Continuous Infusion of Tracer Norepinephrine May Miscalculate Unidirectional Nerve Uptake of Norepinephrine in Humans

Jens H. Henriksen, Niels Juel Christensen, and Helmer Ring-Larsen

In order to evaluate uptake kinetics of norepinephrine (NE) in different tissues, a catheterization study was performed in control subjects (n=6) and patients with enhanced sympathetic nervous activity (cirrhosis, n=12) during constant intravenous infusion of L-[3H]norepinephrine ([3H]NE) for 75 minutes. In spite of a higher NE spillover from kidneys in patients compared with controls (82 vs. 49 ng/min, p<0.01), renal extraction ratios of [3H]NE were similar in the two groups (0.33 vs. 0.32, NS), and no significant change was observed during the time of infusion. In contrast, liver-intestine extraction ratios of [3H]NE decreased significantly and equally with infusion time in patients (from 0.57 to 0.44, p<0.01) and controls (from 0.59 to 0.46, p<0.01). This was observed despite the fact that spillover of NE from this vascular bed was observed only in patients with cirrhosis and not in controls (41 vs. -5 ng/min, p<0.02). In the lower limb, net release of NE was similar in patients and controls, and extraction ratios of [3H]NE decreased almost equally with infusion time (from 0.35 to 0.30, p<0.01 and from 0.40 to 0.24, p<0.1, respectively). Whole-body clearance of [3H]NE decreased over time in patients (—6%, p<0.01) and controls (—20%, p<0.01), but significant difference was not observed between the groups. We conclude that failure to attain a steady state with respect to [3H]NE removal was demonstrated in areas of large tissue volume relative to blood flow. This was found under the condition of high as well as low spillover of NE. Therefore, in addition to neuronal tracer rerelease, delayed distribution of the tracer in some tissues seems to be important for the changing whole-body clearance observed. (Circulation Research 1989;65:388-395)

Norepinephrine (NE), the neurotransmitter released from axon terminals of sympathetic postganglionic neurons, leaks into plasma where its concentration may reflect the neurotransmitter activity. The concentration of endogenous NE in plasma may be accurately measured by enzymatic isotope derivative techniques or high-performance liquid chromatography (HPLC).1 Circulating NE is rapidly removed from plasma by processes having a half-life of a few minutes. However, since a vascular area may release as well as remove NE, quantification of NE kinetics demands a method by which one is able to distinguish both fluxes.2,3 Infusion of tritium-labeled norepinephrine ([3H]NE) has provided valuable results concerning NE kinetics in normal humans as well as in patients.2-7 Inherent problems pertaining to the use of labeled NE are related to 1) the achievement of steady-state conditions during constant tracer infusion and 2) the presence of neuronal rerelease of [3H]NE after uptake in sympathetic axon terminals. The present study was, therefore, undertaken in order to evaluate the effect of distribution and rerelease on the kinetics of the sympathetic neurotransmitter. We focused on kinetics in different organs and tissues. Control subjects and patients with cirrhosis and enhanced sympathetic nervous activity were studied.

Subjects and Methods

Patients and Controls

The study comprised 18 subjects (2 women, 16 men), see Table 1. Twelve were patients with biopsy-verified cirrhosis (42–72 years of age, 58–95 kg body weight). Six subjects (36–68 years of age, 62–99 kg body weight) served as controls. These subjects had no disorders or disorders of minor degree: one with fatty liver, one with unspecific
TABLE 1. Clinical Data, Arterial Norepinephrine, Epinephrine, and Plasma Clearance of Tritium-Labeled Norepinephrine in Supine Resting Patients With Cirrhosis and in Control Subjects

<table>
<thead>
<tr>
<th>Subject and diagnosis</th>
<th>Age (years)/sex</th>
<th>Body weight (kg)</th>
<th>Arterial NE (ng/ml)</th>
<th>Arterial E (ng/ml)</th>
<th>Whole-body plasma clearance of [3H]NE (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with cirrhosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>1 Esophageal varices, ascites, AC</td>
<td>45/F</td>
<td>64</td>
<td>0.80</td>
<td>0.03</td>
<td>1.34</td>
</tr>
<tr>
<td>2 Esophageal varices, ascites, AC</td>
<td>63/M</td>
<td>72</td>
<td>0.50</td>
<td>0.07</td>
<td>1.71</td>
</tr>
<tr>
<td>3 Alcoholic hepatitis, esophageal varices, slight ascites, AC</td>
<td>57/M</td>
<td>73</td>
<td>0.19</td>
<td>0.05</td>
<td>1.66</td>
</tr>
<tr>
<td>4 Ascites, AC</td>
<td>62/M</td>
<td>95</td>
<td>0.52</td>
<td>0.037</td>
<td>1.55</td>
</tr>
<tr>
<td>5 Gastric ulcer, ascites, AC</td>
<td>49/M</td>
<td>78</td>
<td>0.59</td>
<td>0.095</td>
<td>1.44</td>
</tr>
<tr>
<td>6 No ascites, AC</td>
<td>71/M</td>
<td>75</td>
<td>0.74</td>
<td>0.097</td>
<td>1.94</td>
</tr>
<tr>
<td>7 Ascites, AC</td>
<td>72/M</td>
<td>59</td>
<td>0.40</td>
<td>0.097</td>
<td>1.54</td>
</tr>
<tr>
<td>8 Slight ascites, AC</td>
<td>66/M</td>
<td>72</td>
<td>0.39</td>
<td>0.09</td>
<td>1.36</td>
</tr>
<tr>
<td>9 No ascites, AC</td>
<td>56/F</td>
<td>58</td>
<td>0.26</td>
<td>0.037</td>
<td>1.74</td>
</tr>
<tr>
<td>10 Ascites, AC</td>
<td>42/M</td>
<td>57</td>
<td>0.24</td>
<td>0.097</td>
<td>1.47</td>
</tr>
<tr>
<td>11 Ascites, umbilical hernia, AC</td>
<td>61/M</td>
<td>94</td>
<td>0.57</td>
<td>0.107</td>
<td>1.22</td>
</tr>
<tr>
<td>12 Posthepatitic and alcoholic cirrhosis, tense ascites</td>
<td>45/M</td>
<td>52</td>
<td>1.39</td>
<td>0.297</td>
<td>1.63</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td></td>
<td>72</td>
<td>0.50*</td>
<td>0.07†</td>
<td>1.51†‡</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Fatty liver</td>
<td>53/M</td>
<td>84</td>
<td>0.25</td>
<td>0.04</td>
<td>1.75</td>
</tr>
<tr>
<td>2 Unspecific slight cholestasis</td>
<td>56/M</td>
<td>74</td>
<td>0.10</td>
<td>0.033</td>
<td>1.60</td>
</tr>
<tr>
<td>3 No abnormal finding</td>
<td>37/M</td>
<td>70</td>
<td>0.32</td>
<td>0.03</td>
<td>1.61</td>
</tr>
<tr>
<td>4 No abnormal finding</td>
<td>44/M</td>
<td>62</td>
<td>0.37</td>
<td>0.037</td>
<td>2.16</td>
</tr>
<tr>
<td>5 Chronic alcoholism, normal liver biopsy</td>
<td>39/M</td>
<td>71</td>
<td>0.12</td>
<td>0.043</td>
<td>2.03</td>
</tr>
<tr>
<td>6 Slight bronchitis</td>
<td>68/M</td>
<td>99</td>
<td>0.31</td>
<td>0.017</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td></td>
<td>73</td>
<td>0.28</td>
<td>0.035</td>
<td>1.68§</td>
</tr>
</tbody>
</table>

NE, norepinephrine; E, epinephrine; [3H]NE, tritium-labeled NE; AC, alcoholic cirrhosis.

*p<0.025, significantly different from controls.

†p<0.01, significantly different from controls.

‡p<0.001, heterogeneity over time (Friedman test).

§p<0.01, heterogeneity over time (Friedman test).

Changes in liver biopsy, three without abnormal findings, and one with chronic bronchitis. All subjects were considered to be in a stable state.

Patients and controls consented to participate in the following investigations after thorough oral and written explanation. The study was approved by the Ethics Committee for Medical Research in Copenhagen (V.100.286/84). No complications or side effects were seen during or after the investigative procedure.

**Catheterization**

Patients and controls underwent organ-vein and right-heart catheterization to diagnose or exclude portal venous hypertension, organic heart disease, or renal vascular disease. All subjects were studied in the morning after an overnight fast and after at least 1 hour in the supine position. Catheterization of hepatic, right renal, and femoral veins was performed as described earlier. In brief, a Courmand or Swan-Ganz catheter size 7F was guided under local anesthesia to the above locations by the antecubital or femoral route under fluoroscopic control. A small indwelling polyethylene catheter was placed in the femoral artery by the Seldinger technique. Hepatic blood flow was determined by the indocyanine-green constant-infusion technique. Renal blood flow was determined by the [125]Ihippuric acid after correction for renal extraction of hippuran. Renal mass was taken to be 300 g.

**Protocol**

Plasma samples for determination of endogenous NE and epinephrine (E) were simultaneously collected from the femoral artery/right renal vein, femoral artery/hepatic vein, and femoral artery/femoral vein before infusion of tracer NE. Constant intravenous (cubital vein) infusion of tritium-labeled L-norepinephrine (chain-labeled [3H]NE, NEN-337, 20 Ci/mmol [750 Gdps/mmol] specific activity, New England Nuclear, Boston, Massachusetts, pharmaceutically prepared for intravenous

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injection by Isotopapoteket, Copenhagen, Denmark) was maintained by a calibrated pump (Dich, Copenhagen, Denmark). The preparation was stored under nitrogen, and the radiochemical purity was checked by HPLC and found to be greater than 98%. The infusion rate was 0.8 μCi/min (30 kdps/min) equivalent to 7 ng chemical NE/min. After 25, 55, and 75 minutes of constant [3H]NE infusion, plasma samples were collected simultaneously from artery and organ vein in the above-mentioned locations. This was achieved by changing the position of the venous catheter between the three locations under fluoroscopic control. Arterial and venous blood samples from all three areas were collected within 5 minutes. Calculated whole-body radiation dose was less than 200 mrem (2 mSv).

Analysis

Plasma concentrations of endogenous NE and E in artery and veins were determined by an enzymatic isotope-derivative technique.12 Sample-to-sample coefficient of variations for plasma were 7% for NE and 11% for E. [3H]NE in plasma was extracted by alumina, eluted by acid, freeze-dried, and counted in a liquid scintillator as earlier described.4 Deaminated metabolites were measured after ethyl acetate extraction, but only an insignificant number of counts could be ascribed to deaminated products as determined by HPLC.13

Calculations

Whole-body clearance (Cl) of norepinephrine was assessed as infusion rate of [3H]NE divided by the arterial concentration of [3H]NE (C_a[3H]NE).

Tissue extraction ratios (i.e., the fraction of influx extracted during one passage) of E, NE, and [3H]NE were measured as e=(C_v−C_a)/C_a, where C_v and C_a are the plasma concentrations of E, NE, and [3H]NE in artery and organ vein (hepatic, renal, or femoral vein), respectively.

Organ uptake and spillover rates of NE (J_upake and J_spillover) were determined as

\[ J_{\text{spillover}} = \text{plasma flow rate} \times C_{a,\text{NE}} \times \left( 1 - \frac{C_v}{C_a} \right)[3H]NE \]

and

\[ J_{\text{upake}} = \text{plasma flow rate} \times C_{a,\text{NE}} \times \left( \frac{C_v}{C_a} - \frac{C_a}{C_v} \right)[3H]NE \]

Statistical Evaluation

The significance of differences between median values was tested by the Wilcoxon and Mann-Whitney tests for paired or group data, respectively. For repeated measurements, heterogeneity was analyzed by the Friedman test. Values of \( p<0.05 \) were considered significant.

Results

Whole Body

Concentrations of endogenous plasma catecholamines are summarized in Table 1. [3H]NE in arterial plasma in time sequence is illustrated in Figure 1, and values of Cl appear in Table 1. In patients and controls, C_a[3H]NE increased, and Cl decreased with increasing time of infusion (both groups, \( p<0.01 \) by Friedman test). No significant differences were found between patients and controls.

Organs and Tissues

Blood flow and NE uptake and spillover in different organs and tissues are shown in Table 2. In spite of a higher NE release from patient kidneys compared with control kidneys (82 vs. 49 ng/min, \( p<0.01 \)), renal extraction ratios of [3H]NE were similar in the two groups (median 0.33–0.31 vs. 0.32–0.35, NS), and no significant change was observed during the time of infusion (Table 3). In contrast, the arteriohepatic venous extraction ratios of [3H]NE decreased significantly and equally with infusion time in patients (from 0.56 to 0.44, \( p<0.01 \)) and controls (from 0.59 to 0.46, \( p<0.01 \)). This decrease was observed despite the fact that spillover of NE in this vascular bed was observed only in patients with cirrhosis and not in controls (41 vs. −5 pg/min, \( p<0.02 \)). In the lower limb, spillover of NE was similar in patients and controls (Table 2), and extraction ratios of [3H]NE decreased almost equally with infusion time (from 0.35 to 0.30, \( p<0.01 \) and from 0.40 to 0.24, \( p<0.1 \), respectively).

The extraction ratio of [3H]NE in liver-intestine was significantly higher than that of the kidney or lower limb in patients (\( p<0.01 \) and controls

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**Figure 1.** Plots of arterial concentration of tracer norepinephrine ([3H]NE) in plasma over time in six control subjects and 12 patients with cirrhosis. At time zero, a constant intravenous infusion of [3H]NE was started in supine, resting subjects. The numbers refer to subjects’ numbers in the tables. Values of \( p \) show significance levels of heterogeneity over time as tested by Friedman analysis.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Blood flow (ml/min)</th>
<th>Plasma flow (ml/min)</th>
<th>NE uptake (ng/min)</th>
<th>NE spillover (ng/min)</th>
<th>Blood flow (l/min)</th>
<th>Plasma flow (ml/min)</th>
<th>NE uptake (ng/min)</th>
<th>NE spillover (ng/min)</th>
<th>Blood flow (ml/min×10 kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
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<td>564</td>
<td>333</td>
<td>54</td>
<td>81</td>
<td>1.45</td>
<td>927</td>
<td>482</td>
<td>304</td>
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<tr>
<td>2</td>
<td>570</td>
<td>336</td>
<td>69</td>
<td>99</td>
<td>1.43</td>
<td>841</td>
<td>240</td>
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<td>576</td>
<td>357</td>
<td>39</td>
<td>81</td>
<td>1.36</td>
<td>897</td>
<td>124</td>
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<td>230</td>
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<tr>
<td>4</td>
<td>555</td>
<td>366</td>
<td>84</td>
<td>63</td>
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<td>1,473</td>
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<td>81</td>
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<td>Median</td>
<td>573†</td>
<td>363‡</td>
<td>48§</td>
<td>82‡</td>
<td>1.44</td>
<td>903</td>
<td>180§</td>
<td>41‡</td>
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<td>51</td>
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<td>170</td>
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<td>494</td>
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<tr>
<td>Median</td>
<td>897</td>
<td>546</td>
<td>38</td>
<td>49</td>
<td>1.22</td>
<td>705</td>
<td>76#</td>
<td>-5</td>
<td>170</td>
</tr>
</tbody>
</table>

NE, norepinephrine.

*Median from our laboratory as determined by strain-gauge plethysmography (Bülow et al, unpublished observation, n=8).

†p<0.01, significantly different from controls.

‡p<0.02, significantly different from controls.

§p<0.01, size of uptake significantly different from size of spillover.

||p<0.05, significantly different from controls.

††Mean±SD from our laboratory (Astrup et al14).

#p<0.05, size of uptake significantly different from size of spillover.
Another possibility might be accumulation of labeled mixed model. This could be due to the existence of level is larger than expected from a simple well-system), 55 minutes to nine half-lives (99.8%), and 75 minutes to eleven half-lives (99.99%). It is evident that the present change in circulating tracer level is larger than expected from a simple well-mixed model. This change might be due to the existence of areas with slow capillary exchange (i.e., slow perfusion and/or low capillary permeability) or distribution of tracer to a large (intracellular) space. Another possibility might be accumulation of labeled metabolites of NE in plasma not being detected by the analytical procedure. This possibility is considered in detail in the following paragraphs.

Howes et al described the appearance of [3H]dihydroxyphenylglycol ([3H]DHPG) in plasma during infusion of [3H]NE. A substantial proportion of the radioactivity in alumina eluates was due to dihydroxymetabolites of NE in their study. However, Eisenhofer et al found only small increments in plasma DHPG (2-6%) during intravenous infusion of labeled or unlabeled NE. In a previous study, Brown found no increase in DHPG in plasma in human subjects during infusion of NE, whereas a significant increase in plasma DHPG was observed after neuronal release of NE. The studies by Eisenhofer et al and by McCane and Forfar indicate that the radioactivity in alumina extracts is predominantly [3H]NE after intravenous infusion of [3H]NE in humans. Finally, Esler et al found in a recent study that the proportion of DHPG in alumina eluates represented only 2% of the extracted radioactivity. In the present study, no significant radioactive counts could be ascribed to deaminated products. In a recent study in three normal subjects and using HPLC, we found, however, that after 80 minutes' [3H]NE infusion, 0-6% of the radioactivity in alumina extracts of plasma was due to accumulation of [3H]DHPG. Thus, the present finding of increasing C_{spillover} cannot be ascribed to accumulation of labeled metabolites.

To elucidate the questions on failure to attain a steady state of C_{spillover}, we studied tissues with different characteristics. In the kidney, a fast distribution of tracer would be expected due to the high perfusion rate, the permeable vasculature, and the low tissue volume. In patients with cirrhosis, an increased renal sympathetic tone has been established, and as expected, endogenous NE spillover from the kidney in patients was higher than in controls (Table 2). However, we found no signs of progressive neuronal rerelease of tracer since the extraction ratio of [3H]NA remained constant with time in patients as well as in controls. This may be due to the fact that by 25 minutes, the tracer equilibration with the intraneuronal pool is largely complete in the kidney. On the other hand, a very slow equilibration might also give a constant or almost constant extraction ratio. However, this seems less likely in light of the high perfusion and high catecholamine turnover in this tissue.

The splanchnic system, which is complex due to the connection of intestine and spleen in series with the liver, revealed a higher extraction ratio of labeled NE compared with both kidney and lower limb. The high extraction ratio may be caused by perfusion of capillaries in series. Another possibility is the large metabolic capacity of the liver. The present finding of median extraction ratios of NE and [3H]NE close to 0.60 in control subjects is similar to reports of others. The lower extraction of catecholamines in patients with cirrhosis

(p<0.05), but no significant difference was found between the kidney and lower limb. The extraction ratio of endogenous NE was significantly lower than that of [3H]NE in all vascular areas studied (p<0.01) except for liver-intestine of controls. On the contrary, the extraction ratios of E were higher than those of [3H]NE in patients (p<0.01) and controls (p<0.02) with all tissue values combined.

**Discussion**

We investigated an organ with a high blood-flow rate and a low tissue weight, the kidney (high perfusion coefficient); a region with a high blood-flow rate and a high tissue weight, the splanchnic system (medium perfusion coefficient); and a part of the body with a low blood-flow rate combined with a high tissue weight, the lower limb (low perfusion coefficient). A constant extraction ratio indicates slight or constant neuronal tracer rerelease and steady state with respect to transvascular exchange of tracer. In contrast, a progressively decreasing extraction ratio may be seen in case of increasing neuronal rerelease and/or delayed transvascular exchange of tracer. In the following, catecholamine kinetics are discussed in relation to perfusion, capillary exchange, and space of distribution. Substantial differences were recorded between cirrhotic patients and controls. Thus, circulating endogenous catecholamines were significantly higher in the former group; this occurrence also applied to the estimated NE spillover from kidney and liver-intestine and indicated enhanced sympathetic tone in these patients as expected from previous reports. By including patients with cirrhosis and enhanced sympathetic nervous tone, catecholamine kinetics was studied in conditions of normal as well as increased NE spillover.

The slight, but significant, increase in C_{spillover} from 25 minutes to 55 and 75 minutes after starting intravenous tracer infusion (Figure 1) shows that steady state was not completely achieved within 25 minutes. This failure to attain a steady state with respect to circulating tracer concentration may be due to delayed distribution or neuronal rerelease of tracer. It may be assumed that the extracellular space (approximately 15 liters) is the quantitatively most important space of distribution of catecholamines and that Cl is 1.5-2.0 l/min; therefore, half-life of NE will be approximately 5-7 (0.69x15/2.0 to 0.69x15/1.5) minutes. Thus, 25 minutes of infusion corresponds to about four half-lives (95% of steady-state concentration in a well-mixed system), 55 minutes to nine half-lives (99.8%), and 75 minutes to eleven half-lives (99.99%). It is evident that the present change in circulating tracer level is larger than expected from a simple well-mixed model. This could be due to the existence of areas with slow capillary exchange (i.e., low perfusion and/or low capillary permeability) or distribution of tracer to a large (intracellular) space. Another possibility might be accumulation of labeled metabolites of NE in plasma not being detected by the analytical procedure. This possibility is considered in detail in the following paragraphs.

Howes et al described the appearance of [3H]dihydroxyphenylglycol ([3H]DHPG) in plasma during infusion of [3H]NE. A substantial proportion of the radioactivity in alumina eluates was due to dihydroxymetabolites of NE in their study. However, Eisenhofer et al found only small increments in plasma DHPG (2-6%) during intravenous infusion of labeled or unlabeled NE. In a previous study, Brown found no increase in DHPG in plasma in human subjects during infusion of NE, whereas a significant increase in plasma DHPG was observed after neuronal release of NE. The studies by Eisenhofer et al and by McCane and Forfar indicate that the radioactivity in alumina extracts is predominantly [3H]NE after intravenous infusion of [3H]NE in humans. Finally, Esler et al found in a recent study that the proportion of DHPG in alumina eluates represented only 2% of the extracted radioactivity. In the present study, no significant radioactive counts could be ascribed to deaminated products. In a recent study in three normal subjects and using HPLC, we found, however, that after 80 minutes' [3H]NE infusion, 0-6% of the radioactivity in alumina extracts of plasma was due to accumulation of [3H]DHPG. Thus, the present finding of increasing C_{spillover} cannot be ascribed to accumulation of labeled metabolites.

To elucidate the questions on failure to attain a steady state of C_{spillover}, we studied tissues with different characteristics. In the kidney, a fast distribution of tracer would be expected due to the high perfusion rate, the permeable vasculature, and the low tissue volume. In patients with cirrhosis, an increased renal sympathetic tone has been established, and as expected, endogenous NE spillover from the kidney in patients was higher than in controls (Table 2). However, we found no signs of progressive neuronal rerelease of tracer since the extraction ratio of [3H]NA remained constant with time in patients as well as in controls.

This may be due to the fact that by 25 minutes, the tracer equilibration with the intraneuronal pool is largely complete in the kidney. On the other hand, a very slow equilibration might also give a constant or almost constant extraction ratio. However, this seems less likely in light of the high perfusion and high catecholamine turnover in this tissue.

The splanchnic system, which is complex due to the connection of intestine and spleen in series with the liver, revealed a higher extraction ratio of labeled NE compared with both kidney and lower limb. The high extraction ratio may be caused by perfusion of capillaries in series. Another possibility is the large metabolic capacity of the liver. The present finding of median extraction ratios of NE and [3H]NE close to 0.60 in control subjects is similar to reports of others. The lower extraction of catecholamines in patients with cirrhosis

(p<0.05), but no significant difference was found between the kidney and lower limb. The extraction ratio of endogenous NE was significantly lower than that of [3H]NE in all vascular areas studied (p<0.01) except for liver-intestine of controls. On the contrary, the extraction ratios of E were higher than those of [3H]NE in patients (p<0.01) and controls (p<0.02) with all tissue values combined.
may in part be due to intrahepatic shunts. A decreased metabolic capacity seems less likely because the uptake rate of NE was higher in the cirrhotic patients compared with the controls (Table 2). Velasquez and Alexander found in animal experiments that portal venous NE was greater than arterial NE. This finding indicated intestinal sympathetic activation with spillover of NE. In controls, we found no signs of spillover of endogenous NE. Thus, it is unlikely that the decreased extraction ratio of tracer NE with infusion time in control subjects is due to tracer rerelease. An explanation could be delayed distribution to an intracellular space. However, it should be emphasized that the estimation of NE uptake and spillover (Table 2) refers to the first 25 minutes of the study. It cannot be excluded that minor changes in sympathetic nervous activity may have occurred during the study although heart rate and arterial blood pressure remained constant. Moreover, altered rates of NE metabolism (e.g., exhaustion of adenosylmethionine) and changes in uptake by neurons or extraneuronal sites are potential but less likely explanations. In patients with cirrhosis, spillover of endogenous NE was found, and tracer rerelease may contribute to the decreasing extraction ratio. Under normal conditions, the vasculature in intestine and liver is very permeable to low molecular substances. Capillarization of liver sinusoids in patients with cirrhosis may decrease the permeability to larger molecules but not to low molecular substances. Therefore, these changes in the cirrhotic microvasculature are unlikely to delay the capillary exchange of NE in the liver significantly.

In the lower limb, a slow tracer distribution would be expected due to the low perfusion rate here, the less permeable capillaries (compared with kidney and splanchnic system), and the large volume of tissue. In patients as well as in controls, the extraction ratios decreased with increasing infusion time. This is probably due to delayed distribution and neuronal rerelease in combination although the latter may be less important because the spillover of endogenous NE was relatively low in the resting supine condition.

The extraction ratio of endogenous E showed a greater variation compared with [3H]NE, but in the main, extraction of E was slightly higher than that of tracer NE in all tissues combined (Table 3). Because E in general is not reused, this discrepancy may be attributed to neuronal rerelease of tracer NE. Recently, Peronnet et al. applied regional plasma E fractional extraction as an index of NE removal from plasma and found in dogs that the main contributor to catecholamine removal from the circulation appeared to be the hepatosplanchnic vascular bed in rest. During exercise with concomitant enhancement of sympathetic nervous activity, skeletal muscle vascular beds accounted for the major removal of circulating E. We found a substantial catecholamine uptake in liver-intestine in patients with cirrhosis and enhanced sympathetic nervous activity, but the uptake was smaller in the lower limb, and only a small difference was found between patients and controls. Thus, regional differences were demonstrated in the present study, and differences between NE and E with respect to uptake mechanisms and metabolism may exist. Moreover, E may be released from sympathetic fibers in some tissue. Therefore, at present no final preference can be given to E versus [3H]NE with respect to kinetics of catecholamine uptake.

Our findings of different patterns of tracer fractional extraction over time in different tissues and the resulting unsteady state of arterial [3H]NE may question the estimation of overall clearance and appearance of NE as assessed by the method of continuous infusion of [3H]NE. In other words, if the removal of tracer from plasma includes transport to slowly equilibrating tissue spaces without metabolic degradation of tracer, the fractional extraction and, thereby, the clearance may be overestimated. On the other hand, reuse of tracer NE with subsequent rerelease of [3H]NE will lead to underestimation of NE removal from plasma. Obviously, these questions need further clarification.

In summary, we have demonstrated failure to attain a steady state with respect to circulating tracer norepinephrine during 75 minutes intravenous infusion in subjects with different levels of circulating norepinephrine. Almost constant tracer extraction was found in the kidney over time, even in subjects with a high spillover of endogenous norepinephrine. In contrast, in the splanchnic system, decreasing tracer extraction was observed also in subjects with no spillover of endogenous norepinephrine. Thus, similar results may be found under the condition of high as well as low spillover of norepinephrine, and decreasing extraction ratios were observed in areas of large tissue volume relative to blood flow. Therefore, in addition to neuronal rerelease, delayed distribution of the tracer in some tissues seems to be important for the changing whole-body clearance observed.

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References


**Key Words**: catecholamines • neuronal re-release • norepinephrine • nonsteady state • cirrhosis
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J H Henriksen, N J Christensen and H Ring-Larsen

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