Effect of Carrageenan-Induced Acute Peripheral Inflammation on the Electrolyte Disposition to Cerebrospinal Fluid in Rats

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To clarify whether peripheral inflammation has a remote effect on the central nervous system, the electrolyte disposition between the circulating blood and central nervous system was evaluated in rats with carrageenan-induced acute peripheral inflammation (API). \(\lambda\)-Carrageenan was subcutaneously injected in the hind paw of the rat, and lithium was utilized as a surrogate marker of sodium. When the plasma and cerebrospinal fluid (CSF) concentrations of lithium were examined following lithium being intravenously administered, it was revealed that the CSF concentration of lithium in API rats is reduced compared to that in normal rats, while the plasma concentration profile of lithium in API rats is indistinguishable from that in normal rats. The pharmacokinetic analysis showed that the lithium disposition from the plasma to CSF markedly decreased by 35.8% in API rats compared to that in normal rats. On the other hand, when lithium was immediately administered into the lateral ventricle, its elimination profiles in CSF were not different between normal and API rats. It is therefore probable that the lithium disposition from the plasma to CSF alters in API rats, reflecting the process of electrolytes from the circulating blood to brain tissue being suppressed in response to peripheral inflammation.

Key words carrageenan; cerebrospinal fluid; lithium; microdialysis; peripheral inflammation

It has been reported that the influence of peripheral inflammatory disease reaches far beyond the affected region, and it often causes the functional impairment of remote, unrelated organs. For example, the mortality of patients suffering from ischemic heart disease is closely associated with whether they have/do not have periodontal disease.\(^1,2\) The presence of inflammation in dialysis patients increases mortality from cardiovascular disease.\(^3\) Besides these somatic influences, peripheral inflammation may have an adverse effect on the function of the central nervous system. It was demonstrated that turpentine-induced inflammation in the hind paw activates the anterior pituitary gland to increase the plasma adrenocorticotropic hormone (ACTH) concentration in mice,\(^4\) and that inflammatory colitis enhances seizure susceptibility affecting the electrolyte disposition between the circulating blood and brain tissue. In this study, we examined the effect of peripheral inflammation on the electrolyte disposition between the plasma and CSF in rats, in which lithium was utilized as a surrogate marker of sodium,\(^12,13\) and its concentration profile in CSF was monitored with a microdialysis probe placed in the lateral ventricle.\(^4,15\) It is well known that lithium resembles to sodium regarding chemical properties and it is handled by various electrolyte transporters, such as SLC12A2/NKCC1 and SLC9A1/NHE1, in a similar manner to sodium.\(^12,13\) To induce peripheral inflammation in rats, carrageenan administration was employed since this induction method has been established for decades, and there is only a remote chance that the subcutaneously administered carrageenan will contact the brain tissue directly. We also analyzed the lithium elimination profile in CSF to examine the mechanism of an altered lithium disposition related to peripheral inflammation.

MATERIALS AND METHODS

Materials Lithium chloride was purchased from Nacalai Tesque (Kyoto, Japan). \(\lambda\)-Carrageenan was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). An acrylamide monomer, glycine, and other chemicals used were of the finest grade available. Affinity-purified rabbit anti-rat \(\alpha_1\)-acid glycoprotein (AGP) polyclonal antibody was purchased.
from Life Diagnostics Inc. (West Chester, PA, U.S.A.).

Animals Male Wistar rats (220–330 g) were purchased from Charles River Laboratories Japan (Yokohama, Japan). They were housed at 20–25°C and 40–50% humidity with a 12-h light/dark cycle, and were allowed free access to water and a standard diet. All animal experiments were approved by the animal ethics committee of Okayama University (OKU-2011157), and performed in accordance with the institutional guidelines for animal experimentation.

Induction of Acute Peripheral Inflammation (API) in Rats API was induced in rats as follows: After being anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally (i.p.)), rats were treated with λ-carrageenan (i.p.).

Hercules, CA, U.S.A.). The plasma specimen was supplied for the collection plasma specimen was stored at 20°C after its protein content was determined with a protein assay kit (Bio Rad, Hercules, CA, U.S.A.). The plasma specimen was collected. The lithium concentration in the plasma specimen was determined as described later.

For the collection of plasma specimens, blood drawing was performed under anesthesia with ethyl ether just before the rats were treated with λ-carrageenan or PBS, and 6, 24, and 48 h after the treatment, in which 200 µL of blood was drawn from the jugular vein with a 26-gauge needle, piercing through the pectoral muscle from the skin surface. Blood was then centrifuged at 12000 x g, 4°C for 10 min, and the collected plasma specimen was stored at −20°C after its protein content was determined with a protein assay kit (Bio Rad, Hercules, CA, U.S.A.). The plasma specimen was supplied for western blot analysis as previously reported.

Evaluation of the Lithium Disposition from the Plasma to CSF in Rats The lithium disposition from the plasma to CSF was evaluated by comparing their lithium concentration profiles as previously reported. Briefly, to examine the lithium concentration profile in the plasma, each rat was fixed on its back after being anesthetized with pentobarbital, and the lithium chloride solution prepared at a concentration of 375 mM was gently administered into the right femoral vein with an infusion pump. The lithium dose administered was set as 0.75 mmol/kg, and the infusion rate was adjusted to 0.1 mL/min.

Then, 100 µL of blood was drawn from the jugular vein at 5, 15, 40, 60, 120, and 180 min following the start of administration. The lithium concentration in the collected effluent was determined as described later, including the permeation coefficient of the probe assembly that was determined prior to each experiment. To determine the probe’s permeation coefficient, the probe was first immersed in the lithium solution (donor solution) prepared at a concentration of 1 µg/mL (144 µM) with Ringer’s solution. Its inlet and outlet tubes were connected to the syringe infusion pump and the fraction collector, respectively. The probe was then perfused with Ringer’s solution at a rate of 0.5 µL/min, and the perfusion effluent from the outlet was collected every 15 min until 120 min. The donor solution was collected at 0 and 120 min. The permeation coefficient was calculated by dividing the lithium concentration in the effluent by that in the donor solution.

The mean values and standard errors of the permeation coefficients for the probes used in the experiments with normal and API rats were 0.233 ± 0.021 and 0.233 ± 0.016, respectively, not being different between the groups.

To evaluate the lithium disposition from the plasma to CSF, the index of the lithium concentration in the plasma and CSF was determined in a model-dependent manner, assuming that lithium instantaneously distributes to be equilibrated between the plasma and CSF. The following equations were employed to characterize the lithium concentration profiles in the plasma and CSF:

\[ C_p(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (a > b) \] (1)

\[ C_{CSF}(t) = K_{eq} \cdot C_p(t) \] (2)

where \( C_p(t) \) and \( C_{CSF}(t) \) are the lithium concentrations in the plasma and CSF at time \( t \) following intravenous lithium administration, respectively. \( K_{eq} \) is the equilibrium index regarding the lithium disposition between the plasma and CSF. Four pharmacokinetic parameters (\( A, B, \alpha, \) and \( \beta \)) and the index \( K_{eq} \) were simultaneously determined with a nonlinear, least-squares method, in which the observed concentration profiles were fitted to the equations. After the parameters were determined, the area under the lithium concentration profile in the plasma \((AUC_p)\) was obtained with a definite integral of Eq. 1 from 0 to infinite, if necessary. The total plasma clearance of lithium (\( CL_p \)) was calculated by dividing the lithium dose by \( AUC_p \) and the volume of the lithium distribution (\( V_p \)) was obtained by dividing \( CL_p \) by the parameter \( \beta \).

Evaluation of Lithium Elimination from CSF in Rats

To evaluate the lithium elimination process from CSF, lithium chloride solution was immediately administered into the cerebroventricle, and the lithium concentration in CSF was monitored with the microdialysis method as described above. The lithium chloride solution was prepared at a concentration of 12.5 mM, and administered at an infusion rate of 0.5 µL/min via the guide cannula set for probe insertion. The infusion rate
was chosen as about one tenth of the CSF drainage rate so as not to perturb the CSF hydrodynamics. The lithium dose administered was set as 0.12 $\mu$mol/kg. After administration, the microdialysis probe assembly was inserted into the ventricle via the guide cannula, and the lithium concentration in CSF began to be monitored in the same manner as described above. The permeation coefficients of the probes used in the experiments with normal and API rats were determined to be 0.214 $\pm$ 0.005 and 0.229 $\pm$ 0.005, respectively, not being different between the groups.

The lithium concentration profile in CSF was characterized in a model-dependent manner with the equation below:

$$C_{\text{icv, CSF}}(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

where $C_{\text{icv, CSF}}(t)$ presents the lithium concentrations in CSF at time $t$ following lithium administration into the cerebroventricle. After the parameters in Eq. 3 ($A$, $B$, $\alpha$, and $\beta$) were determined, the area under the lithium concentration profile in CSF ($AUC_{\text{CSF}}$), along with the lithium clearance from CSF ($CL_{\text{CSF}}$) and the volume of the lithium distribution in CSF ($V_{\text{CSF}}$), was obtained as described earlier.

**Lithium Determination** Lithium concentrations in the plasma specimens and effluents were determined using an atomic absorption spectrometer equipped with a furnace atomizer (AA6300/GFA-EX7i, Shimadzu Co., Kyoto, Japan) at a wavelength of 670.8 nm. For determination, the specimens and effluents were diluted with 10 mmol/L nitric acid as instructed. The lowest lithium concentration detectable in the collected samples was 0.18 $\mu$mol/L in this study.

**Data Analysis** Data are shown as the means $\pm$ S.E. of 3–5 experiments, unless indicated otherwise. Significant differences were evaluated with Student’s $t$-test when data were normally distributed; otherwise the Mann–Whitney $U$-test was employed for evaluation. A $p < 0.05$ was considered to indicate significance.

**RESULTS**

**Induction of API in Rats** As shown in Fig. 1, $\lambda$-carrageenan treatment of the hind paws of rats causes an increase in the plasma AGP level. Although the AGP level in rats treated with carrageenan was not different from that in those with PBS at 6 h after treatment, it significantly increased at 24 h, being 3.6 times higher than that in rats with PBS. In the rats treated with carrageenan, an increased AGP level was also noticeable at 48 h, but it is uncertain whether the inflammatory status at 48 h further exacerbates as compared with that at 24 h, since the median value of the AGP levels given at 48 h is nearly the same as that at 24 h (Fig. 1). It was also observed that the values at 48 h more markedly vary compared with those at other time points (Fig. 1). Based on these results, the rats treated with carrageenan for 24 h were used as API rats in this study.

**Evaluation of the Lithium Disposition from the Plasma to CSF in Rats** The lithium concentration profiles in the plasma and CSF following intravenous lithium administration were examined, and the lithium disposition from the plasma to CSF was evaluated. In normal rats, it was shown that the lithium concentration in plasma decreases in a two-exponential manner, in which the early phase of the profile gradually reduces into the late phase (Fig. 2A). The total plasma clearance of lithium was calculated as 3.8 mL/min/kg, and the volume of the lithium distribution in the plasma was estimated to be 1.1 L/kg in normal rats. Figure 2A also shows that the lithium concentration in CSF decreases in a two-exponential manner, in which the early phase of the profile gradually reduces into the late phase (Fig. 2A). The total plasma clearance of lithium was calculated as 3.8 mL/min/kg, and the volume of the lithium distribution in the plasma was estimated to be 1.1 L/kg in normal rats.
observed in normal rats, the profile in CSF is clearly lowered compared to that observed in normal rats, yet their decreasing trends appear to be similar (Fig. 2A). These observations were reflected in the pharmacokinetic parameters. That is, the parameters obtained for API rats are comparable with those obtained for normal rats, except for the lithium equilibrium index $K_{eq}$ (Table 1). The index in API rats markedly decreases from the value in normal rats, indicating that the lithium disposition from the plasma to CSF is suppressed by 38.5% in API rats compared to that in normal rats (Table 1).

**Evaluation of Lithium Elimination from CSF in Rats**

The lithium concentration profile in CSF was then examined following lithium being immediately administered into the cerebroventricle. Regarding the lithium concentration profile in normal rats, it was also shown to decrease in a two-exponential manner, in which the lithium concentration in an early phase is rapidly reduced compared to that observed following lithium being intravenously administered (Figs. 2A, B). As a result, the parameters obtained for the lithium profile in CSF with the intracerebroventricular administration in normal rats are markedly different from those obtained with intravenous lithium administration in normal rats (Table 1). Based on the parameters, the volume of the lithium distribution in CSF is 760 µL/kg, and the lithium clearance from CSF is 8.9 µL/min/kg in normal rats. As for the CSF concentration profile of lithium in API rats, it is indistinguishable from that in normal rats, indicating that lithium is cleared from CSF in the same manner in normal and API rats (Fig. 2B).

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**Table 1. Pharmacokinetic Parameters Determined with the Lithium Concentration Profiles in Plasma and CSF**

| Pharmacokinetic Parameters | Intravenous administration | Intracerebroventricular administration |
|-----------------------------|----------------------------|---------------------------------------|
|                             | Normal rats | API rats | Normal rats | API rats |
| Lithium dose                | 0.75 mmol/kg | 0.12 µmol/kg |
| Number of experiments       | 4 | 4 | 5 | 3 |
| $A$ (mmol/L)                | 0.737±0.143 | 0.720±0.155 | 1.475±0.360$^b$ | 1.528±0.312 |
| $a$ (min$^{-1}$)            | 0.051±0.027 | 0.047±0.027 | 0.178±0.041$^b$ | 0.189±0.037 |
| $B$ (mmol/L)                | 0.648±0.166 | 0.603±0.185 | 0.060±0.037$^b$ | 0.076±0.033 |
| $\beta$ (min$^{-1}$)        | 0.004±0.002 | 0.003±0.002 | 0.012±0.007 | 0.013±0.005 |
| $K_{eq}$ (dimensionless)    | 0.148±0.013 | 0.091±0.010$^c$ | —$^d$ | —$^d$ |

$^a$ Data are shown as the mean±S.E. $^b$ Significantly different from the corresponding normal value in the intravenous administration study ($p<0.05$). $^c$ Significantly different from the value in the normal rats ($p<0.05$). $^d$ Not applicable.
We examined whether the electrolyte disposition from the plasma to CSF alters in response to peripheral inflammation employing lithium as a surrogate marker of sodium. As shown in Fig. 2A, although there appears to be no detectable change in the plasma concentration profiles of lithium between normal and API rats, the CSF concentration profile of lithium in API rats markedly decreases compared to that in normal rats. The pharmacokinetic analysis indicated that the lithium disposition from the plasma to CSF is reduced by 35.8% in API rats (Table 1). As a factor involved in the decrease, increased lithium efflux from CSF to the plasma was considered, and, therefore, the lithium concentration profile in CSF was examined with lithium being administered into the cerebroventricle. As a result, there was no difference in the lithium concentration profiles in CSF between normal and API rats (Fig. 2B), indicating that the process of lithium efflux from CSF is not affected in API rats. With these findings, it is conceivable that the decreased lithium disposition from the plasma to CSF observed in API rats is caused by suppression of the lithium entry process from the plasma to CSF.

Regarding the mechanism underlying the decreased lithium disposition, we could not fully reveal it in this study, but it seems to be probable that electrolyte transporters expressed on the brain capillary epithelia have an impaired function in API rats, in which sodium transporters, such as SLC12A2/NKCC1, SLC9A1/NHE1, and SLC4A10/NCBE, are likely to be involved in the decreased disposition, since they are known to handle lithium in a similar manner to sodium. Sodium/K+ -ATPase has been reported to barely recognize lithium as a substrate, and, therefore, it may not be involved in the decreased disposition. Sodium transporters are also expressed on the choroid plexus epithelia, and they play a primary role in producing CSF while regulating and maintaining its electrolyte composition. It is therefore likely that an altered function of the choroid plexus is involved in the decreased lithium disposition from the plasma to CSF in API rats, although the interior surface of the brain capillary contacting the choroid plexus is about one five-thousandth that making up the blood–brain barrier in the brain tissue. Regarding the factors causing the suppressed activity of those transporters, inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, can be thought as candidates, since these substances are known to be released into the circulating blood with carrageenan treatment in rats.

A promoted release of interferon (INF)-γ from lymphocytes is also demonstrated in carrageenan-induced inflammation. As the released mediators suppress the expression of electrolyte transporters and/or their mRNA, it is plausible that the inflammation mediators that are released into the circulating blood affect the capillary epithelia to suppress the expression of electrolyte transporters in API rats. This decreases sodium entry to the brain tissue, which is observed as a decrease in the lithium disposition from the plasma to CSF. Besides the speculated mechanism described above, we can consider another mechanism accounting for the decreased lithium disposition in API rats whereby the nociceptive neural afferent signal accompanying peripheral inflammation causes functional alteration of the brain capillary epithelia and choroid plexus. Additionally, further study is required to clarify whether the handling of nutrients in the brain tissue also varies in API rats, since various nutrients such as glucose, amino acids, and organic acids, are known to be transported in a sodium-coupled manner.

It was demonstrated in normal rats that the lithium concentration in CSF following its administration into the cerebroventricle decreases in a different manner compared to that following intravenous administration (Figs. 2A, B). As the plasma and CSF concentration profiles of lithium observed following intravenous lithium administration vary in parallel (Fig. 2A), lithium appears to rapidly distribute from the circulating blood to brain tissue, allowing its concentration in CSF to be instantaneously equilibrated with that in the plasma. The rapid distribution may be related to the fact that the brain capillary network efficiently provides nutrients and oxygen throughout the brain tissue. Since the brain tissue is closely associated with the lateral ventricle that retains CSF, the lithium concentration in CSF is thought to reflect the average lithium concentration in the brain tissue (Fig. 2A). As for the lithium profile in CSF following intracerebroventricular administration (Fig. 2B), it can be inferred that lithium firstly diffuses in the cranium from the site of administration to distribute in the brain tissue, being reflected by the rapid decrease in the concentration observed in an early phase of the profile. After the distribution has equilibrated, the lithium concentration in CSF gradually decreases as lithium in the brain tissue is removed via the brain capillary network and the drainage. The gradual decrease is likely to be reflected in the late phase of the profile (Fig. 2B). With the lithium profile in CSF, the volume of distribution of lithium in CSF was estimated to be 760 μL/kg, being comparable with the volume of distribution of inulin in CSF that was reported to be around 240 μL in rats. The lithium clearance from CSF was also obtained to be a similar value to the bulk flow rate of CSF in rats (5.4–8.9 μL/min). These things suggest that lithium in CSF diffuses in the extracellular fluid of the brain tissue without entering the brain cells, and, therefore, it can be considered that the lithium profile in CSF following intracerebroventricular administration mainly reflects the lithium's diffusion process in the cranium and its elimination process from CSF.

In summary, the effect of API on the electrolyte disposition from the plasma to CSF was examined with lithium being utilized as a surrogate marker of sodium in rats. It was revealed that the lithium concentration profile in CSF following intravenous administration is reduced in API rats, while there was no alteration regarding the concentration profile following intracerebroventricular administration in API rats. Therefore, the sodium disposition from the plasma to CSF seems to be affected in API rats. In addition, it is rational to think that the disposition regarding other electrolytes is also affected in a similar manner, inferring that electrolyte entry from the circulating blood to brain tissue is suppressed in API rats. These findings provide useful information for individual dose-optimization according to a patient’s clinical condition and symptoms.

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