Technique for Repeated Collection of Cerebrospinal Fluid from Cisterna Magna of Anesthetized Strain 13 Guinea Pigs[1] (43273)

C. T. LIU[2] AND Z. M. GUO[3]

Department of Clinical and Experimental Physiology, Disease Assessment Division, United States Army Medical Research Institute of Infectious Disease, Fort Detrick, Frederick, Maryland 21702-5011

Abstract. **To study biochemical changes in cerebrospinal fluid (CSF), we developed a reliable technique for repeated collection of CSF in anesthetized strain 13 guinea pigs. The animal's head was mounted in a stereotaxic instrument with ventral tilt at 30°, and cisternal puncture was made with an L-shaped, 23-gauge needle through the shaved skin. Clear CSF was collected in a 1-ml syringe surrounded by crushed ice. Each collection procedure lasted for 3 min, and three consecutive collections produced about 0.2 ml of CSF. Sampling was repeated at 3-hr intervals. With intravenous saline infusion (10 ml/kg·hr), a total volume of 0.6–1.0 ml of CSF was collected over 6 to 12 hr. Animals maintained a mean blood pressure, heart rate, and minute volume, with few changes during CSF sampling for the entire collection.** [P.S.E.B.M. 1991, Vol 197]

The pathogenesis of Pichinde viral infection has been studied in the strain 13 guinea pig model (1). To understand the neurochemical mechanisms of this and other similar viral diseases, biochemical analyses of cerebrospinal fluid (CSF) have become increasingly important, and techniques for repeatedly sampling CSF from the inbred guinea pig are essential.

Several techniques of cisternal puncture for collecting CSF from guinea pigs have been reported by other investigators (2–3). With these established approaches, only a small volume (30–330 μl) of CSF can be collected from the guinea pig. Although a chronic cannulation of the cisterna magna makes repeated collection of CSF possible, complicated surgical operations are required, and a large volume (about 1 ml) of CSF required by certain biochemical analyses could not be collected daily (2). Furthermore, cardiopulmonary responses of the guinea pig to procedures of CSF collection were not measured.

Because the undesirable metabolism in the CSF sample may alter the measurements of neuromediators (4), we considered that it was imperative to avoid the presence of continuing chemical reactions during CSF sampling. The objective of this study was to develop a reliable and simple technique for collecting a larger volume (about 1 ml) of CSF from the anesthetized strain 13 guinea pig, and also to limit disturbances of cardiopulmonary functions during repeated CSF collection over 6 to 12 hr.

Materials and Methods

Animal Anesthesia and Surgery. Six male strain 13 guinea pigs, each weighing 500–700 g, were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg). During the experiment, a supplemental dose of sodium pentobarbital (one tenth of the original dose) was administered when needed. Rectal temperatures of anesthetized guinea pigs were maintained at 36–37°C with a homeothermic blanket (Harvard Apparatus, Ltd., Edenbridge, KY). To measure pulmonary functions, we performed a tracheotomy, and tracheal tubing was connected to a Fleisch pneumotachograph (Dynasciences, Blue Bell, PA). The left common carotid artery was cannulated to measure blood pressure, and the left external jugular vein was cannulated for isotonic saline infusion. With sufficient collateral circulation to the brain, the change in cerebral blood flow might be

[1] This work was presented at the American Physiological Society Annual Meeting (Physiologist 33:A129, 1990) on October 10, 1990 in Orlando, Florida.

[2] To whom requests for reprints should be addressed.

[3] Senior postdoctoral associate, supported by an award from the National Research Council, Washington, DC.

Received January 30, 1991. [P.S.E.B.M. 1991, Vol 197]
Accepted April 4, 1991.

0037-9727/91/1974-0400$3.00/0

negligible after unilateral cannulation of a common carotid artery.

Apparatus for CSF Sampling. The cisternal puncture device was an L-shaped 23-gauge needle, connected to a 2-cm piece of polyethylene tubing (PE 50, i.d. 0.58 mm, 0.d. 0.965 mm), and fitted to a 1-ml glass syringe. The syringe and needle adapter were wrapped with a rubber membrane cut from a surgical glove finger and filled with crushed ice. We used a stereotaxic instrument (Kopf Instruments, Tujunga, CA) to stabilize the animal's head. To ensure precision in needle placement, the L-shaped needle was fixed with the standard electrode carrier of the stereotaxic instrument.

Cisternal Puncture. The animal's nape was completely shaved by using clippers and hair remover (Carter Products, New York, NY). The head of the guinea pig was mounted in a stereotaxic instrument with ventral declivity at approximately 30°. While the tubercle of atlas was pressed with the forefinger, skin covering the dorsal margin of the atlanta tubercle was punctured with a 20-gauge needle. After the 20-gauge needle was withdrawn, the multiple-direction drives of the stereotaxic instrument were adjusted precisely to insert the L-shaped, 23-gauge needle tip into the cisterna magna via the punctured skin hole. A drop of saline, used as a marker, was placed in the PE 50 tubing connecting the puncture needle to the sampling 1-ml syringe. When the puncture needle was inserted into the neck musculature, the plunger of the sampling syringe was pulled gently to create a slight negative pressure. Once the puncture needle tip entered the cisterna magna (Fig. 1), the driving of the puncture needle was stopped as the saline drop began to move forward to the sampling syringe.

CSF Collection. Cerebrospinal fluid was collected slowly by pulling the plunger of the sampling syringe for 3 min, after which the sample syringe with the needle adapter was separated from the PE 50 tubing. The PE 50 tubing was reconnected to another needle adapter fitted with an empty syringe at the 0-ml mark. Cerebrospinal fluid in the syringe was transferred into a glass vial, sealed with a tight cap, and placed in liquid nitrogen. The collection was repeated consecutively three times. Cerebrospinal fluid was resampled at 3-hr intervals for 6–12 hr. Fractional CSF formation rate at a 3-hr interval was calculated as CSF vol (μl) per 180 min. If collected CSF was used for β-endorphin radioimmunoassay (Incstar Corp., Stillwater, MN), cerebrospinal fluid samples from an animal were pooled in the same glass vial, which was placed in a boiling water bath for 10 min to abolish the proteinase activity. The heat-treated CSF samples were stored at −70°C until use. Otherwise, no heat treatment was applied.

Saline infusion was accomplished via a venous catheter connected to a Razel infusion pump (Razel Scientific Instruments, Inc., Stanford, CT) fitted with a 30-ml syringe filled with isotonic saline. Immediately after the first CSF sample was withdrawn, the saline infusion was started at a rate of 10 ml/kg·hr. Before, during, and immediately after CSF sampling, the heart rate, mean blood pressure, and minute volume were measured by using a Gould polygraph and a pulmonary mechanics analyzer (Buxco Electronic, Inc., Sharon, CT).

Results

Volume of CSF Samples. After cardiopulmonary functions were stabilized from the general surgery and cisternal puncture, about 0.2 ml of clear CSF was sampled in three consecutive collections (each collection lasted for 3 min). With an intravenous saline infusion for 3 hr, the cisterna magna was refilled, and approximately 0.2 ml (200 μl) more of CSF was taken. Repeatedly sampled CSF volumes for 12 hr and fractional CSF formation rates starting at 3 hr are illustrated in Figure 2. About 1.0 ml of CSF was collected from

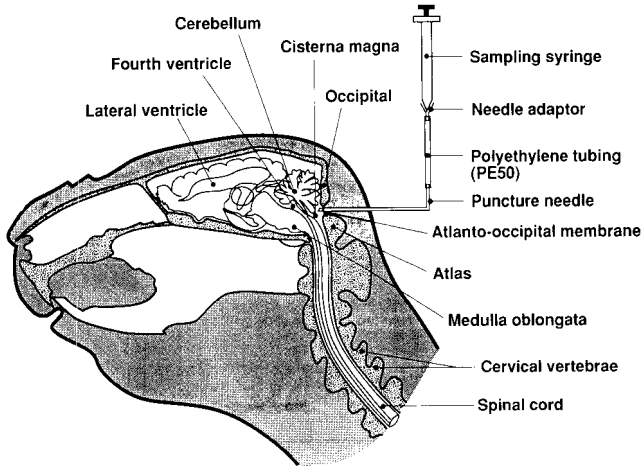

Figure 1. A diagram of the essential anatomy of a guinea pig's head, showing the needle location for collecting CSF from the cisterna magna.

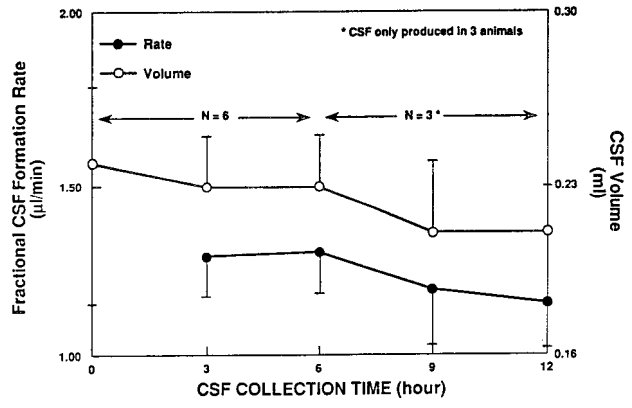

Figure 2. Fractional CSF formation rate and CSF volume collected over 12 hr from the cisterna magna of anesthetized strain 13 guinea pigs.

three of the six guinea pigs (50%) over 12 hr. After 6-hr repeated sampling, we could not collect any more CSF from the other three animals. A total volume of about 0.6 ml of CSF was collected during the first 6 hr in these three guinea pigs (Fig. 2).

Cardiopulmonary Functions. Figure 3 shows the variations of cardiopulmonary functions over the 12 hr. When compared with values prior to and immediately after sampling, heart rate, mean blood pressure, and minute volume were relatively constant during CSF sampling. Because the animals were anesthetized for more than 10 hr during the experiment, cardiopulmonary functions changed slightly with time toward to the end.

Discussion

Jones and Robinson (2) withdrew CSF samples repeatedly from conscious guinea pigs. This approach produced only 100 μl of CSF daily. This volume is not sufficient for certain biochemical analyses requiring 1 ml of CSF, such as β-endorphin determination. In 1983,

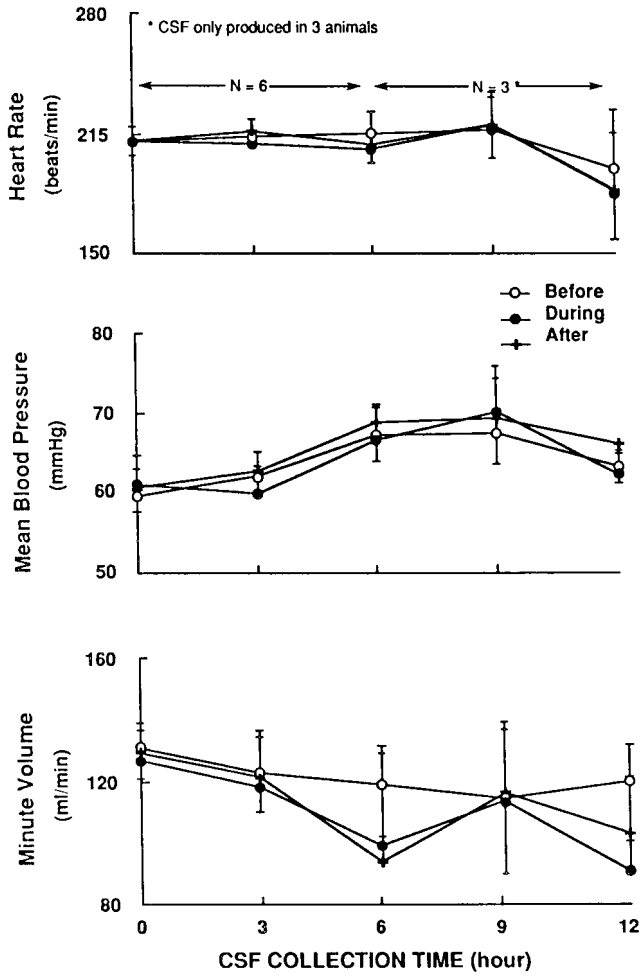

Figure 3. Effects of CSF collections over 12 hr on heart rate, mean blood pressure, and minute volume in anesthetized strain 13 guinea pigs.

Reiber and Schunck (3) reported a cisternal puncture technique for collecting CSF from anesthetized strain 13 guinea pigs without surgical operations. A 23- or 24-gauge needle was manually inserted into the cisterna magna through the skin of the neck, muscles, and the atlanto-occipital membrane. Although the cisternal puncture could be repeated weekly, only a small volume of CSF (30–330 μl) was collected in each puncture.

The technique developed in our study remarkably improves the cisternal puncture procedure, which we made stereotaxically without surgical operation. The puncture needle was easily and precisely inserted into the cisterna magna by slowly adjusting the posterior-anterior drive of the stereotaxic instrument. With this approach, brain tissues were not touched or injured. Because the head of the guinea pig was mounted by using a stereotaxic instrument with ear bars, the tip of the puncture needle was maintained stably within the cisterna magna for 6 to 12 hr. Finally, a volume as large as 0.6 to 1.0 ml of clear CSF was collected. When about 1 ml of CSF from the same animal within 12 hr is required for biochemical analyses, this technique is preferable.

Lai *et al.* (5) estimated that the total CSF volume of the rat is about 400–500 μl. The formation rate of CSF in the rat brain is about 2.2 μl/min, and about 100 μl of CSF could be taken every hour. Spector and Johnson (6) indicated that in mammals, the total CSF volume is about 15% of the brain weight, and the choroid epithelial cells per gram of brain tissue manufacture CSF at the rate of about 1 μl/min. We observed that the brain weight of 500–700 g strain 13 guinea pigs ranged from 3 to 4 g (unpublished data), that the total CSF volume might be estimated as 450–600 μl, and that the total CSF formation rate could be 3–4 μl/min. In this study, the fractional CSF formation rate sampling at the region of cisterna magna was about one fourth (1.2 μl/min) the estimated CSF formation rate. Only about 200 μl of CSF was collected at each 3-hr interval to prevent cardiopulmonary disturbances. The reason for cessation of CSF formation in the brain after 6-hr repeated sampling in three animals is unknown, because the puncture needle was removed, flushed, and repositioned into the cisterna magna.

We collected CSF samples repeatedly for 6–12 hr. During this period, water was lost from the skin, lung, and kidney. Therefore, after collection of the first CSF sample, isotonic saline was infused intravenously at 10 ml/kg·hr to compensate for the water loss of the animal. Frankmann (7) suggested that the concentrations of relevant molecules in CSF might be modified by rapid repeated sampling of CSF. The continued production of new CSF may have diluted the biological substances in CSF. However, Suckling and Reiber (8) found contrary results: When CSF was sampled consecutively from guinea pigs, the albumin concentration in

the first CSF sample was lower than in the later CSF samples. We measured the β-endorphin concentrations in the CSF samples collected at different periods with intravenous saline infusion. β-Endorphin concentrations in the first CSF sample and in subsequent samples did not differ significantly (unpublished observation).

Withdrawal of CSF produces a decrease in intracerebroventricular pressure, which may affect the activities of the central nervous system in modulating cardiovascular and pulmonary functions. However, in our studies, cardiopulmonary functions of the anesthetized strain 13 guinea pigs were not significantly modified within 6 to 12 hr by repeated collection of CSF, indicating the superiority of our developed technique.

1. Peters CJ, Jahrling PB, Liu CT, Kenyon RH, Mckee KT Jr, Barrera Oro JG. Experimental studies of arenaviral hemorrhagic fever. Curr Top Microbiol Immunol **134**:5–68, 1987.
2. Jones PM, Robinson IC. A method for repeated sampling of cerebrospinal fluid from conscious guinea pigs. J Neurosci Methods **3**:295–300, 1981.
3. Reiber H, Schunck O. Suboccipital puncture of guinea pigs. Lab Anim **17**:25–27, 1983.
4. Fridland GH, Desidero DM. Profiling of neuropeptides using gradient reversed-phase high-performance liquid chromatography with novel detection methodologies. J Chromatogr **379**:251–268, 1986.
5. Lai YL, Smith PM, Lamm WJE, Hildebrandt J. Sampling and analysis of cerebrospinal fluid for chronic studies in awake rats. J Appl Physiol **54**:1754–1757, 1983.
6. Spector R, Johanson CE. The mammalian choroid plexus. Sci Am **11**:68–74, 1989.
7. Frankmann SP. A technique for repeated sampling of CSF from the anesthetized rat. Physiol Behav **37**:489–493, 1986.
8. Suckling AJ, Reiber H. Cerebrospinal fluid sampling from guinea pigs: Sampling volume-related changes in protein concentration in control animals and animals in the relapsing phase of chronic relapsing experimental allergic encephalomyelitis. Lab Anim **18**:36–39, 1984.