Higher Dopamine Level in Lymph from the Cervical Lymph Trunk than in Plasma Following Intravenous Bolus Injection of L-Dopa in Rats

Jun-ichi SUDO,* Hiroaki IWASE, Jun TERUI, Taiji HAYASHI, and Momoko SOYAMA

Department of Clinical Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-02, Japan. Received November 9, 1994; accepted January 23, 1995

To clarify the mechanism(s) responsible for nausea and vomiting induced by l-dopa administration, dopamine levels in the plasma and lymph of rats were investigated in the 60-min period following an intravenous bolus of l-dopa (2.5 mg/kg body weight). The dopamine level in plasma from the femoral artery was the highest at 5 min immediately after the l-dopa injection, and was eliminated thereafter. Showing the same tendency as the plasma, the lymph from the thoracic duct showed a maximal increase of dopamine at 0 to 10 min, and a rapid decrease later. In contrast, the dopamine level in the lymph from the cervical lymph trunk increased, peaked at 10 to 20 min, and fell gradually thereafter. The dopamine level in the cervical lymph was higher than that in the thoracic lymph. When these data were kinetically analyzed, the cervical lymph had a larger area under the dopamine concentration-time curve than the thoracic lymph. Both the cervical lymph and the thoracic lymph had longer values of dopamine mean residence time than the plasma. Our findings revealed that when l-dopa was administered with an intravenous bolus, dopamine was higher and remained longer in the cervical lymph than in the rest of the body.

Key words l-dopa; dopamine; lymph; cervical lymph trunk; thoracic duct

l-Dopa is a therapeutic agent used for Parkinson’s disease.1-4 Despite its usefulness, this agent has various adverse effects including nausea, vomiting, hypotension, cardiac irregularities, abnormal involuntary movements, and psychiatric disturbances.1-4

In this study, to obtain some information concerning these adverse effects of l-dopa, we investigated the time-dependent alterations of lymphatic flow, and the levels of L-dopa and dopamine in the lymph from the cervical lymph trunk and from the thoracic duct of rats that had received an intravenous bolus injection of l-dopa. We also investigated the time-dependent alterations of l-dopa and dopamine in the plasma, and carried out kinetic analysis of both the plasma and lymphatic data.

MATERIALS AND METHODS

General Procedure Male Wistar strain rats (Sankyo Labo Service; Tokyo), weighing 250±10 g, received an intraperitoneal injection of thiobutabarbital (B.Y.K.; Hamburg, Germany), at 100 mg/kg body weight, for anesthesia and immobilization,5 and were intubated for free respiration. The right femoral vein was then catheterized with a polyethylene tube (PE-50) for the continuous infusion of saline at an infusion rate of 0.5 ml/min/kg body weight. L-Dopa (1-3,4-dihydroxyphenylalanine; Sigma Chemicals; St Louis, MO, U.S.A.), dissolved in saline at a concentration of 2.5 mg/ml, was injected at a dose of 2.5 mg/kg body weight through this route. This dose was smaller than the doses (11, 25, and 50 mg/kg body weight) used by Tyce and Owen6 and by Landsberg et al.7

Collection of Blood After the animals were operated on as described above, the left femoral artery was catheterized with a polyethylene tube (PE-50) that had been filled with 0.2 M EGTA (Wako Pure Chemical Industries; Osaka) dissolved in saline. The blood was collected into chilled tubes containing 40 µl of a solution containing 0.2 M EGTA and 0.2 M reduced glutathione (GSH; Sigma Chemicals); the total volume collected was restricted to within 2 ml to avoid possible physiological alterations in concentrations of the amines induced by the loss of blood. Plasma, obtained by centrifugation (1700 × g, 10 min, 4°C) of the blood, was used for the determination of l-dopa and the amines.

Collection of Lymph Experiments for the collection of lymph were performed with rats other than those used for the procedure described above. The animals were anesthetized and catheterized as described above. Catheterization of the left cervical lymph trunk was performed separately from that of the thoracic duct. The catheterization was done by the cervical approach procedure described by Reinhard et al.9 A polyethylene tube (PE-10) that had been filled with 0.2 M EGTA dissolved in saline was inserted into the left cervical lymph trunk or into the thoracic duct. The lymph that flowed out of the catheter was collected sequentially and continuously –10 to 0 (before), 0 to 10, 10 to 20, 20 to 30, 30 to 45, and 45 to 60 min after the injection of l-dopa. The lymph was collected into chilled tubes in which 10 µl of a solution containing 0.2 M EGTA and 0.2 M GSH had been placed beforehand.8 After the volume of lymph was measured (1 mg = 1 µl), it was centrifuged (10062 × g, 10 min, 4°C) to obtain the supernatant used for the determination of l-dopa and the amines.

Determination of l-Dopa and Catecholamines For determination of l-dopa and amine levels in the plasma and lymph fluid, the samples were treated using the method of Eriksson and Persson.8 l-Dopa and the amines were determined electrochemically by the high-performance liquid chromatographic method of Yamazaki and Sudo,10 with some modification: column, reversed-phase type (Wakosil-II 5C18 HG; particle size, 5 µm; 4.6 mm × 25 cm; Wako Pure Chemical Industries); mobile phase, mixture of 100 mM NaH2PO4, 0.08 mM EDTA-2Na and 150 mg/l sodium octyl sulfate (Kanto Chemicals; Tokyo) (pH 3.1, adjusted with concentrated phosphoric acid); flow rate, 1.0 ml/min; column temperature, 40°C; voltage for de-

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tection, +700 mV.

**Kinetic Analysis** The kinetic parameters of L-dopa and dopamine were estimated by the model-independent moment method of Yamaoka et al. In terms of plasma data, 5 rats corresponding to 5 time-points of 5, 15, 25, 37.5, and 52.5 min following the L-dopa injection, were regarded as one group (with a total of 6 groups using 30 rats). The values for lymph corresponding to the 5 periods of 0 to 10, 10 to 20, 20 to 30, 30 to 45, and 45 to 60 min following the L-dopa injection, were obtained from an identical animal (in total, 6 rats, respectively, with lymph collection from the thoracic duct and from the cervical lymph trunk).

**Statistics** Results are given as means ± S.E.M. Following an analysis of variance, statistical significance was assessed by t-test (Student's or Aspin-Welch's) for unpaired samples in terms of 2 groups, or by Bonferroni's method in terms of 3 groups: p values of less than 0.05 were considered significant.

**RESULTS**

**Effect due to Operative Invasion** The following two groups that did not receive intravenous injections of L-dopa were prepared: a “control group,” in which rats were only anesthetized; and an “operated group,” in which rats were anesthetized, and the junction of the left cervical lymph trunk and the thoracic duct was ligated, with the thoracic duct being cut peripherally to the ligated point so that the lymph could flow out freely. Blood was taken after completion of the operation.

In comparison to the “control group,” the plasma in the “operated group” revealed a decrease in dopamine and increases in norepinephrine and epinephrine (Table 1).

Next, blood and lymph were collected, respectively, at 60 min and for 60 min after the commencement of lymph collection in rats that had received thoracic duct-catheterization. Compared with the plasma of the “operated group” shown in Table 1, the plasma in the thoracic duct-catheterized rats showed further increases in L-dopa, dopamine, norepinephrine, and epinephrine (Table 2). Also, the thoracic duct-catheterized rats showed lower levels of dopamine, norepinephrine, and epinephrine in the lymph than in the plasma (Table 2).

**Lymph Flow in L-Dopa Administration** Lymph flow was determined following an intravenous bolus injection of L-dopa (2.5 mg/kg body weight).

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**Table 1. Changes in Levels of L-Dopa and Catecholamines in Plasma due to Operative Invasion**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Operated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Dopa</td>
<td>424 ± 32</td>
<td>398 ± 39</td>
</tr>
<tr>
<td>Dopamine</td>
<td>542 ± 44</td>
<td>172 ± 16e</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>127 ± 7</td>
<td>243 ± 22e</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>111 ± 14</td>
<td>200 ± 19e</td>
</tr>
</tbody>
</table>

“Control group,” anesthetized only; “operated group,” the rats were anesthetized, the junction of the left cervical lymph trunk and the thoracic duct was ligated, and the thoracic duct was cut peripherally to the ligated point so that lymph fluids could flow out freely. Blood was taken after completion of the operation. Values, means ± S.E.M. (n = 6). Statistics: a) in p < 0.01, compared with “control group.”

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There was no significant difference in lymph flow in the cervical lymph trunk between the control and the L-dopa-administered groups during the same observation periods (Fig. 1).

The lymph flow in the thoracic duct, in contrast, was lower in the L-dopa-administered group than in the control group during the periods of 30 to 45 and 45 to 60 min after the administration of L-dopa (Fig. 1).

**Plasma and Lymphatic Levels of L-Dopa and Dopamine**

**Table 2. Levels of L-Dopa and Catecholamines in Plasma and Lymph in Thoracic Duct-Catheterized Rats**

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Lymph from thoracic duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Dopa</td>
<td>651 ± 8b,2a</td>
<td>682 ± 21b,2o</td>
</tr>
<tr>
<td>Dopamine</td>
<td>422 ± 19b,2o</td>
<td>141 ± 6b,2o</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>558 ± 38b,2o</td>
<td>198 ± 4b,2o</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>455 ± 22b,2o</td>
<td>209 ± 12b,2o</td>
</tr>
</tbody>
</table>

The rats were anesthetized, and the thoracic duct was catheterized. Blood and lymph were taken, respectively, at 60 min and for 60 min after the commencement of lymph fluid collection. Values, means ± S.E.M. (n = 6). Statistics: a) in p < 0.05 and b) in p < 0.01, compared to the plasma of the “control group” in Table 1; c) in p < 0.01, compared to the plasma of “operated group” in Table 1; d) in p < 0.01, compared between plasma and lymph in thoracic duct-catheterized rats.

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**Fig. 1. Changes of Lymph Flow in the Cervical Lymph Trunk (A) and Thoracic Duct (B) Following an Intravenous Bolus of L-Dopa (2.5 mg/kg Body Weight)**

“Control,” control group; “L-Dopa,” L-dopa-administered group. Points and bars, means ± S.E.M. (n = 6). Statistics, between “control” and “L-Dopa”: a) p < 0.01. ---O---, control; - - - - , L-Dopa.
in L-Dopa Administration  Levels of L-dopa and amines in the plasma and the lymph were determined, after an intravenous bolus injection of L-dopa (2.5 mg/kg body weight).

Values for epinephrine and norepinephrine in the plasma were within the range of 1000 pg/ml. We tried to determine the L-dopa and amines in lymph that had been sampled consecutively from individual rats, as described above. However, since the volume of the samples was very small, only L-dopa and dopamine were detected in lymph samples obtained from the rats that had received L-dopa. Thus, in the findings described below, all describe L-dopa and dopamine in rats that had received the L-dopa administration.

L-Dopa in the plasma and the lymph from the thoracic duct reached the highest concentrations immediately after the L-dopa injection, at 5 min and 0 to 10 min, respectively, and then fell with elapsing time (Fig. 2). In contrast, L-dopa in the lymph from the cervical lymph trunk remained at a constant level (Fig. 2).

The values for dopamine were higher in the lymph from the cervical lymph trunk during the periods of 0 to 10, 10 to 20, and 45 to 60 min, compared with the levels in the thoracic lymph (Fig. 3). Dopamine levels in the cervical lymph increased, peaked at 10 to 20 min, and gradually fell thereafter (Fig. 3). Dopamine levels in the plasma and in the thoracic lymph showed the highest values immediately after the L-dopa injection, at 5 min and 0–10 min, respectively, and fell with time (Fig. 3). The dopamine levels in the plasma and the thoracic lymph were similar throughout the entire experimental process.

Kinetic Analysis of L-Dopa and Dopamine in Plasma and in Lymph in L-Dopa Administration  By analysis with a model-independent moment method, there were no significant differences in the area under the L-dopa concentration–time curve for the plasma, the thoracic lymph and the cervical lymph (Table 3). The cervical lymph had a longer mean residence time for L-dopa than the thoracic lymph (Table 3).

For dopamine, the cervical lymph had a larger area

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Lymph from thoracic duct</th>
<th>Lymph from cervical lymph trunk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng·h/ml) 199.7 ± 10.9</td>
<td>58.7 ± 4.4</td>
<td>389.8 ± 162.9</td>
</tr>
<tr>
<td>MRT (h) 0.318 ± 0.011</td>
<td>0.280 ± 0.014</td>
<td>0.448 ± 0.063</td>
</tr>
</tbody>
</table>

AUC, area under L-dopa concentration–time curve; MRT, mean residence time. Each parameter was calculated using values obtained at the time-points of 5, 15, 25, 37.5, and 52.5 min after L-dopa injection for plasma, and at periods of 0–10, 10–20, 20–30, 30–45 and 45–60 min after the injection for lymph. Values, means ± S.E.M. (n = 6). Other explanations, as described in Materials and Methods. Statistics: a) in p < 0.05, compared to “lymph from cervical lymph trunk.”

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Lymph from thoracic duct</th>
<th>Lymph from cervical lymph trunk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng·h/ml) 4.8 ± 0.2</td>
<td>6.2 ± 0.7</td>
<td>165.5 ± 55.9</td>
</tr>
<tr>
<td>MRT (h) 0.231 ± 0.015</td>
<td>0.311 ± 0.017</td>
<td>0.328 ± 0.026</td>
</tr>
</tbody>
</table>

AUC, area under dopamine concentration–time curve; MRT, mean residence time. Other explanations, as described in Table 3. Values, means ± S.E.M. (n = 6). Statistics: a) in p < 0.05, compared to “plasma”; b) in p < 0.01, compared to “lymph from cervical lymph trunk.”

Fig. 2. Changes in L-Dopa Levels in Plasma and in Lymph Obtained from the Cervical Lymph Trunk and Thoracic Duct Following an Intravenous Bolus of L-Dopa (2.5 mg/kg Body Weight)


Table 3. Kinetic Parameters of L-Dopa Concentration–Time Curves Based on the Model-Independent Moment Method

Table 4. Kinetic Parameters of Dopamine Concentration–Time Curves Based on the Model-Independent Moment Method
under the dopamine concentration–time curve than the thoracic lymph (Table 4). Both the cervical lymph and the thoracic lymph had longer values of mean residence time of dopamine than the plasma (Table 4).

**DISCUSSION**

Methodologically, the operative invasion induced by lymph duct-catheterization had a significant effect on the plasma levels of endogenous L-dopa and catecholamines (Tables 1 and 2). Nevertheless, our study, with the intravenous injection of a large dose of exogenous L-dopa (2.5 mg/kg body weight), revealed that levels of L-dopa and dopamine in the plasma and in the lymph (Figs. 2 and 3) were higher by two or more orders of magnitude than those found in the "operated group" (Tables 1 and 2). Thus, we concluded that we could ignore the alterations of L-dopa and dopamine in the plasma and the lymph fluid induced by the operative invasion in our study in which a large dose of exogenous L-dopa was administered.

Among the adverse effects of L-dopa, the most important early side effects are nausea and vomiting. One classic hypothesis for the manifestation of nausea and vomiting is the following: dopamine that is formed from L-dopa peripherally in the body stimulates the chemoreceptor trigger zone. If so, the dopamine level needs to be investigated in the interstitial fluid, which is in direct contact with the cells of the chemoreceptor trigger zone. However, in practice, it is technologically difficult to obtain interstitial fluid from this location. The collection of peripheral lymph, on the other hand, can be relatively easily achieved. Peripheral lymph is considered to provide a close approximation of the interstitial fluid. Thus, we considered that the lymph from the cervical lymph trunk partially reflected the interstitial fluid in the chemoreceptor trigger zone, and that lymph from the thoracic duct would reveal the extent of the peripheral formation of dopamine from L-dopa in organs including intestine, liver, and kidneys.

We found that L-dopa levels in the plasma and the thoracic lymph fluid were the highest immediately after the L-dopa injection, at 5 min and 0 to 10 min, respectively, and that L-dopa was eliminated from the plasma and from the thoracic lymph with a similar time-course (Fig. 2).

Regarding the similarity of the L-dopa elimination pattern between the plasma and the thoracic lymph, L-dopa is not bound to plasma proteins, and freely passes through the vessel walls into the interstitial spaces of peripheral tissues. This ease of L-dopa in passing through the vessel walls is not found in the blood-brain barrier. Accordingly, these findings and our result seem to imply that interstitial L-dopa also freely passes through the lymph vessel walls and diffuses into the lymph in the body, excluding the blood-brain barrier system.

Dopamine in the plasma and the thoracic lymph was the highest immediately after the L-dopa injection, at 5 min and 0 to 10 min, respectively, and was eliminated thereafter (Fig. 3). Further, the dopamine levels in the plasma and the thoracic lymph were similar throughout the entire experimental process (Fig. 3). Regarding the transformation of L-dopa to dopamine in the body, L-dopa is taken up into various organs, including the intestine, liver, and kidneys, through a "neutral amino acid transport system," and aromatic L-amino acid decarboxylase activity is high in these organs. This enzyme is present in the plasma, though it is not well known to what extent it is present in lymph. In the plasma, the prompt appearance of dopamine immediately following L-dopa injection can be attributed to the decarboxylation of L-dopa by the enzyme in the plasma rather than in any organs. On the other hand, with regard to thoracic lymph, our finding that the dopamine levels in plasma and lymph were similar immediately following L-dopa injection (Fig. 3), leads us to surmise that the enzyme is present in the lymph at the same level as it is in the plasma, and/or that the dopamine transformed from L-dopa passes freely through both blood vessel walls and the lymphatic vessel walls, resulting in equal diffusion into the plasma and lymph.

The effects of L-dopa on cervical lymph flow showed no significant differences between the control and the L-dopa-administered groups. In contrast, thoracic lymph flow was depressed during the periods 30 to 45 and 45 to 60 min following L-dopa administration (Fig. 1). Dopamine depresses lymphatic flow by constricting lymphatic smooth muscle. We surmised that our finding of a depressed thoracic lymph flow could be ascribed to the elevated levels of dopamine (Fig. 3). However, this did not seem to be applicable to the case of the cervical lymph duct, although the dopamine level was much higher in the cervical lymph than in the thoracic lymph (Fig. 3).

Our main findings were: (i) dopamine levels in the lymph from the cervical lymph trunk were higher than those from the thoracic duct during the periods of 0 to 10, 10 to 20, and 45 to 60 min after L-dopa administration (Fig. 3), (ii) the area under the dopamine concentration–time curve was larger in the cervical lymph than in the plasma or the thoracic lymph (Table 4), and (iii) the mean residence time of dopamine was longer in both the cervical lymph and the thoracic lymph than in the plasma (Table 4). Although the cervical lymph trunk is a terminal lymphatic vessel that gathers lymphatic and interstitial fluids of the head portion, the extent to which the lymph fluid contains lymphatic and interstitial fluids that are in contact with the chemoreceptor trigger zone remains to be investigated. We could not find any precedent reports concerned with this point, whereas our data and the anatomical location of the cervical lymph trunk indicate that the extent of content of the target fluids in cervical lymph is not negligible.

Further, our finding revealed that, following the L-dopa-administration, the dopamine level in cervical lymph was more than 10 times higher than that in thoracic lymph or in plasma (Fig. 3). It is known that aromatic L-amino acid decarboxylase shows high activity in brain capillaries, and that this enzyme in capillary endothelial cells plays a role in the enzymic blood-brain barrier, due to which little L-dopa reaches the parenchyma. Thus, high levels of dopamine remain outside the blood-brain barrier, although part of the dopamine is oxidized there by monoamine oxidase. Further, we found here that
cervical lymph flow was far slower than thoracic lymph flow (Fig. 1). In addition, the chemoreceptor trigger zone in the area postrema of the medulla is functionally outside the blood-brain barrier. Accordingly, the above reports and our findings (Fig. 3 and Table 4) suggest that: (i) when a large amount of L-dopa is intravenously injected, the high level of dopamine in the cervical lymph fluid reflects the higher level of dopamine outside the blood-brain barrier, and (ii) the chemoreceptor trigger zone is exposed longer with a higher concentration of dopamine. These may be among the mechanisms responsible for the clinically problematic early adverse effects of nausea and vomiting that occur when L-dopa is administered.

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REFERENCES