An Improved Method for Using Cisternal Cerebrospinal Fluid in Conscious Rats for Application in the Measurement of Catecholamines

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Summary This paper describes an improved method for the application of cerebrospinal fluid (CSF) in conscious rats. The modified polyethylene catheter was chronically inserted into the cisterna magna through a hole drilled in the skull of an anesthetized rat. Fifty microliters of CSF was taken from the recovered conscious rat for radioenzymatic analysis of catecholamines. The values of noradrenaline were 540 ± 142 pg/ml (mean $\pm$ S.D., $n = 50$) in Wistar rats and 494 ± 175 (42) in spontaneously hypertensive rats. However, values of adrenaline and dopamine were less than the lower limits for detection.

Key words: cerebrospinal fluid, conscious rats, catecholamine.

The method of taking cerebrospinal fluid (CSF) from the cisterna magna in conscious rats was determined with implantation of the steel chronical catheter by BOUMAN and VAN WIMERSMA GREIDANUS (1979) and SWARTZ and STENBERG (1980). The method using the flexible polyethylene catheter by SARNA *et al.* (1983) had overcome the deficiency of the steel catheter, which previously occasionally caused brain tissue damage and signs of encephalitis. However, even with the method by SARNA *et al.* (1983), when taking samples it was difficult to maintain good conditions for long after the rats returned to active movement, even if the outflow of CSF was fixed and verified at the time of implantation under anesthesia. After the catheter is inserted into the cisterna magna through the hole drilled close to the occipital crest, the outer side of the catheter is fixed on the skull with dental cement. The free inner tip of the catheter is thought to move and come into contact with the tissue around it when the rat moves its head actively. Therefore, the polyethylene catheter was modified to avoid such tissue contact, which would stop the flow of CSF. The tip of the modified catheter would be inserted deeply into the cisterna magna and accordingly raise the CSF volume to be taken.

This method was further applied to measure catecholamines in CSF in conscious rats by the radioenzymatic method, in which more CSF was needed for analysis. Since little is known about the value of catecholamines in CSF in conscious rats, not much can be said about its physiological or pathophysiological significance in rats. In hypertensive patients, CSF noradrenaline levels increased significantly (CUBEDDU *et al.*, 1984; LAKE *et al.*, 1981). Therefore, catecholamines in CSF in conscious Wistar rats and spontaneously hypertensive rats (SHR), as models of hypertensive rats, were measured in order to ascertain whether some difference between the two states of blood pressure existed in the rats. Other than for the measurement of catecholamine, the CSF taken from conscious rats is useful not only for general physiological and pathophysiological studies but also for biochemical studies.

The catheter for the cisterna magna, as shown in Fig. 1, consists of polyethylene tubes SP19 and SP10 (Natsume), which were fused together using a hot air stream; the tip of SP10 was then bent to between a 120–130° angle. The fused knob was useful as a stopper to the bone. The bending tip was bevelled at the position of

Fig. 1. A modified intracisternal catheter and its position for insertion.

2–2.5 mm from the bending point and a few holes made on the opposite side using a needle. A wire longer than the catheter was inserted into the catheter then bevelled at the SP10 side. The wire at the SP19 side was slightly bent as the mark showing the depth of the inner tip. The end of SP19 was circumferentially marked with a pen in the same direction as the tip of SP10.

Wistar rats and SHR of both sexes, weighing 200–500 g, were anesthetized intraperitoneally with thiamylal (50 mg/kg). The occipital hairs of the rats, which were placed in a prone position, were removed with clippers and an incision was made along the sagittal line around the interparietal bone. After the tissues on the surface of the bone were removed, a hole was drilled at the position of the arrow as shown in Fig. 1. The catheter was further inserted into the cisterna magna and oriented caudally, and finally the inner wire was pulled out. After verification of withdrawal of CSF from the end of SP19 by syringe, the catheter was fixed on the bone with dental cement (Shofu, HY-BOND GLAS IONOMER-F). In the SHR, a small screw fastened on bone was used for firm settlement of the catheter.

Immediately after implantation, a flow of CSF containing blood from the catheter was sometimes noticed. When this flow stopped, the stainless steel stopper (diameter 0.4 mm, length 1 cm) was fixed to the end of the catheter.

After the operation, the rats were kept individually in plastic cages measuring $35 \times 30 \times 17$ (depth) cm and containing wood chips. Two days later, after the rat began to take ample water and pellets, CSF was withdrawn from the conscious rats in the home cage through the cisternal catheter connected to another longer cannula (cotents: 50 microl, length: 50 cm) with a syringe as unobtrusively as possible. The CSF was immediately moved to a tube cooled to 4°C. Five microlitres of CSF for dead volume was collected in a shorter cannula connected to the longer one and then discarded.

Fifty microl of CSF was taken for analysis of catecholamines. The rat was calm and quiet during this collection. The time taken to collect 50 microl of CSF depended on the condition of the tip of the cisternal cannula but ranged from 1 to 10 min. When more than 10 min was needed, the sampling was abandoned. The volume needed for this analysis depended on the lower limit of the substance to be analyzed. If more quantity was required, it was possible to take around 200 microl, but the rat would notice such a change. Catecholamines were measured by a radioenzymatic assay (PEULER and JOHNSON, 1977), using 40 microl of CSF. Concentrations of adrenaline and dopamine in CSF were under than the lower limits for detection.

An example of repeated CSF sampling is shown in the following. When CSF was taken every day as a preliminary experiment for the study of the circadian rhythm of noradrenaline in CSF in a conscious Wistar rat, its values, based on a daily taking of 50 microl of CSF, were 464 pg/ml at 14:00 on the first day, 384 at 0:00 on the second day, 808 at 5:00 on the third day, 394 at 19:00 on the fourth day, and 414 at 10:00 on the fifth day. On the fifth day, when CSF was taken continuously for 10 min at 10:00, the values were 414 pg/ml, 368, 400, 482, and

Table 1. Noradrenaline concentrations of cerebrospinal fluid in conscious Wistar rats and spontaneously hypertensive rats (pg/ml, mean $\pm$ S.D., sample number).

	Male	Female	Total
Wistar	545 $\pm$ 145 (28)	534 $\pm$ 144 (22)	540 $\pm$ 142 (50)
SHR	486 $\pm$ 184 (13)	498 $\pm$ 177 (29)	494 $\pm$ 175 (42)

443. Because 50 microl of CSF implies about 20% of a rat's total CSF volume (CSERR, 1965; BURNS *et al.*, 1976), and because removal of this volume may affect circadian rhythm, sampling was performed once a day rather than every 4–5 h. Further study is needed for interpretation of these values in the context of circadian rhythm.

The values of noradrenaline in CSF taken by the improved method in conscious Wistar rats and SHR are shown in Table 1. Central noradrenaline is involved in the development and maintenance of hypertension in animal models of hypertension (MAJOR *et al.*, 1984). However, the difference in noradrenaline concentrations in CSF between conscious Wistar rats and SHR was not significant, although the SHR had a tendency to show a lower value. This tendency may imply that decreases in central noradrenaline are related to the hypertensive state in SHR, because a central α -2 adrenoceptor agonist, clonidine, has an antihypertensive effect.

Noradrenaline concentration in CSF is 120–200 pg/ml in conscious monkeys (ZIEGLER *et al.*, 1976) and 127–311 pg/ml in humans (LAKE *et al.*, 1981; KAWANO *et al.*, 1982; CUBEDDU *et al.*, 1984). The value in conscious rats, 540 pg/ml, was quite high in comparison, and the reason for this remains unknown.

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