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Enhancement of Blood–brain Barrier Permeability by Sodium Caprate

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Abstract

We examined the effect of sodium caprate on the integrity of the tight junctions of brain capillary endothelial cells, using a modified in-situ brain perfusion technique. Model hydrophilic compounds used were [^{3}H]mannitol, [^{14}C]sucrose, [^{3}H]PEG900, [^{3}H]PEG4000, FITC-dextran 4000, FITC-dextran 20 000 and FITC-dextran 70 000.

The brain distribution volume of [^{14}C]sucrose was significantly increased by sodium caprate in a concentration-dependent manner. The effective minimum concentration was 10 mM. Furthermore, the effects of sodium caprate on the distribution volumes of hydrophilic compounds, [^{3}H]mannitol, [^{14}C]sucrose, [^{3}H]PEG900, [^{3}H]PEG4000, FITC-dextran 4000, FITC-dextran 20 000 and FITC-dextran 70 000, showed a molecular weight dependence. A plot of apparent permeation clearance against diffusion coefficient values suggested that 20 mM sodium caprate induced pores so large that the above compounds could pass through the blood–brain barrier with negligible friction within the pore.

Our results showed that intracarotid sodium caprate perfusion could enhance the permeation of hydrophilic compounds through the blood–brain barrier.

The blood–brain barrier, which is mainly composed of brain capillary endothelial cells, is characterized by the presence of intercellular tight junctions in a continuous nonfenestrated layer of endothelial cells (Cornford 1985). The blood–brain barrier serves to restrict the transfer of exogenous compounds, especially hydrophilic drugs, into the brain. To overcome this limitation, some researchers have tried to enhance the blood–brain barrier permeability to hydrophilic drugs in order to enhance the therapeutic effect in brain. One possible approach is to open transiently the tight junctions of the brain capillary endothelial cells. Infusion of a mannitol solution of high osmotic pressure into brain arteries is known to open the tight junctions (Neuwelt & Rapoport 1984). Some other compounds such as a bradykinin agonist (RMP-7) (Inamura et al 1994; Bartus et al 1996) and histamine (Butt 1995) are also effective. Recently, Saija et al (1997) reported that sodium dodecyl sulphate, an anionic surfactant, enhanced the blood–brain barrier permeability of [^{14}C]aminoisobutyric acid, but the mechanism was not established. Our group has found that sodium caprate and palmytoylcarnitine enhanced the intestinal absorption of hydrophilic compounds by opening the tight junction of intestinal epithelial cells (Tomita et al 1996). In this study, we have examined the effect of sodium caprate on the blood–brain barrier permeability to a series of hydrophilic compounds.

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Materials and Methods

Animals

Male Wistar rats weighing 200–250 g (Japan SLC Co., Shizuoka, Japan) were used. They were allowed free access to food and water until the experiment.

Chemicals

[^{14}C(U)]Sucrose (4·7 mCi mmol^{-1}), [^{3}H]PEG900 (1·8 mCi mmol^{-1}) and [^{3}H]PEG4000 (1·5 mCi

$mmol^{-1}$) were purchased from New England Nuclear (Boston, MA). [^{3}H]Mannitol (15 Ci $mmol^{-1}$) was from American Radiolabeled Chemicals Inc. (St Louis, MO). Sodium caprate, FITC-dextran 4000 (MW 4400), FITC-dextran 20 000 (MW 19 600) and FITC-dextran 70 000 (MW 71 200) were from Sigma Chemical Co. (St Louis, MO). All other chemicals were commercial products of reagent grade.

Experimental procedure

The effect of sodium caprate on brain capillary endothelial cell tight junctions was measured by the internal carotid artery perfusion technique (Takasato et al 1984) with minor modifications as follows. Adult male rats were anaesthetized with ketamine (235 mg kg^{-1}, i.m.) and xylazine (2·3 mg kg^{-1}, i.m.). After coagulation of branching vessels, the right external carotid artery was catheterized for retrograde perfusion with polyethylene catheters (PE-50) filled with sodium heparin (100 int. units mL^{-1}). For the measurements, the right common carotid artery was ligated approximately 1 s before perfusion. Perfusion fluid (in mM: NaCl 142·0, $NaHCO_3$ 28·0, KH_2PO_4 4·2, $CaCl_2$ 1·7, $MgSO_4$ 1·0) containing a radiolabelled compound and an FITC-dextran was then infused at a constant rate (1 mL min^{-1} instead of 4·98 mL min^{-1} (Takasato et al 1984)) by a perfusion pump. Immediately after the start of perfusion, the left common carotid artery was ligated. Rats were decapitated at appropriate times, and the amount of the test compound in the brain was assayed. The distribution volume in brain (Vd), extraction ratio (E), and the apparent permeation clearance (PS_{app}) were calculated by the use of equations 1, 2 and 3, respectively:

$$Vd = C_{brain}/C_{puf} \quad (1)$$

$$E = C_{brain}/(F \times C_{puf} \times T) \quad (2)$$

$$PS_{app} = -F \times \ln(1 - (E_{treat} - E_{control})) \quad (3)$$

where C_{brain} and C_{puf} are the concentration of the substrate in the brain (nCi $(g\,brain)^{-1}$ or ng $(g\,brain)^{-1}$), and in the perfusate (nCi mL^{-1} or ng mL^{-1}), respectively, and F and T are the perfusion rate (mL min^{-1} $(g\,brain)^{-1}$) and the perfusion period (min), respectively. E_{treat}, extraction in the sodium caprate-treated group, and $E_{control}$, extraction in the control, are the measured extractions with or without sodium caprate, respectively. The PS_{app} was corrected for the apparent vascular space estimated from $E_{control}$. We expressed the perfusion rate as the pump flow rate divided by brain weight, because the perfusion rate was not directly measured in this study.

Assay method

For animals treated with radiolabelled compounds, the ipsilateral hemisphere of the perfused side of the brain was weighed and solubilized in 2 mL Soluene-350 (Packard Ins. Co., Meriden, CT) at 60°C for 3 h. The sample was decolourized with 30% H_2O_2 and neutralized with 5 M HCl. Ten millilitres of scintillation fluid (Clear-sol I; Nacalai Tesque, Kyoto, Japan) was then added to the sample for the determination of the radioactivity on an Aloka 3000 liquid scintillation counter. For FITC-dextrans, the ipsilateral hemisphere of the perfused side was weighed and homogenized in 50 mM Tris buffer solution (pH 7·4). The homogenate was centrifuged (3000 rev min^{-1}, 30 min), methanol was added to the supernatant (1 : 1), and the mixture was centrifuged again under the same conditions. The fluorescence intensity in the supernatant was determined with a Hitachi 650 fluorospectrometer (emission 450 nm, excitation 560 nm).

Statistical analysis

The data were statistically analysed using Dunnett's test after one-way analysis of variance. The criterion of significance was taken as $P < 0{\cdot}05$.

Results

Effect of perfusion period on the distribution volume of [^{14}C]sucrose

A distribution volume that is larger than the control value indicates that a compound permeates out of the brain vessels. Thus, the effect of perfusion period on the distribution volume was examined. A [^{14}C]sucrose solution was infused into rat brain from the external carotid artery and the distribution volume of [^{14}C]sucrose was calculated (eqn 1). Since the perfusion rate was low, the brain was not filled with perfusate immediately (Figure 1A). Thus, the contra-lateral carotid artery was ligated immediately after perfusion started. As shown in Figure 1B, the distribution volume of [^{14}C]sucrose did not change until 5 min after the start of perfusion of isotonic sucrose solution in the absence of sodium caprate, even though the perfusate would have filled the brain within 1 min. The obtained volume of 4–5 μL $(g\,brain)^{-1}$ was smaller than the reported values (8–15 μL $(g\,brain)^{-1}$) of the volume of blood vessels. This may be because of

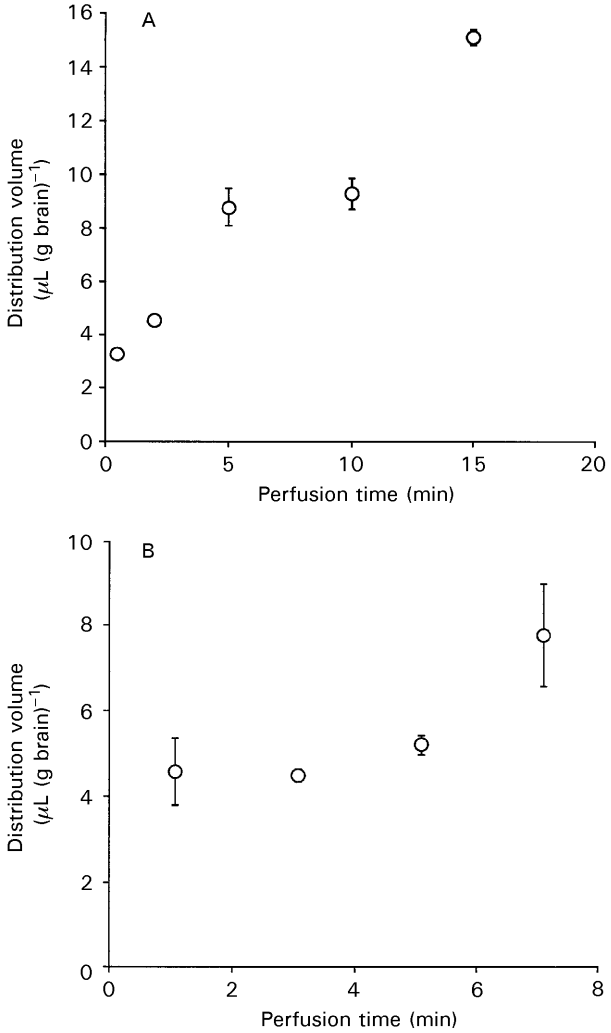

Figure 1. Effect of perfusion period on the distribution volume of [^{14}C]sucrose during in-situ brain perfusion. A. The distribution volume of [^{14}C]sucrose is plotted against the perfusion time. The perfusion flow rate was 1·0 mL min^{-1}. B. The perfusion flow rate was 1·0 mL min^{-1}. Immediately after the start of perfusion, the left common carotid artery was ligated. Each point represents the mean ± s.e. of three to four animals. When the s.e.m. is not indicated by a bar, it is smaller than the symbol.

the low perfusion pressure. However, the observed distribution volume tended to increase after 7-min perfusion, and therefore a 5-min perfusion time was adopted in the following experiments.

Effect of sodium caprate on the distribution volume of [^{14}C]sucrose

The distribution volumes were determined after 5-min perfusion of [^{14}C]sucrose solution containing various concentrations of sodium caprate. The distribution volume was increased by concentrations of sodium caprate greater than 10 mM (Figure 2).

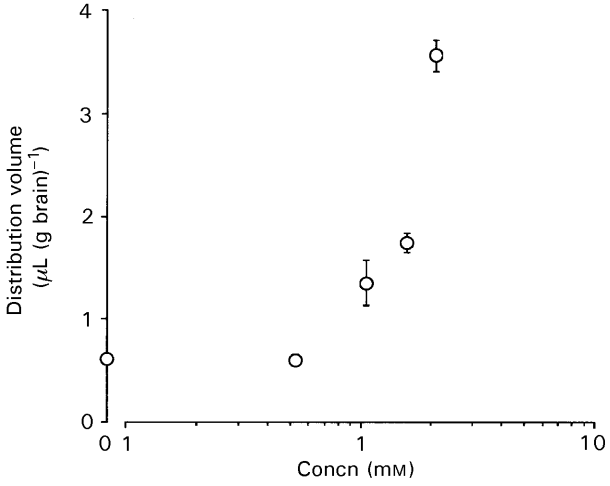

Figure 2. Dependence of the distribution volume of [^{14}C]sucrose on the concentration of sodium caprate. Each point represents the mean ± s.e. of three to twenty-one animals.

Effect of sodium caprate on the distribution volumes of hydrophilic compounds

The effects of sodium caprate on the distribution volumes of various hydrophilic compounds were determined in the same manner as described for [^{14}C]sucrose (Table 1). When 20 mM sodium caprate was infused, the distribution volumes of all the tested compounds were significantly increased from the control value, though the ratio of the increase over the control tended to decrease as the molecular weight increased. Apparent permeation clearances of the hydrophilic compounds, calcu-

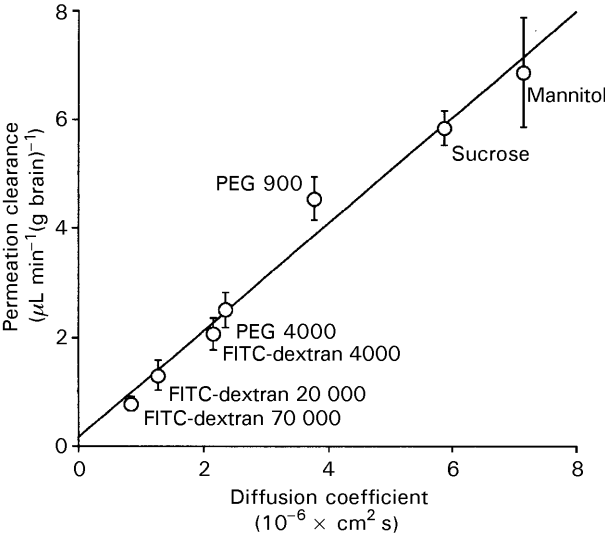

Figure 3. The relationship between permeation clearance and diffusion coefficient. The diffusion coefficients were calculated from the Stokes–Einstein equation for equivalent spheres as described by Adson et al (1996). The regression line was calculated by the least-squares method ($y = 0{\cdot}98x - 0{\cdot}14$, $r^2 = 0{\cdot}981$). Each point represents the mean ± s.e. of three to twenty-one animals.

Table 1. The distribution volumes of several hydrophilic compounds.

Substrate	MW	Distribution volume (μL (g brain)$^{-1}$)	
		Control	Sodium caprate (20 mM)
Mannitol	182·2	5·65 ± 0·37	39·6 ± 5·1*
Sucrose	342·3	5·52 ± 0·29	35·1 ± 1·5*
PEG900	900	5·51 ± 0·69	30·7 ± 1·1*
PEG4000	4000	4·90 ± 0·51	17·6 ± 1·7*
FITC-dextran 4000	4400	4·43 ± 0·58	14·8 ± 1·4*
FITC-dextran 20 000	19 600	3·07 ± 0·24	9·93 ± 1·08*
FITC-dextran 70 000	71 200	3·02 ± 0·23	6·61 ± 0·70*

Each value represents the mean ± s.e. of three to four experiments. *$P < 0·05$.

lated by using equation 3, were plotted against the corresponding diffusion coefficients according to Adson et al (1996) (Figure 3). A linear regression line passing near the origin was obtained.

Discussion

The tight junctions of brain capillary endothelial cells strictly restrict the permeation of hydrophilic compounds through the blood–brain barrier. The present results show that the blood–brain barrier permeability to hydrophilic compounds was enhanced by sodium caprate. Our group had found that sodium caprate could open the tight junctions of intestinal epithelial cells (Tomita et al 1996), and our present result suggests that it also opens those of brain capillary endothelial cells.

Several mechanisms can account for increased blood–brain barrier permeability. These include increased vesicular transport, increased transcellular penetration, and increased opening of the tight junctions of brain capillary endothelial cells. Increased vesicular transport should affect the permeability of the tracers to similar extents, as vesicles should not distinguish molecules with radii of less than approximately 1/10 of the vesicular radius. In contrast, increased opening of the tight junctions of brain capillary endothelial cells should affect the permeability of the tracers in a molecular size-dependent manner. Table 1 shows that the increase of permeability caused by sodium caprate declines with increasing molecular size. Therefore, this result suggests that the tight junctions of brain capillary endothelial cells were opened by sodium caprate. Since the E_{treat} of FITC-dextran 70 000 was significantly increased over the control, it appears that the tight junctions opened widely enough to allow permeation of a compound of 30 Å radius (calculated by use of the Stokes–Einstein equation). The linear plot that passes near the origin in Figure 3 suggests that 20 mM sodium caprate generated pores in the blood–brain barrier that were wide enough for the compounds to pass though with negligible friction within the pore. Accordingly, the pore radius produced with 20 mM sodium caprate could not be calculated using the Renkin function.

Interestingly, in this study, the minimum effective concentration of sodium caprate was 10 mM. In our previous study (Tomita et al 1996), more than 5 mM sodium caprate opened the tight junctions of the intestine. Thus, sodium caprate had essentially the same effect on tight junctions of brain capillary endothelial cells and intestinal epithelial cells.

In conclusion, the present results obtained by using an in-situ rat brain perfusion technique show that intracarotid sodium caprate perfusion can enhance the permeation of hydrophilic compounds through the blood–brain barrier. Further study is needed to examine the time course of recovery of the blood–brain barrier integrity.

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