Capillary diffusion capacity and tissue distribution of pancreatic procolipase in rat

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Rippe, Catarina, Bengt Rippe, and Charlotte Erlanson-Albertsson. Capillary diffusion capacity and tissue distribution of pancreatic procolipase in rat. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1179–G1184, 1998.—The permeability-surface area product of procolipase and its apparent distribution volume in rat tissues were assessed using a tissue uptake technique. Procolipase was investigated together with 51Cr-EDTA, used as an inert extracellular marker, and 131I-albumin, used as a plasma volume marker. The tissue uptake of procolipase seemed to occur by passive transport in most of the organs studied, such as in muscle, liver, lung, adipose tissue, adrenal glands, colon, and skin. However, throughout the gastrointestinal tract, except in the colon, there was a high uptake of procolipase, greatly exceeding that of 51Cr-EDTA. This was especially evident in the colon, and 131I-albumin, used as a marker of the plasma volume. Because 51Cr-EDTA is a small solute (molecular radius ~ 4.7 Å), it is widely used to measure extracellular fluid volume. On the other hand, albumin is a protein that does not easily pass through the capillary walls. Therefore, it is essentially retained in the circulation and can be used as a marker of the plasma volume. Procolipase is a small protein with a molecular mass of 10 kDa (molecular radius ~ 17 Å). Hence, a tissue uptake technique (6, 24) should be ideal to study its transcapillary transport capacity [permeability-surface area product (PS)], and this method was used in the present study.

METHODS

Male Wistar rats weighing 267 ± 22 g were obtained from Møllegaard (Denmark). Until the experiments were performed, the animals had free access to food and water. Anesthesia was introduced by intraperitoneal injection of pentobarbital sodium (50 mg/kg). The tail artery was cannulated with a polyethylene catheter (PE-50) and used for blood pressure registration (via a pressure transducer coupled to a polygraph; model 7B, Grass Instruments). The left carotid artery and the left jugular vein were also cannulated with PE-50 catheters, the former used for blood sampling and the latter for tracer injections. To maintain a constant body temperature, the rats were placed on a heating pad.

Labeling procedures. Procolipase was labeled with 125I using the chloramine-T method (10). The reaction mixture contained 10 μg porcine procolipase (15), 130 MBq 125I, and 2 μg chloramine-T and was incubated for 5 min on ice. 125I-procolipase was separated from free 125I on a PD-10 Sephadex G-25 M column (Pharmacia, Uppsala, Sweden). 51Cr-EDTA was purchased from Amersham and 131I-albumin from Kjeller. Before each experiment, 131I-albumin and 125I-procolipase were purified from free iodine, using centrifugal filtration (Microcon 30 and Microcon 10, Amicon).

Experimental procedures. Three groups of animals were used in the experiment, as indicated in Table 1. One group (group A) was given 131I-procolipase and 51Cr-EDTA simultaneously, using 51Cr-EDTA as an extracellular marker. Another group (group B) was administered with 125I-procolipase together with 131I-albumin to evaluate the plasma volume in the tissue simultaneously with the uptake of procolipase. The last group (group C) was given 131I-albumin alone for complementary information on albumin distribution. We gave ~ 70 kBq 125I-procolipase as a bolus dose together with either ~ 70 kBq 131I-albumin (group B) or ~ 50 kBq 51Cr-EDTA (group A). Simultaneously, an infusion of the tracers diluted in physiological saline was given (3 ml/h) to maintain the plasma concentration of the injected tracers constant over time. The
The plasma equivalent space vs. time curve was used as $V_0$ in the equation
\[ V(t) = V_0 + V_E (1 - e^{-k t}) \] (1)

where $V_0$ represents the distribution volume of tracer at time 0, which, in the absence of endothelial binding, equals the regional plasma volume. The intercept with the $y$-axis of the albumin space vs. time curve was used as $V_0$ in the equation above. $V_E$ is the equilibration distribution volume, which was integrated to get the area under the curve (AUC). To calculate the tissue uptake, the accumulated amount of tracer in the tissue ([counts per minute (cpm)]/g) was divided by the average plasma concentration of tracer (cpm/ml) obtained from the AUC, to yield the "plasma equivalent" space. This space was then followed as a function of time. The plasma equivalent space for a molecule as large as albumin can with a small space for a molecule as large as albumin can with a small

The tracer plasma equivalent space as a function of time to an arbitrary mathematical function, which was approximated to $Q/V_E$ (12).

where $k$ approximates to $Q/V_E$ (12).

Statistics. The plasma equivalent space vs. time curve was assessed by fitting the experimental data $V(t)$ to Eq. 1, using nonlinear least-squares regression analysis (Microcal Origin 4.0, Microcal Software). The values for the parameters $V_E$ and $PS$ are given with their respective standard errors.

RESULTS

Plasma concentration curves of tracers. Figure 1 shows the plasma concentration of the radiolabeled proteins as a function of time. The plasma concentration of $^{131}$I-albumin decreased with a rate of 19%/h, which was slightly larger than we expected, and was due to the equilibration with the extravascular space. For both $^{125}$I-procolipase and $^{51}$Cr-EDTA, a decrease in concentration was observed over the first 20 min followed by an increase in plasma concentration that reached a stable value 40 min after the start of tracer infusion.

$V_E$ and $PS$. $V_E$ and $PS$ for the different tissues are shown in Table 2. The rate of $^{51}$Cr-EDTA accumulation was lower than expected for a molecule having a radius of 4.7A, conceivably due to blood flow limitation. Therefore only an apparent $PS (V_E k)$ is given for $^{51}$Cr-EDTA in Table 2.

In muscle tissue (biceps femoris, gastrocnemius, and tibialis anterior), the uptake of procolipase seemed to be entirely passive in nature. $^{51}$Cr-EDTA reached a blood-tissue equilibrium ($V_E$) after 20–30 min, whereas $^{125}$I-procolipase, which had a much slower uptake, achieved equilibrium first after 80–100 min (Fig. 2). Both $^{125}$I-procolipase and $^{51}$Cr-EDTA had similar $V_E$ and their uptake rates were as large as expected considering their different sizes assuming diffusive transport. However, the albumin space can be regarded as an indicator of the $V_D$.

Similar passive distribution patterns were achieved for colon, lung, adrenal glands, skin, and adipose tissue (not shown). In these tissues there was no indication of

![Fig. 1. Relative plasma concentrations of tracers (means ± SE) as a function of time. Plasma concentrations of tracers were standardized to the given dose. $^{125}$I-procolipase and $^{51}$Cr-EDTA were given as a bolus dose followed by continuous infusion, whereas $^{131}$I-albumin was given as a bolus dose only.](http://apgi.physiology.org/)

<table>
<thead>
<tr>
<th>Infusion Time, min</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>255 ± 8 (5)</td>
<td>263 ± 6 (5)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>277 ± 9 (4)</td>
<td>244 ± 8 (3)</td>
<td>254 ± 1 (2)</td>
</tr>
<tr>
<td>25</td>
<td>271 ± 25 (4)</td>
<td>251 ± 0 (2)</td>
<td>271 ± 27 (3)</td>
</tr>
<tr>
<td>40</td>
<td>270 ± 3 (4)</td>
<td>274 ± 9 (3)</td>
<td>250 (1)</td>
</tr>
<tr>
<td>80</td>
<td>236 ± 38 (4)</td>
<td>237 ± 12 (4)</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>252 ± 37 (4)</td>
<td>283 ± 6 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; $n$ is the no. of rats given in parentheses. Group A, rats infused with $^{125}$I-procolipase and $^{131}$Cr-EDTA. Group B, rats infused with $^{125}$I-procolipase and $^{131}$I-albumin. Group C, rats injected with $^{125}$I-albumin alone.

infusion contained either $^{125}$I-procolipase alone or $^{125}$I-procolipase and $^{51}$Cr-EDTA. Because albumin is largely retained in the circulation and disappears at ∼12–17%/h (23, 28), there is no obvious need for any infusion of the protein. Blood samples (25 µl) were collected at 1, 3, 5, 8, 16, 25, 40, 60, 80, 120, and 160 min after tracer infusion. The blood was transferred into tubes and counted in the gamma counter. Before and during the experiment, hematocrit (50 µl) was measured to convert blood concentration to plasma concentration.

Before and during the experiment, hematocrit (50 µl) was measured to convert blood concentration to plasma concentration. The animals were killed at 3, 8, 25, 40, 80, and 160 min by an intravenous injection of saturated KCl. The following tissues were dissected: biceps femoris muscle, gastrocnemius muscle, tibialis anterior muscle, stomach, pancreas, ileum, duodenum, colon, lung, liver, adrenal glands, skin, adipose tissue, and brain (cerebrum and cerebellum). All tissues were immediately collected in tarred test tubes, weighed, and counted in a gamma counter. The number of animals in each experimental group and the body weights are shown in Table 1.

Calculations. The average plasma concentration was calculated by fitting the plasma concentration curve as a function of time to an arbitrary mathematical function, which was integrated to get the area under the curve (AUC). To calculate the tissue uptake, the accumulated amount of tracer in the tissue ([counts per minute (cpm)]/g) was divided by the average plasma concentration of tracer (cpm/ml) obtained from the AUC, to yield the "plasma equivalent" space. This space was then followed as a function of time. The plasma equivalent space for a molecule as large as albumin can with a small error be regarded as the intravascular distribution volume ($V_0$). Thus a blood-rich organ will have a large albumin space, which should be accounted for. The tracer plasma equivalent space as a function of time can be described as

![Table 1. Body weight in different experimental groups](http://apgi.physiology.org/)
specific uptake of procolipase, even though the $V_E$ of procolipase seemed higher than that of $^{51}$Cr-EDTA at 180 min (shown in Table 2). However, there was no difference at 80 min.

In the liver, after a high immediate uptake into Disse's space of $^{51}$Cr-EDTA, $^{131}$I-albumin, and $^{125}$I-procolipase, the subsequent uptake of $^{125}$I-procolipase and $^{51}$Cr-EDTA was very low, exhibiting a clearance of 0.50 and 0.30 ml min$^{-1}$·100 g$^{-1}$, respectively.

Figure 3 shows the plasma equivalent space as a function of time in stomach, pancreas, duodenum, and ileum. In these tissues, we found a much higher uptake of $^{125}$I-procolipase than of $^{51}$Cr-EDTA. This was especially prominent in the stomach, in which a high plasma equivalent space for $^{125}$I-procolipase was detected. Actually, $^{125}$I-procolipase did not attain equilibrium during the experimental period, i.e., during 160 min. The pancreas, duodenum, and ileum showed a similar tissue uptake pattern, with a higher uptake of $^{125}$I-procolipase than of $^{51}$Cr-EDTA. In these tissues, there was, however, indication of saturation after 160 min. The uptake of $^{125}$I-procolipase in these organs differs from the passive behavior of procolipase in muscle, suggestive of active transport in the gastrointestinal tract (except for the colon).

There was no significant uptake across the blood-brain barrier (BBB) of $^{51}$Cr-EDTA or $^{131}$I-albumin during the experimental period. The uptake of $^{125}$I-procolipase was, however, considerably higher than that of $^{131}$I-albumin. The PS in cerebral and cerebral was 0.052 ± 0.005 and 0.043 ± 0.009 ml min$^{-1}$·100 g$^{-1}$, respectively. The apparent $V_E$ for cerebral and cerebral during the observation period was 2.1 ± 0.09 and 2.5 ± 0.3 ml/100 g, respectively, which was much higher than the plasma volume (0.46 ± 0.06 and 0.89 ± 0.06 ml/100 g). This indicates a selective transport of procolipase into the brain tissue of the rat (Fig. 4).

**DISCUSSION**

We have collected data strongly suggesting that pancreatic procolipase, when injected into the bloodstream, has specific transport mechanisms in the capillaries of the upper gastrointestinal tract, and surprisingly, also in the brain. In the other tissues examined, e.g., muscle tissue, the uptake of procolipase was completely passive. In the liver, an instantaneous equilibration of $^{125}$I-procolipase, $^{51}$Cr-EDTA, and $^{131}$I-albumin occurred, probably with Disse's space. This was followed by a slower uptake, presumably across the interstitium, of both $^{125}$I-procolipase and $^{51}$Cr-EDTA, which could be due to their excretion into the bile (16).

Throughout the gastrointestinal tract (the stomach, pancreas, duodenum, and ileum), there was a much higher uptake of $^{125}$I-procolipase than of $^{51}$Cr-EDTA. Procolipase was hence rapidly taken up to these tissues and its $V_E$ greatly exceeded that of $^{51}$Cr-EDTA, a criteria for active transport. Because $V_E$ was larger for $^{125}$I-procolipase than for $^{51}$Cr-EDTA, $^{125}$I-procolipase seemed to be transported against a concentration gradient, although there is also a slight possibility of tissue binding of tracer. This suggests that there may be active transport of procolipase from the bloodstream to the gastrointestinal tract and/or tissue binding. Fenestrated capillaries exist throughout the gastrointestinal tract and offer a large surface area for transport of small and large solutes but not a reduced selectivity to macromolecules and polypeptides. This is in line with the results of our study. There was indeed a higher uptake of $^{51}$Cr-EDTA in the gastrointestinal tract than,

Table 2. Distribution volume and permeability-surface
area product

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$V_E$, ml/100 g</th>
<th>PS, ml·min$^{-1}$·100 g$^{-1}$</th>
<th>$V_E$, ml/100 g</th>
<th>$V_{k1}$, ml·min$^{-1}$·100 g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior muscle</td>
<td>6.5 ± 0.5</td>
<td>0.15 ± 0.02</td>
<td>5.0 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Biceps femoris muscle</td>
<td>6.0 ± 0.4</td>
<td>0.15 ± 0.02</td>
<td>5.3 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>6.4 ± 0.5</td>
<td>0.19 ± 0.02</td>
<td>5.6 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Stomach</td>
<td>NSAT</td>
<td>NSAT</td>
<td>17.0 ± 0.8</td>
<td>4.5 ± 1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>32 ± 2</td>
<td>0.68 ± 0.008*</td>
<td>16 ± 2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Duodenum</td>
<td>39 ± 3</td>
<td>0.82 ± 0.2*</td>
<td>11 ± 0.4</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Ileum</td>
<td>32 ± 5</td>
<td>0.50 ± 0.1*</td>
<td>11 ± 1</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Colon</td>
<td>23 ± 3</td>
<td>0.61 ± 0.2</td>
<td>17 ± 2</td>
<td>3.9 ± 2</td>
</tr>
<tr>
<td>Lung</td>
<td>27 ± 5</td>
<td>0.67 ± 0.2</td>
<td>16 ± 0.9</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>32 ± 7</td>
<td>0.50 ± 0.2†</td>
<td>24 ± 7</td>
<td>0.30 ± 0.1†</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>20 ± 2</td>
<td>0.50 ± 0.06</td>
<td>12 ± 2</td>
<td>1.7 ± 0.9</td>
</tr>
<tr>
<td>Skin</td>
<td>43 ± 2</td>
<td>0.82 ± 0.04</td>
<td>31 ± 3</td>
<td>4.8 ± 2</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>11 ± 2</td>
<td>0.091 ± 0.01</td>
<td>9 ± 1</td>
<td>0.38 ± 0.1</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.5 ± 0.3</td>
<td>0.043 ± 0.009</td>
<td>NU</td>
<td>NU</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>2.1 ± 0.09</td>
<td>0.052 ± 0.005</td>
<td>NU</td>
<td>NU</td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_E$, equilibration volume; PS, permeability-surface area product; NSAT, nonsaturable active transport. NU, no uptake to tissue. *Saturable active transport or tissue binding. †Values for liver presumably represent equilibration between Disse's space and the interstitium.
for example, in muscle. However, the much higher uptake of $^{125}$I-procolipase compared with that of $^{51}$Cr-EDTA cannot be explained by an increased permeability of fenestrated capillaries. A significant finding of the present study is the high amount of $^{125}$I-procolipase taken up by the stomach. Actually, this uptake did not reach saturation within the experimental period (160 min). Instead, there was a linear uptake as a function of time. This again suggests that procolipase is actively transported from the circulation to the stomach. Recently, Sörhede et al. (25, 26) showed, with in situ hybridization and immunohistochemical methods, that procolipase is produced in chief cells of gastric fundus. It could be speculated that circulating procolipase acts as a feedback signal on the production of gastric procolipase.

A major finding of the present study was the data supporting the uptake of procolipase across the BBB. Passive diffusion of procolipase over the BBB is not likely to occur due to the tight junctions that characterize BBB selectivity. Instead, a specific mechanism is suggested to mediate the uptake of procolipase to the brain. In recent years, receptors for peptides and other macromolecules have been identified in isolated brain capillaries (19) and hypothesized to mediate transcytosis. Furthermore, recent studies have indicated that circulating peptides or proteins may undergo receptor-mediated transport through the BBB. This has been suggested for ligands, such as insulin (7), transferrin (9) and leptin (1). A comparison of results with those from another study (20), in which the cerebellar uptake of a number of proteins and polypeptides was examined, shows that the PS for procolipase is 2.4 times higher than that for transferrin but three times lower than for insulin (20). The influx of leptin into the brain was in the same range as for procolipase and was also saturable (1). Although, it has been known for some
time that insulin and certain other hormones can be selectively transported across the BBB, the present results were completely unexpected. It can only be speculated as to why procolipase, which is present in serum in very low concentrations, has a certain specific uptake pathway across the BBB. A specific uptake may have implications for conditions when a large circulating pool of pancreatic enzymes occurs, such as during acute pancreatitis. Pancreatic proteins are normally secreted toward the apical region but constitutive-like pathways also direct secretion toward the basolateral membrane (5). This secretion could explain how pancreatic proteins enter the circulation. A recent study (5) has shown that this secretion could be increased by maximal stimulation with CCK-8. Hence, procolipase may have some yet unknown actions in the CNS, related to lipid metabolism or postprandial conditions after high-fat feeding. Alternatively, procolipase in the brain may serve as a precursor for the peptide enteropeptin mediating satiety through central mechanisms (2).

Among physiologists, active transendothelial transport of macromolecules is a controversial issue. Several authors have persistently argued against active transcytosis as a major mechanism of protein transport between blood and tissue (21, 22, 27). Their evidence is based on a large number of lymph flux analyses and tissue uptake studies in various organs. It should, however, be pointed out that the studies on which these conclusions were reached were all concerned with transcapillary passage of "bulk" plasma proteins, mostly albumin, fibrinogen, α2-macroglobulin, and some immunoglobulins. However, with regard to insulin, for example, it was pointed out that it differed markedly in its transport from inert probes, such as inulin, which is of equal size but has no specific function in the human body (11). Above all, it has been shown that insulin can strongly bind to the endothelium even in vivo (11) and it can be transported more or less intact across the endothelium by active mechanisms (14). With regard to procolipase, it seemed to be specifically transported or bound to the endothelium of the upper gastrointestinal tract and the CNS, which strongly indicates specialized modes of action in these target organs.

As expected, 125I-procolipase was taken up passively in muscle (Fig. 2). The rate of tissue uptake was eightfold higher for 51Cr-EDTA than for 125I-procolipase. In an aqueous solution, 51Cr-EDTA diffuses at a rate 8.7-fold higher than 125I-procolipase, depending on its smaller size (molecular radius 4.7 Å for 51Cr-EDTA vs. ~17 Å for 125I-procolipase). According to the theory of restricted diffusion through a membrane having cylindrical pores (18), an eightfold lower transport of a 17 Å molecule compared with that of 51Cr-EDTA can be expected, if the equivalent capillary pore radius were 75 Å (6). However, since 51Cr-EDTA was most likely flow limited in its transport, this pore radius is an overestimate. The measured PS for 51Cr-EDTA in muscle was thus only 1.2 ml·min⁻¹·100 g⁻¹, whereas the expected value is 2–3 ml·min⁻¹·100 g⁻¹ in nonvasoilated tissue (6, 17). From this comparison, one can calculate that plasma flow would have been 1.1–1.2 ml·min⁻¹·100 g⁻¹ in our rats and that PS for 51Cr-EDTA was markedly underestimated, whereas PS for 125I-procolipase was near zero. Thus if plasma flow (Q) was marked, then PS/Q for procolipase is near zero, which by being <0.33 ensures nonflow-limited conditions (6).

In conclusion, the present study has demonstrated specific uptake of circulating pancreatic procolipase in the gastrointestinal tract and brain tissue of the rat. In most other tissues, the uptake was passive according to current concepts. The specific uptake of procolipase across the BBB is surprising and intriguing and therefore deserves further investigation.

We are grateful to Anna Rippe (Departments of Nephrology and Physiology, University Hospital of Lund) for excellent technical assistance.

This work was made possible through grants from the Swedish Medical Research Council (07094 and 08285), A. Pålsson’s Foundation, and The Swedish Nutrition Foundation.

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Received 23 February 1998; accepted in final form 24 July 1998.

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