Transcutaneous Cisternal Puncture for Sampling of Cerebrospinal Fluid in Awake Rat

Yoshihiro TAKASUGI1), Toru SHIRAI1), Koichi FUTAGAWA2), Yoshihisa KOGA1), Kentaro EGAWA3), Shinsuke WATANABE3), and Takashi UMEDA4)

1)Department of Anesthesiology, Kinki University School of Medicine, 377–2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, 2)Department of Anesthesiology, Nara Hospital, Kinki University School of Medicine, 1248–1 Otoda, Ikoma, Nara 630-0293, 3)Life Science Research Institute, Kinki University School of Medicine, 377–2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, and 4)Department of Clinical Acupuncture, Osaka Kansai College of Oriental Medicine, 2–11–1 Wakaba, Kumatori-cho, Sennan, Osaka 590-0482, Japan

Abstract: Reported cisternal puncture methods require the anesthetization and fixation of an animal within a stereotaxic frame. To determine the effect of anesthesia and animal fixation on the central nervous system (CNS), amino acid concentrations of cerebrospinal fluid (CSF) sampled by transcutaneous cisternal puncture were compared among awake rats, pentobarbital-anesthetized rats and pentobarbital-anesthetized rats fixed in a stereotaxic frame. Although the concentrations of many amino acids in the CSF of pentobarbital-anesthetized rats were lower than in awake rats, use of the stereotaxic frame resulted in significantly increased amino acid concentrations in the CSF. These data indicate that CSF sampling by transcutaneous cisternal puncture from awake rats is a suitable method for serial measurement of drug effects on the CNS.

Key words: amino acids, awake rat, cisternal puncture

Analysis of substances in the cerebrospinal fluid (CSF) of rats has been used to investigate metabolism and neurotransmitters in the brain and for the long-term evaluation of the effects of drug therapy on the central nervous system (CNS). CSF sampling can be achieved by various methods, including cisternal cannulation [1, 4, 8], intraventricular cannulation [5], and cisternal puncture [2, 7, 9]. Although, these techniques allow for serial monitoring of changes in CSF, surgical intervention, anesthesia or noxious stimuli might confound the results [6].

In the present study, rats were trained to remain immobile under manual fixation, and CSF was collected by transcutaneous cisternal puncture while the animals were awake and without use of a stereotaxic frame. Concentrations of CSF amino acids were assessed and compared among awake rats (control group), pentobarbital-anesthetized rats (pentobarbital group), and pentobarbital-anesthetized rats that were fixed in a stereotaxic frame (stereotaxic group).

The experimental protocol was approved by the Committee on the Ethics of Animal Experimentation of

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Address corresponding: Y. Takasugi, Department of Anesthesiology, Kinki University School of Medicine, 377–2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
Kinki University School of Medicine. Ten male Sprague Dawley rats (10 to 12 weeks of age; weight 340–420 g) were used for each animal group. Rats were bred in the Life Science Research Institute of Kinki University School of Medicine, maintained under controlled conditions (temperature 23 ± 0.5°C; humidity 55%; 12/12h light/dark cycle), and fed a commercial diet, CE-2 (Clea Japan Inc., Tokyo, Japan), with ad libitum access to tap water. Rats in the control group were trained to remain immobile by exposing the animal to various manual manipulations for ten to fifteen minutes every day for two weeks (Fig. 1).

In the control group, rats received 0.2 ml of 1% lidocaine injected into the posterior cervical skin using a 27-gauge needle ten minutes prior to CSF sampling. Rats were placed in the prone position, and 70 to 100 µl of CSF were aspirated from the cisterna magna by advancing a 27-gauge butterfly needle connected to a 1-ml syringe through the muscle layer overlying the atlanto-occipital membrane and into the cisternal compartment (Fig. 2). In the pentobarbital group, rats were anesthetized intraperitoneally with pentobarbital sodium (37.5 mg/kg) and the loss of righting reflex was confirmed 10 to 15 min later. CSF samples were obtained using the same technique described for non-anesthetized rats. In the stereotaxic group, CSF samples were collected by the cisternal puncture procedure while the anesthetized rats were fixed in a stereotaxic frame without withdrawal reflex. All CSF samples were quick-frozen in liquid nitrogen and stored at –80°C until assayed.

CSF samples were filtered through a 10,000 NMWL ultrafilter (UFC3 LGC00, MCMEDICAL Inc., Tokyo, Japan), followed by centrifugation at 3,000 rpm for 10 min. Concentrations of free amino acids were determined by high-performance liquid chromatography using a coulometric array electrochemical detector (Model 5600A CoulArray® System, ESA, Chelmsford, MA, USA). Ninety microliters of the CSF were diluted in 20 µl of o-phthalaldehyde and injected into a reverse phase C18 column by the isocratic method, followed by analysis by four of sixteen sensors in the detector that was set at working electrode potentials of +200 mV and +520 mV at intervals of 140 mV. CSF samples were analyzed for the levels of various amino acids, including aspartate (Asp), asparagine (Asn), glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA), glycine (Gly), taurine (Tau), alanine (Ala), arginine (Arg) and threonine (Thr). Some of the sample were visibly contaminated with blood (control group, n=2; pentobarbital group, n=1; stereotaxic group, n=1) and were excluded from the analysis.

Data are expressed as mean ± standard deviation. Analysis of variance and Tukey’s HSD post-hoc test were employed to determine the inter-group differences of amino acid concentrations in the CSF. Statistical analysis was performed using Statistica for MS Win-
Fig. 3. Comparison of amino acid concentrations in the CSF among awake rats (control group), pentobarbital anesthetized rats (pentobarbital group) and pentobarbital-anesthetized rats fixed in a stereotaxic frame (stereotaxic group). The results are expressed as mean ± standard deviation. * P<0.05, compared by Tukey’s HSD post hoc test.

dows Ver. 5.05J (StatSoft). A value of p<0.05 was selected to indicate statistical significance.

Figure 3 shows amino acid concentrations in the CSF in the three groups. CSF concentrations of Asn, Glu, Gly and Tau were significantly lower and that of Arg was higher in the pentobarbital group than in the control group. CSF concentrations of Asp, Gln, Arg, Ala and Thr were higher and that of Glu was lower in the stereotaxic group than in the control group. CSF concentrations of all amino acids except GABA were significantly higher in the stereotaxic group than in the pentobarbital group. CSF concentrations of GABA were lower in the pentobarbital group (0.016 ± 0.007 nmol/mL) and the stereotaxic group (0.018 ± 0.009 nmol/
ml) than in the control group (0.028 ± 0.013 nmol/ml; p=0.23, 0.14).

The cisterna magna is the largest CSF compartment lying between the cerebellum and the upper medulla. Although the cisternal cannulation method is invasive, it has the advantage of not requiring repeated anesthesia for serial or continuous CSF sampling. However, the surgery is complicated, and cannulation leaves the rat highly susceptible to infections [3, 9]. Further, the cannula can easily become occluded [3]. In contrast, transcutaneous cisternal puncture is not invasive and is well tolerated by the rat. These properties allow long-term and repeated experiments and decrease the number of animals needed to demonstrate statistically significant effects. However, cisternal puncture methods described in the literature require the anesthetization and fixation of an animal within a stereotaxic frame, which may alter the release of neurotransmitters in the CNS and confound experimental results. The results of the present experiment indicate that pentobarbital anesthesia decreases the levels of amino acids and fixation in a stereotaxic frame induces noxious stimuli resulting in increased levels of amino acids.

Using the method described in this study, 70 to 100 µl of CSF could be aspirated from awake rats without fixation and with a relatively low rate of contamination with blood (10%). Further, van den Berg and colleagues [9] reported that this rate of contamination can be decreased with experience and proficiency in performing the cisternal puncture method.

The CSF volume in the rat is estimated as approximately 400 µl, with a CSF formation-rate of approximately 2.2 µl/min [4]. Thus, the 70 to 100 µl volume aspirated in the present study should be regenerated within 1 h. Based on these data and the time required for healing of the atlanto-occipital membrane, CSF collection at 24 h intervals appears appropriate for serial measurement of drug effects on the CNS.

In summary, the present study demonstrated that pentobarbital anesthesia decreases the concentrations of many amino acids in the CSF and that stimulation by the stereotaxic frame results in significant increases in amino acid concentrations. Further, CSF sampling by transcutaneous cisternal puncture from awake rats is a suitable method for serial measurement of drug effects on the CNS.

References