_{50} \) calculated in the apical uptake experiments. These results further suggest that the uptake of serotonin at the apical membrane of Caco-2 cells occurs through a SERT-mediated mechanism.

In relation to the uptake of serotonin at the basolateral membrane of Caco-2 cells, the results obtained in the trans-epithelial permeability experiments support the conclusion that it is also SERT-mediated. Indeed, desipramine (0.4 μM) and fluoxetine (0.02 μM) significantly reduced the cellular uptake and the \( P_{\text{app}} \) to [³H]serotonin in the basolateral-to-apical direction. Moreover, the cellular uptake of [³H]serotonin was also clearly Na⁺-dependent (although no such dependence could be demonstrated in relation to the \( P_{\text{app}} \)).

The results concerning the permeability of serotonin across Caco-2 cell monolayers suggest that the apical-to-basolateral and basolateral-to-apical transepithelial flux of serotonin across Caco-2 monolayers occurs mainly through the trans-cellular route, because a reduction in the \( P_{\text{app}} \) is accompanied by a similar reduction in the cellular content of [³H]serotonin. However, an exception was found with the Na⁺ omission results. In both apical-to-basolateral and basolateral-to-apical experiments, Na⁺ omission produced a significant reduction in the cellular uptake of [³H]serotonin, but did not change the \( P_{\text{app}} \). This suggests that other mechanisms of [³H]serotonin transepithelial flux (e.g., paracellular route) may be operating in Caco-2 monolayers.

The putative participation of transporters belonging to the OCT family of Na⁺-independent transporters (see Introduction) in the uptake and transepithelial permeability of [³H]serotonin was also assessed. For this purpose, we tested the effect of decynium-22 and corticosterone, two known inhibitors of OCT1, OCT2, and EMT. The \( k_i \) values in relation to OCT1, OCT2, and EMT are, for decynium-22, 500, 51, and 17 nM (Russ et al., 1992; Martel et al., 1996; Gründemann et al., 1997), and, for corticosterone, 72, 0.67, and 0.12 μM, respectively (Martel et al., 1996; Gründemann et al., 1997, 1998). The results obtained in this work suggest that transporters of the OCT family are not involved in either the apical or basolateral uptake of [³H]serotonin by Caco-2 cells. Indeed, in the apical uptake experiments, corticosterone (0.01–100 μM) did not affect the uptake of [³H]serotonin, and decynium-22 (0.001–10 μM) reduced it, but with a very low potency, compared with its \( k_i \) values in relation to OCT1, OCT2, or EMT (see above). Moreover, corticosterone (100 μM) did not affect the uptake and the \( P_{\text{app}} \) of [³H]serotonin in both the apical-to-basolateral and basolateral-to-apical permeability experiments. Concerning the inhibitory effect of decynium-22, two explanations seem feasible at this time. First, decynium-22 may exhibit some toxicity on Caco-2 cells (recently, our group found that micromolar concentrations of this compound have some cytotoxic effects on the JAR human placental choriocarcinoma cell line; Martel and Keating, 2003). Second, it is possible that high (micromolar) concentrations of decynium-22 exert some inhibition of SERT (it was previously reported that 3 μM of this compound caused a 15% inhibition of NAT; Russ et al., 1993).

In conclusion, the results of this study suggest that the human intestinal epithelial (Caco-2) cells possess a functionally active SERT, capable of removing serotonin from the extracellular space. So, similarly to what happens in rat and guinea pig mucosa (Takayanagi et al., 1995; Wade et al., 1996; Chen et al., 1998), SERT seems to be present in human intestinal mucosa. In contrast, SERT was not functionally detected in rabbit enterocytes (Alcalde et al., 2000). Moreover, our results also suggest that in Caco-2 cells, SERT is present both at the basolateral and apical cell membranes, thus being able to remove both intraluminal and interstitial serotonin. Interestingly enough, 5-hydroxytryptamine contained in EC cells is liberated into both the intestinal lumen and interstitial space (Ahlman et al., 1984; Grönstad et al., 1988). Inhibition of SERT (e.g., with fluoxetine) caused a decrease in the cellular uptake of [³H]serotonin (when serotonin was added to either the apical or basolateral medium) and in the apical-to-basolateral and basolateral-to-apical transepithelial flux of [³H]serotonin across Caco-2 cell monolayers. So, SERT present in epithelial cells is probably able to effectively decrease the luminal and interstitial concentration of serotonin in the human intestine, thus constituting an effective means of inactivating serotonin. In accordance with this, inhibition of SERT (with fluoxetine) causes a potentiation of physiological responses mediated by serotonin in the guinea pig intestine (Wade et al., 1996; Chen et al., 1998).

Because serotonin plays an important role in the regulation of gastrointestinal physiology, affecting epithelial secretion, muscular contraction, and neuronal transmittance, such a mechanism of inactivation is expected to be extremely important in the physiology of the intestine, and may contribute to the gastrointestinal side effects (e.g., nausea and diarrhea) commonly observed with serotonin-selective reuptake inhibitors (Spigset, 1999).

References


J Pharmacol Exp Ther 354:220–236.


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