Metabolic Requirements for 5-Hydroxytryptamine Uptake by the Isolated Lung

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The lung plays an important role in determining the concentrations of circulating 5-hydroxytryptamine (5-HT) in the body. The pulmonary role in removal of 5-HT is accomplished by uptake and then metabolism to 5-hydroxyindoleacetic acid (5-HIAA). The uptake process is sodium- and temperature-dependent and is inhibited by tricyclic antidepressants. With increasing concentrations of extracellular 5-HT, uptake by lung approaches a constant rate suggesting a saturable process. These characteristics suggest that 5-HT uptake by lung does not occur by passive diffusion, but rather by a carrier-mediated transport process. In order to determine if this transport process is energy-dependent, the effects of metabolic inhibitors on 5-HT uptake were examined in an isolated guinea pig lung.

Methods

Male guinea pigs (Hartley strain) weighing 300-350 g were anesthetized with intraperitoneal pentobarbital (50 mg/kg). A tracheostomy was performed and the animal was ventilated with a rodent respirator at a tidal volume of 2.5 ml, a rate of 80/min, and an end-expiratory pressure of 2 cm of H2O. The thorax was opened, the heart transected and a catheter placed in the pulmonary artery. Krebs-Ringer bicarbonate solution, pH 7.4, was infused to clear the lungs of blood. The lungs were removed from the thorax and transferred to a water-jacketed Plexiglas incubation chamber maintained at 37°C. The perfusion medium now consisted of Krebs-Ringer bicarbonate solution containing 4 percent (w/v) fatty acid-free bovine serum albumin (Sigma, Cohn fraction V) and 5 mM glucose. Flow was maintained into the pulmonary artery at 10 ml/min by a peristaltic pump. The perfusate dripped freely from the transected left atrium into the incubation chamber, was recirculated through a flow cuvette where pH, PO2, and PCO2 could be monitored by electrodes (Radiometer), through an aerator, and then back to the peristaltic pump. The perfusate was aerated with the same gas mixture used for ventilation of the lungs. For control experiments, 95 percent O2 5 percent carbon dioxide was used for ventilation. Oxygen tension in the expired gas and in the perfusate was greater than 500 mm Hg. For anoxic experiments, 95 percent N2: 5 percent CO2 was used. Oxygen tension was less than 5 mm Hg in the expired gas. Perfusion pH in all experiments was 7.35-7.42. Ventilation pressure and mean perfusion pressure were monitored. Lungs were preincubated for 15 minutes with or without an inhibitor in the perfusate and then (14C) 5-HT (0.25×10-6M) was added to the perfusate.

Aliquots of perfusate were removed at 5, 10, 20 and 30 minutes after the addition of (14C) 5-HT and the perfusion was then terminated. The lung was weighed immediately to determine wet weight and then lyophilized to obtain dry weight. In some experiments, after preincubation for one hour using the recirculating system, a once-through system was used to evaluate 5-HT uptake. (14C) 5-HT was infused by a syringe pump into the pulmonary artery, recirculation was stopped, and the effluent from the transected left atrium was collected at one minute intervals.

(14C) 5-HT was separated from its metabolic product, (14C) 5-HIAA by the method of Jonsson and Lewander and aliquots were subsequently analyzed on a scintillation counter (Packard Tri-Carb).

A semilogarithmic plot of (14C) 5-HT concentration in the perfusate vs time of perfusion showed a linear decrease during the 30 minutes of perfusion after the addition of (14C) 5-HT. The ratio between the disappearance rate of (14C) 5-HT in the presence of inhibitor and the disappearance rate in control lungs, multiplied by a 100, gave percent 5-HT uptake by lung. Uptake by control lungs was considered to be 100 percent. In those experiments using the once-through system, net uptake of (14C) 5-HT by the lung was calculated from the difference between the inflow and outflow concentrations multiplied by the flow rate. Uptake of (14C) 5-HT in the presence of inhibitor was compared to uptake by control lungs and again expressed as percent of control. Data were analyzed by the t test for independent samples and the level of statistical significance taken as <0.05.

Results

In order to study the metabolic requirements for (14C) 5-HT uptake by lung, the disappearance of (14C) 5-HT from the perfusate was examined in the presence of various metabolic inhibitors. Glucose me-
metabolism was altered in several ways. When glucose was omitted from the perfusing medium, there was mild inhibition of (14C) 5-HT uptake (Table 1). In the presence of 2-deoxyglucose (5×10^-4M), an inhibitor of phosphoglucoisomerase, and in the presence of iodoacetate (10^-4M), an inhibitor of glyceraldehyde phosphate dehydrogenase, there was a more marked inhibition of uptake (Table 1). Inhibition of oxidative metabolism by either anoxia or cyanide (10^-4M) also resulted in a marked reduction of uptake of (14C) 5-HT (Table 1). (14C) 5-HT uptake by lung was also examined in the presence of oxidizable substrates other than glucose. Lungs were preincubated with the glycolytic inhibitor deoxyglucose, and pyruvate (10^-2M) or palmitate (5×10^-4M) were used as substrates. With either of these substrates, (14C) 5-HT uptake returned to normal (Table 1).

**DISCUSSION**

A reduction of (14C) 5-HT uptake by the lung in the presence of either inhibitors of glycolytic or oxidative metabolism suggests a requirement for metabolic energy by the uptake process. Glycolysis could be contributing to overall ATP synthesis or providing substrate for oxidative metabolism. Evidence that the latter may be true is the normal uptake of 5-HT in the presence of other oxidizable substrates. Additional evidence that oxidative metabolism is required is the marked inhibition of 5-HT uptake in the presence of anoxia or cyanide. An energy requirement for 5-HT uptake provides additional evidence that this is an active transport process.

Thus, 5-HT uptake by lung resembles transport of this amine by cells from the central nervous system, platelets and, mast cells. Transport of 5-HT across their plasma cell systems, the amine is then transported across an intracellular membrane to enter a storage vesicle. How-ever, after 5-HT is taken up by the lung, it is rapidly metabolized to 5HIAA.

(14C) 5-HT uptake by lung is reduced by inhibitors of glycolytic or oxidative metabolism and thus is an energy requiring process. The uptake of 5-HT closely resembles biogenic amine transport in other cell systems.

**REFERENCES**


**Discussion**

Dr. Tierney: Apparently glycolytic and oxidative metabolism are required. Does the oxidative metabolism require an intermediate from glucose metabolism or can other substrates such as fatty acids substitute?

Dr. Steinberg: I mentioned that we've used palmitate and in the presence of glycolytic inhibitor and in the presence of 2-deoxyglucose we've depressed glycolysis and then added palmitate. We were able to support transport with palmitate.

**Factors in Glucose Oxidation by Alveolar Macrophages: Glucose Transport and Glycogenolysis**

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Phagocytosis by alveolar macrophages (AM) is an important pulmonary defense mechanism and is associated with increased glucose conversion to CO₂. Glucose oxidation is also an important source of energy and reducing equivalents in AM. Previous studies have shown that a group of drugs presumed to elevate cyclic AMP (theophylline, dibutaryl cyclic AMP, PGE₁ and PCF₃) and cytochalasin B, which interferes with the function of contractile microfilaments, all diminished glucose conversion to CO₂ by both resting and phagocytosing cells. We present evidence concerning four general mechanisms for this impairment of glucose oxida-

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