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Insulin increases central apolipoprotein E levels as revealed by an improved technique for collection of cerebrospinal fluid from rats

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Abstract

Cerebrospinal fluid (CSF) provides an invaluable analytical window to the central nervous system (CNS) because it reflects the dynamically changing complement of CNS constituents. We describe an improved method for sampling CSF in rats that is easy to perform. It has a 96% success rate of CSF collection and consistently yields large volumes (150–200 μ l) of CSF. The blood contamination rate is also low (6%) as determined by both visual inspection and the lack of molecular detection of apolipoprotein B, a plasma-derived protein, which is absent in the CNS. This improved method of CSF sampling can have broad applicability in physiological and pharmacological evaluation for diverse CNS targets. We used this technique to provide proof of principle by examining the effect of intraperitoneal insulin on the level of apolipoprotein E (apoE) in the CSF. Insulin (0.5 and 1 U/kg) led to a significant increase of insulin in both plasma and CSF at 2 h after intraperitoneal administration and decreased blood glucose for at least 2 h. ApoE concentrations in CSF, but not in plasma, were also significantly increased, and its time-course was inversely correlated with the alterations in blood glucose over 2 h. These results provide a pharmacological validation of the novel CSF sampling and validation procedure for sampling rat CSF.

Keywords

Cerebrospinal fluid collection; cisternum magnum; insulin; apolipoprotein E

1. Introduction

The cerebrospinal fluid (CSF), which is produced by the choroid plexus and interstitial fluid efflux from the brain, is crucial for nutrition and circulation of the brain and spinal cord

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(Johnston and Papaiconomou, 2002). Because the bulk flow of the brain interstitial fluid is toward the CSF, changes in the levels of specific constituents within the CSF often reflect biological and functional changes in brain activity under various physiological and pathological states (de, Diamant et al., 1993). In humans, analysis of the constituents and their fluctuations in CSF is commonly used for the diagnosis and determination of disease stage and/or grade (Lindquist, Linne et al., 1988; Wahlund and Blennow, 2003). Although the importance of these investigations is well recognized in the neuroscience field, studies using CSF collected from small animals, such as rats, are not common, in large part due to the difficulty of obtaining CSF samples of sufficient volume and that are not contaminated with blood.

Several methods have been devised for the collection of CSF samples from rats, including direct lumbar puncture (De la Calle and Paino, 2002; Wang, Kimura et al., 2005), the surgical implantation of chronic or acute cannulas or catheters into the cisternum magnum (Huang, Saljo et al., 1995; Westergren and Johansson, 1991), and percutaneous CSF collection (Sharma, Schultze et al., 2006). All suffer to some extent from either or both of the two technical problems. There is a limited volume (30–100 μ l) of CSF that can be reliably collected, and there is often contamination from blood (10–30% or more of the samples contain blood cells) (Cserr, 1965; De la Calle and Paino, 2002; Ylitalo, Heikkinen et al., 1976), compromising interpretation of experimental results. In the present article, we describe a modified method for harvesting CSF in rats that is easy to perform, that allows the reliable collection of large volumes of CSF (up to 200 μ l), and that is associated with a lower average proportion (6%) of blood-contaminated samples. We further describe a technique that sensitively and reliably screens for even trace amounts of blood contamination using detection of apolipoprotein B (apoB), a plasma-derived protein that is normally absent in the CNS (Takechi, Galloway et al., 2010). As a proof of principle, we used these techniques to demonstrate that CSF apoE levels are significantly up-regulated following the intraperitoneal (ip) administration of insulin.

2. Materials and Methods

2.1. Animals

Adult male Long-Evans rats (300–400 g, Harlan, Indianapolis, IN) were individually housed in a temperature-controlled vivarium on a 12/12 h light/dark cycle (lights on at 0600 h) with *ad libitum* access to pelleted rodent chow and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

2.2. Materials

An electrode holder with a 90° bend at its distal end (Kopf, Tujunga, CA, Model 1769, Fig. 1A) was attached to a rat stereotaxic instrument (Stoelting, Wood Dale, IL). Micro-Renathane tubing was obtained from Braintree Scientific, Inc. (Cat. No. MRE 033, Braintree, MA). A Swiss Boy lab jack for raising the stereotaxic instrument was purchased from Sigma-Aldrich (Cat. No. Z635359, St. Louis, MO).

2.3. Procedures

The CSF sampling procedure is a modification and improvement of our previous technique (Stein, Dorsa et al., 1983). The blunt end of a 25-gauge (1½-inch) needle (removed from its hub) is connected to the Micro-Renathane tubing and mounted onto the right-angle electrode holder and adjusted so that it is exactly perpendicular to the ear bars and thus parallel to the long axis of the stereotaxic apparatus. A 1.0-ml syringe containing normal saline is attached via a 25-gauge disposable needle to the distal end of the tubing. After saline is flushed through the tubing and the needle, slight negative pressure is applied to create a small air

space extending 0.5 cm behind the blunted needle, allowing for an air-bubble barrier to form between the CSF and the normal saline.

Rats are anesthetized with ip ketamine (87 mg/kg body weight) plus xylazine (13 mg/kg). The area between the shoulders and below the skull is shaved. The rat is then placed prone on the stereotaxic instrument, which is elevated by a Swiss Boy lab jack so that the ear bars are approximately 15 cm above the surface of the table (Fig. 1B). The rat is then placed into the ear bars and the incisor bar is adjusted to the lowest possible position so that the rat's head is maximally ventroflexed. The surgical site is swabbed with 10% povidone, followed by 70% ethanol. A mid-sagittal incision of the skin, approximately 2 cm in length, is made inferior to the occipital crest. The subcutaneous tissue and occipital muscles are separated by blunt dissection with forceps to expose the atlanto-occipital membrane at the base of the skull.

After the atlanto-occipital membrane is exposed, the surrounding area is gently cleaned with sterile saline-soaked cotton swabs to remove any residual blood or other interstitial fluid. The sharp tip of the needle (Fig. 1D and 1E) is advanced slowly in an anterior direction by the worm-gear-driven drives to reach the surface of the membrane. Increased resistance occurs as the needle slowly penetrates the dura mater, and it diminishes as the tip of the needle enters the cisternum magnum (Fig. 1C). CSF flow is then spontaneously initiated and the air bubble moves toward the syringe. Gentle negative pressure is applied to the syringe to continue the flow of CSF until the air bubble enters the collection syringe. The syringe is then disconnected and the CSF is collected via gravity drainage into a 500 μ l microtube, which is positioned below the rat (Fig. 1B). This method typically allows collection of 150–200 μ l CSF within a 5-min period when it is used as a terminal procedure. After each collection, the entire collection apparatus is flushed with sterile saline. If the experiment is to end after CSF sampling is complete, the rat is sacrificed by decapitation and blood is collected. The collected CSF and plasma samples are immediately frozen on dry ice and stored at -80°C .

Nine rats were used for repeating the procedure at one-week intervals for a total of 3 samplings, and only 100 μ l of CSF was collected from those rats at each procedure. In the 1st procedure, CSF was withdrawn from all of the rats. At the time of the 2nd procedure, 1 week later, the 9 rats were divided into 2 groups of equal body weight. One group ($n = 5$) was used for CSF re-sampling, and the other ($n = 4$) was used as a sham control, in which the rats were anesthetized and the skin over the dorsum of the neck re-opened, but no needle penetration occurred and no CSF was withdrawn. After each CSF-sampling, the muscles were re-aligned and loosely sutured and the skin was closed with 4-0 suture, and 3 ml of 0.9% NaCl was injected ip to prevent dehydration. At the 3rd procedure, 1 week later, CSF (150–200 μ l) was withdrawn from all rats, and the rats were then sacrificed. Body weight, food and water intake, and their behavior were monitored daily.

2.4. Western blot of apoB and apoE

Two-fold serial dilutions of plasma, corresponding to 2.5–0.078 % plasma in water (v/v), were prepared as standards using rat plasma. 20 μ l of the standards and individual rat CSF samples (20 μ l) were heat-denatured, and separated by 4 to 15% SDS-PAGE gradient gels and then electro-transferred to PVDF membranes (Millipore, Bedford, MA). After incubating in blocking buffer, membranes were incubated with a polyclonal rabbit anti-rat apoB antibody (1:5,000 dilution) (Lo, Nordskog et al., 2008) overnight at 4°C with gentle shaking. After washing 3 times for 10 min in PBS, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000, Santa Cruz Biotechnology, Inc., CA) for 1 h at room temperature. The amount of immune complexes was quantified using an enhanced chemiluminescence detection system (GE healthcare,

Little Chalfont, Buckinghamshire, UK). The reacted membranes were exposed to x-ray film (Kodak Scientific, Rochester, NY). Film density, measured as transmittance, was expressed as volume-adjusted optical density (OD).

The conditions and procedures for determining apoE levels in 20 μ l of CSF using Western blot were described previously (Shen, Tso et al., 2008)

2.5. Application of the method to determine the effects of ip insulin on apoE in CSF

Naïve rats were divided into 2 groups ($n = 7$ rats/group) with equal body weight. Group 1 was injected ip with saline (1 ml/kg), and Group 2 was injected ip with insulin (Humulin N, Eli Lilly and Company, Indianapolis, IN) at a dose of 1 U/kg. Two h later, CSF was withdrawn. The rats were then sacrificed and blood collected, and CSF and plasma were stored at -80° C.

To determine the time-course of changes of CSF apoE level in response to ip insulin, 3 groups of rats were used, receiving 0, 0.5 and 1 U/kg insulin, respectively. CSF (120–150 μ l/rat) was collected at 0, 15, 30, 60, 90, or 120 min relative to the injections ($n = 5$ –7 rats/time point/group). The rats were then sacrificed and blood collected.

2.6. Measurements of insulin and apoE

Insulin concentrations in both CSF and plasma were measured using an *Ultra Sensitive Rat Insulin ELISA Kit* (Cat. No. 90060, Crystal Chem., Inc., Downers Grove, IL) according to the manufacturer's instructions. Briefly, a sample of CSF, plasma, or a rat-insulin standard (5 μ l/sample) was added to an antibody-coated microplate. After incubation for 2 h at 4° C, 100 μ l of anti-insulin enzyme conjugate was dispensed to each well and incubated at room temperature for 30 min. After washing, 100 μ l of enzyme substrate solution was added to each well, and then incubated for 40 min. The reaction was stopped by adding stop solution, and insulin concentrations were determined by subtracting the absorbance at 630 nm from the absorbance at 450 nm.

ApoE concentrations in plasma and CSF were measured with an ELISA. A 96-well high binding ELISA plate (Corning, Corning, NY) was coated with 100 μ l of goat antiserum against rat apoE (1:300) and incubated overnight at 4° C. After washing in 10 mM PBS containing 0.05% Tween 20 (PBS-Tween 20), nonspecific protein binding sites were blocked by the addition of 1% BSA in PBS-Tween 20. 100 μ l of standard (0–35 ng/ml apoE), CSF samples (1:200 diluted in PBS-Tween 20 containing 1% BSA) and plasma samples (1:10,000 dilution) were added in duplicate to the coated wells and incubated overnight at 4° C. After washing, 100 μ l of rabbit polyclonal antibody against rat apoE (1:3,000 diluted in PBS-Tween 20 containing 1% BSA) were added and incubated for 2 h at 37° C. After washing, 100 μ l of peroxidase conjugated anti-rabbit IgG (1:200 diluted in PBS-Tween 20 containing 1% BSA, DAKO) was added and incubated for 1 h at 37° C. Color development was initiated by the addition of 200 μ l of O-phenylenediamine dihydrochloride peroxidase substrate (Sigma, St. Louis, MO). After 30 min in the dark at room temperature, 50 μ l of 3M HCl was added to stop the reaction, and the absorbance at 492 nm was measured.

2.7. Statistics

Data were analyzed using parametric statistics (SigmaPlot, version 11; SyStat, San Jose, CA). For comparisons of apoE levels between two groups (insulin and saline treatments), Student's t-test was used. Two-way ANOVA followed by Tukey's test was used for analysis of time-course changes in apoE and glucose levels following the treatments with different doses of insulin. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. CSF sampling procedure parameters

CSF was collected from 152 rats, the volume ranging from 150~200 μ l collected within 12 min per rat, including the period from skin incision to closure; and 5 min from needle insertion to completion of CSF sampling. The temporal limitation of 5 min was imposed to minimize the possibility that continued drainage of the CSF might affect its composition.

For the experiment in which CSF was re-sampled at weekly intervals, 100 μ l of CSF was reliably obtained from each rat at each time point. We also monitored the time-course changes in body weight, food and water intake over the re-sampling procedure. Compared with measurements obtained prior to the first procedure (i.e., pre-surgery), rats lost some body weight (less than 3% of total body weight) and ate slightly less food over the first 2 days after CSF sampling, but they increased food intake and regained their body weight quickly, and had restored pre-surgery weight within 5 days (Fig. 2A and B). All rats drank more water after the CSF was withdrawn, and water consumption quickly returned to normal (Fig. 2C). To determine whether the repeated withdrawal of CSF had adverse effects on body weight or food or water intake half the rats were re-sampled at Procedure 2 and half used as sham controls. Consistent with what we observed after the first procedure, the rats in both groups had comparable transient reductions of body weight and food intake, the only difference being that the rats undergoing CSF-withdrawal drank more water than Sham rats (Fig 2), but the difference was not significant statistically. During the entire experimental period, the animals' general behaviors (posture, walking, grooming, etc.) appeared normal.

3.2. Determination of blood contamination in CSF samples

By visual inspection, more than 96% of the CSF samples were clear, i.e. only 6 in 152 CSF samples appeared to have blood contamination. This was further evaluated using Western blotting for apoB, a protein found in high concentration in plasma, but absent in the CNS (Takechi, Galloway et al., 2010). We conducted Western blot using plasma diluted in water, spanning a range of concentrations corresponding to 2.5-0.078%, as standards. As depicted in Fig. 3, this immunoassay was able to detect apoB levels as low as 0.078% of aqueous solutions. The lack of apoB-immunoreactive bands in the great majority of CSF samples 1 (Fig. 3) indicates the absence of detectable plasma contamination. In one instance, CSF Sample 2, although the CSF appeared clear visually, the apoB method was nonetheless able to detect a slight blood contaminant (less than 0.156%, Fig. 3). An additional 2 samples were found with detectable apoB bands and were not used in the experiments assessing CSF apoE. Therefore, the total blood contamination rate was 6 % $[(6 + 3)/152 = 5.9\%]$.

3.3. Ip insulin increases apoE in CSF

As depicted in Fig. 4, ip insulin (1 U/kg) increased plasma insulin by nearly 3-fold (Fig. 4A) and increased CSF insulin around 2-fold (Fig. 4B) 2 h after the injection. While apoE concentrations in plasma remained essentially unchanged (Fig. 4C), the insulin treatment significantly increased the levels of apoE in the CSF (Fig. 4D), compared with saline-injected controls.

3.4. Time-course of changes of CSF apoE levels following ip insulin

Consistent with previous reports (Satoh, Nguyen et al., 2004), insulin at both doses significantly decreased blood glucose during the 2-h observation period (Fig. 5A). The insulin significantly increased apoE levels in the CSF starting at 15 min and lasting until 2 h, especially at the higher dose of insulin, compared to the apoE levels in saline-treated controls at the same time points (Fig. 5B). No significant differences in CSF apoE levels were found between the 2 doses of insulin (0.5 and 1 U/kg) at any time point.

4. Discussion

CSF is frequently analyzed in clinical populations for assessing disease progression in the CNS or biomarkers of drug efficacy (Koch, Donarski et al., 2001). However, obtaining CSF from pre-clinical species, particularly rodents, can be challenging due to small body sizes, and consequently low volumes of CSF. In recent decades, the advances in the development of highly sensitive detection techniques, such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA), have made it feasible to detect biological changes in the CSF of small animals such as rats. Several techniques for sampling CSF from the rat have been reported, and rat CSF is increasingly being used in pharmacological and biochemical research (Barzilai, Wang et al., 1997; Cassar, Tovcimak et al., 2010; De la Calle and Paino, 2002; Huang, Saljo et al., 1995; Sanvitto, Azambuja et al., 1987; Shapiro, Stiteler et al., 2011; Wang, Kimura et al., 2005). Compared with previously established methods, the procedure we describe here has proven to be superior in several aspects: 1) There is a higher success rate (96%); 2) larger volumes (up to 200 μ l) of CSF can be obtained within a short operation window (12 min); 3) there is a lower blood contamination rate (6 %) of CSF samples; and 4) a molecular screening for apoB can be used to detect minute blood contamination of rat CSF samples, allowing the ability to exclude blood-contaminated CSF samples and thereby avoid misinterpretation of experimental data.

CSF collection from rats has commonly been performed via the lumbar cistern or the cisternum magnum. De La Calle et al. developed a method for collecting CSF by direct lumbar puncture in rats (De la Calle and Paino, 2002), and this was later modified by Wang et al. (Wang, Kimura et al., 2005). This method is of particular relevance when analyzing neurotransmitters and their metabolites whose concentrations may vary in the lower spinal cord with respect to cranial levels (De la Calle and Paino, 2002). This CSF sampling procedure, however, yields limited volume (30–70 μ l) of CSF and a high blood contamination rate (24.9%) (De la Calle and Paino, 2002) and (11%) (Wang, Kimura et al., 2005) due to injury of the vertebral blood vessels caused by the inserted needle. Damage to the spinal cord or to the dorsal roots at the level of the puncture might also be a concern. An important shortcoming of the lumbar technique is that the biochemical composition in the CSF collected from lumbar area may not fully reflect neurological activity occurring in the brain.

The cisternum magnum has the largest volume of any pool of CSF in the CNS of adult rats. Bouman and van Wimersma Greidanus described a method for implanting chronic metal cannulas in rats such that the cannula tip was in the cisternum magnum and the other end was anchored to the dorsum of the skull (Bouman and van Wimersma Greidanus, 1979). While this technique allows sampling CSF from awake, relatively unrestrained rats, we found it to be extremely difficult for many reasons including a low rate of successful cannula implantation, unreliable CSF flow from otherwise successfully implanted cannulas, and frequent blood contamination. Nonetheless, with perseverance, we were able to use the method to assess uptake of insulin from plasma to CSF in rats (Steffens, Scheurink et al., 1988; Strubbe, Porte, Jr. et al., 1988). The present findings that CSF insulin is increased following ip insulin are consistent with those results.

Westegren and Johansson used a similar technique and found a progressive increase over time of blood cell and albumin contamination in the CSF (Westegren and Johansson, 1991). Huang et al. later modified the technique by implanting a catheter such that its tip was affixed to the atlanto-occipital membrane over the cisternum magnum. For sampling, a needle was inserted through the catheter so that it punctured the membrane and CSF (up to 50 μ l in 5 min) was obtained by natural flow (Huang, Saljo et al., 1995). The rat was anesthetized for the sampling procedure, eliminating the primary advantage of the Bouman

and van Wimersa Greidanus (Bouman and van Wimersma Greidanus, 1979) technique. Chronically implanted devices allow the repeated sampling of CSF from rats at specific points of time, which is useful to monitor the changes in substances that are extremely labile. However, some of the disadvantages associated with permanently implanted cannulas include the time-consuming process of preparation of the cannula/catheter system and surgical procedure, bleeding, nervous tissue damage, temporary breakdown of the blood-brain barrier, and potential for infection and cannula/catheter obstruction (Halonen, Pitkanen et al., 1991; Patsalos, Alavijeh et al., 1992; Westergren and Johansson, 1991). In comparison, the technique we describe here does not require an implanted cannula and has minimal adverse consequences such as bleeding, tissue damage or infections. The method is fast, minimally invasive and consistently yields large volumes of blood-free CSF.

Recently, Nirogi et al. described a different approach. They used percutaneous CSF collection by directly inserting a needle into the cisternum magnum without making an incision in the skin (Nirogi, Kandikere et al., 2009); i.e., the skin and musculature overlying the cisternum magnum is left intact, and the CSF is thus sampled without direct visual confirmation of the location of the cisterna magna. However, the needle puncture can obviously cause inadvertent damage to vessels and nervous tissue, leading to high rate of blood contamination. Because of this shortcoming, those authors used a 25-cm length of PE-50 tubing to collect the CSF, so that the portion of tubing containing the contaminated blood could be cut off to limit contamination of the sample (Nirogi, Kandikere et al., 2009).

The atlanto-occipital membrane, which lies between the base of the occipital bone and the first cervical vertebra, forms a window over the cisterna magna. Pegg et al. reported a method for sampling CSF from rat cisternum magnum by incising the atlanto-occipital membrane with a dissecting instrument to directly expose the still-intact dura mater (Pegg, He et al., 2010). While the exposed dura provides a translucent “viewing window,” through which vasculature can be visualized and avoided with a puncture, this procedure nonetheless had 10% blood contamination (Pegg, He et al., 2010). An additional drawback of this procedure is that it is impossible to obtain repeated samples from the same rat.

The production of CSF in rats is about 2.2 $\mu\text{l}/\text{min}$ (Cserr, 1965) and the total volume is 400–550 μl (Lai, Smith et al., 1983; Meek and Neff, 1973). It has been estimated that the CSF volume in the cisternum magnum and the adjacent subarachnoid space is 190 and 246 μl , respectively (Burns, London et al., 1976). Therefore, it is feasible to withdraw up to 200 μl from a rat as a terminal procedure without severely depleting the system, and it allows re-sampling of 100 μl CSF at one-week intervals in the same animal. Supported by our detailed observations (Fig. 2), the CSF resampling had no significant adverse consequences, the rats displaying normal behavior including body weight and food intake.

CSF is derived in part from the extracellular fluid in the CNS. It is segregated from plasma by the blood-CSF barrier. It is estimated that 80% of CSF proteins come from the plasma, but the protein concentration of whole blood is 200- to 400-fold higher than that in CSF (Cserr, 1965; Thompson, 1995). As a result, even a small percentage of blood contamination during CSF collection can dramatically alter the content profile, leading to the misinterpretation of the experimental data. Conventionally, blood contamination in CSF is examined by visual observation, counting red blood cells, or measuring the total protein concentration (Hurtt and Smith, 1997; Jeong, Hahm et al., 2006; Nirogi, Kandikere et al., 2009; Pegg, He et al., 2010). Some investigators have simply used blood-contaminated CSF after centrifugation of the samples (Cassar, Tovcimak et al., 2010). However, these measurements are not sufficiently sensitive to assess a low level of blood contamination in CSF. Here we describe a highly sensitive molecular method to screen for potential blood contamination of the CSF by measuring apoB, a plasma-derived protein that is absent in the

CNS (Takechi, Galloway et al., 2010). In order to estimate the degree of contamination, dilutions of rat plasma were initially analyzed. As depicted in Fig. 3, our detection limit reached as low as 0.0781% (v/v) blood contamination in CSF by Western blot. The lack of apoB-immunoreactive bands in the great majority of the CSF samples 1 (Fig. 3) therefore indicates the absence of detectable plasma contamination. Interestingly, one CSF sample that appeared clear by visual inspection was found to have < 0.0781% plasma contamination as determined by measuring apoB. Thus, when no apoB is detected, there is a high degree of confidence that the sample is uncontaminated with blood.

Using our technique, we analyzed apoE protein levels in the CSF after ip insulin in rats. We first focused on the levels of apoE concentration in plasma and CSF at 2 h after ip insulin (1 U/kg), and found that the treatment significantly elevated apoE in the apoB-absent CSF samples, but not in plasma. Concomitant with this, insulin was increased in both CSF and plasma (Fig. 4). In a follow-up experiment, insulin was administered and blood glucose and plasma and CSF apoE assessed over time. As depicted in Fig. 5, blood glucose was significantly decreased at the first sampling time (15 min) and remained low for up to 2 h, the decrease being greater with the higher dose of insulin. ApoE levels were increased in the apoB-absent CSF, but not in plasma (Fig. 5). Since apoE is mainly produced in brain astrocytes, the elevations of CSF apoE at 15, 30 and 60 min suggests that insulin or insulin-induced hypoglycemia (or some other central effect of ip insulin) increases the secretion of apoE.

ApoE is a constituent of several lipoprotein particles and is involved in lipid transport and metabolism (Mahley, 1988). It is produced in several organs including the liver and the brain (Boyles, Pitas et al., 1985). Recently, we demonstrated that apoE acts as a satiation factor in the hypothalamus. Intracerebroventricular (icv) administration of apoE significantly decreased food intake without causing malaise, whereas icv infusion of apoE antibody stimulated feeding, implying that endogenous apoE reduces food intake (Shen, Tso et al., 2008). One of the mechanisms mediating apoE's anorectic action in the hypothalamus is increased PI3K/Akt signaling, which is the same signaling pathway activated by insulin (Niswender, Morrison et al., 2003). Insulin plays an important role in the control of energy and glucose homeostasis (Begg and Woods, 2012; Ryan, Woods et al., 2012), and determining whether insulin's catabolic actions in the brain are related to the increased brain apoE, how apoE is up-regulated by systemic insulin, and from which brain tissue the increased apoE is secreted or produced are interesting studies for the future.

In conclusion, an improved procedure for CSF sampling from the cisternum magnum in rats has been described. Compared to existing techniques, this procedure provides the advantages of consistently yielding large volumes of blood-free CSF and sensitive detection of blood contamination using an assay for apoB. This method was used to demonstrate that the peripheral administration of insulin increases CSF apoE levels. Understanding the mechanisms as to how insulin up-regulates central apoE levels, and determining whether the increased apoE is involved in insulin's central actions on body energy and glucose homeostasis, might yield new insights into the prevention and treatment of obesity and diabetes.

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Highlights

- We describe an improved method for sampling CSF in rats.
- This technique has a 96% success rate and yields up to 200 μ l of CSF within 12 min.
- Blood contamination rate is low (6%), and a molecular examination of apoB is used to detect trace amounts of blood contamination in CSF samples.
- Using this technique, we demonstrated that ip insulin significantly increased apolipoprotein E level in rat CSF.
- This improved method can have broad applicability in physiological and pharmacological evaluation for diverse CNS targets.

Fig. 1.

(A) Photo of the stereotaxic instrument with an electrode holder bent at 90° at its distal end. (B) Position of the rat during the operation. The stereotaxic device is raised above the surface and the incisor bar is positioned so that the rat's neck is maximally dorsoflexed. The rat's weight is supported by the floor of the apparatus. (C) Close-up view of the penetration of a needle into the exposed cisternum magnum. (D & E) Two views of the v-shaped puncture needle tip.

Fig. 2.

Mean body weight (A), food intake (B) and water intake (C) in rats anesthetized 3 times, one week apart. Arrows indicate the days when the procedure was performed. Mean $\pm$ S.E., $n = 9$ in the 1st and 3rd weeks, $n = 5$ CSF re-sampling rats and $n = 4$ Sham rats in the 2nd week. No significant difference was found between the CSF-rats and sham rats following the second procedure.

Fig. 3.

Determination of apoB by Western blot. Two-fold serial dilutions of plasma, corresponding to 2.5-0.078 % plasma in water (v/v), were prepared as standards using the plasma from one rat. These standards were run in parallel. A level as low as 0.078% blood contamination (Lane 1) was observed as clear bands. Undiluted CSF samples (Lanes 8 and 9, 20 μ l/lane) from 2 individual rats, which were visually clear, were used as examples to determine potential blood contamination. The apoB-immunoreactive bands (apoB100 and apoB48) were observed only in CSF Sample-2 (Lane 9), but not in CSF Sample-1 (Lane 8), indicating slight blood contamination in Sample 2.

Fig. 4.

Insulin and apoE levels after peripheral administration of saline or insulin in rats. Two h after ip insulin (1 U/kg), immunoreactive insulin was increased significantly in both plasma (A) and apoB-negative CSF (B). ApoE concentration was significantly elevated in the CSF (D), but not in the plasma (C). Mean $\pm$ S.E., $n = 5-8$, * $P < 0.05$, ** $P < 0.01$, compared with saline controls.

Fig. 5.

Blood glucose and apoB-negative CSF apoE levels after ip insulin. Both doses of insulin (0.5 and 1 U/kg) significantly reduced blood glucose (A), but increased apoE levels in the CSF (B). $n = 6\sim 7$ rats per group at each time point, Mean $\pm$ S.E., * $P < 0.05$, and ** $P < 0.01$, compared to saline controls at the same time point; and # $P < 0.05$, compared to low dose of insulin (0.5 U/kg) at the same time point.