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Myocardial serotonin exchange: negligible uptake by capillary endothelium

T. C. MOFFETT, I. S. CHAN, and J. B. BASSINGTHWAIGHTE

Center for Bioengineering, University of Washington, Seattle, Washington 98195

Abstract

The extraction of serotonin from the blood during transorgan passage through the heart was studied using Langendorff-perfused rabbit hearts. Outflow dilution curves of ^{131}I - or ^{125}I -labeled albumin, $[^{14}\text{C}]$ sucrose, and $[^3\text{H}]$ serotonin injected simultaneously into the inflow were fitted with an axially distributed blood-tissue exchange model to examine the extraction process. The model fits of the albumin and sucrose outflow dilution curves were used to define flow heterogeneity, intravascular dispersion, capillary permeability, and the volume of the interstitial space, which reduced the degrees of freedom in fitting the model to the serotonin curves. Serotonin extractions, measured against albumin, during single transcappillary passage, ranged from 24 to 64%. The ratio of the capillary permeability-surface area products for serotonin and sucrose, based on the maximum instantaneous extraction, was 1.37 ± 0.2 ($n = 18$), very close to the predicted value of 1.39, the ratio of free diffusion coefficients calculated from the molecular weights. This result shows that the observed uptake of serotonin can be accounted for solely on the basis of diffusion between endothelial cells into the interstitial space. Thus it appears that the permeability of the luminal surface of the endothelial cell is negligible in comparison to diffusion through the clefts between endothelial cells. In 18 sets of dilution curves, with and without receptor and transport blockers or competitors (ketanserin, desipramine, imipramine, serotonin), the extractions and estimates of the capillary permeability-surface area product were not reduced, nor were the volumes of distribution. The apparent absence of transporters and receptors in rabbit myocardial capillary endothelium contrasts with their known abundance in the pulmonary vasculature.

Keywords

myocardial endothelium; serotonin uptake; ketanserin; imipramine; modeling

UPTAKE OF SEROTONIN (5-hydroxytryptamine, 5-HT) by endothelial cells has been studied extensively in the lung: indicator dilution studies by Gillis et al. (10), Catravas and Gillis (4), and Rickaby et al. (24) have demonstrated the high extraction capacity of a carrier-mediated transport system in canine, rabbit, and human pulmonary capillary endothelium. The binding sites of the endothelial transport system are similar to at least one of the two groups of serotonin receptors (5-HT₁ and 5-HT₂ receptors) proposed by Peroutka and Snyder (22). Catravas and Gillis (4) and Hageman et al. (12) found that addition of imipramine and cyproheptadine, 5-HT₂ blockers, to the perfusate was effective at blocking

pulmonary vasoconstriction and in decreasing serotonin extraction. Whether or not any serotonin receptors are also transport sites remains to be seen.

Serotonin can, in some species, also directly influence coronary function. Serotonin injected into an isolated, perfused rabbit heart produces inotropic and chronotropic effects that are blocked by adrenergic blockers but not by blockers of either of the two 5-HT receptor groups (8, 9). Although these effects may be independent of any action by the endothelium, there are strong indications that the 5-HT₂ sites on the coronary arterial endothelium actively participate in serotonin-induced vasodilation (5). Shepro and Hechtman (25) have pointed out that aortic endothelial cells take up serotonin passively, in contrast to the facilitated saturable uptake by adipose and pulmonary endothelial cells. There is no direct evidence concerning myocardial capillary endothelium.

In this study we examined the exchange of serotonin during passage through the capillaries, not arteries or veins, of isolated rabbit hearts. Kinetic data on serotonin transport were obtained in the form of outflow dilution curves. The instantaneous extraction of serotonin, calculated by reference to albumin that does not traverse the capillary wall to any significant extent, gives a measure of the sum of passive transport via the endothelial clefts plus carrier-mediated transport across the endothelial plasmalemma. The outflow dilution curve of serotonin in conjunction with those of two reference tracers, one intravascular and one interstitial, was analyzed with a three-region multiple-pathway mathematical model of a capillary-interstitial fluid (ISF)-cell unit. Because regional myocardial blood flows show a severalfold variation (14) the intravascular tracer, iodinated albumin, was used to account for vascular dispersion and flow heterogeneity. The curve for [¹⁴C]sucrose, an inert extracellular marker, was used to estimate the volume of the interstitial space, further reducing the number of free parameters used in the fitting of the serotonin curve. Via the modeling analysis, the binding to or transport of serotonin across the endothelial layer can be evaluated by comparing model solutions with the experimental data. The hydrophilic nature of serotonin is a strong indication that any rapid uptake must occur by an endothelial carrier-mediated process, already shown to be the case in the lung. Model results obtained in this manner showed that uptake of serotonin by endothelial cells was insignificant. This conclusion was confirmed by the experimental observations that serotonin extraction was not reduced in the presence of serotonin transporter or receptor blockers.

These data indicate that myocardial capillary endothelium is dramatically different from pulmonary capillary endothelium, where serotonin uptake is exceedingly rapid and retention great. It also contrasts with the observations of Cohen et al. (5) on the endothelium of large coronary arteries where serotonin has a vasodilator effect. Because arterial surface area is so small compared with that of capillaries, the apparent absence of inhibitable uptake in our studies does not conflict with there being significant uptake by arterial endothelium. Consequently, we conclude that not only do cardiac capillary endothelial cells differ with respect to serotonin uptake from those in other organs but also from those lining the large coronary vessels of the same organ. Similarly, uptake of norepinephrine by dog cardiac capillary endothelium is small or negligible (6), whereas that of pulmonary capillary endothelium is large (21, 23). That such localization of function occurs should not be surprising when one considers that endothelial cells should serve the functions of the tissue

locally. Thus endothelial cells are not to be considered as a “newly discovered” widely distributed vascular organ, but as components modulating the functions of the organ in which they are located.

METHODS

Heart preparation

The experiments were performed on isolated, nonworking, spontaneously beating rabbit hearts. The hearts weighed 6.9–10.9 g and were perfused through an aortic cannula with oxygenated saline solution with a composition of (in mM) Na 147.4, K 5.4, Ca 1.8, Mg 0.5, Cl 133.1, HCO_3^- 23.8, $H_2PO_4^-$ 0.4, EDTA 0.01, glucose 5, with added insulin (10 U/l) and bovine serum albumin (0.1 mM). The perfusion rate was controlled with a roller pump on the inflow line. Perfusate temperature was maintained at 37–38°C¹ with a counter-current heat exchange system supplied by a constant temperature water bath. Heart rate and perfusion pressure were monitored and recorded continuously. Drainage from the coronary sinus, right atrium, and right ventricle was collected via a small cannula through the right apex. Left ventricular drainage was removed via a small cannula through the apex. Flow rates through both the right and left ventricular cannulas were recorded before each tracer injection. Each experiment consisted of 3–4 injections separated in time by ~10 min to allow complete washout of labeled sucrose and serotonin from the heart. The first injection of any experiment always occurred in the absence of any competitors or blockers. In following runs a solution containing the blocker was exchanged for the untreated perfusate. Only one blocking agent was used during the course of any one experiment. At the end of each experiment the wet weight of the myocardium devoid of fatty tissue was measured.

Tracers

Immediately prior to each experiment a dose of three tracers was prepared. The following radiotracers were used: 1) ¹³¹I- or ¹²⁵I-labeled human serum albumin, 10 μCi; 2) [¹⁴C(U)]sucrose (mol wt 342.3), 2 μCi, $D_{25} = 0.5209 \times 10^{-5} \cdot \text{cm}^2 \cdot \text{s}^{-1}$ (19); 3) 5-[1, 2-³H(N)]hydroxytryptamine creatinine sulfate (mol wt 387.4), 1.0 μCi, sp act 28.3 Ci/mmol. Radiochemical purity was tested by using paper chromatography; the results showed 98% purity for [¹⁴C]sucrose and 96% purity for the [³H]5-hydroxytryptamine creatinine sulfate.

Injections

The timing of the injections into the arterial inflow, lasting 0.5–1.5 s, was recorded and the midpoint used at zero time, $t = 0$. The injectate contained an intravascular reference substance (albumin), an extracellular reference (sucrose), and the test substance (serotonin). The radioactive tracers were diluted with physiological saline; the volume of the injectate was 0.1–0.36 ml.

¹Experiment series 02024 was performed at 20°C.

Competitors and inhibitors

Agents used to block serotonin receptors or transport sites were unlabeled 5-hydroxytryptamine creatinine sulfate (Sigma Chemical), desipramine (gift from USV Pharmaceuticals, Tuckahoe, NY), and ketanserin (kindly provided by Janssen R & D, New Brunswick, NJ). Unlabeled serotonin was added to the perfusate solution in concentrations of 0.01, 1.0, and 10 μ M. Perfusate containers were covered to prevent the light-activated decomposition of 5-hydroxytryptamine creatinine sulfate. Similarly, ketanserin (tartrate form), a highly specific antagonist for 5-HT₂ receptors (17, 27), was prepared in concentrations of 1, 10, and 100 μ M. Desipramine was used at a concentration of 80 μ M, far higher than usual pharmaceutical levels.

Sample collection and preparation

The perfusate samples were collected from coronary sinus outflow into the right ventricle at intervals of 1.0–2.0 s for the first 30 samples. In one series of injections an additional set of 30 samples was taken at 2.0-s intervals so that the total collection period varied from 26 to 88 s. A volume of 0.1 ml of each outflow sample, each standard, and background sample was pipetted into glass vials prefilled with Aquasol (New England Nuclear) liquid scintillation cocktail.

Previous literature (4, 24) showed that contamination of outflow dilution samples by serotonin metabolites during single-pass bolus injections in canine and rabbit lungs was negligible. These studies, performed on an organ well known for its high capacity uptake and degradation of biogenic amines, found that even when extraction is as high as 80%, sample contamination out to 30 s by labeled 5-hydroxyindoleacetic acid, the primary product of serotonin metabolism, contributed <4% of sample ³H activity in the outflow. The reasons were determined to be: 1) cell uptake is the rate-limiting step in serotonin metabolism; and 2) the release of 5-hydroxyindoleacetic acid is very slow compared with the rate of serotonin entry because it is polar and a specific transporter is lacking. The uptake that these investigators observed in the lung far surpasses that seen in the heart; we confirmed this in isolated lung dilution curves from the lungs of a rabbit of the same species as those that provided the hearts that we report here. For these reasons, and because of the distinction that can be made between the kinetics of extracellular and permeating, transformed substrates using careful modeling analysis, we did not do chemical separation analysis on the outflow samples.

Radioisotope counting

Counting was done in a liquid scintillation counter (Beckman Instruments, LS 5800) using three windows. Four sets of quench-spillover correction curves (background, ³H, ¹⁴C, and ¹³¹I or ¹²⁵I) of 15–25 data points each were made for each experiment. The correction curves provided coefficients for a matrix to estimate the true counting rates of the ³H, ¹⁴C, and ¹³¹I or ¹²⁵I labels in each sample using a Gaussian elimination technique to invert the matrix. Experimental samples were recounted after the iodinated tracer had decayed by at least eight half-lives (2 mo for ¹³¹I, 16 mo for ¹²⁵I). Contrasting the data from the triple- and dual-label counting showed that the differences, expressed as a percentage of the dual-label curve, remained constant over the time course of the curve indicating the absence of

systematic error, since the relative concentrations of the labels changed continuously over the time course of the curves. In the recounted data the change for sucrose averaged <5%, and the serotonin curve averaged 10% higher than the triple-label curve. Since dual-label counting of ^3H and ^{14}C is inherently more accurate than triple-label counting, the recounted sucrose and serotonin curves were used in the analysis.

Calculation of dilution curves

The dilution curves contained relatively little statistical counting noise, since the peak counting rate was usually >100,000 counts/5 min for each tracer. Each curve was terminated at 0.5% of its peak value, so counting errors ordinarily had standard deviations <3%. Each dilution curve was normalized with respect to injected activity so that it is the fraction of the injected tracer emerging per second, $h(t)$. For a given tracer

$$h_i(t) = F \frac{\text{cpm}_i}{\text{cpm}_{\text{dose}}} \quad (1)$$

where $h_i(t)$ is the fraction of dose exiting per second, F is the flow of perfusate in milliliters per second, cpm_i is the counts per minute per milliliter of effluent for sample i , and cpm_{dose} is the counts per minute of the injected dose.

Methods of analysis

The methods are in general the same as those described in detail by Kuikka et al. (16). The area of the outflow dilution curve and the mean transit time through the heart for each tracer were calculated from the normalized curves. In those cases where the sampling period ended before the entire curve had been collected the residual area was approximated by fitting a monoexponential curve through the tail of the curve and extrapolating the curve to 0.1% of the peak height. Extractions of sucrose and serotonin were calculated using the formula

$$E(t) = 1 - \frac{h_D(t)}{h_R(t)} \quad (2)$$

where $E(t)$ is the instantaneous extraction at time t , $h_D(t)$ is the fraction of injected diffusible tracer exiting per unit time, and $h_R(t)$ is the similar value calculated for the intravascular reference tracer. These extractions were used to estimate the capillary permeability-surface area product, PS_c , for the diffusible tracers using the Crone-Renkin equation (7)

$$PS_c = -F_s \ln(1 - E_{\text{max}}) \quad (3)$$

where E_{max} is the maximal instantaneous extraction, estimated by smoothing through the peak of the curve of $E(t)$.

Analysis of the outflow dilution data with two-region (capillary-ISF) and three-region (capillary-ISF-parenchymal cell) blood-tissue exchange models is described in detail by Kuikka et al. (16). In all forms the capillary region is nondispersive plug or piston flow with transit time = $V_{\text{pl}}/F_{\text{pl}}$. The same approach was used in applying the four-region capillary-endothelium-ISF-myocyte model described by Gorman et al. (11). Parameters used in fitting the data are shown in Fig. 1 and defined along with related terms used in the text as follows

F_S flow of solute-containing perfusate ($= F_{pl}$ when hematocrit is zero), $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

G_{ec} first order clearance in the endothelial cell, without return, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

G_{pc} first order clearance in the parenchymal cell, without return, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

PS_c total capillary permeability surface area product, a sum of $PS_g + PS_{ec}$, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

PS_g permeability surface area product for passive transport through the gaps between the endothelial cells, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

PS_{ec} luminal permeability surface area product for the endothelial cells, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

PS_{eca} antiluminal permeability surface area product for the endothelial cells, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

PS_{pc} permeability surface area product for parenchymal cells (myocytes), $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$

V_{pl} intracapillary volume of distribution, ml/g

V'_{isf} interstitial fluid volume of distribution, ml/g

V'_{pc} parenchymal cell volume of distribution, ml/g

The albumin curve, $h_R(t)$, was fitted first. Since albumin does not leave the vascular space, its outflow dilution curve was fitted with an axially distributed capillary model with no exchange with the interstitial space. On the assumption that the regional flow heterogeneity was well approximated by a 5–9 pathway truncated Gaussian distribution with a relative dispersion between 20 and 35%, the albumin fit was used to define the input concentration-time curves of simultaneously injected tracers by deconvolution. By this approach we account for the distribution of regional flows rather well, as has been described in the methods of Kuikka et al. (16) on the principles given in detail by Bassingthwaite and Goresky (2). This dispersion is in accordance with the distributions of flows in isolated perfused rabbit hearts observed by Little et al. (18). Distributions of relative regional flows were assumed to be constant over the observed ranges of mean flows in accord with the observations of Knopp et al. (15) and King et al. (14). Although this phenomenon has not been demonstrated for isolated rabbit hearts over a wide range of mean flows, it seems more likely to be true for these vasodilated preparations than for in vivo hearts.

The data were fitted with a weighted nonlinear least-squares routine with the weighting factor for each point of $1/\sqrt{h_D(t)}$. This gives the points following the peak a greater contribution to the fit than an ordinary least-squares fitting. Sensitivity functions for each parameter were used in the optimization of the fit to identify those parameters which most affected the shape of each portion of the curve.

The second phase of the strategy was to fit the sucrose indicator dilution curve with a two-region model allowing permeation through the interendothelial cellular clefts so that PS_g and V_{isf} are finite. The values of PS_g and of V'_{isf}/V_{pl} were assumed to be the same for all regions, the regions differing only in terms of the perfusate flow. The assumption of

uniformity of *PS* products and volumes is the standard assumption made by workers in the field, but nevertheless should be recognized as unproven and untested.

The third phase of the strategy was to determine if the serotonin dilution curves could be fitted with the same two-region model using estimates of PS_g calculated from the PS_g (sucrose), and further, assuming transiently that there was no entry of serotonin into either the endothelial or parenchymal cells. This approach was indicated by the lack of any significant uptake by the endothelium as measured against sucrose. Additionally, the low extraction of the heart compared to that of the lung points to a markedly reduced capacity for amine uptake. In the absence of steric hindrance in the intracellular clefts, the PS_g for serotonin would be expected to be ~1.39 times that for sucrose, this being the ratio of free diffusion coefficients estimated from the square root of the reciprocal of molecular weights (26). In this calculation, the molecular weight (176.2) of the active alkyl amine moiety was used

$$1.39 = \frac{\sqrt{342}}{\sqrt{176}} \quad (4)$$

The value of V'_{isf} (serotonin) was set to that found in the modeling of sucrose although it was recognized that sucrose, a compound with a molecular weight twice that of serotonin, is likely to be excluded from areas of the interstitium more accessible to the smaller serotonin molecule. So in the two-region model V'_{isf} (sucrose) effectively serves as a lower bound for estimating V'_{isf} (serotonin). This strategy provided model curves that were closely fitted to the original data, but the model solution was too straight in the late portion of the tail to fit the slight curvature of the observed dilution curves.

The fourth element of the strategy was to consider entry of serotonin from the ISF into a further volume of distribution. This could be plasmalemmal binding sites on myocytes, endothelial cells or nonspecific binding in the interstitium. By providing access to another region the model solution could fit the curvature of the data better. In the model the additional volume was represented by the parenchymal cell; sensitivity analysis indicates that parenchymal cell PS_{pc} and V'_{pc} exert effects in this part of the curve (1). Since the two-region model fits near the peak of the curve, the portion most strongly affected by endothelial uptake, were quite close, model parameters in the four-region model describing endothelial membrane flux, PS_{ec1} and PS_{eca} , were set to zero. In one set of analyses, reported in Table 2, PS_g (serotonin) was set to $1.39 \times PS_g$ (sucrose), V'_{isf} (serotonin) was set equal to V'_{isf} (sucrose) and V'_{pc} was fixed at estimates of parenchymal cell volumes obtained from Kuikka et al. (16). Consumption terms, G_{pc} and G_{ec} , were set to zero with the justification that serotonin recovery was always equal to or very close to the recovery of the nonmetabolized sucrose. One parameter, PS_{pc} for serotonin, was left free for the model to optimize in fitting the data. During optimization, the value of PS_{pc} was driven to values close to zero, effectively depriving serotonin access to V'_{pc} and indicating that the solute really did not enter this volume.

In the end the best fit was achieved by allowing the interstitial volume of distribution for serotonin to increase ~3% above that for sucrose. This version of the model analysis is equivalent to stating that the interstitial volume of distribution is augmented by a volume continuous with but excluded to sucrose or by equilibrative binding (rapid kinetics and probably nonspecific) of 3% of the interstitial serotonin.

RESULTS

Outflow dilution data

Eighteen sets of outflow dilution curves were obtained from experiments using a total of six isolated rabbit hearts. Experimental conditions are listed in Table 1. The normalized outflow dilution curves from one experiment are shown in Fig. 2. These curves possess features consistently found in the entire set of experiments.

Albumin, an unextracted intravascular reference tracer, has a relatively narrow high peak and rapid decline relative to a more diffusible substance such as sucrose. Sucrose, an extracellular marker, diffuses into the interstitial fluid via the clefts between the endothelial cells but does not enter cells. The escape of sucrose beyond the vascular space is reflected in the decreased peak height of the sucrose curve relative to albumin, and in the subsequent crossover of the two curves 3–5 s after the peak. Both phenomena are the result of the larger volume of distribution for sucrose, the sum of vascular plus interstitial spaces.

Serotonin is extracted slightly more than sucrose, as evidenced by the serotonin curve being lower than that of sucrose. Rapid backdiffusion of serotonin from the extravascular space results in the serotonin curve crossing above the albumin and sucrose curves 2–4 s after the peak. This crossover is to be expected because the higher diffusivity of serotonin will enhance its departure from the interstitial space. Twenty to 50 s after the peak the serotonin and sucrose curves converge rather closely. In three experiments, a second crossover was observed to occur 22–32 s following the peak when the serotonin curve dropped below that for sucrose. Following a second crossover, the sucrose and serotonin curves remained very close to each other. Thus these data are consistent with a higher rate of escape from the capillary for serotonin than for sucrose. Although the volumes of distribution of sucrose and serotonin, as estimated from the mean transit times, appear to be quite similar, explanation for the forms of the tails of the curves requires more formal analysis.

The shape of dilution curves did not change with the addition of blocking agents to the perfusate. Features which would have exhibited change, such as relative heights and time of initial crossover, were unaffected by all blocking agents used. Figure 3 shows two sets of curves on the same heart, one control and one blocked with 1 μ M unlabeled serotonin. The lack of a saturable serotonin transport system is indicated by the virtual overlap of the [3 H]serotonin curves even in the presence of serotonin at a concentration equal to the estimated Michaelis constant (K_m) of serotonin transport in the lung (20) and that would presumably reduce the cellular uptake by 50%. A 10-fold higher serotonin concentration similarly had no effect on the relative extraction of serotonin and sucrose. The stability of the preparation is demonstrated by the reproducibility of the 131 I-labeled albumin curves. Much higher doses of other putative blockers (six tests, Table 2) similarly had no effect.

Tracer recoveries

Termination of sample collection before all tracer had exited the heart resulted in recoveries of <100%. Monoexponential extrapolation of the tails of the curves gave <3.5% additional tracer for both sucrose and serotonin and <0.3% for albumin. Inaccuracies caused by errors in flow rate measurements or dose calibrations are correctable because all tracers are equally affected. However, persistent problems were experienced with iodoalbumin recovery, which in six experiments was lower than either the recovery of [³H]serotonin or [¹⁴C]sucrose. The shape of the albumin curve was shown to be correct because the percent difference between the diffusible curves obtained by dual and triple label counting was constant and did not change as a function of the iodine count rate.

Consequently, the areas of nonmetabolized albumin and sucrose curves, with tail extrapolation, were scaled to 1.0 for analysis and modeling to satisfy the conservation of mass requirements of the model. To preserve the relationship between the diffusible curves, the serotonin and sucrose curve were always scaled by the same factor. This failure to achieve 100% (see Table 2) creates a small error in absolute, but not relative, scaling and does not influence parameter estimation from model fitting.

Uptake: controls and blocked

Peak extraction of serotonin, $E_{\max}$, occurring during the early upslope and peak phase of the curve, averaged $44\% \pm 9\%$ ($n = 9$) for control studies and $48\% \pm 11\%$ ($n = 9$) in experiments in the presence of blockers or competitors (Table 2). Sucrose had an average $E_{\max}$ of $37\% \pm 9\%$ ($n = 18$) from all experiments. Corresponding estimates of PS_c from the Crone-Renkin equation, equal to PS_g in the absence of endothelial uptake, yielded an average (all experiments) PS_c (serotonin) of 2.26 ± 0.78 ($n = 18$) and PS_c (sucrose) of 1.70 ± 0.63 ($n = 18$). From these values, the mean ratio of PS_c (serotonin) to PS_c (sucrose), 1.37, is only slightly less than the expected ratio of 1.39 based on molecular weights (see METHODS).

Agents that traditionally block 5-HT₂ transport, imipramine and desipramine, and unlabeled serotonin, which must compete for any transporter, caused no reduction in extraction. This was anticipated completely, for the comparison with sucrose attributed all the extraction to a pathway available to sucrose, presumably the interendothelial clefts. Likewise, the extraction in the presence of ketanserin (a receptor blocker) averaged $56\% \pm 7\%$ ($n = 3$), not different from a control extraction of 57%.

Model transport parameters

Results for model fits of each experiment are presented in Table 2. The values for PS_c are more accurate than those estimated via the Crone-Renkin expression (Eq. 3), since backdiffusion is accounted for by fitting the entire curve, including the tail, in the automated parameter estimation routine. A set of model solutions from a control experiment of average goodness of fit are shown in Fig. 4.

Estimates of PS_g and V'_{isf} for sucrose averaged 1.61 ± 0.7 ($n = 18$) $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and 0.32 ± 0.08 ($n = 18$) ml/g , respectively. The only free parameters used in fitting the sucrose outflow

dilution curves were PS_g and V'_{isf} . These parameter values were used in two ways in the fitting of the serotonin curves. The V'_{isf} (sucrose) was used as the initial value for V'_{isf} (serotonin) that was allowed to vary in the optimization to obtain the best model fit to the data. PS_g results for serotonin and sucrose were obtained in one set of analyses as independent free parameters, with the overall result that the ratio PS_g (serotonin)/ PS_g (sucrose) averaged 1.64 ± 0.2 ($n = 18$). Because this is close to the ratio of free diffusion coefficients, 1.39, in a second analysis strategy PS_g (serotonin) was fixed at $1.39 \times PS_g$ (sucrose) and not allowed to vary. This is a test of whether or not the data are less well fitted when a stringent constraint was imposed. By this strategy there are only three free parameters, PS_g and the two V'_{isf} values used in the combined fitting of the whole of the three outflow dilution curves for albumin, sucrose, and serotonin. The system is strikingly overdetermined by the data, that is, there are several more independent information items in the data than there are degrees of freedom. Estimates of V'_{isf} for serotonin averaged 0.32 ± 0.09 ($n = 9$) ml/g for control runs and 0.34 ± 0.09 ($n = 9$) ml/g for test runs. The coefficients of variation (CV) were no worse for the constrained fitting (average CV = 0.21 ± 0.05) than for the unconstrained fitting (average CV = 0.14 ± 0.05 , not reported in Table 2).

Use of a four-region (capillary-endothelial cell-interstitial fluid-parenchymal cell) model, with its additional shaping parameters, allowed the opportunity to get slightly closer fits of the model to the data than were obtained with the two-region model (capillary-ISF) with one free variable for serotonin alone. The effect of permeability parameters, PS_{eca} , PS_{ecl} , and PS_{pc} , on the goodness of fit were tested by fixing PS_g (serotonin)/ PS_g (sucrose) = 1.39 to account for permeation through the clefts and determining whether or not letting these cell PS s at the three surfaces take values >0 contributed to a reduction in the coefficient of variation. Opening up PS_{ecl} contributed nothing to the fit of the upslopes and peaks, the regions of greatest sensitivity to PS_{ecl} . (This observation is in tune with the lack of influence of blocking agents at concentrations well above the K_m observed for serotonin transport in the lung.) Allowing even a small degree of parenchymal cell uptake by opening the PS_{pc} resulted in a poorer fit, especially in the tail. Overall, increasing the degrees of freedom in the model produced no substantial reductions in the coefficients of variation. What this means is that systematic deviations of the two-region, extracellular model solutions from the data points are so small that using more flexible model forms with greater degrees of freedom does not improve the fit. This in turn implies that the residuals are random, and not systematic with respect to the models in their different forms.

DISCUSSION

These experiments indicate that, unlike the lung, the heart does not possess a significant, high-affinity, saturable uptake mechanism for serotonin in either the coronary capillary endothelium or in the surrounding parenchyma. Transcoronary extraction of serotonin relative to sucrose did not change from control values when either blockers or competitors were present in the perfusate. Further, serotonin extraction was very close to the value predicted from the sucrose extraction corrected for the difference in diffusivities due to serotonin's smaller molecular mass. Therefore, it is apparent that serotonin leaves the blood,

as does sucrose, primarily by diffusion through interendothelial clefts. The mean instantaneous extraction for serotonin is also similar to that in data from isolated perfused (20) and in vivo (4) lungs in the presence of blockers of endothelial uptake. Pulmonary extraction of serotonin is ~80% in control runs and falls to 20–40% with μM amounts of serotonin in the perfusate (20). Although lungs appear consistent in taking up serotonin, other organs are not. For example, bovine aorta does not show the existence of a saturable uptake mechanism (3).

The lack of a transport system suggests that the serotonin-mediated inhibitory effect of the coronary endothelium on serotonin-induced vasoconstriction (5) is probably not controlled by intracellular serotonin. This would also preclude such a role for serotonin metabolites as access to mitochondrial-bound monoamine oxidase (A), [MAO(A)], would be denied. Indeed, Cohen et al. (5) found that MAO(A) inhibitors had no effect on the inhibitory action of the endothelium. The combination of their results with ours would suggest that the vasodilatory effect might be initiated at the endothelial cell surface by receptors with rapid association/dissociation rates.

Model estimates of the V'_{isf} of sucrose and serotonin were ~0.32 ml/g. These results compare closely with the 0.31 ml/g obtained by Kuikka et al. (16) on the modeling of D- and 2-deoxy-D-glucose in similarly perfused rabbit hearts, but are roughly twice that seen for the interstitial sucrose space of in vivo dog heart preparations (13). The disparity between the interstitial volumes of the isolated and in vivo states is due to the formation of tissue edema in the isolated heart. By virtue of its smaller mass and lesser space from which it is excluded in ISF, serotonin should and did have a slightly larger volume of distribution than sucrose.

Conclusion

Capillary exchange of serotonin in the heart occurs by passive diffusional transport through the clefts between endothelial cells. The absence of substantial endothelial uptake is consistent with its modest pharmacological effects observed in the rabbit heart. Although this illustrates that the behavior of endothelial cells can differ from one organ to another, it does not clarify the distinction between endothelial cells of arteries, arterioles, capillaries, and veins and does not touch on differences between species.

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FIG. 1.

Full form of the model used for analysis of indicator-dilution curves. Analysis showed that data could be fitted with a much reduced form, lacking both endothelial and parenchymal cell entry. (From Ref. 11.)

FIG. 2.
Coronary sinus outflow dilution curves for [^3H]serotonin, [^{14}C]sucrose, and ^{125}I -labeled albumin.

FIG. 3.

Two sets of outflow dilution curves for [3 H]serotonin and 131 I-labeled albumin from the same heart under control conditions and with 1 μ M unlabeled serotonin in the perfusate during the dilution curve and for 10 min preequilibration. The close similarity of the two sets of curves indicates that 1 μ M serotonin had no inhibitory effect on the tracer exchange and suggests a passive transport mechanism only.

FIG. 4. Outflow dilution curves for $[^3\text{H}]$ serotonin, $[^{14}\text{C}]$ sucrose, and ^{125}I -labeled albumin and the two-region model solutions fitted to them. The model parameters were: V'_{isf} (sucrose) = 0.41 ml/g, V'_{isf} (serotonin) = 0.43 ml/g, PS_g (sucrose) = 2.0, and PS_g (serotonin) = 2.8 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$.

TABLE 1

Experimental conditions

Expt. No.	F_s , ml · min ⁻¹ · g ⁻¹	Blocker	Inj Vol, ml	Serotonin concentration, μ M	HR, beats/min	Press, mmHg
0202841	2.9		0.36	1.1	180	40
0202842	2.9		0.18	1.1	190	63
0202843	2.9	DMI 80 μ M	0.15	1.1	190	69
0602841	3.5		0.10	3.3	156	37
0602842	5.2		0.10	3.3	123	56
0602843	3.4		0.10	3.3	111	40
0602844	3.6	DMI 80 μ M	0.10	3.3	irr	13
0902841	3.8		0.15	2.3	110	41
0902842	3.8	Imipr 10 μ M	0.15	2.3	105	32
0902843	3.9		0.15	2.3	irr	55
2604841	3.9		0.10	1.4	146	57
2604842	3.9	Ketan 1 μ M	0.10	1.4	144	70
2604843	3.9	Ketan 10 μ M	0.10	1.4	112	82
2604844	3.9	Ketan 100 μ M	0.10	1.4	irr	137
1408841	2.9		0.10	1.4	170	42
1408842	2.9	5-HT 0.01 μ M	0.10	1.4	180	41
1408843	2.9	5-HT 1.0 μ M	0.10	1.4	156	40
1408844	2.9	5-HT 10 μ M	0.10	1.4	189	41

Inj vol, total injectate volume in milliliters; HR, heart rate in beats/min; Press, perfusion pressure in mmHg; irr, irregular or indeterminate heart rate; serotonin concentration, concentration of labeled serotonin in the injectate. The reported heart rates and perfusion pressures are those measured 5 s prior to injection.

TABLE 2

Data summary and model solution

Expt. No.	$F_s, \text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	Recovery*		E_{max}		$V_{\text{site}} \text{ ml/g}$		$P^S_{\text{g}} \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$		CV§	Blocker
		Sucrose	Serotonin	Sucrose	Serotonin	Sucrose	Serotonin†	Sucrose	Serotonin†		
0202841	2.9	0.98	1.00	0.19	0.32	0.26	0.24	0.59	0.83	0.25	Control
0202842	2.9	0.93	1.00	0.23	0.30	0.25	0.21	0.55	0.76	0.21	Control
0202843	2.9	0.94	1.00	0.20	0.24	0.22	0.20	0.46	0.64	0.18	DMI 80 μM
0602841	3.5	0.95	1.00	0.39	0.46	0.30	0.32	1.47	2.04	0.27	Control
0602842	5.2	0.95	1.00	0.36	0.45	0.41	0.43	2.02	2.81	0.18	Control
0602843	3.4	0.96	1.00	0.41	0.51	0.25	0.27	1.76	2.45	0.28	Control
0602844	3.6	0.96	1.00	0.39	0.49	0.27	0.32	1.60	2.22	0.27	DMI 80 μM
0902841	3.8	0.85	1.00	0.44	0.41	0.31	0.30	1.56	2.17	0.07	Control
0902842	3.8	1.00	0.98	0.31	0.44	0.35	0.46	1.42	1.97	0.24	imipramine 10 μM
0902843	3.9	0.96	1.00	0.40	0.49	0.48	0.49	2.07	2.88	0.15	Control
2604841	3.9	0.95	1.00	0.43	0.57	0.26	0.31	2.61	3.63	0.22	Control
2604842	3.9	0.95	1.00	0.44	0.55	0.27	0.30	2.71	3.76	0.23	ketanserin 1 μM
2604843	3.9	0.92	1.00	0.41	0.49	0.24	0.30	1.64	2.27	0.18	ketanserin 10 μM
2604844	3.9	0.90	1.00	0.54	0.64	0.45	0.49	3.03	4.21	0.29	ketanserin 100 μM
1408841	2.9	1.01	0.94	0.36	0.48	0.38	0.33	1.36	1.89	0.20	Control
1408842	2.9	0.90	0.94	0.46	0.55	0.33	0.31	1.46	2.03	0.17	5-HT 0.01 μM
1408843	2.9	0.97	0.94	0.42	0.48	0.43	0.39	1.58	2.19	0.23	5-HT 1.0 μM
1408844	2.9	0.97	0.96	0.30	0.44	0.33	0.33	1.06	1.47	0.18	5-HT 10 μM
Control ($n = 9$)											
Means±SD	3.61	0.95	0.99	0.36	0.44	0.32	0.32	1.55	2.16	0.20	
0.72		0.04	0.02	0.09	0.09	0.08	0.09	0.67	0.93	0.07	
Blocked ($n = 9$)											
Means±SD	3.42	0.94	0.98	0.39	0.48	0.32	0.34	1.66	2.31	0.22	
0.51		0.03	0.03	0.10	0.11	0.08	0.09	0.78	1.09	0.04	
All runs ($n = 18$)											
Means±SD	3.51	0.95	0.99	0.37	0.46	0.32	0.33	1.61	2.23	0.21	
0.61		0.04	0.02	0.09	0.10	0.08	0.09	0.71	0.99	0.05	

F_s , flow of perfusate; $E_{\max}$, maximum extraction; V_{isf} , interstitial fluid volume distribution; PS_g , permeability-surface area for passive transport through gaps between endothelial cells; CV, coefficient of variation.

* Scaled recoveries as described in RESULTS section

$\dagger PS_g$ for serotonin was fixed at $1.39 \times PS_g$ sucrose in modeling

$\ddagger$ coefficient of variation for model fit of serotonin outflow dilution curve

$\S V_{isf}$ of serotonin was the free parameter used in modeling.