PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 361–365 (1984)

Kinetics of Serotonin Uptake by the Dog Lung Is pH Independent in the Physiological Range (41807)

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Abstract. Given the strong pH dependence of platelet serotonin uptake, the many similarities between platelet and pulmonary endothelial serotonin uptake and the potential importance of a pH dependence on the interpretation of changes in pulmonary endothelial serotonin uptake in diseased lungs, we examined the influence of pH on the kinetics of serotonin uptake in isolated dog lung lobes using a multiple indicator technique. A range of pH values from about 7.2 to 8.0 were obtained by ventilating the lobes with gas containing different concentrations of CO_2. Over the pH range studied, no significant changes in serotonin uptake were observed, nor were significant changes detected in the kinetic parameters, K_m and V_{max}, for the uptake process. The lack of a significant pH effect represents a difference between platelet and lung endothelial serotonin uptake. It also indicates that correlations between P_{CO_2} and serotonin uptake which might occur in diseased lungs do not imply causality.

It has been suggested that serotonin uptake might provide a sensitive indicator of pulmonary endothelial damage (1, 2). If serotonin uptake by pulmonary endothelial cells were dependent on P_{CO_2} or pH, it could be of considerable importance in the interpretation of the changes in serotonin uptake which might occur in diseased lungs. Several studies have demonstrated that serotonin uptake by human (3, 4) and dog (5) platelets is quite dependent on pH with a decrease in uptake with increasing pH above pH 7. The platelet and endothelial transport mechanisms are similar in several respects (3–13) suggesting a common transport mechanism. On the other hand, over a range of pH wherein platelet serotonin uptake has been shown to be inversely related to pH, Eiseman *et al.* (14) found no relationship between pH and the metabolism of serotonin in isolated dog lungs. Since uptake by the endothelial cell is generally considered to be the rate-limiting step in the lung metabolism of serotonin (8, 9), these results suggest a possible difference between platelet and endothelial uptake mechanisms in this regard. Therefore, in the present study we investigated the influence of pH on the kinetics of serotonin uptake by isolated dog lung lobes using an indicator dilution method in an attempt to further evaluate this possible interrelationship between lung gas exchange function and serotonin uptake.

Materials and Methods. The experiments were performed using a dog lung lobe preparation previously described in detail (15). Twelve dogs (21.7 ± 3.6 (SD) kg body wt) were anesthetized with pentobarbitol sodium (30 mg/kg, iv) heparinized (1250 IU/kg), and exsanguinated through a carotid artery to provide approximately 900 ml of autologous plasma or blood which was used to prime the perfusion system. The lower lobe of the left lung was removed, placed in a perfusion chamber, and the vessels and bronchus connected to the perfusion and ventilation systems, respectively. The blood (used for six of the lobes) or plasma (used for six of the lobes) perfusate, maintained at 37°C, was pumped into the lobar artery from a reservoir into which the perfusate drained from the lobar vein. The perfusate flowrate was set at 5.0 ± 0.38 ml/sec and held constant for each lobe studied. For plasma perfused lobes, the first 250 ml of the effluent plasma, containing most of the blood cells trapped in the lobe, was collected and recentrifuged. Lobar arterial and venous pressures measured relative to the top surface of the lobe were monitored throughout the perfusion period. For the kinetic analysis used in this study, it is important that pulmonary hemodynamics be unaffected by the doses of serotonin used. Therefore, either papaverine hydrochloride (50 μg/ml perfusate; six experiments) or methysergide maleate

0037-9727/84 $1.50

(0.67 μg/ml perfusate; six experiments) was added to the perfusion system to block the vasoactive response to serotonin. Control conditions were established by ventilating (Harvard piston respirator) with a gas mixture of about 16% O_2 and 5.8% CO_2 in N_2. The tidal volume was 150 ml, the frequency was eight breaths/min, and end-inspiratory and -expiratory pressures were about 5 and 1.0 Torr, respectively.

Serotonin uptake by the lobe was determined as follows: Ventilation was halted in end-expiration, and venous outflow was diverted to sampling tubes of a Gilson-Escargot fraction collector. A 0.39-ml bolus containing approximately 6.5 μCi of [^{3}H]serotonin, variable amounts of unlabeled serotonin, and 0.5 or 1.0 mg of indocyanine green dye (lower amount for plasma perfusate) was then rapidly injected by means of an injector placed in the tubing leading to the arterial cannula. Samples were collected at a rate of 1.67 samples/sec for approximately 20 sec. As many as eight injections could be performed with each lobe before the supply of perfusate was exhausted. Two or three bolus injections, each with a different dose of serotonin, were made during each condition studied. One bolus contained a trace dose of <10 nmole, providing capillary concentrations which were low relative to K_m. Unlabeled serotonin was added to the second and, in some cases, the third bolus so that these boluses contained 30 or 100 nmole of serotonin. These doses provided sufficiently high vascular concentrations to reveal the saturation phenomenon of the uptake mechanism (Figs. 1 and 2). A minimum of 15 min elapsed between injections during any one experimental condition. Following the last bolus injection, the lobe was removed from the perfusion system, allowed to drain, and weighed to determine wet weight.

A 1-ml aliquot of each sample collected was prepared for analysis by adding 4 ml ethanol (0°C). The ethanolic supernatants were analyzed for indocyanine green dye spectrophotometrically and ^{3}H by liquid scintillation counting as previously described (15). In addition, in some studies (at least one for each of the experimental conditions studied), samples obtained near the mean transit time were analyzed for 5-hydroxyindoleacetic acid as previously described (15). This was done to verify that under each of the conditions studied, the effluent ^{3}H in the form of the serotonin metabolite was small enough to be ignored in the data analysis.

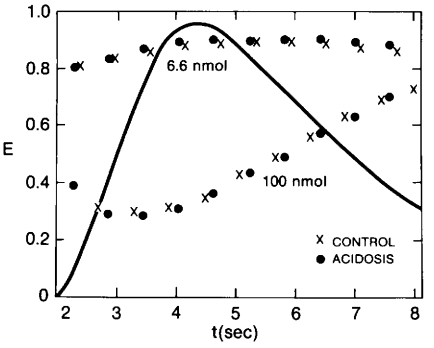

FIG. 1. Extraction ratio, E, curves obtained following a series of two injections into an isolated lobe during control conditions (pH = 7.37) and during acidosis (pH = 7.17). A representative dye curve in arbitrary units is included to put timing in perspective.

Each lobe was studied under control conditions and during exposure to one or both of the experimental conditions under study. Alkalosis was induced by changing the ventilating gas to room air. Acidosis was produced by ventilating the lobes with approximately 16% O_2 and 11% CO_2 in nitrogen. In the initial studies, the experimental conditions were bracketed by injections made during ventilation with the normal gas mixture. When it became evident that the experimental conditions did not cause irreversible changes in the serotonin uptake kinetics, the second set

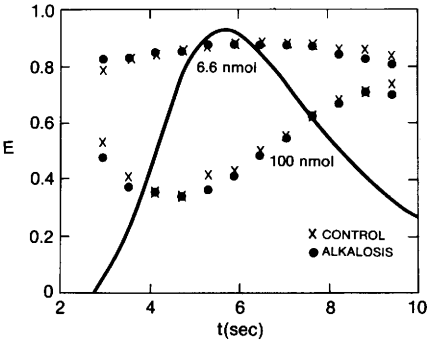

FIG. 2. Extraction ratio, E, curves obtained following a series of two injections into an isolated lobe during control conditions (pH = 7.37) and during alkalosis (pH = 8.08). A representative dye curve in arbitrary units is included to put timing in perspective.

of injections under normal conditions were eliminated and the order of the normal and experimental conditions was randomized. The time taken to allow the gas composition to equilibrate following a change in the ventilating mixture was from 10 to 15 min.

We have previously developed a model for the transport of serotonin from the perfusate during passage through the lungs, assuming that the transport mechanism follows saturation kinetics (15). This model yields the following equation:

$$C_D(t) - C_P(t) = K_m \ln [C_P(t)/C_D(t)] + V_{max}(1 - \alpha)/\dot{Q} + V_{max}\alpha t/\dot{Q}\bar{t}, \quad (1)$$

where C_D (the outflow plasma dye concentration times the mass of serotonin injected divided by the mass of dye injected), C_P (the plasma concentration of serotonin in nmole/ml), $\dot{Q}$ (the plasma flow rate in ml/sec), t (the emergence time in sec), and $\bar{t}$ (the lung mean transit time in sec) are obtained from experimental data (15). Equation (1) is suitable for calculating the kinetic parameters V_{max} (the maximum transport rate of serotonin in nmole/sec) (15), K_m (the plasma concentration which results in a transport rate of $V_{max}/2$ in nmole/ml), and a dimensionless parameter, α, related to the relative amount of the total bolus dispersion which takes place in the capillary bed (15).

To provide estimates of K_m, V_{max}, and α, Eq. (1) was used in a multiple linear regression analysis in the form:

$$C_D(t) - C_P(t) = \beta_0 \ln (C_P(t)/C_D(t)) + \beta_1 + \beta_2 t/\bar{t}. \quad (2)$$

The estimates of the kinetic parameters are found from the coefficients of the multiple regression as follows:

$$K_m = \beta_0, \quad (2a)$$

$$V_{max}/\dot{Q} = \beta_1 + \beta_2, \quad (2b)$$

and the parameter, α, is calculated from:

$$\alpha = \beta_2/(\beta_1 + \beta_2). \quad (2c)$$

The data from the two or three bolus injections made during each condition were used in the regression analysis. Since only the data collected early in time are consistent with the model used to derive Eq. (1), the data set to be used was determined by carrying out the multiple regression analysis starting with the data points obtained earliest in time and then repeating the regression analysis after adding the next point from each curve. Points were added in this fashion until the coefficient of determination (r^2) reached the maximum value before beginning to fall. The values of K_m and V_{max} and α obtained with those data providing the maximum value of r^2 were assumed to be obtained with the optimal set of data. This method has been described previously (16).

The instantaneous serotonin extraction ratio, E, was calculated as:

$$E(t) = 1 - (C_P(t)/C_D(t)). \quad (3)$$

The percentage of serotonin taken up by the lobe during passage of the bolus (% U) was calculated as:

$$\% U = \left(\frac{\dot{Q}}{m}\int_0^a [C_D(t) - C_P(t)]dt\right) \times 100, \quad (4)$$

where $\dot{Q}$ is the plasma flow rate in ml/sec, m is the amount of serotonin injected, and a is the time at which $C_D(t)$ had fallen to 1% of its peak value.

For each group of experiments, comparisons between control and treatment conditions were made using a paired t test.

Results. Neither acidosis nor alkalosis produced significant changes in serotonin uptake or in the calculated values of K_m and V_{max} (Table I). The extraction ratios from individual experiments during control conditions and during acidosis or alkalosis are shown in Figs. 1 and 2, respectively. For the low-dose injections, the serotonin extractions were high, while, when the dose was high, saturation of the uptake mechanism became evident as indicated by the lower extractions. The extraction ratios were not significantly affected by changing the P_{CO_2} and pH.

During the course of the experiments, there were no apparent differences in recorded hemodynamic parameters (Table II) among the conditions studied. The mean value of the perfusion parameter, α, was 1.07 ± 0.05 (SE) and was not significantly changed by pH within the range studied.

TABLE I. SEROTONIN UPTAKE AND KINETIC PARAMETERS

Condition	N	U (%)	K_m (μM)	V_{max}[a] (nmole/sec/g)	r^2
Control	7	74.1 ± 4.0	0.76 ± 0.11	0.25 ± 0.04	0.91 ± 0.02
Alkalosis	7	71.2 ± 3.2	0.71 ± 0.08	0.22 ± 0.03	0.94 ± 0.01
Control	8	74.5 ± 1.8	0.69 ± 0.08	0.19 ± 0.02	0.90 ± 0.02
Acidosis	8	72.5 ± 2.2	0.71 ± 0.07	0.19 ± 0.02	0.90 ± 0.02

Note. The U is the percentage uptake of serotonin from trace dose; r^2 is the coefficient of determination from the multiple regression analysis using the $C_P(t)$ and $C_D(t)$ data and Eq. (2). Values are means ± SE.

[a] The value of V_{max} calculated according to Eq. (2b) has been normalized by dividing by the lobe wet weight.

Discussion. The present study was carried out to test the hypothesis that given the similarities between platelet and pulmonary endothelial serotonin uptake and the well-documented effects of pH on serotonin uptake by platelets, pulmonary endothelial uptake might also be pH dependent. The implications of such a pH dependence would be that in diseased lungs it might be difficult to separate the effects of changes in alveolar gas composition and/or a heterogeneous distribution of alveolar P_{CO_2} from changes in endothelial cell function induced by some other cause related to the lung damage. We found instead that the endothelial uptake was relatively insensitive to changes in P_{CO_2} and pH over the range studied, indicating that in this regard platelet uptake and endothelial uptake are different and that correlations between P_{CO_2} and serotonin uptake which might occur in diseased lungs will not imply causality.

Platelet serotonin uptake has an optimum pH of about 7.0 (5). As the pH increases over the range from 7.2 to 8.0, uptake decreases by about 60% in dog (5) and 40% in human (3) platelets. In many other respects, the platelet and endothelial serotonin transport mechanisms have similar characteristics. Both involve Na^+- and K^+-dependent active transport (8, 11), with reported K_m values for platelet serotonin uptake (Table III) generally near those which we have found for endothelial uptake (Table I). Platelet and endothelial uptake are temperature sensitive, with Q_{10} values around 2.3 (3, 5, 6, 8). Serotonin uptake in both platelet and endothelial cells is significantly reduced by metabolic inhibitors including cyanide and iodoacetate (5, 6, 10, 12) and by certain transport inhibitors, including imipramine, oubain, and chlorpromazine (3, 7–13). The different responses to changes in hydrogen ion concentration may be related to the fate of serotonin once it is transported into the cell rather than transport across the cell membrane itself. In platelets, serotonin is sequestered in storage organelles (9, 11, 17) while in endothelial cells it is rapidly converted to 5-hydroxyindoleacetic acid which is then released from the cell (8, 12, 14, 15). Two distinct serotonin transport systems operate in platelets, one across the cell membrane and one across the membrane of the serotonin storage organelles (9, 11, 17). It has been shown that the transport of serotonin across the mem-

TABLE II. HEMODYNAMIC AND BLOOD GAS DATA

Condition	N	$\bar{t}$ (sec)	Q (ml)	P_a (Torr)	P_v (Torr)	Lobe weight (g)	pH	P_{CO_2} (Torr)	P_{O_2} (Torr)
Control	7	7.26 ± 0.40	37.3 ± 1.7	5.4 ± 0.6	1.0 ± 0.3	45.0 ± 2.1	7.38 ± 0.01	38.7 ± 1.1	102 ± 3
Alkalosis	7	7.20 ± 0.42	37.1 ± 2.0	5.4 ± 0.6	1.1 ± 0.3		7.96 ± 0.08*	8.6 ± 1.7*	135 ± 6*
Control	8	6.67 ± 0.46	32.5 ± 2.3	5.7 ± 0.6	0.5 ± 0.3	40.1 ± 3.0	7.37 ± 0.01	39.0 ± 0.5	107 ± 4
Acidosis	8	6.90 ± 0.54	33.5 ± 2.7	5.5 ± 0.5	0.6 ± 0.3		7.17 ± 0.01*	77.1 ± 2.0*	104 ± 4

Note. Q is the vascular volume obtained by multiplying the lobar mean transit time, ($\bar{t}$), by the perfusate flow rate; P_a is the lobar arterial pressure; P_v is the lobar venous pressure. P_{O_2}, P_{CO_2}, and pH are values obtained from the equilibrated perfusion system. Values are means ± SE.

* Control versus treatment difference is significant at $P < 0.01$.

TABLE III. K_m VALUES FOR THE UPTAKE OF SEROTONIN BY PLATELETS

K_m (μM)	Platelet source	Reference
0.3	Human	6
1.0	Human	7
0.45	Guinea pig	9
0.7	Rat	7
0.64	Pig	13
0.16	Dog	5

brane of the storage organelle is coupled to the pH gradient across that membrane (17). Thus, the pH dependence of platelet uptake may be somehow related to this subcellular transport.

Our results are consistent with those of Eiseman *et al.* (14), who used a different method to measure serotonin metabolism by perfused rat lungs, and were not able to detect a change in the metabolic rate within the pH range of 7.2–7.8. The results are also consistent with the observation that several epithelial Na^+-dependent substrate transport systems (e.g., in renal tubules) are not influenced by pH in this range unless the change in hydrogen ion concentration changes the concentration of the ionized substrate (18, 19). The pK'_1 and pK'_2 for serotonin are about 4.9 and 9.8, respectively (20), indicating little change in the ionization of serotonin in the pH range studied.

The authors thank Victoria M. Wagner-Weber, Jacqueline Owenby, and Julie A. Dunham for their technical assistance and Mary McDermott for typing the manuscript. This study was supported in part by the Veterans Administration and National Heart, Lung and Blood Institute, Research Grant HL-24349.

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Received September 27, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted November 28, 1983.