The nocturnal increase in human cerebrospinal fluid production is inhibited by a β_1-receptor antagonist

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Nilsson, Christer, Freddy Ståhlberg, Peter Gideon, Carsten Thomsen, and Ole Henriksen. The nocturnal increase in human cerebrospinal fluid production is inhibited by a β_1-receptor antagonist. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36): R1445–R1448, 1994.—A circadian variation in human cerebrospinal fluid (CSF) production has recently been demonstrated using magnetic resonance phase imaging. A nightly peak in CSF production was found at ~0200, when production is approximately twice the daytime values. In the present study, we have investigated the effect of a β_1-receptor antagonist, atenolol, on the production of CSF, specifically the nocturnal production peak. CSF production was measured in fourteen healthy volunteers of both sexes in the time interval 1500–1800, with or without drug administration (100 mg orally) at 1800, and a second measurement was made in the time interval 2300–0200. In the absence of drug administration, all nine volunteers showed a significant increase in CSF production at night, from 0.34 ± 0.06 ml/min in the time interval 1500–1800 to 0.61 ± 0.05 (SE) ml/min ($P < 0.005$), confirming the presence of a circadian variation in these individuals. One week later, the experiment was repeated in five of these volunteers, plus an additional five volunteers, but with the administration of 100 mg atenolol orally immediately after the first measurement (at 1800). In five of the volunteers a decrease in CSF production was seen at midnight compared with daytime production values; in two volunteers CSF production remained unchanged, while three volunteers showed increased production. The average CSF production was 32% lower at night (0.27 ± 0.10 ml/min) compared with the afternoon (0.40 ± 0.07 ml/min), after administration of atenolol ($P = 0.37$). Analysis of the data in a 2 × 2 contingency table showed that the effect of atenolol on the nocturnal rise in CSF production was highly significant ($P < 0.005$). In conclusion, the present results further support the concept of a circadian variation in human CSF production and indicate that a β_1-receptor-mediated mechanism might be involved.

flow; phase; magnetic resonance imaging; atenolol; sympathetic; circadian

CEREBROSPINAL FLUID (CSF) is produced by the choroid plexus in the four brain ventricles, flowing out into the cranial and spinal subarachnoid spaces from where it is absorbed into the bloodstream. The main roles of the CSF are to protect the brain mechanically and to provide a suitable chemical environment for the central nervous system (4). In addition, the CSF provides a route of distribution for nutrients and hormones within the brain (18, 23). Although the cellular basis of CSF secretion is fairly well understood, little is known about the physiological regulation of total CSF production, especially in humans.

Several investigators have described a pulsatile flow of CSF through the cerebral aqueduct between the third and fourth ventricles (1, 2, 6, 14). We have described a method for quantifying CSF production by calculating the net flow of CSF through the cerebral aqueduct using magnetic resonance (MR) phase imaging (24, 25). The linearity of the MR phase signal vs. velocity relation in this type of experiment has been theoretically postulated (17, 22) and has been experimentally verified for a large range of flow velocities (7, 24).

Using this method, we have recently demonstrated a circadian variation in human CSF production with a maximum at ~0200 (19). In an attempt to elucidate the mechanism underlying this circadian variation we have studied the effect of the β_1-receptor antagonist atenolol on the nocturnal rise in CSF production.

METHODS

The method for calculating CSF velocities, flow, and production has been described in detail previously (19, 24, 25). This method utilizes the phase information obtained after subtracting two MR images with different phase vs. velocity sensitivity. In this study, the measurements were made in a 1.5-T Magnetom SP 4000 MR scanner (Siemens, Karlsruhe, Germany) using quadrature detection in the head coil and a specially designed interleaved gradient-echo sequence (echo time = 13 ms, repetition time = 59 ms) with additional pulsed gradients for flow encoding to determine CSF velocities in the cerebral aqueduct at between 20 and 32 equally spaced points in the cardiac cycle (Fig. 1). The field of view was 250 mm, and a 256 × 256 matrix was used, giving a volume element (voxel) size of 0.98 × 0.98 × 8 mm^3. The velocity sensitivity in the phase map was determined to be 20 $rad \cdot m^{-1} \cdot s^{-1}$, and the in vivo phase noise was ~0.04 rad, corresponding to a linear velocity of 2 mm/s. To reduce errors in the calculation of CSF production due to variations in heart rate during measurements, continuous registration of heart rate was done, and the average heart rate for the whole measurement period was used.

For determination of whether a β_1-adrenoreceptor antagonist has an effect on the circadian variation in human CSF production, fourteen healthy volunteers (8 males, 6 females) aged 21–32 yr were used. In each volunteer, CSF production was first measured in the time interval 1500–1800, followed by a second measurement in the interval 2300–0200, with or without oral administration of 100 mg atenolol (Atenolol Fermenta, Merckle/Fermenta, Sweden) at 1800. In five of the volunteers (3 males, 2 females), CSF production was measured both with and without treatment with atenolol. The measurements with atenolol treatment followed 1 wk after the control measurements. In total, 9 measurements were made without and 10 with atenolol. In all but four of the untreated volunteers each time point was represented by the average of two consecutive measurements, rather than by a single measure-

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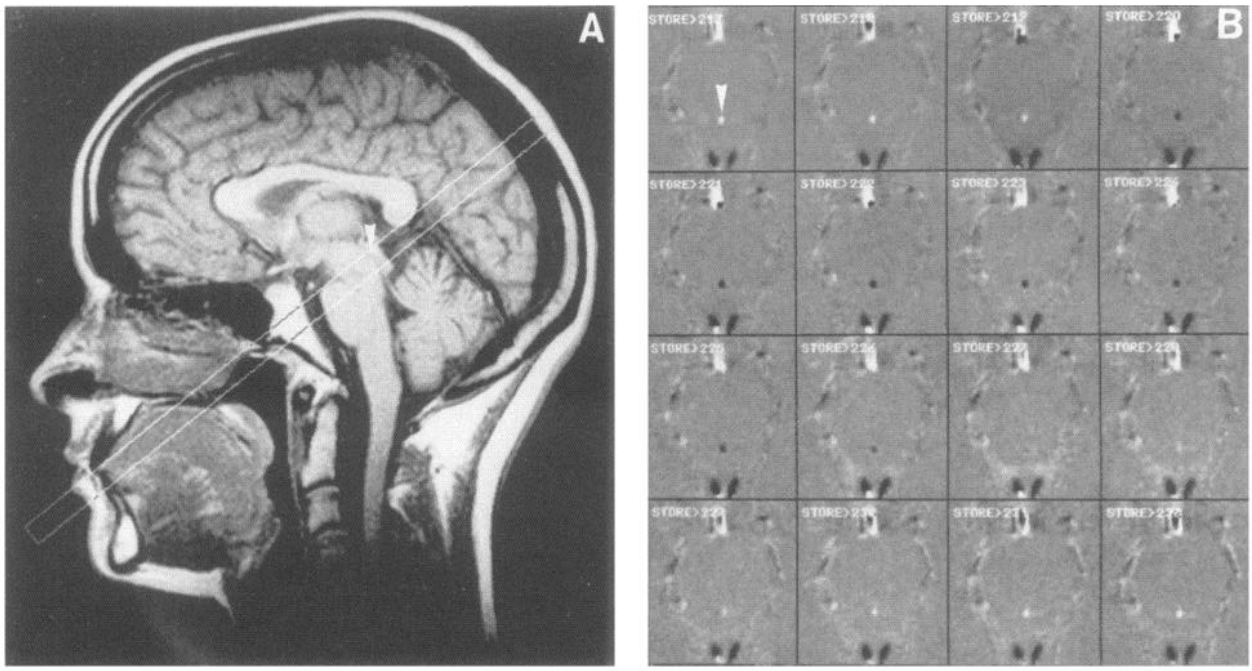

Fig. 1. *A*: Magnetic resonance image in the midsagittal plane of a healthy volunteer. Area within the box was divided into 3 8-mm-thick sections perpendicular to the cerebral aqueduct (arrowhead). After inspection of the 3 sections, the subject was repositioned, if required, and flow measurement was started. *B*: flow phase images of the cerebral aqueduct (arrowhead), showing a sequence of images covering ~1.3 heart cycles. The difference between aqueduct and brain tissue phase signal is proportional to the velocity of the CSF, while the direction of the flow can be seen as either a lighter (cranial flow) or darker aqueduct (caudal flow).

ment. The two-tailed Student's t-test for paired samples was performed in the untreated and treated groups, respectively, to determine whether an increase in CSF production occurred at night and whether the heart rate of the volunteers changed. The effect of atenolol on the nocturnal increase in CSF production was evaluated statistically by a 2 × 2 contingency table for small samples (27). For this purpose the effect on CSF production was calculated as follows. Unchanged production was defined as ≤10% change from the daytime value, and increased/decreased CSF production was defined as >10% change. The study was approved by the local Ethics Committee for Research.

RESULTS

In the nine untreated volunteers, the average CSF production was 0.34 ± 0.06 (SE) ml/min (range 0.13–0.66 ml/min) in the time interval 1500–1800, and it increased significantly by 80% to 0.61 ± 0.05 ml/min in the time interval 2300–0200 (range 0.30–0.79 ml/min; $P < 0.005$) (Fig. 2). In contrast, only 3 of 10 volunteers treated with atenolol showed increased CSF production at night, while the other 7 volunteers showed unchanged (2 volunteers) or decreased (5 volunteers) production. Treatment with atenolol resulted in a decrease in mean CSF production by 32%, from 0.40 ± 0.06 to 0.27 ± 0.10 ml/min (Fig. 2). This change was not statistically significant ($P = 0.37$, 2 tailed). The night-to-day ratio for each individual volunteer was calculated (expressed as ±%change) and used to confirm that atenolol inhibited the nocturnal increase in CSF production seen in untreated volunteers. These data were analyzed in a 2 × 2 contingency table. In a two-tailed test, atenolol had a highly significant, inhibitory effect ($P < 0.005$) on the nightly increase in CSF production (Table 1). As atenolol is a β_1-receptor antagonist, it has a negative chronotropic effect on the heart. Accordingly, the heart rate fell by 19% ($P < 0.005$), from 60 ± 4 to 48 ± 2 beats/min between 1500–1800 and 2300–0200 in the treated volunteers, confirming that the drug had been absorbed. These data were compared with the heart rates in the nine untreated volunteers in the corresponding time intervals, to exclude the possibility that the decrease in heart rate was due to time of day. There was no significant decrease in heart rate in

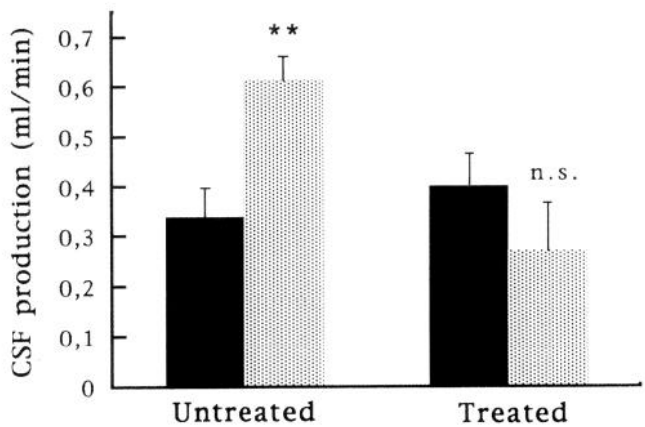

Fig. 2. CSF production in volunteers untreated ($n = 9$) or treated ($n = 10$) with 100 mg atenolol orally. Filled bars, CSF production in the time interval 1500–1800; stippled bars, production at night (2300–0200. Values are presented as means ± SE. Statistical analysis of the data was made using 2-tailed Student's t-test for paired samples. $^{**}P < 0.005$ vs. 1500–1800 value; NS, not significant.

Table 1. *Effect of atenolol on night-to-day ratio in human CSF production*

	CSF Production at Night		Total
	Increased	Unchanged or decreased	
Untreated	9	0	9
Treated	3	7	10
Total	12	7	19

A 2 × 2 contingency table analysis of the effect of atenolol on the nocturnal increase in human cerebrospinal fluid (CSF) production; values are nos. of volunteers. Unchanged production was defined as ≤ 10% change from daytime value; increased/decreased CSF production was defined as > 10% change. In a 2-tailed test, the difference between untreated and treated groups was calculated as highly significant ($P < 0.005$).

untreated volunteers between 1500–1800 (59 ± 2 beats/min) and 2300–0200 (58 ± 3 beats/min).

DISCUSSION

The method used in this study for the measurement of human CSF production has been extensively characterized in our laboratory (9–11, 19, 24, 25). The values of CSF production obtained are extrapolations of the average net caudal CSF flow through the cerebral aqueduct during 8 min. These values are within previously determined physiological production values (3, 16, 21). Furthermore, the extrapolated total production values for the whole 24-h period are similar for most individuals in spite of the pronounced intraindividual variation underlying the observed circadian rhythm (19). A problem encountered previously concerns the precision of the method, with a variation in consecutive production measurements of up to 30%, assumed to consist of intrinsic phase noise, variations in electrocardiogram rhythm over the examination time, and errors caused by nonorthogonal tilting of the imaging slice relative to the aqueduct (25). In this study, improved hardware enabled the use of a circularly polarized transmit-receive system, giving a maximum improvement in signal-to-noise ratio with a factor of 1.4, as well as the use of tilted imaging slices that could be placed at right angles to the length axis of the cerebral aqueduct independently of the positioning of the head of the volunteer. To further reduce noise, we increased the number of velocity measurement points from 10 to between 20 and 32, and the mean of two consecutive measurements was used for each production calculation. Residual experimental error due to the variation in heart rate during the period of measurement was reduced by continuous registration of heart rate and subsequent software correction of variations.

Although there have been speculations on the possibility of circadian variation in CSF production (8), such variation has been difficult to verify with the methods previously available. The results from the present study where all of the nine untreated volunteers showed almost double CSF production at night compared with the afternoon strengthens our previous hypothesis (19). This suggests that CSF production in humans is relatively stable during daytime but increases in the late evening with a peak after midnight. Indirect evidence for circadian variation in humans may be found in continuous recordings of intracranial pressure in children with acute hydrocephalus, which show daily cyclic variations with nighttime peaks absent in children with chronic hydrocephalus (12).

The choroid plexus epithelium contains various receptors for neurotransmitters, as well as blood- and CSF-borne hormones, e.g., vasopressin, atrial natriuretic peptide, and 5-hydroxytryptamine (18). There is a rich supply of noradrenergic, sympathetic nerve fibers to the choroid plexus and corresponding β_1-adrenergic receptors in the epithelium (13). Furthermore, in the rabbit, stimulation of the sympathetic cervical ganglia inhibits CSF production, while sympathectomy results in an increase in production (13). Sympathetic nerves also have an essential role in the secretion of the pineal gland hormone, melatonin. On activation by the hypothalamic pacemaker nucleus suprachiasmaticus, a specific branch of sympathetic nerves, originating in the superior cervical ganglia, stimulates the synthesis and activity of the melatonin-synthesizing enzyme *N*-acetyltransferase in pinealocytes after binding to β_1-receptors on these cells (20). This occurs at nighttime, resulting in a pronounced increase in melatonin secretion and a rise in the concentration of melatonin in serum and CSF (20). Although based on indirect evidence, a stimulatory role for melatonin in the control of CSF production has been suggested (5, 15), and the nocturnal increase in melatonin closely resembles the pattern of circadian variation in CSF production.

In an attempt to elucidate the mechanism underlying the circadian variation in CSF production observed by us, we hypothesized that the sympathetic nervous system might be involved, either directly by stimulation of the choroid plexus epithelium or indirectly via the β_1-adrenoceptor-stimulated secretion of melatonin from the pineal gland (20). We found that average CSF production values at night were not significantly lower compared with daytime values after treatment with atenolol. In terms of basal CSF secretion these results are difficult to interpret as the study was designed to investigate the effect of atenolol on the demonstrated rise in CSF production between the two periods of measurement. Therefore they cannot be compared directly to previous studies in experimental animals on the role of the sympathetic nervous system in the regulation of CSF production (13). However, our results indicate that atenolol inhibits the nocturnal increase in CSF production. It should be noted that in three volunteers the nocturnal increase in CSF production was not affected by atenolol. As these volunteers showed a decrease in heart rate in response to atenolol, the results cannot be explained by failure to absorb the drug. The difference could be explained by biological variation between volunteers and might suggest that other factors could be involved in the regulation of human CSF production.

Atenolol has previously been shown to abolish the nocturnal surge of melatonin in serum (26), at the same dose that in this study inhibited the expected rise in CSF

production. Atenolol is an essentially lipid-insoluble substance, precluding central effects. Both the pineal gland and the choroid plexus lack a blood-brain barrier, however, making these structures accessible to atenolol in the blood. Thus activation of β_1-receptors on either the choroid plexus epithelium or pinealocytes could be the direct or indirect cause, respectively, for a circadian variation in human CSF production. The present results cannot distinguish between these two possible mechanisms. In summary, this study provides additional evidence for circadian variation in human CSF production and indicates that a β_1-adrenergic mechanism is involved.

Perspectives

CSF production measurements in humans by MR imaging offer the advantage of being completely noninvasive, in contrast to ventriculolumbar perfusions in neurosurgical patients (3, 21) or the modified indirect method of Masserman (see Ref. 16), which involves lumbar puncture. The method has recently been used in our laboratory to study CSF flow dynamics and production in both normal aging and in patients with increased intracranial pressure (9–11). We believe that the method is also useful for functional evaluation of drugs.

Although the function of circadian variation in CSF production is unknown, it might have clinical implications. The chemical analysis of CSF would have to be related to the time of sampling, as compounds present in the CSF would be differently diluted during day and night, respectively. Circadian variation in CSF production could also be important in the management of patients with impaired CSF drainage. Finally, we expect that MR imaging measurements of CSF production will continue to be of importance for the study of human CSF physiology.

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