Site and Mechanism of Uptake of $^3$H-/-Norepinephrine by Isolated Perfused Rat Lungs

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ABSTRACT

The uptake of $^3$H-/-norepinephrine was examined in isolated perfused rat lungs, and the mechanism of uptake was compared with that previously found for 5-hydroxytryptamine. Most of the norepinephrine taken up by the lungs was rapidly deaminated and O-methylated, and the metabolic products were subsequently returned to the perfusate. The presence of iproniazid and tropolone markedly inhibited this metabolism; because the metabolites were released from the lungs more rapidly than was norepinephrine itself, these inhibitors appeared to increase the rate of uptake of the amine. The uptake of norepinephrine was a saturable, temperature-dependent process with a $K_m$ of $1.11 \times 10^{-8}$ M and a $V_{max}$ measured during metabolic inhibition of $2.78 \times 10^{-8}$ moles/g min$^{-1}$. Uptake was inhibited by cocaine, imipramine, ouabain, and potassium-free medium as well as by decreasing the sodium concentration or increasing the potassium concentration of the medium. Although normetanephrine reduced the uptake of norepinephrine, metaraminol had no effect. Autoradiography indicated that norepinephrine was taken up into endothelial and smooth muscle cells, but the mechanism of the uptake appeared to combine features characteristic of both extraneuronal and neuronal uptake. The mechanism of uptake of norepinephrine by the lungs is compatible with a sodium-dependent, carrier-mediated transport, but several differences appear to exist between the transport and the site of uptake of norepinephrine and 5-hydroxytryptamine.

KEY WORDS

pulmonary catabolism  sodium-coupled transport  normetanephrine  serotonin  temperature dependence  endothelial cells  amine metabolism  pulmonary autoradiography

It is now well established that the lungs of many species can take up and metabolize biogenic amines (1-4). Hughes et al. (2) have demonstrated that norepinephrine is actively taken up during one passage through the pulmonary circulation by a saturable process which can be blocked by cocaine. Similar findings have been reported by Junod (3) for 5-hydroxytryptamine; the uptake of this amine by the lungs is compatible with a sodium-dependent, carrier-mediated transport process (3) that has previously been described for norepinephrine and 5-hydroxytryptamine transport in brain (5), platelets (6), and nerve endings (7). Strum and Junod (8) have presented autoradiographic evidence indicating that the uptake of 5-hydroxytryptamine from the pulmonary circulation is into endothelial cells.

The present study was undertaken to determine whether the mechanism for the uptake of norepinephrine in the lung is similar to that for the uptake of 5-hydroxytryptamine.

Methods

SOLUTIONS AND CHEMICALS

The millimolar composition of the Krebs bicarbonate solution was: NaCl 118, KCl 4.75, KH$_2$PO$_4$ 1.19, MgSO$_4$ 1.19, CaCl$_2$ 2.54, NaHCO$_3$ 25, and glucose 5. In addition, bovine serum albumin (fraction V) was added up to a final concentration of 4.5%, and the solution was adjusted to pH 7.4 with IN NaOH; the final sodium concentration in the perfusion medium was 161 mM. The solution was equilibrated with 95% O$_2$-5% CO$_2$. Sodium-free albumin was prepared by adjusting the pH of a 30% albumin solution to 5.0 and then dialyzing the solution extensively against twice-distilled water at 4°C. When sodium-free medium was used, NaHCO$_3$ was replaced with Tris buffer and NaCl was replaced with isotonic sucrose. A potassium-free medium was obtained by replacing the potassium salts with their sodium equivalents. When an elevated potassium concentration was required, KCl was added; the sodium concentration remained the same. The concentrations of sodium and potassium in the medium were verified using a flame photometer.

$7^-^3$H-/-Norepinephrine (specific activity 6.4 c/mmole) was obtained from both New England Nuclear and Amersham/Searle Corporation. The compounds 3-(3, 4-dihydroxyphenyl)-/-alanine (2, 5, 6-$^3$H-ring) (specific activity 1 c/mmole) 1, 2-$^3$H-3, 4-dihydroxyphenylethyla-
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mine hydrochloride (specific activity 4.5 c/mmole), 7-3H-3-methoxy-4-hydroxy-dl-mandelic acid (specific activity 2.5 c/mmole), and 7-3H-dl-noradrenaline (specific activity 5.7 c/mmole) were obtained from Amersham/Searle Corporation. 7-3H-d/-norepinephrine (specific activity 9.9 c/mmole) was obtained from New England Nuclear. All of these compounds were diluted in 0.01M HCl containing 5% Na₂S₂O₃ and stored at 4°C. Suitable amounts of unlabeled compounds were added to the labeled compounds when a concentration above 7 x 10⁻⁷M was used. 7-3H-norepinephrine, dl-noradrenaline, 3, 4-dihydroxymandelic acid, 3-methoxy-4-hydroxymandelic acid, ascorbic acid, ethylenediaminetriacetic acid (EDTA), and tropolone were obtained from commercial sources. Imparimine HCl was donated by Geigy Pharmaceuticals and iproniazid phosphate was donated by Hoffman LaRoche. Unless otherwise specified, the drugs were added to the perfusion medium.

PERFUSION METHODS

The perfusion methods were identical to those previously described (3). Briefly, male Sprague-Dawley rats (250-300 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip), tracheotomized, and artificially ventilated with 95% O₂-5% CO₂. The thorax was then opened, and cannulas were placed in the pulmonary artery via the right ventricle and in the left ventricle. Then, without interruption of the circulation, the lungs were perfused with Krebs solution, using a peristaltic pump at 10 ml/min. The lungs were finally removed from the thorax and placed in a closed chamber kept at 35°C for 30 minutes at 4°C. The lobes of the lungs were dissected free, blotted dry, weighed, and homogenized at 4°C; 60-90 seconds elapsed between the end of the perfusion and homogenization.

ANALYTICAL PROCEDURES

Samples (0.2 ml) of inflow, effluent, and lung homogenate were dissolved in NCS solubilizer (Amersham/Searle), and their radioactive contents were measured after the addition of 10 ml of scintillation fluid (7 g of 2, 5-diphenyloxazole [POPOP] and 0.1 g of p-bis-[O-methylnitro]-benzene [Bis-MSB] per liter of toluene). Efficiency of counting was monitored by the addition of internal standard, and the results were corrected accordingly. The metabolites of 3H-norepinephrine were analyzed according to two methods. In the first method, the lungs and 1-ml samples of inflow and effluent were homogenized in 6- and 2-ml volumes, respectively, of methanol-acetone (1:1) solution, containing 0.5% ascorbic acid at 4°C. The homogenate was then centrifuged at 10,000 g for 30 minutes at 4°C; a 1-ml sample of the supernatant solution was evaporated to dryness under nitrogen, taken up with 0.2 ml of 95% ethanol, and finally centrifuged for 10 minutes at 1,000 g to remove the turbidity. A 10-ml sample of the supernatant solution was then analyzed by thin-layer chromatography using glass plates coated with cellulose (9). A butanol-methanol-acetic acid (3:1:1) solvent system was used, and the plate was run twice to obtain good separation between norepinephrine, normetanephrine, 3,4-dihydroxymandelic acid, and 3-methoxy-4-hydroxymandelic acid. The spots of these compounds were detected under ultraviolet light, and the spots were scraped into counting vials containing 4% Cab-O-Sil in the scintillation fluid (7 g of PPO and 1 g of Bis-MSB per liter of toluene). Recovery of tritium with this method was calculated by adding a known amount of tritiated compound to a lung homogenate and determining the amount finally recovered from the thin-layer plate. The low recovery of 25% was consistent for normetanephrine, 3-methoxy-4-hydroxymandelic acid, and norepinephrine; it resulted because the tritiated compound remained partly adsorbed on the cellulose when the cellulose was suspended in the scintillation fluid. This adsorption had a much greater quenching effect than was indicated by the addition of the 3H-toluene internal standard. Despite the low recovery, we believe that this method provided valid quantitative results because of the consistency of the results and the similarity between the results obtained by methods 1 and 2. In the second method, 0.4 nmol perchloric acid extracts of lung containing 0.05% ascorbic acid and 0.05% EDTA were adjusted to pH 6.0 by the addition of 1N KOH. A sample was then passed through a 10 mm x 0.55-mm glass column (Amberlite CG 50, 100-200 mesh, sodium-form) and equilibrated with 0.4M sodium phosphate buffer. The eluate and the 20-ml washout contained the deaminated products; norepinephrine was eluted with 10 ml of 2% boric acid, and catechol-O-methyltransferase products were finally eluted with 0.125N HCl (10). Recoveries using this method were again determined by adding a known amount of tritiated compound to a lung homogenate; they were consistently greater than 90%. Using these two methods, the distribution of radioactivity among the various compounds expressed as a percent was essentially identical, and, consequently, the results were pooled. Analyses were not made of the form in which tritium was present in the lungs following an infusion of 3H-dopamine or 3H-dopa; only the total radioactivity present was determined.

AUTORADIOGRAPHY

The lungs were perfused for 6 minutes in the presence of iproniazid and tropolone with a medium containing 1 x 10⁻⁷M 3H-norepinephrine. They were subsequently fixed by a 10-minute perfusion with 2.5% glutaraldehyde in 0.133M sodium phosphate buffer. The postfixation and the dehydration steps, and the sectioning, and the autoradiography were identical to those reported by Strum and Junod (8). The uptake of 3H-norepinephrine in the presence of tropolone and iproniazid during a 6-minute infusion of 1 x 10⁻⁷M norepinephrine was 6.7 ± 0.79% (se) (the experiment) calculated as the amount of radioactivity in the lungs following the infusion expressed as a percent of the total amount of radioactivity infused during the 6 minutes. This low percent reflects saturation of the uptake mechanism. Approximately 11.5% of this total amount of 3H-norepinephrine taken up was lost during the 10-minute fixation period with glutaraldehyde as measured from the radioactivity in the effluent. Subsequent losses from the fixed pieces of lung during the processing were very small and similar to those reported by Strum and Junod (8) for 3,5-dihydroxytryptamine: 0.15% was lost during the additional 2-hour glutaraldehyde fixation, 0.23% was lost during the 18-hour storage in cold buffer, and 2.7% was lost during dehydration in a graded series of ethanol.

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These values are percents of the radioactivity remaining in the pieces of lung following complete processing of the tissue.

Results

The relationship between the concentration of \(^3\)H-\(l\)-norepinephrine in the perfusion medium and the rate of accumulation of radioactivity in the lungs is illustrated in Figure 1. The rate of uptake apparently increased when the metabolic inhibitors iproniazid and tropolone were added to the perfusion medium, as evidenced by the significant differences \((P < 0.05)\) between the uptakes with all but the highest concentration of norepinephrine.

One concentration of norepinephrine is considered in Table 1. In the presence and the absence of the inhibitors, the uptake appeared to be saturable; the linearity of the Lineweaver-Burk plot confirmed this impression. Even with the highest concentration of norepinephrine used, there was no increase in perfusion pressure. The uptake mechanism did not show any optical specificity; the rate of uptake during a 2-minute infusion of \(2 \times 10^{-4}\) M dl-norepinephrine was \(1.65 \pm 0.44 \text{ (sE) moles/g min}^{-1}\) (five lungs) and the rate for a similar infusion of \(l\)-norepinephrine was \(1.55 \pm 1.8 \text{ moles/g min}^{-1}\) (five lungs).

Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Uptake of Norepinephrine ((\times 10^{-4}) moles/g min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no inhibitors)</td>
<td>6 (2.80 \pm 0.40^*)</td>
</tr>
<tr>
<td>Control (with inhibitors)</td>
<td>8 (3.89 \pm 0.23)</td>
</tr>
<tr>
<td>Cold (4°C)</td>
<td>3 (0.47 \pm 0.06^t)</td>
</tr>
<tr>
<td>Glucose-free medium</td>
<td>4 (3.71 \pm 0.42)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>3 (1.02 \pm 0.12^t)</td>
</tr>
<tr>
<td>5 (\times 10^{-4}) M</td>
<td>3 (0.52 \pm 0.07^t)</td>
</tr>
<tr>
<td>1 (\times 10^{-4}) M</td>
<td>3 (0.45 \pm 0.06^t)</td>
</tr>
<tr>
<td>Imipramine</td>
<td>5 (0.39 \pm 0.04^t)</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td>4 (2.52 \pm 0.18^t)</td>
</tr>
<tr>
<td>2 (\times 10^{-4}) M</td>
<td>7 (2.77 \pm 0.16^t)</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>4 (2.21 \pm 0.28^t)</td>
</tr>
<tr>
<td>5 (\times 10^{-4}) M</td>
<td>4 (3.65 \pm 0.28)</td>
</tr>
<tr>
<td>Metaraminol</td>
<td>5 (\times 10^{-4}) M</td>
</tr>
</tbody>
</table>

The lungs were equilibrated for 10 minutes and then perfused for 2 minutes with \(2.0 \times 10^{-4}\) M \(^3\)H-\(l\)-norepinephrine. Drugs were present during the entire perfusion. Unless otherwise noted, iproniazid (\(5 \times 10^{-4}\) M) and tropolone (\(2 \times 10^{-4}\) M) were always present. Results are expressed as means \(\pm\) SE.

* \(P < 0.05\).
† \(P < 0.001\).
‡ \(P < 0.01\).
from the action of monoamine oxidase and catechol-O-methyltransferase. The percents of the metabolites in the lung were unrelated to either the period of infusion (1-12 minutes) or the concentration of \(^3\text{H}\)-l-norepinephrine in the perfusion medium (0.7-20 \(\times\) \(10^{-7}\)M). The presence of iproniazid and tropolone markedly, but not completely, inhibited the action of these enzymes. Analysis of lungs which had been perfused with dl-norepinephrine revealed a significant \((P < 0.05)\) increase in the percent of O-methylated metabolites over that found following perfusion of l-norepinephrine (Table 2): norepinephrine 17.35 \(\pm\) 1.79%, normetanephrine 16.69 \(\pm\) 1.16%, 3,4-dihydroxymendelic acid 34.05 \(\pm\) 1.51%, and 3-methoxy-4-hydroxymendelic acid 30.04 \(\pm\) 2.86% (five lungs). The chromatographic analysis also showed that the radioactivity in the effluent during a 2-minute perfusion of l-norepinephrine contained up to 9% metabolites of norepinephrine, which must have been released from the lungs. This figure was calculated from the analysis of samples of inflow and effluent so that any spontaneous oxidation of norepinephrine in the solution was taken into account. If correction is made for the release of \(^3\text{H}\)-l-norepinephrine metabolites, the uptake values were similar in the presence and the absence of iproniazid and tropolone. Therefore, the rate limiting step in the uptake of \(^3\text{H}\)-l-norepinephrine was not the subsequent metabolism of the amine. Figure 1 also indicates that cold abolished the uptake of \(^3\text{H}\)-l-norepinephrine. Neither \(^3\text{H}\)-dopamine nor \(^3\text{H}\)-dopa (4.7 \(\pm\) 0.8 \(\times\) \(10^{-11}\) moles/g min\(^{-1}\)) for a 2-minute infusion of \(^3\text{H}\)-dopamine or 2 \(\times\) \(10^{-9}\) \(^3\text{H}\)-dopa) was concentrated in the lungs. Iproniazid and tropolone were absent in these latter experiments.

In subsequent experiments, iproniazid and tropolone were added to the perfusion medium so that the radioactivity present in the lungs could be considered to represent more closely the transport of \(^3\text{H}\)-l-norepinephrine into the lung tissue. Since the rate of uptake of \(^3\text{H}\)-l-norepinephrine was a linear function of the duration of the infusion for periods up to at least 2 minutes, the rate of uptake at this time could be considered to be the initial rate of uptake and the Michaelis-Menten equation could be applied to characterize the transport of \(^3\text{H}\)-l-norepinephrine. The uptake \(\times\) \(10^{-10}\) moles/g min\(^{-1}\) of lung \(\pm\) SE was as follows: 1 minute = 3.9 \(\pm\) 0.28, 2 minutes = 7.5 \(\pm\) 0.48, 6 minutes = 14.3 \(\pm\) 1.11, 9 minutes = 26.7 \(\pm\) 3.08, and 12 minutes = 33.7 \(\pm\) 2.16; these data were from four to nine lungs with a medium concentration of \(7 \times\) \(10^{-6}\)M \(^3\text{H}\)-l-norepinephrine. A Lineweaver-Burk plot was drawn from the data in Figure 1; \(K_m\) was 1.11 \(\times\) \(10^{-4}\)M and \(V_{\text{max}}\) was 2.78 \(\times\) \(10^{-9}\) moles/g min\(^{-1}\) (Fig. 2). The maximum values found for the tissue-medium ratio and the percent uptake over the concentrations of norepinephrine and the durations of infusions used were 16.8 and 36.8%, respectively. However, these values were contingent on the concentration of norepinephrine and the period of infusion.

The effect of various drugs and experimental conditions on the uptake of \(^3\text{H}\)-l-norepinephrine following a 2-minute infusion of 2 \(\times\) \(10^{-9}\)M norepinephrine is reported in Table 1. Although perfusion with a glucose-free medium for 12 minutes had no effect on the uptake, imipramine and cocaine, drugs which markedly inhibit membrane transport of norepinephrine in various cellular systems, had comparable inhibitory effects on the uptake of \(^3\text{H}\)-l-norepinephrine by the lungs. The effect of 5-hydroxytryptamine on the uptake of norepinephrine was interesting in that it resulted in partial competitive inhibition regardless of the concentration of 5-hydroxytryptamine used (Fig. 2 and Table 1). Normetanephrine inhibited the uptake of \(^3\text{H}\)-l-norepinephrine, but metaraminol was without effect at a high concentration.

Table 3 summarizes the effects of ouabain and changes in the ionic concentration of the perfusion...
Effect of Sodium and Potassium Ions and Ouabain on the Uptake of \( ^3 \text{H}-\text{L}-\text{norepinephrine} \) from a Medium Containing 2 x 10^-10 M \( ^3 \text{H}-\text{L}-\text{norepinephrine} \) during a 2-Minute Infusion

<table>
<thead>
<tr>
<th>Perfusion medium</th>
<th>N</th>
<th>% (^3 \text{H}-\text{L}-\text{norepinephrine} ) uptake lost in 12 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no inhibitors)</td>
<td>5</td>
<td>95.2 ± 1.44*</td>
</tr>
<tr>
<td>Control (with inhibitors)</td>
<td>4</td>
<td>33.9 ± 1.07</td>
</tr>
<tr>
<td>+ 1 x 10^-4 M cocaine</td>
<td>3</td>
<td>25.2 ± 7.84</td>
</tr>
<tr>
<td>+ 1 x 10^-4 M 5-hydroxytryptamine</td>
<td>4</td>
<td>27.4 ± 4.86</td>
</tr>
<tr>
<td>Sodium-free medium</td>
<td>4</td>
<td>96.8 ± 1.12*</td>
</tr>
</tbody>
</table>

Following a 10-minute equilibration period, the lungs were infused for 6 minutes with 2 x 10^-10 M \( ^3 \text{H}-\text{L}-\text{norepinephrine} \). The normal Krebs solution was then replaced by a medium containing cocaine, 5-hydroxytryptamine, or no sodium, as required, and perfusion was continued for 12 more minutes in the absence of norepinephrine. Iproniazid (5 x 10^-10 M) and tropolone (2 x 10^-10 M) were present for the entire perfusion except when otherwise noted. The uptake of \( ^3 \text{H}-\text{L}-\text{norepinephrine} \) was taken as that found during previous experiments following a 6-minute infusion of 2 x 10^-10 M \( ^3 \text{H}-\text{L}-\text{norepinephrine} \). The values represent the total loss of tritium over the 12-minute period and were calculated from the tritium remaining in the lung at the end of the period. Results are expressed as means ± SE.

* \( P < 0.01 \).
Light microscope autoradiograph of a section from a lung infused via the pulmonary circulation with $^3$H-$\text{-}l$-norepinephrine. Black silver grains, which indicate $^3$H-$\text{-}l$-norepinephrine uptake, overlie the wall of a small vessel (V) that communicates with unlabeled capillaries (arrows).

electron micrographs as either arteries or veins according to the nature of their vascular wall. Veins were characterized by the presence of only a few layers of highly organized smooth muscle cells and the absence of elastic tissue in the wall (Fig. 5); arteries were characterized by a vascular wall containing less well-organized layers of smooth muscle cells with intervening elastic tissue. Table 5

Electron microscope autoradiographs of a portion of two endothelial cells lining a capillary venule in a $^3$H-$\text{-}l$-norepinephrine-infused lung. Silver grains (black threads) overlie the cytoplasm and the nucleus (primarily the heterochromatin areas) of the endothelial cells.
FIGURE 5
Electron microscope autoradiograph of the wall of a large vein in a lung infused with \textsuperscript{14}H-l-norepinephrine. Silver grains overlie the endothelial cells (arrow) and the smooth muscle cell (SM). An unlabeled capillary (C) can also be seen.

FIGURE 6
Electron microscope autoradiographs of two capillary lumens, each lined by a layer of endothelial cells, from a lung infused with \textsuperscript{14}H-l-norepinephrine. The upper endothelial cells are heavily labeled, but the lower ones contain only a single silver grain.

summarizes the distribution of labeling among the different types of vessels.

**Discussion**

In the present study, we have shown that the uptake of norepinephrine by the rat lung is a saturable process characterized by Michaelis-Menten kinetics and inhibited by cocaine, imipramine, and hypothermia in a manner similar to that previously found for 5-hydroxytryptamine (3). Hughes et al. (2) appear to have erred by a factor of ten in their calculation of \( V_{\text{max}} \); on correction, their \( V_{\text{max}} \) and \( K_m \) values are very similar to those found in the present study and those reported for rabbit lung (4). Following uptake, norepinephrine was rapidly deaminated and O-methylated. If \( dl \)-norepinephrine instead of \( l \)-norepinephrine was infused through the lung, the percent of O-methylated products increased toward the value reported following perfusion of racemic norepinephrine through the rat lung (2). This change in the distribution of metabolic products apparently resulted from the optical specificity of monoamine oxidase for the levo form of the amine (11) and the...
lack of optical specificity of catechol-O-methyltransferase (12). The fact that we were apparently unable to completely block metabolism of norepinephrine with iproniazid and tropolone would be explained in part if the amine was oxidized to any degree while it was in the perfusion medium. Analysis of the radioactivity in the inflow medium, which was sampled immediately prior to the pulmonary artery, revealed that 94% was norepinephrine; the remaining 6% could have been oxidation products. This possibility would have been avoided if EDTA had been present in the medium. Alternatively, the apparent loss of 6% could reflect a shortcoming of our extraction and separation process.

Sodium and potassium ions appear to participate in the uptake of norepinephrine as they do in the uptake of 5-hydroxytryptamine in the lung (3), the uptake of norepinephrine in synaptosomes (13), and the uptake of norepinephrine in heart slices (7). Our results strongly suggest that the uptake of norepinephrine in the lung is consistent with a sodium-coupled uptake mechanism that depends on an optimal concentration of potassium as proposed by Bogdanski and Brodie (7) to account for norepinephrine uptake in the heart. The inhibitory effects of hypothermia, cocaine, and imipramine are consistent with the concept of such an energy-requiring amine transport mechanism and so confirm the recent results with rabbit lungs (4).

The inhibitory effects of ouabain and ionic modifications of the perfusate on the uptake of \(^{3}H\)-l-norepinephrine were not due to a primary inhibition of glucose uptake, since perfusion of the lungs with a glucose-free medium did not result in a depression of uptake of the amine; these experiments, however, do not preclude the possibility that glucose is the main source of energy for the maintenance of the sodium gradient. Similarly, Iwasawa et al. (4) have reported that the omission of glucose is without effect on norepinephrine uptake in rabbit lungs.

The kinetic constants determined in our study closely resemble those found by Iversen (14) to describe what he has termed Uptake, in the heart, an uptake into neuronal tissue. The pulmonary uptake of norepinephrine further resembles Uptake, in that it is sodium-dependent and can be inhibited by cocaine and imipramine; however, the similarity appears to end at this point. In other ways, the pulmonary uptake resembles the extraneuronal uptake termed Uptake, by Iversen (15). Evidence for this relationship with Uptake, is as follows. First, norepinephrine taken up by the lung was rapidly metabolized. This process would not have occurred if Uptake, had been involved, because a further uptake of the amine into vesicles would have provided protection from the metabolizing enzymes. Second, the uptake of norepinephrine into lung was inhibited by normetanephrine, a compound reported by Burgen and Iversen (16) to specifically inhibit Uptake, although a high concentration of metaraminol, which specifically inhibits Uptake, had no effect. We cannot explain why Alabaster and Bakhle (17) failed to show an inhibitory effect of the same concentration of normetanephrine on the uptake of normetanephrine by rat lungs. Third, dopamine, which has a high affinity for Uptake, in the heart, did not appear to be taken up to any extent by the lung. However, an alternative explanation for this finding is that the low concentrations of dopamine and dopa found in the lung following perfusion reflected a more rapid metabolism rather than a lower rate of uptake, in comparison with norepinephrine.

Finally, our results provide strong evidence that the norepinephrine taken up by the lung is concentrated in endothelial cells of the pulmonary vessels and that little appears in the very sparse adrenergic endings. This conclusion is reinforced by our observation that, in contrast to neuronal uptake (14), the uptake mechanism in the lung revealed no chemical stereospecificity (4). However, the similarity between Uptake, in the heart and the uptake of norepinephrine in the lung cannot be taken too far. The kinetic constants for Uptake, in the heart are approximately a thousandfold greater than those that we found for the uptake in the lung. Moreover, the norepinephrine taken up by Uptake, in the heart is rapidly washed from the tissue,
whereas the norepinephrine in the lung is only slowly removed. Finally, Uptake, in the heart favors the uptake of epinephrine, but epinephrine passes through the lung almost unaffected (2, 18). Therefore, it appears that the distinction between Uptake, and Uptake, cannot be strictly applied to the uptake of norepinephrine in pulmonary tissue.

Although the mechanism of uptake of norepinephrine in the lung appears to resemble that previously reported for 5-hydroxytryptamine by Junod (3), several differences have emerged. We found that the uptake of norepinephrine was more sensitive than that of 5-hydroxytryptamine to the inhibitory action of ouabain. This finding is consistent with the observation (19) that in rabbit synaptosomes ouabain directly inhibits the uptake of norepinephrine in addition to inhibiting the sodium-potassium–adenosinetriphosphatase (Na–K–ATPase). This direct inhibition occurs to a much lesser extent with 5-hydroxytryptamine. The observations of Iwasawa et al. (4) that ouabain equally inhibits the removal of norepinephrine and 5-hydroxytryptamine by the rabbit lung may not be inconsistent with our own results, because the rat is a species known for its resistance to the Na–K–ATPase blocking action of glycosides (20). Although the rate of uptake of norepinephrine bears a linear relationship to the sodium concentration in the present experiments and thus resembles the uptake of norepinephrine in synaptosomes (13, 21), Junod (3) has found that the relationship between the rate of uptake of 5-hydroxytryptamine in the lung and the sodium concentration is hyperbolic over the same range of sodium concentrations.

Our results appear to substantiate the speculations of Alabaster and Bakhle (17) and Iwasawa and Gillis (22) in that, although the mechanism of uptake for norepinephrine resembles that for 5-hydroxytryptamine, two distinct carriers or sites of uptake are involved. One possible explanation for the limited degree of competitive inhibition which we observed between these two amines is the partial sharing of one of these sites by norepinephrine and 5-hydroxytryptamine with the other site restricted to norepinephrine alone. Extending this theory, only one component of norepinephrine uptake would be inhibited by 5-hydroxytryptamine in low concentrations. Alabaster and Bakhle (17) reported no competition when they used low concentrations of 5-hydroxytryptamine; however, they did not perform an adequate number of experiments on which to apply statistical analysis and draw such a conclusion. The partial inhibition of 5-hydroxytryptamine uptake by large concentrations of norepinephrine observed by Junod (3) is probably a nonspecific phenomenon.

Previous results have suggested that the principal site of uptake of norepinephrine in the lung is the endothelial cell. Hughes et al. (2) concluded from light microscope autoradiography that thinly muscled blood vessels and capillaries were the main sites of labeling in rat lung following 3H-l-norepinephrine perfusion. Subsequently, Iwasawa et al. (4) reported that, following infusion of norepinephrine through the rabbit lung, intense fluorescence appeared in the endothelial cells of capillaries and postcapillary venules. The results of the present study represent the first attempt to quantify the distribution of norepinephrine using both light and electron microscope autoradiography.

Autoradiographs of lung infused with 3H-l-norepinephrine were similar to those of lung infused with 3H-5-hydroxytryptamine (8) in that the endothelial cells of vessels were labeled. However, the lung infused with 3H-l-norepinephrine differed from the lung infused with 3H-5-hydroxytryptamine in several ways: (1) its pattern of labeling was not uniform throughout the lung, (2) the small distended vessels communicating with capillary beds were consistently labeled, (3) only about one-third of the capillaries were labeled, and (4) the smooth muscle cells in the walls of large veins were labeled, but large arteries rarely showed labeling. It appears that there is some overlap in the sites for uptake of norepinephrine and 5-hydroxytryptamine since the endothelial cells of vessels are labeled in both instances. However, 3H-5-hydroxytryptamine is taken up primarily by pulmonary capillary endothelial cells, whereas most capillaries remain unlabeled in a lung treated in an identical manner but infused with 3H-l-norepinephrine. Whether the same lung capillary would label with both amines is unknown. The labeling of vascular smooth muscle observed with 3H-l-norepinephrine is not observed with 3H-5-hydroxytryptamine.

We believe that the nonuniform regional labeling pattern seen in the lungs infused with norepinephrine is a true representation of the uptake sites of this biogenic amine and is not the result of incomplete perfusion, because the capillaries had widely patent lumens and were free of blood cells. Although 3H-l-norepinephrine is a diffusible substance, glutaraldehyde acts to stabilize it in the
sites where uptake has occurred. It has been reported that glutaraldehyde has the ability to bind certain amino acids to tissue (23) and to react with the primary amino group of norepinephrine to form a polymer that is bound in situ in tissue sections (24). As indicated in our autoradiographs, \(^{3}H\)-l-norepinephrine not associated with tissue sites is completely washed out during the dehydration procedure; thus, radioactivity is not found indiscriminately associated with other structures in the lung. The labeling of nuclei in the presence of \(^{3}H\)-l-norepinephrine is also observed with \(^{3}H\)-5-hydroxytryptamine (7); this finding agrees with studies showing that 5-hydroxytryptamine can bind to the bases of nucleic acids (25, 26). Whether this phenomenon is also true for norepinephrine is unknown, but it is conceivable that, if \(^{3}H\)-l-norepinephrine is not firmly bound within the cytoplasm of the cell, it could become available for binding to the nucleus during perfusion fixation with glutaraldehyde. It is pertinent to note that our interest was in the cellular not the subcellular site of labeling.

Although most capillaries in the \(^{3}H\)-l-norepinephrine-infused lung were not labeled, the small distended vessels leading into or out of the capillary beds (Fig. 3) were heavily labeled. These numerous small vessels often lacked a complete smooth muscle investment and frequently communicated with unlabeled capillaries, suggesting a regional specificity in the uptake of \(^{3}H\)-l-norepinephrine by the lung vasculature. These labeled small vessels probably represent the "distended thinly muscled blood vessels" that Hughes et al. (2) reported were labeled with \(^{3}H\)-norepinephrine and the venules that Iwasawa et al. (4) suggested as a site of norepinephrine binding. In our autoradiographs, 90% of these vessels were labeled. In contrast, relatively few capillaries (31%) showed radioactivity, although this percent represents a large surface area. A labeling of "thinly muscled terminal bronchioles" reported by Hughes et al. (2) was not observed in our perfused lungs. Mast cells and other cell types were not labeled, but one group of unmyelinated nerve fibers near the vessels in the hilus of the lung did contain a few silver granules. All other \(^{3}H\)-l-norepinephrine radioactivity above the level of background was associated exclusively with blood vessels. The absence of sympathetic nerve fibers was not unexpected, because the blood vessels of rat lungs are known for their sparse innervation (8, 27, 28) compared with those of other species. Our conclusion that the sympathetic nerves played little part in the uptake of norepinephrine by the lungs is substantiated by the results of Iwasawa and Gillis (22) who used lungs from rabbits treated with 6-hydroxydopamine.

The perfusion of lungs used for autoradiography was carried out in the presence of iproniazid and tropolone, because in the absence of these inhibitors norepinephrine was rapidly metabolized and the products quickly washed from the lungs. Although the possibility exists that these two agents influenced the localization of the amine, it seems more likely that Iwasawa et al. (4) failed to observe fluorescence following infusion of rabbit lungs with norepinephrine because of this very rapid metabolism and the lack of accumulation of norepinephrine (Table 4).

Although we have demonstrated that some norepinephrine is taken up into vascular smooth muscle cells, such uptake contributes little to the overall uptake in the lung. Gillespie and Towart (29) have reported that in the rabbit ear artery the \(K_{m}\) of uptake into smooth muscle is \(4.9 \times 10^{-8}\) M, which is on the order of a factor of three greater than that which we found for uptake in the lung.

References

10. Minard FN, Grant DS: Convenient method for the chroma-
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TERENCE E. NICHOLAS, JUDY M. STRUM, LYNNE S. ANGELO and ALAIN F. JUNOD

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