Modulation of Serotonin Uptake Kinetics by Ions and Ion Gradients in Human Placental Brush-Border Membrane Vesicles†

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ABSTRACT: The modulation of serotonin uptake kinetics by Na^+, Cl^-, H^+, and K^+ was investigated in brush-border membrane vesicles prepared from normal human term placentas. The presence of Na^+ and Cl^- in the external medium was mandatory for the function of the serotonin transporter. In both cases, the initial uptake rate of serotonin was a hyperbolic function of the ion concentration, indicating involvement of one Na^+ and one Cl^- per transport of one serotonin molecule. The apparent dissociation constant for Na^+ and Cl^- was 145 and 79 mM, respectively. The external Na^+ increased the V_{max} of the transporter and also increased the affinity of the transporter for serotonin. The external Cl^- also showed similar effects on the V_{max} and the K_t, but its effect on the K_t was small compared to that of Na^+. The presence of an inside-acidic pH, with or without a transmembrane pH gradient, stimulated the NaCl-dependent serotonin uptake. The effect of internal $[H^+]$ on the transport function was to increase the V_{max} and decrease the affinity of the transporter for serotonin. The presence of K^+ inside the vesicles also greatly stimulated the initial rates of serotonin uptake, and the stimulation was greater at pH 7.5 than at pH 6.5. This stimulation was a hyperbolic function of the internal K^+ concentration at both pH values, indicating involvement of one K^+ per transport of one serotonin molecule. The apparent dissociation constant for K^+ was 5.6 mM at pH 6.5 and 4.0 mM at pH 7.5. The effects of internal $[K^+]$ on the uptake kinetics were similar to those of internal $[H^+]$. It increased the V_{max} but decreased the affinity for the transporter for serotonin. In addition, the effects of internal $[H^+]$ on the kinetic parameters were masked by the presence of K^+ inside the vesicles, indicating competition between internal K^+ and internal H^+.

We have recently provided evidence for the existence of a transport system specific for serotonin in brush-border membranes isolated from normal human term placentas (Balkovetz et al., 1989). The transport system is energy dependent, has an absolute requirement for external Na^+ and Cl^-, and is energized by an inwardly directed NaCl gradient. Serotonin is a neurotransmitter and has profound effects on the function of the vascular system (Houston & Vanhoutte, 1986; Hollenberg, 1988). Human placenta is a highly vascularized tissue, and serotonin thus has the potential to modulate the placental function under physiological conditions. The role of serotonin in the growth and development of the fetus is unknown and remains unexplored. The brush-border membrane of the placental syncytiotrophoblast is in direct contact with the maternal blood and forms the first link between the mother and the developing fetus. The presence of an active transport system for serotonin in this membrane may have important implications in the function of the human placenta and hence in fetal growth and development. In the present study, we have characterized this potentially important transport system in detail using purified human placental brush-border membrane vesicles, with emphasis on the modulation of uptake kinetics by various ions and ion gradients.

The mechanism of the plasma membrane serotonin transporter has been extensively studied in the past in two other tissues, namely, platelet and brain (Ross, 1982). Even though platelets are often considered as a model for serotonergic neurons (Stahl & Meltzer, 1978), it is becoming increasingly evident that the serotonin transporters present in these two tissues may be similar but not identical. Important differences exist between platelet and brain serotonin transporters, and these include Na^+ stoichiometry (Talvenheimo et al., 1983; O'Reilly & Reith, 1988), involvement of H^+ and H^+ gradient (Keyes & Rudnick, 1982; Reith et al., 1989), and mode of regulation (Arora et al., 1983; Abel et al., 1985). The results of the present investigation show that the activity of the serotonin transporter in human placental brush-border membrane vesicles is modulated by Na^+, Cl^-, K^+, and H^+ and by concentration gradients of these ions across the membrane. A comparison of these data with those already available on platelet and brain serotonin transporters reveals that the placental transporter exhibits more similarities with the platelet transporter than with the neuronal transporter.

Materials and Methods

5-[1,2-^{3}H(N)]Hydroxytryptamine binoxalate (specific radioactivity, 30.4 Ci/mmol) was purchased from Dupont-New England Nuclear. Serotonin and imipramine were obtained from Sigma. All other chemicals were of analytical grade.

Brush-border membrane vesicles were prepared from normal human term placentas by a Mg^{2+} aggregation method as previously described (Ganapathy et al., 1985; Balkovetz et al., 1986). These membranes were found to be enriched 20–25-fold in brush-border marker enzymes, alkaline phosphatase (EC 3.1.3.1) and 5′-nucleotidase (EC 3.1.3.5), in comparison with the washed placental tissue. The membrane vesicles were preloaded with the buffer of desired pH and composition during the isolation procedure. The protein concentration of the final membrane suspension was adjusted to 6 mg/mL after which the suspension was divided into small aliquots and stored in liquid N_2 until use.

Uptake measurements were made by using a rapid filtration technique (Ganapathy et al., 1981). Millipore filters (DAWP type, pore size 0.65 μm) were employed in these studies.

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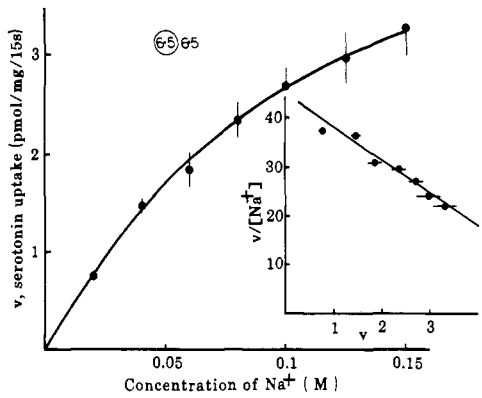

FIGURE 1: Dependence of initial uptake rate of serotonin on extravesicular Na^+ concentration. Membrane vesicles were preloaded with 20 mM MES/Tris buffer, pH 6.5, containing 375 mM mannitol. Uptake of serotonin (final concentration, 20 nM) was measured with a 15-s incubation in 20 mM MES/Tris buffer, pH 6.5, containing 187.5 mM Cl^- and varying concentrations of Na^+ and Li^+. Osmolality of the uptake buffer was maintained equal to that of the preloading buffer (400 mosm). Final concentration of Cl^- during uptake was 150 mM, and that of Na^+ ranged between 0 and 150 mM. Inset: Hill-type plot (v versus $v/[Na^+]^n$ with a value of $n = 1$).

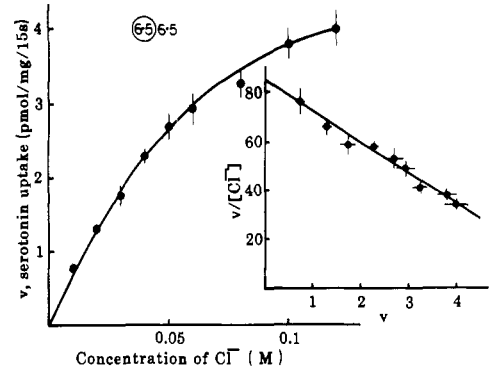

FIGURE 2: Dependence of initial uptake rate of serotonin on extravesicular Cl^- concentration. Membrane vesicles were preloaded with 20 mM MES/Tris buffer, pH 6.5, containing 300 mM mannitol. Uptake of serotonin (final concentration, 20 nM) was measured with a 15-s incubation in 20 mM MES/Tris buffer, pH 6.5, containing 150 mM Na^+ and varying concentrations of Cl^- and gluconate. Osmolality of the uptake buffer was maintained equal to that of the preloading buffer (325 mosm). Final concentration of Na^+ during uptake was 120 mM, and that of Cl^- ranged between 0 and 120 mM. Inset: Hill-type plot (v versus $v/[Cl^-]^n$ with a value of $n = 1$).

Uptake was initiated by rapidly mixing 40 μL of the membrane suspension with 160 μL of uptake buffer containing radiolabeled serotonin. After incubation for a desired time at room temperature (21–22 °C), uptake was terminated by adding 3 mL of ice-cold stop buffer [155 mM KCl, buffered with either 5 mM 4-morpholineethanesulfonic acid (MES)[1]/Tris, pH 6.5, or 5 mM HEPES/Tris, pH 7.5], and the mixture was filtered. After washing with the stop buffer (3 × 5 mL), the radioactivity associated with the filter was counted. Specific serotonin uptake, which invariably represented greater than 95% of the total uptake, was calculated by subtracting the uptake measured in the presence of 100 μM imipramine from the total uptake.

Each experiment was done with 2–10 different membrane preparations. Uptake measurements were made in duplicate or triplicate, and the variation among the replicate values was always less than 10% of the mean value. Initial uptake rates were determined by using either a 15-s or a 30-s incubation (uptake rate was found to be linear at least up to 45 s). Kinetic constants, K_t (substrate concentration which produces a half-maximal transport velocity) and V_{max} (maximal transport velocity), were calculated by using Eadie–Hofstee plots (initial uptake rate/substrate concentration versus initial uptake rate). The stoichiometry of Na^+, Cl^-, and K^+ was determined by the "activation" method described by Turner (1983). The Hill coefficient, n, which is an estimate of the number of reactive binding sites, was calculated by analyzing the experimental data according to the following Hill-type equation:

$$v = \frac{V_{max}[x]^n}{K_t^n + [x]^n}$$

where v is the initial uptake rate and x is the ion (Na^+, Cl^-, or K^+) whose stoichiometry is being determined. The results are expressed as the mean ± SE.

Results

Influence of Na^+ and Cl^- on Serotonin Uptake Kinetics. We have previously shown that serotonin uptake in human placental brush-border membrane vesicles exhibits an absolute requirement for Na^+ and Cl^- (Balkovetz et al., 1989). In the present study, we have investigated the influence of Na^+ and Cl^- on the kinetics of serotonin uptake. We first studied the dependence of the initial uptake rate of serotonin on the extravesicular concentration of Na^+ in the presence of a fixed concentration of extravesicular Cl^-. The concentration of Na^+ was varied between 20 and 150 mM, and the concentration of Cl^- was kept constant at 150 mM. As can be seen in Figure 1, the uptake rate increased with increasing concentrations of Na^+, and the rate was a hyperbolic function of the extravesicular concentration of Na^+. The data were used to construct a Hill-type plot (v versus $v/[Na^+]^n$), and the plot was found to be linear when n was assigned a value of 1 (Figure 1, inset). This indicates a 1 Na^+:1 serotonin stoichiometry. The apparent $K_{t(Na^+)}$ was 145 ± 15 mM.

Similar experiments were done with Cl^-. The concentration of extravesicular Cl^- was varied between 10 and 120 mM while keeping the extravesicular concentration of Na^+ constant at 120 mM. Figure 2 shows that the initial uptake rate was hyperbolically related to Cl^- concentration, indicating a 1 Cl^-:1 serotonin stoichiometry. A linear plot (Figure 2, inset) when v was plotted against $v/[Cl^-]$ provides supporting evidence for this stoichiometry. The apparent $K_{t(Cl^-)}$ was found to be 79 ± 5 mM.

We then studied the influence of extravesicular Na^+ and Cl^- on the kinetic parameters (K_t and V_{max}) of serotonin uptake. To evaluate the effects of Na^+, initial uptake rates of serotonin were measured at a fixed concentration of Cl^- (120 mM), but at two different concentrations of Na^+ (120 and 40 mM). To evaluate the effects of Cl^-, uptake rate measurements were made at a fixed concentration of Na^+ (120 mM), but at two different concentrations of Cl^- (120 and 40 mM). Serotonin concentration was varied between 20 and 200 nM. The results, when plotted according to the method of Eadie–Hofstee (initial uptake rate/serotonin concentration versus initial uptake rate), gave linear plots ($r^2 > 0.98$). This indicates that the uptake rate was a saturable function of the serotonin concentration under all of the above described experimental conditions. The effects of Na^+ and Cl^- on K_t and V_{max} of the serotonin uptake process as assessed from these plots are summarized in Table I. Extravesicular Na^+ increased the V_{max} and decreased the K_t. At 40 mM Na^+, the V_{max} decreased to 77% of the V_{max} at 120 mM Na^+, and, at the same time,

[1] Abbreviations: MES, 4-morpholineethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

Table I: Effects of Ions and Ion Gradients on Serotonin Uptake Kinetics

ion	conc (mM)	pH_i	pH_o	$V_{max} \pm SE$ [pmol (mg of protein)$^{-1}$ (15 s)$^{-1}$]	$K_t \pm SE$ (nM)
sodium					
Na_{out}	120	6.5	6.5	13.74 ± 0.32	56.8 ± 2.6
Na_{out}	40	6.5	6.5	10.57 ± 0.27	116.1 ± 4.7
chloride					
Cl_{out}	120	6.5	6.5	13.74 ± 0.32	56.8 ± 2.6
Cl_{out}	40	6.5	6.5	10.11 ± 0.56	80.6 ± 8.0
proton					
no gradient		6.5	6.5	8.21 ± 0.11	47.2 ± 1.4
		7.5	7.5	2.15 ± 0.14	15.1 ± 2.1
gradient		5.5	7.5	7.73 ± 0.19	70.9 ± 3.2
potassium					
K_{in}	0	6.5	6.5	8.21 ± 0.11	47.2 ± 1.4
K_{in}	50	6.5	6.5	51.80 ± 0.78	231.5 ± 9.3
K_{in}	0	7.5	7.5	2.15 ± 0.14	15.1 ± 2.1
K_{in}	50	7.5	7.5	40.31 ± 3.40	242.9 ± 31.2
K_{in}	0	5.5	7.5	7.73 ± 0.19	70.9 ± 3.2
K_{in}	50	5.5	7.5	13.11 ± 0.50	119.7 ± 8.0

the K_t increased about 104%. Thus, Na^+ not only increases the affinity of the transporter for serotonin but also enhances the translocation rate of the transporter complex. Extravesicular Cl^- also affected both kinetic parameters of serotonin uptake. At 40 mM Cl^-, the V_{max} was decreased and the K_t was increased compared to the corresponding values at 120 mM Cl^-. However, while the influence of Cl^- on the V_{max} was comparable to that of Na^+, the influence of Cl^- on the K_t was very small compared to the change in the K_t caused by Na^+.

Influence of H^+ on Serotonin Uptake Kinetics. We then probed the role of H^+ in the modulation of serotonin uptake. We investigated the effect of H^+ concentration as well as the effect of a transmembrane H^+ gradient on the uptake process. Figure 3 describes the time course of NaCl gradient-driven serotonin uptake under three experimental conditions: (a) $pH_i = pH_o = 7.5$; (b) $pH_i = pH_o = 6.5$; and (c) $pH_i = 5.5$, $pH_o = 7.5$. In each case, a transient active accumulation of serotonin inside the vesicles against a concentration gradient was evident (the "overshoot" phenomenon) in the presence of an inwardly directed NaCl gradient. However, this NaCl gradient-dependent uptake was found to be greatly influenced by H^+ concentration as well as by a transmembrane H^+ gradient. In the absence of a H^+ gradient ($pH_i = pH_o$), the initial uptake rate of serotonin was 2–3 times greater at pH 6.5 than at pH 7.5. Similarly, the intravesicular concentration of serotonin at the peak of the overshoot was also 2–3-fold higher at pH 6.5 than at pH 7.5. However, there was no change in the equilibrium concentration of serotonin (3-h incubation), indicating that the observed changes in the initial uptake rates were not due to pH-induced alterations in the vesicle volume and/or integrity. When the NaCl-dependent serotonin uptake was measured at a constant extravesicular pH of 7.5, the presence of an acidic pH inside the vesicles markedly stimulated the uptake. Again, the stimulation was not the result of the changes in the vesicle volume or integrity because the equilibrium value remained the same in the presence and in the absence of the H^+ gradient.

Kinetic determinations, summarized in Table I, revealed that the K_t and the V_{max} of the uptake system were dramatically modified by H^+ concentration and by the H^+ gradient. In the absence of a H^+ gradient, increased H^+ concentration (pH 6.5 versus pH 7.5) increased the V_{max} approximately 4-fold but also decreased the affinity of the transporter for serotonin approximately 3-fold. The presence of an outwardly directed H^+ gradient had similar effects on the K_t and the V_{max}, except

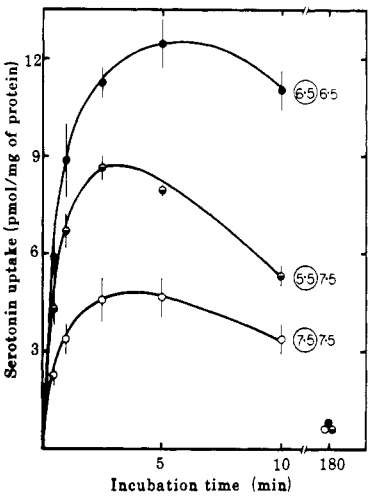

FIGURE 3: Influence of pH and pH gradient on serotonin uptake. Membrane vesicles were preloaded with 300 mM mannitol, buffered with 25 mM MES/Tris, pH 5.5 or 6.5, or 20 mM HEPES/Tris, pH 7.5. Uptake of 20 nM serotonin was measured in 150 mM NaCl, buffered with 20 mM HEPES/Tris, pH 7.75 or 7.5, or 25 mM MES/Tris, pH 6.5. In all cases, the final concentration of NaCl during uptake was 120 mM. Serotonin uptake in the presence of an outwardly directed H^+ gradient was measured by diluting the membranes suspended in pH 5.5 buffer with the pH 7.75 uptake buffer. Due to carry-over of the preloading buffer, the extravesicular pH during uptake was 7.5 in this experiment.

that the change in the affinity was even more dramatic. The affinity for serotonin was found to be approximately 5-fold less in the presence than in the absence of the H^+ gradient. It has to be mentioned here that since the kinetic parameters were determined under nonsaturating concentrations of Na^+ ($[Na^+]_o = 120$ mM; $K_{t(Na^+)} = 145 \pm 15$ mM), the reported V_{max} represents only approximate rather than absolute values.

Influence of Intravesicular K^+ on Serotonin Uptake Kinetics. In our earlier study on the serotonin transporter of the human placental brush-border membrane, we observed that the uptake system was stimulated by intravesicular K^+ (Balkovetz et al., 1989). However, the stimulation decreased as higher K^+ concentrations, the maximal stimulation occurring at 20–40 mM K^+. Nelson and Rudnick (1979) have reported that the platelet serotonin transporter was stimulated by intravesicular K^+ and that the stimulation increased with increasing concentrations of K^+ over the range of 0–100 mM. Similar results have recently been obtained with the serotonin transporter of the plasma membrane derived from brain (Reith et al., 1989). One important difference between these two studies and ours is the potassium salt employed to vary the concentrations of K^+. We had used potassium sulfate whereas Nelson and Rudnick (1979) and Reith et al. (1989) had used potassium phosphate. It appeared to us that the anions sulfate and phosphate might have contributed to the observed differences. Human placental brush-border membranes possess a sulfate transport system which operates via sulfate/anion exchange (Cole, 1984; Boyd & Shennan, 1986). When the placental brush-border membrane vesicles are preloaded with K_2SO_4 and then diluted in a sulfate-free uptake medium to measure serotonin uptake as it was done in our earlier study, it is likely that sulfate transport down its concentration gradient produces an inside-alkaline pH gradient across the membrane under these experimental conditions as a result of sulfate/hydroxyl exchange. Since we have shown in the present investigation that the NaCl gradient-dependent serotonin uptake is affected by transmembrane pH gradients, the sulfate salt of K^+ may not be suitable to study the effect of K^+ on the uptake process.

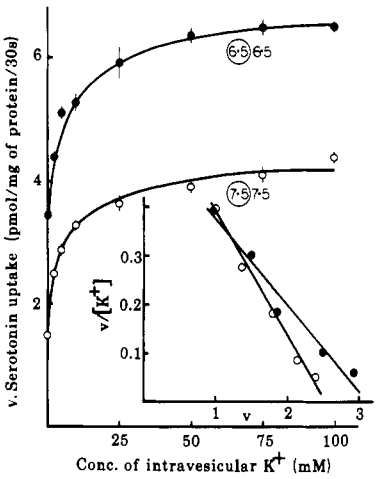

FIGURE 4: Effect of intravesicular K^+ concentration on serotonin uptake. Membrane vesicles were preloaded with either 20 mM MES/Tris buffer, pH 6.5, or 20 mM HEPES/Tris buffer, pH 7.5, containing varying concentrations of K^+ and mannitol. Osmolality was maintained at 325 mosm. The phosphate salt of K^+ was used in this experiment. Uptake of 20 nM serotonin was measured with a 30-s incubation in 150 mM NaCl buffered with either 20 mM MES/Tris buffer, pH 6.5, or 20 mM HEPES/Tris buffer, pH 7.5. Inset: Hill-type plot (v versus $v/[K^+]^n$ with a value of $n = 1$).

We have therefore reinvestigated the influence of intravesicular K^+ on serotonin uptake in these vesicles using potassium phosphate. The results are given in Figure 4. The experiments were carried out at two pH values, 6.5 and 7.5, but in the absence of a transmembrane pH gradient. At both pH values, intravesicular K^+ stimulated the NaCl-dependent serotonin uptake. The stimulation increased with increasing concentration of K^+ over a concentration range of 0–100 mM. In contrast to our earlier study in which the stimulation was found to decrease at K^+ concentrations higher than 30–40 mM when potassium sulfate was used, the present results demonstrate that there was no such decrease when potassium phosphate was used. The data given in Figure 4 were further analyzed to determine the K^+ stoichiometry and the $K_{t(K^+)}$. A Hill-type plot (V versus $v/[K^+]^n$) using only the K^+-dependent uptake rates (i.e., uptake rate in the presence of K^+ minus uptake rate in the absence of K^+) was found to be linear at both pH values when n was assigned a value of 1. This indicates a 1 K^+:1 serotonin stoichiometry (Figure 4, inset). The apparent $K_{t(K^+)}$ was 5.6 ± 0.6 mM at pH 6.5 and 4.0 ± 0.2 mM at pH 7.5.

We then studied the effect of intravesicular K^+ on the kinetic parameters of the NaCl gradient-dependent serotonin uptake in these membrane vesicles. Since the uptake process is modulated by H^+, the experiments with K^+ were done at two pH values, 6.5 and 7.5, but in the absence of a transmembrane pH gradient. The results of these experiments are summarized in Table I. At both pH values, intravesicular K^+ dramatically increased the V_{max} but decreased the affinity of the transporter for serotonin. At pH 6.5, the increase in the V_{max} was 6-fold, and the decrease in the affinity was 5-fold. At pH 7.5, the increase in the V_{max} was almost 20-fold, and the decrease in the affinity was 16-fold. We also determined the influence of internal K^+ on the kinetic parameters of the serotonin transporter when an outwardly directed H^+ gradient was present across the membrane. These studies showed that, even under these conditions, the effect of internal K^+ was to increase the V_{max} and reduce the affinity of the transporter for serotonin.

Discussion

This report describes for the first time a systematic study on the modulation of serotonin uptake kinetics by ions and ion gradients in brush-border membrane vesicles derived from human term placentas. Na^+ and Cl^- were found to be obligate for the function of the serotonin transporter in these membrane vesicles. H^+ and K^+, though not obligate, had profound effects on the kinetics of the uptake system. The influence of these ions on the uptake of serotonin has been investigated in platelet plasma membrane vesicles and more recently in brain plasma membrane vesicles. In contrast to platelets where Na^+-dependent uptake represents almost all of the high-affinity serotonin uptake (Rudnick, 1977), in brain there is a significant component (about 50%) of high-affinity serotonin uptake which is not inhibited by classical serotonin uptake inhibitors such as fluoxetine (O'Reilly & Reith, 1988). In this respect, human placental brush-border membrane vesicles resemble the platelet plasma membrane vesicles because in both systems there is little or no high-affinity serotonin uptake in the absence of Na^+. Therefore, only the characteristics of the Na^+-dependent serotonin uptake of the brain plasma membrane vesicles are used in the following discussion on the differences and similarities among the serotonin transport systems in brain, platelet, and human placenta.

Sodium and Chloride. Extravesicular Na^+ increases the affinity of the transporter for serotonin as well as the translocation rate of the transporter complex in platelets (Talvenheimo et al., 1983) as it does in the human placenta. The relationship between the K_t or the V_{max} and Na^+ has not been studied in brain owing to the presence of a large component of the Na^+-independent high-affinity serotonin uptake. The Na^+ to serotonin stoichiometry in the placenta is 1:1 which is the same as reported in platelets (Talvenheimo et al., 1983). In contrast, two Na^+ ions have been shown to be involved in the transport of one serotonin molecule in the brain (O'Reilly & Reith, 1988). External Cl^- increases the V_{max} and decreases the K_t (Nelson & Rudnick, 1982). Though the placental serotonin transporter exhibits similar characteristics, the effect of Cl^- on the K_t is very small in the placenta compared to the effect seen in platelets. There is no available information on the influence of Cl^- on the kinetic parameters of the brain serotonin transporter. The apparent $K_{t(Na^+)}$ for the platelet and the brain transporters is 52 and 118 mM, respectively, and the corresponding value for the placental transporter is 145 ± 15 mM. The apparent $K_{t(Cl^-)}$ in the brain is 18 mM whereas it is 79 ± 5 mM in the placenta. It thus appears that there are significant tissue differences in the affinities of the serotonin transporter for Na^+ and Cl^-, but it cannot be ruled out at this time that these differences result from varying experimental conditions employed in these studies.

Proton and Potassium. The role of protons in the NaCl gradient-driven serotonin transport has been studied in detail in plasma membrane vesicles prepared from platelets (Keyes & Rudnick, 1982). An inside-acidic pH greatly stimulates the initial uptake rate. In the absence of a transmembrane NaCl gradient ($[NaCl]_i = [NaCl]_o$), an outwardly directed H^+ gradient is able to drive serotonin uptake into the vesicles against a concentration gradient. On the basis of these results, Keyes and Rudnick (1982) have concluded that the presence of an outwardly directed H^+ gradient energizes the NaCl-dependent serotonin uptake by coupling the NaCl–serotonin transport with countertransport of H^+. The involvement of H^+ in serotonin transport in brain plasma membrane vesicles has been recently investigated (Reith et al., 1989). The results of this study are strikingly different form the above-described results in the platelet. An inside-acidic pH is unable to stimulate the NaCl-dependent serotonin uptake in the brain. In fact, the uptake is drastically inhibited by an inside-acidic pH.

The data from the present investigation indicate that, with respect to the role of H^+, the placental serotonin transporter behaves similar to the platelet system. The NaCl-dependent serotonin uptake in placental brush-border membrane vesicles is markedly stimulated by an inside-acidic pH as well as by an inside-acidic transmembrane pH gradient.

Even though coupling of transmembrane H^+ gradients to NaCl-dependent serotonin uptake has been unambiguously demonstrated in platelet plasma membrane vesicles, the influence of H^+ on the kinetic parameters of the uptake system has not been investigated. The present study describes for the first time the modulation of serotonin uptake kinetics by H^+ and the H^+ gradient. Internal H^+ increases the V_{max}, but decreases the affinity of the transporter for serotonin. These effects on the V_{max} and the K_t are observed in the presence as well as in the absence of a transmembrane pH gradient.

Intravesicular K^+ is known to accelerate the NaCl-dependent serotonin transport both in platelet (Nelson & Rudnick, 1979; Keyes & Rudnick, 1982) and in brain (O'Reilly & Reith, 1988; Reith et al., 1989) plasma membrane vesicles. Similarly, the human placental serotonin transporter is also stimulated by intravesicular K^+. The apparent $K_{t(K^+)}$ value for the platelet and brain serotonin transporters are 20 and 2 mM, respectively (Nelson & Rudnick, 1979; Reith et al., 1989), the experimental pH being 6.7 in both cases. The apparent $K_{t(K^+)}$ in the placenta is 5.6 mM at pH 6.5 and 4.0 mM at pH 7.5. In the platelet, competition between internal H^+ and K^+ has been demonstrated (Keyes & Rudnick, 1982). The stimulation caused by internal K^+ increases as the concentration of internal H^+ decreases. Internal K^+ stimulates the uptake only minimally when the internal H^+ concentration is high (pH 5.6). Our results demonstrate a similar competition between internal H^+ and K^+ in the placenta. The maximal stimulation observed at 100 mM internal K^+ is 2.9-fold at pH 7.5 whereas the stimulation decreases to 1.9-fold at pH 6.5. The competition between H^+ and K^+ is also evident from the $K_{t(K^+)}$ values calculated at different pHs. The affinity of the transporter for internal K^+ decreases as the concentration of internal H^+ increases. Moreover, analysis of the effects of H^+ and K^+ on the serotonin uptake kinetics also indicates competition between these ions. Internal K^+ has been shown to increase the V_{max} and decrease the affinity for serotonin in platelet plasma membrane vesicles (Nelson & Rudnick, 1979). Similar results have been obtained in placental brush-border membrane vesicles. The presence of K^+ inside the vesicles markedly increases the values of both the V_{max} and the $K_{t(serotonin)}$. The effects of internal K^+ and of internal H^+ on the kinetic parameters are qualitatively very similar. Moreover, the influence of internal H^+ on the kinetic parameters tends to disappear in the presence of internal K^+.

Considering all available information on the effects for Na^+, Cl^-, H^+, and K^+ on the initial uptake rate and on the kinetic constants of the serotonin transporter, it appears that the human placental transporter resembles more closely the platelet transporter than the brain transporter. The Na^+ stoichiometry and the influence of H^+ are the most important characteristics which distinguish the platelet and the placental transporters from the neuronal transporter.

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