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NEW EXPERIMENTAL MODEL OF ACUTE AQUEDUCTAL BLOCKAGE IN CATS: EFFECTS ON CEREBROSPINAL FLUID PRESSURE AND THE SIZE OF BRAIN VENTRICLES

M. KLARICA,[a] D. ORE̊ŠKOVIĆ,[b]* B. BOŽIĆ,[c] M. VUKIĆ,[d] V. BUTKOVIĆ[e] AND M. BULAT[a]

[a]*Department of Pharmacology and Croatian Institute for Brain Research, School of Medicine University of Zagreb, Šalata 11, Zagreb, HR 10 000, Croatia*

[b]*Ruđer Bošković Institute, Bijenička 54, Zagreb, HR 10 000, Croatia*

[c]*Department of Neurosurgery, "Sestre milosrdnice" General Hospital, Vinogradska 29, Zagreb, HR 10 000, Croatia*

[d]*Department of Neurosurgery, School of Medicine, University of Zagreb, Kišpatiæeva 12, Zagreb, HR 10 000, Croatia*

[e]*Department of Radiology, School of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, HR 10 000, Croatia*

Abstract—It is generally assumed that cerebrospinal fluid (CSF) is secreted in the brain ventricles, and so after an acute blockage of the aqueduct of Sylvius an increase in the ventricular CSF pressure and dilation of isolated ventricles may be expected. We have tested this hypothesis in cats. After blocking the aqueduct, we measured the CSF pressure in both isolated ventricles and the cisterna magna, and performed radiographic monitoring of the cross-sectional area of the lateral ventricle. The complete aqueductal blockage was achieved by implanting a plastic cannula into the aqueduct of Sylvius through a small tunnel in the vermis of the cerebellum in the chloralose-anesthetized cats. After the reconstitution of the occipital bone, the CSF pressure was measured in the isolated ventricles via a plastic cannula implanted in the aqueduct of Sylvius and in the cisterna magna via a stainless steel cannula. During the following 2 h, the CSF pressures in the isolated ventricles and cisterna magna were identical to those in control conditions. We also monitored the ventricular cross-sectional area by means of radiography for 2 h after the aqueductal blockage and failed to observe any significant changes. When mock CSF was infused into isolated ventricles to imitate the CSF secretion, the gradient of pressure between the ventricle and cisterna magna developed, and disappeared as soon as the infusion was terminated. However, when mock CSF was infused into the cisterna magna at various rates, the resulting increased subarachnoid CSF pressure was accurately transmitted across the brain parenchyma into the CSF of isolated ventricles. The lack of the increase in the CSF pressure and ventricular dilation during 2 h of aqueductal blockage suggests that aqueductal obstruction by itself does not lead to development of hypertensive acute hydrocephalus in cats.

Key words: cerebrospinal fluid formation, cerebrospinal fluid pressure, transmantle gradient, acute hydrocephalus, obstruction of the aqueduct of Sylvius.

*Corresponding author. Tel: +385-1-468-0-218; fax: +385-1-456-1-177.
E-mail address: doresk@irb.h (D. Orešković).

Abbreviations: BV, brain ventricle; CM, cisterna magna; CSF, cerebrospinal fluid; ICP, intracranial pressure; ISF, interstitial fluid.

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According to the generally accepted hypothesis of the cerebrospinal fluid (CSF) dynamics, CSF is produced within the cerebral ventricular system, and it circulates slowly from the brain ventricles (BV) toward the subarachnoid space to be absorbed into the venous sinuses and/or into lymphatics via perineural sheets of cranial and spinal nerves (Brodbelt and Stoodley, 2007; Johanson et al., 2008). It is believed that CSF is formed mainly by the secretory activity of the choroid plexuses in the BV, and that the majority of the remaining CSF is probably produced by the ependyma. The endothelium of the choroid plexus capillaries is fenestrated, and the first stage in the CSF formation is the passage through the endothelium of a plasma ultrafiltrate, facilitated by a hydrostatic pressure. During the second stage of the CSF formation, the ultrafiltrate passes through the choroidal epithelium at the surface of the choroid plexus and into the ventricle. The passage through the choroidal epithelium is an active metabolic process, which transforms the ultrafiltrate into secretion (CSF) (Davson et al., 1987; Brown et al., 2004). Since this second stage is an active process, the CSF formation rate should not be significantly altered by moderate changes in the intracranial pressure (ICP) (Heisey et al., 1962; Rubin et al., 1966; Cutler et al., 1968; Sklar et al., 1980; Pollay et al., 1983). It is believed that CSF is passively absorbed (under pressure gradient between CSF and blood) through arachnoid villi of the dural venous sinuses. In addition, there is a large amount of literature data which suggest that significant absorption of CSF from the subarachnoid space to the lymphatic system takes place (Johnston et al., 2004, 2005; Koh et al., 2006). According to all the above, it is generally accepted that CSF should flow unidirectionally (forced by pulsations of vessels) from BV to subarachnoid space with exchange of various substances (more or less manifested) between CSF and interstitial compartments (Johanson et al., 2008). This hypothesis, with minor modifications, represents a common point of reference in scientific papers, review articles and in numerous textbooks, and it is proffered as an unquestionable fact. The hypothesis is applied to explain the removal of cerebral metabolites, an increase in ICP and the development of hydrocephalus.

According to the abovementioned hypothesis, if the CSF system is blocked between the ventricular and subarachnoid space, a significant increase in the ventricular pressure and dilation of BV is expected to take place. Namely, the actively produced CSF should be accumulated inside the BV because it could not be absorbed into the venous sinuses or lymphatics from the subarachnoid space.

However, in light of our former investigations we did not expect that the blockade of cerebrospinal spaces between the BV and the subarachnoid space should necessarily result in a significant intraventricular pressure increase due to the accumulation of the CSF formation volume inside the BV. We have shown (Orešković et al., 1991) that at a physiological ICP, the CSF production and absorption are in balance in isolated BV. Furthermore, when labeled water is infused into the lateral ventricle, it is not distributed to the cisterna magna (CM) but rather absorbed into periventricular capillaries, indicating that the CSF volume (water) is absorbed in the ventricles (Bulat, 1993; Bulat et al., 2008). In addition, it was shown that molecules with different molecular weight (organic anions such as brain metabolites and ^{3}H-benzylpenicillin, and larger molecules such as ^{3}H-inulin and horseradish peroxidase) could be distributed rapidly from BV to interstitium and finally absorbed into blood via cerebral capillaries (Rennels et al., 1985; Vladić et al., 2000, 2008; Zmajević et al., 2002). Moreover, when the aqueduct of Sylvius was cannulated, no CSF outflow was observed from the isolated ventricle at a normal CSF pressure suggesting that no net formation of CSF took place in the ventricles (Orešković et al., 2001, 2002). In addition to that, Milhorat et al. (1976) have shown that surgical removal of choroid plexuses in hydrocephalus cases failed to improve the patients' condition, and that CSF composition and formation remained similar to those in hydrocephalus-free individuals. This has been observed in an experimental model of the disease (Milhorat, 1969). The concept suggesting that the choroid plexus also participates in the absorption of CSF is a rather old idea (Foley, 1921; Hassin, 1924). It has been suggested that the choroid plexus probably acts as two-way traffic (Dodge and Fishman, 1970). In some children shunted due to obstructive hydrocephalus, the shunts became occluded over time without any signs of hydrocephalus progression which indicated that a balance was reached between the CSF formation and absorption in the isolated BV (Holtzer and de Lange, 1973).

In light of these findings, our experiments were designed to detect whether the acute blockade of the aqueduct of Sylvius itself would result in an increase CSF pressure and ventricular dilation. For this reason we have developed a new experimental model featuring a complete aqueductal blockade in cats, which allows simultaneous measurement of CSF pressure inside the BV (in front of the blockade) and subarachnoid spaces (CM; behind the blockade). X-ray ventriculography was performed to explore if the aqueductal occlusion affected the size of the isolated ventricles.

Finally, using the aforementioned model, we have infused mock CSF into the lateral BV at different rates of the hypothetical physiological CSF formation rate to evaluate if our new model is sensitive enough to detect an accumulation of fluid volume in isolated ventricles, and if CSF pressure would change under these conditions. Under such experimental conditions the gradient of CSF pressure was observed in the cranium, and the mechanism of its development was analyzed. This investigation challenges the traditional assumption that a blockade of CSF pathways itself would increase the CSF pressure and dilate the BV, as well as the classic hypothesis of CSF physiology related to secretion and absorption inside the BV.

EXPERIMENTAL PROCEDURES

Animals

The experiments were performed on adult cats, unselected in terms of age and sex, ranging in weight from 1.8–4.0 kg. All experimental procedures were performed in accordance with the European Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, and the Law on Animal Rights and Protection of the Republic of Croatia and with the approval of the institutional Ethical Committee. Every attempt was made to minimize both the number and the suffering of animals used in these experiments. The animals were anesthetized with an i.p. injection of chloralose (α-chloralose, Fluka Chemika, Buchs, Switzerland; 100 mg/kg). The femoral artery was cannulated, blood pressure was recorded via a "T"-connector and samples of blood were taken for the analysis of blood gases. No significant changes either in the blood pressure or blood gases were observed in these experiments in cats, which continued breathing spontaneously under chloralose anesthesia. Physiological saline was applied via the cannulated femoral vein as necessary to maintain the blood pressure, and an overdose of thiopentone was injected at the end of the experiment to euthanize the animals.

Aqueductal occlusion and CSF pressure measurement

In the preliminary experiments, the fourth ventricle was surgically exposed after the opening of CM to occlude the aqueduct of Sylvius, which was followed by a partial removal of the occipital bone and removal of a part of the cerebellum. A polyethylene tubing (i.d. 1.12 mm, o.d. 1.55 mm; Clay-Adams, USA) was heated and pulled so that a narrow tip ($\sim$0.5 mm o.d.) was obtained. After filling the tubing with mock CSF, its narrow tip was covered on the outer side by cyanoacrylate gel glue (Superattack-gel, Loctite, Munich, Germany) for about 2 mm length, and under ocular supervision pushed slightly into the exposed opening of the aqueduct. After 10 s, the complete occlusion of the aqueduct was obtained so that CSF in the third and lateral ventricles communicated with the mock CSF in the polyethylene cannula, but not with the fourth ventricle. This was tested by infusing mock CSF containing 2% Trypan Blue into the lateral ventricle and positioning the outflow of the cannula up to 40 cm above the interaural line. No leakage of Trypan Blue could be detected from the aqueduct into the fourth ventricle under such hydrostatic pressure, since polyethylene tubing was firmly attached to the aqueductal tissue. However, a drawback of this surgical approach was the CSF leakage from the subarachnoid space, as the closing of the surgical wound could not be prevented. To prevent the CSF leakage, a different surgical approach to the aqueduct was used in these experiments, as described in detail in our previous paper (Miše et al., 1996). In short, a burr hole (10 mm in diameter) was made in the midline of the occipital bone and the exposed dura was incised. A tunnel was made through the vermis of the cerebellum (1.5–2.0 cm long and 0.6–0.8 cm wide) with vacuum suction and the opening of the aqueduct in the fourth ventricle was exposed. Under direct vision, the tip of a polyethylene cannula covered with cyanoacrylate gel glue was positioned into the aqueduct as described above. Thereafter, the tunnel in the cerebellum was filled with Gelfoam, the cannula was fixed to the occipital bone by dental cement and the bony hole was covered by dental acrylate so that a hermetic closure was obtained preventing any CSF leakage and blocking the influence of atmospheric pressure.

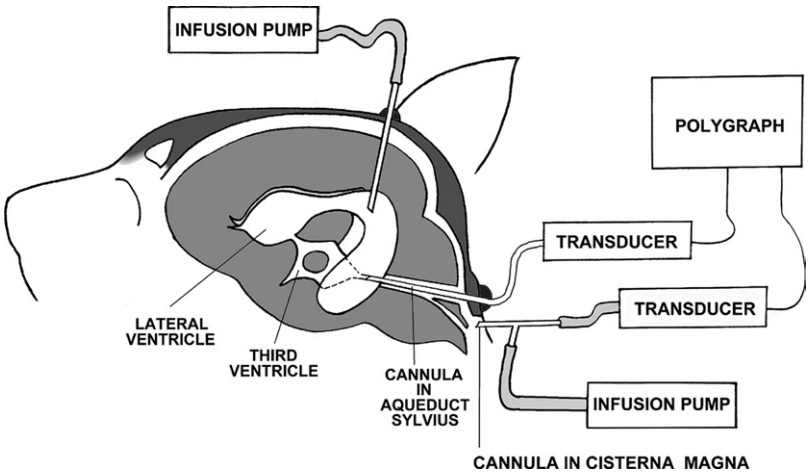

Fig. 1. Scheme of an experimental model showing the position of the cannulas for the CSF pressure recording in the aqueduct of Sylvius and the CM, as well as a cannula in the lateral ventricle and "T" connection to the cannula in the CM used for an intraventricular or intracisternal infusion of the mock CSF, respectively.

A stainless steel cannula (22-gauge) was micromanipulated into the lateral ventricle at coordinates 4.5 mm anteriorly and 9.0 mm laterally from the zero point of the stereotaxic atlas (Snider and Niemer, 1961), and about 10 mm vertically from the dural surfaces, until free communication with CSF in the ventricle was established (Bulat and Živković, 1978). The cannula was connected with an infusion pump via a polyethylene tubing (Harvard M-975, USA) (Fig. 1.), and served to infuse mock CSF (Merlis, 1940) at 7.0 μL/min, 13.0 μL/min, 26.0 μL/min, and 52.0 μL/min to simulate the formation of CSF in the ventricles of the animals before and after the blocking of the aqueduct of Sylvius, as well as for the application of Trypan Blue at the end of the experiment to verify the occlusion of the aqueduct.

CM was also cannulated by a direct puncture with a stainless steel cannula (22 gauge), which was fixed in position by a holder and connected with the plastic tubing filled with mock CSF. Aqueductal and cisternal plastic cannulas were connected to pressure transducers (P23, Gould Electronics, USA) and a polygraph (R511A, Beckman, USA) (Fig. 1) so that CSF pressures could be simultaneously recorded in both the isolated ventricles and CM before and after the aqueductal occlusion. The pressure transducers were calibrated at the level of interaural line taken as the zero reference pressure using water column, and CSF pressure was presented as cm H_2O. The cannula in the CM was connected to the infusion pump via a "T"-connector (Fig. 1) in order to infuse the mock CSF into the CM at different rates (7.0 μL/min; 13.0 μL/min; 52.0 μL/min; 100.0 μL/min).

The impermeability of the aqueductal blockage in our new model was tested at different CSF pressure values and in different time intervals. What we were able to observe in the three preliminary experiments was that the pressure in the ventricles of up to 40 cm H_2O did not result in the breakthrough of the blockage which remained complete 4 h after the placement of cannula in aqueduct. In addition, in the next three experiments we examined the duration of such obstruction and after a prolonged period (24 h) we observed that the tissue in the vicinity of the cyanoacrylic glue became necrotic and that the blockage was leaking.

The rectal temperature was recorded during the experiment and maintained at about 37 °C using a heating pad. To verify that the aqueduct remained successfully occluded in all the experiments presented here (see Results), at the end of each experiment isolated ventricles were perfused with 2% Trypan Blue in saline (26.0 μL/min) for 20 min from the cannula in the lateral ventricle to the aqueductal cannula with its open end positioned 25 cm above the interaural line. Thereafter, the animals were sacrificed by an i.v. overdose of thiopentone. After the careful partial opening of the occipital bone and dissection of the cerebellum, the cannula in the aqueduct was exposed so that any leakage of Trypan Blue from isolated ventricles into the fourth ventricle could be easily detected.

Ventriculography

To explore whether the aqueductal occlusion affected the size of isolated ventricles, X-ray ventriculography was performed in cats. After the placement of aqueductal and ventricular cannulas (see above), a wooden holder was fixed in the cat's mouth, and the animal was set in the sphinx position. From the aqueductal cannula, 100 μL of CSF was removed and the same volume of contrast (Omnipaque, Sanofi Winthrop Pharmaceuticals) applied via a ventricular cannula; this procedure was repeated 10 times during 1 min until 1 mL of contrast was applied. The application of contrast by this microvolume exchange method prevented any significant oscillation in intraventricular pressure and potential changes of ventricular size (Klarica et al., 1994).

After the contrast application, the pressures in the cannulas were adjusted to a normal CSF pressure (8.0 cm H_2O above the interaural level), the cannulas closed and a control X-ray ventriculogram made with an X-ray apparatus (Philips Type Dane 1001) using a mammography film (18×24 cm). The film was fixed close to the lateral side of the cat's head and 90 cm from the X-ray apparatus. The current of 1.5 kW and 20 mA/s was used for recording. Two hours after the control ventriculogram, the second ventriculogram was obtained. The absence of contrast substance behind obstruction (fourth BV and subarachnoid space) shows complete obstruction of the aqueduct of Sylvius during 120 min after blockade (Fig. 2). After that, a bolus of 800 μL of contrast was injected into one cat via an intraventricular cannula and an X-ray ventriculogram was obtained immediately. Namely, 800 μL is of somewhat smaller volume than a newly formed CSF in the isolated ventricles supposed to occur during the period of the observation, i.e. 120 min (see Discussion).

After the scanning of the X-ray films (ScanMaker X 12 USL, Microtek), the ventriculograms were stored in a digital form on a compact disc. Using the ISSA program (Vams, Zagreb) for the planimetric measurement, the total area of the lateral ventricle was delineated and calculated in mm^2.

Statistical analysis for all of the results was performed using paired Student's *t*-test.

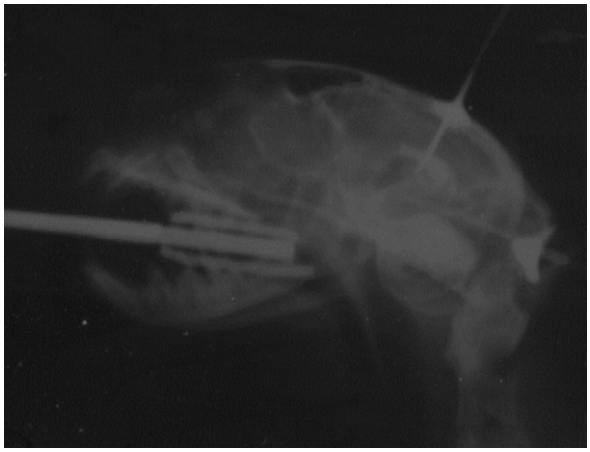

Fig. 2. The cat's ventriculogram 120 min after aqueductal blockage. Stain steel cannula in lateral ventricle is used for application of contrast. Contrast is seen in lateral and third ventricles, and in cannulas. Plastic cannula, which is positioned in aqueduct of Sylvius, causes the complete aqueductal occlusion (there is no contrast in the fourth ventricle and subarachnoid space).

RESULTS

In these experiments we explored whether CSF pressure changed in isolated ventricles over 2 h, whether isolated ventricles changed their size under such conditions, how the mock CSF infusion into isolated ventricles affected the CSF pressure in the ventricles and CM, and how the mock CSF infusion into the CM affected the CSF pressure in isolated ventricles. Fig. 3A shows that the CSF pressure in isolated BV and the CM does not differ significantly over the 120 min period of aqueductal occlusion. In one cat the CSF pressures were measured up to 145 min, and in another up to 190 min after aqueductal occlusion, but

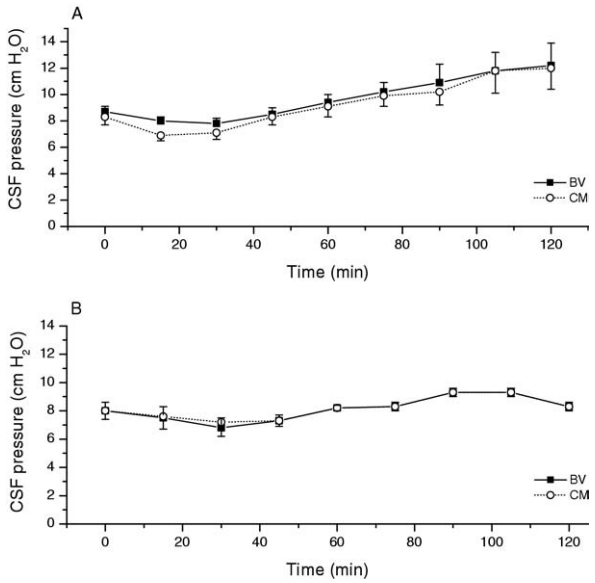

Fig. 3. CSF pressure (cm H_2O) in the BV (black symbols) and the CM (open symbols) in cats with occluded aqueduct (A, $n=5$) and those without such an occlusion (B, $n=5$) during 120 min. The values are mean±S.E.M.

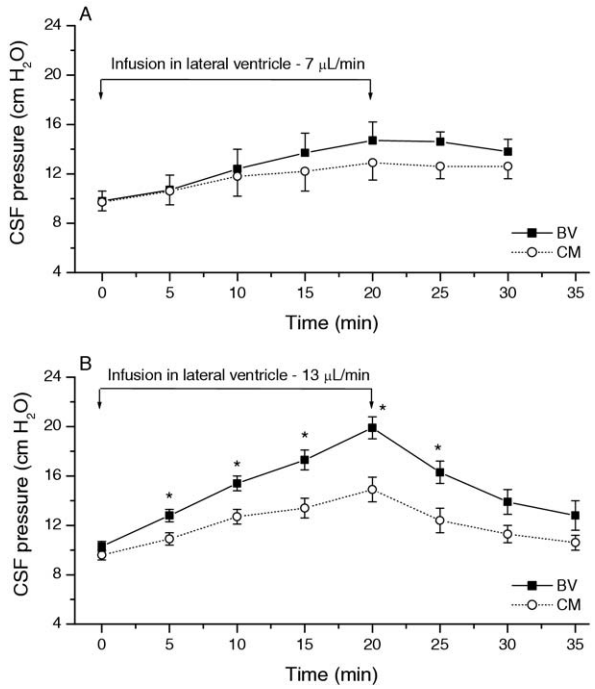

Fig. 4. CSF pressures (cm H_2O) in cats in isolated BV (black symbols) and in the CM (open symbols) during infusion of the mock CSF (the arrows show the start and the end of an infusion) into the lateral ventricle and thereafter. (A) The rate of the infusion was 7.0 μL/min ($n=5$). (B) The rate of the infusion was 13.0 μL/min ($n=5$). The values are mean±S.E.M. * $P<0.05$.

neither CSF pressure increase nor transmantle pressure gradient was observed.

A similar phenomenon was observed in control animals without an aqueductal occlusion (Fig. 3B). In both cases, small fluctuations of CSF pressures were observed over time but no significant difference between the ventricular and cisternal CSF pressures developed at any time interval. The fact that the CSF pressure in animals with the aqueductal occlusion also remained relatively constant and within a physiological range for more than 2 h suggests that no net CSF formation took place in the BV.

To estimate whether the ventricular size changes when the aqueduct is occluded, the cross-sectional area of the lateral ventricle was measured by X-ray ventriculogram and planimetry. Immediately after the aqueductal occlusion the cross-sectional area was 162±7.1 mm^2 (mean±S.E.M.) and 2 h later the same area was 166±7.6 mm^2. Thus, the cross-sectional area of the lateral ventricle did not change significantly over the 2 h of aqueductal occlusion ($P>0.1$). At the end of one of these experiments a bolus of mock CSF (800 μL) was injected into the lateral ventricle and its dilation was evident.

In the next group of experiments featuring the occlusion of the aqueduct, we imitated the CSF formation in isolated ventricles by infusing mock CSF at an infusion rate of 7.0 ($n=5$) and 13.0 μL/min ($n=5$) over 20 min (Fig. 4A and B). During the infusion of mock CSF at a rate of 7.0 μL/min (Fig. 4A), the ventricular CSF pressure was increased slightly faster than the cisternal CSF pressure. It

should be emphasized that a pressure gradient was observed in each experiment although no statistically significant difference was established between the ventricular and cisternal pressures. However, when mock CSF was infused at a rate 13.0 μL/min (Fig. 4B), the ventricular CSF pressure increased more significantly than the cisternal CSF pressure so that at the end of the infusion, i.e. in the 20th minute, the ventricular and cisternal pressures were 19.9±0.9 (mean±S.E.) and 14.9±0.9 cm H_2O, respectively. After the end of the infusion, both CSF pressures returned to the control values during the following 15 min. Thus, it appears that a transmantle pressure gradient of 5.0 cm H_2O was generated during the induced CSF formation of 13.0 μL/min. The return of ventricular CSF pressure toward the control value indicates that most of the CSF added volume was absorbed in isolated ventricles under an increased CSF pressure.

To explore whether the transmantle pressure gradient can develop in an open CSF system, we imitated the CSF formation in the BV with an open CSF system at infusion rates of 7.0 μL/min (n=3; 20 min); 26.0 μL/min (n=4; 20 min); 52.0 μL/min (n=6; 5 min) and 100.0 μL/min (n=4; 4 min) (Fig. 5). An increase in ICP was observed at all infusion rates except at 7.0 μL/min, but the transmantle gradient pressure between the ventricles and the CM was not observed at any rate of perfusion, not even at 100.0 μL/min.

In the group of experiments shown in Table 1, we infused mock CSF in front of (in the lateral ventricle) and behind the blockage (in the CM) in the animals with occluded aqueducts during a short time interval (5 min), and monitored the pressure in the isolated ventricles and the CM to test the development of a transmantle pressure gradient. When mock CSF was infused into the CM no pressure gradient occurred between the isolated ventricles and the CM at any rate of infusion (7.0; 13.0; 52.0 μL/min). However, during the infusion of mock CSF into the lateral ventricle significant changes in CSF pressure did not occur only at the rate of 7.0 μL/min, whereas an increased pressure and gradient developed at the rates of 13.0 and 52.0 μL/min (P<0.05) and were rate-dependent; at the rates of 13.0 μL/min and 52 μL/min, the mean pressure gradient was 2.5 cm H_2O and 14 cm H_2O, respectively (Table 1).

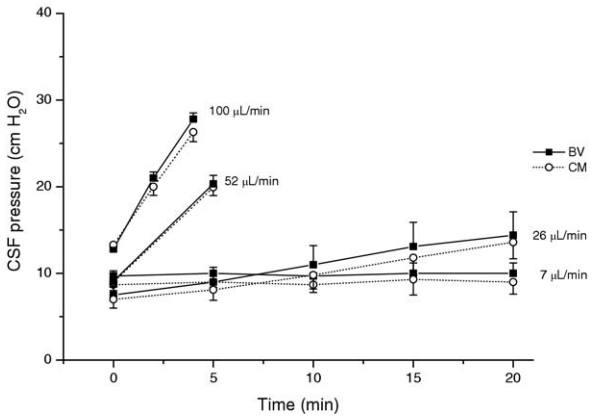

Fig. 5. CSF pressures (cm H_2O) of control cats in the BV (black symbols) and the CM (open symbols) during infusion of the mock CSF into the lateral ventricle at 7.0 μL/min (n=3); 26.0 μL/min (n=4); 52.0 μL/min (n=6) or 100.0 μL/min (n=4) rates of infusion. The values are mean±S.E.M.

Table 1. CSF pressure (cm H_2O) in the BV and in the CM in animals with occluded aqueduct under the control conditions (control) and 5 min after the beginning a mock CSF infusion (7.0, 13.0 and 52.0 μL/min) either into the CM or the lateral ventricle

Infusion rate		CSF pressure (cm H_2O)	
		Control	5 min
Infusion into the CM			
7.0 μL/min	BV	10.0±0.8	11.0±0.8
(n=3)	CM	10.0±0.9	11.3±1.0
13.0 μL/min	BV	10.3±0.2	12.8±0.4
(n=4)	CM	9.3±0.4	12.4±0.4
52.0 μL/min	BV	10.8±0.8	23.0±1.3
(n=4)	CM	10.3±0.9	22.5±1.2
Infusion into the lateral ventricle			
7.0 μL/min	BV	9.8±0.8	10.7±1.2
(n=5)	CM	9.7±0.7	10.6±1.1
13.0 μL/min	BV	10.3±0.4	12.8±0.5*
(n=5)	CM	9.6±0.4	10.3±0.5
52.0 μL/min	BV	11.3±1.5	37.5±0.9*
(n=4)	CM	9.8±1.2	23.5±3.8

* P<0.05 when compared to CM.

DISCUSSION

We have designed a set of experiments in order to obtain an acute and complete obstruction of the aqueduct of Sylvius and thus measure CSF pressure in front of (BV) and behind (subarachnoid spaces, i.e. CM) the obstruction to detect whether it would lead to an increased CSF pressure in isolated ventricles, the development of the transmantle pressure gradient and/or their dilation. The occlusion of the aqueduct in our model was achieved using a cannula of the same width as the aqueduct so that the cannula exerted no pressure on the adjacent tissue, and local disturbance in the blood circulation or venous pathway was avoided. Furthermore, the hole in the occipital bone was hermetically closed to prevent CSF leakage from the subarachnoid space and the influence of atmospheric pressure. This way, we obtained the first animal model in which a complete obstruction was effectively achieved with a normal CSF pressure (Fig. 3).

Fig. 3A shows that the CSF pressures in the isolated ventricles and CM were practically equal over 120 min, and similar to the pressures recorded when the aqueduct was not occluded (Fig. 3B). These experimental data contradict the classic hypothesis according to which the CSF secreted in the ventricles cannot be absorbed, due to aqueductal occlusion, at hypothetic CSF absorption sites outside the ventricles (i.e. arachnoid villi or perineural sheaths of cranial nerves) so that the CSF accumulation in the ventricles should lead to a significant rise in the CSF pressure. Actually, according to the data obtained by the

perfusion method, CSF secretion in cats (Pollay, 1974) ranges from 15 do 25 μL/min. If only the CSF amount occurring within the isolated BV (two lateral and the third ventricle) is taken into account, 900–1200 μL of CSF should have been secreted during the 2 h when the obstruction was present. Since the volume of both lateral ventricles and the third ventricle is about 1300 μL in cats (Levinger and Edery, 1968), the newly emergent CSF would be expected to cause a significant increase in CSF pressure. As the aqueduct of Sylvius was completely blocked in our model, a question arises as to why there was no pressure increase. In view of all the aforementioned facts, the absence of the CSF pressure rise in the isolated BV over time strongly suggests that the formation and absorption of CSF are equal, i.e. that there is no net formation of CSF in the ventricles.

To imitate the net formation of CSF in isolated ventricles, mock CSF was infused at rates of 7.0 μL/min (Fig. 3A) and 13.0 μL/min (Fig. 4B) over 20 min. During the mock CSF infusion at a rate of 7.0 μL/min, a somewhat higher pressure increase was observed in the isolated ventricles than in the CM, but no statistical difference between these pressures was detected ($P>0.1$). However, when the infusion rate was 13.0 μL/min, the clear transmantle pressure gradient developed and subsequently declined once the infusion was discontinued so that both pressures returned toward normal values. The increased pressure in the isolated ventricles should speak in favor of the absorption of the CSF volume into the periventricular capillaries (Bulat et al., 2008), which would also explain the dissipation of the CSF pressure increase after the infusion of the mock CSF was stopped (Fig. 4A and B). However, when the aqueduct was eventually opened, the intraventricular infusion of mock CSF, even at very high rates (Fig. 5), did not generate the pressure gradient since pressure was immediately transmitted to the other CSF compartments as may be expected according to Pascal's law of hydrodynamics.

The question arises as to how transmantle pressure is transmitted from the cortical CSF to the CSF in isolated ventricles in comparison to its transmission in the opposite direction. Table 1 shows CSF pressures in the CM and isolated ventricles under control conditions and 5 min after the intracisternal infusion of mock CSF at different rates (7.0, 13.0 and 52.0 μL/min). For comparative purposes, the infusion of mock CSF in isolated ventricles at the same rates for 5 min is added in Table 1. The difference in the pressure transmission is especially evident at the infusion rate of 52.0 μL/min during 5 min. At that infusion rate into the CM, the pressures in the CM and isolated ventricles doubled but showed no evidence of the transmantle pressure difference. On the contrary, during the infusion of mock CSF into the isolated ventricles, intraventricular CSF pressure increased much more than that in the CM, so that the transmantle pressure gradient of 14 cm was generated.

These results indicate that pressure transmission from the isolated ventricles to the cortical subarachnoid space is different than the transmission taking place in the opposite direction, which may be due to several factors. The fluid pressure transmitted from isolated ventricles to the cortical subarachnoid CSF should displace a part of the cortical CSF to the spinal CSF due to the distensibility of the spinal dura mater (Martins et al., 1972; Tunturi, 1978, 1980). That way, the cortical CSF pressure increase is partly compensated. Furthermore, according to Hakim and Hakim (1984) the lines of pressure from the small surface area of isolated ventricles toward the large cortical surface area should be dissipated. On the other hand, during the infusion of mock CSF into the CM, the spinal compensation of CSF pressure is rapidly exhausted, and the lines of pressure from the large cortical spherical surface area toward the centrally located small ventricular surface area should be concentrated, so that the pressure is rapidly transmitted from cortical to ventricular CSF. This is the probable reason why an increase in cortical CSF pressures is faithfully transmitted into the isolated BV and so the gradient of transmantle pressure is not developed.

Our X-ray measurements of the cross-sectioned area of the lateral ventricle immediately after the aqueductal occlusion and 2 h later (Fig. 2) did not disclose any significant dilation of the ventricle (see Results). These results are contrary to the results of Milhorat et al. (1970) in monkeys and dogs who used a different experimental approach to induce the isolation of the BV. Namely, they opened the atlantooccipital membrane, introduced a Foley catheter into the fourth ventricle and filled it with saline (1.0–1.5 mL) to obstruct communication of CSF between the aqueduct and the fourth ventricle. Under such conditions, dilation of the ventricles was evident after 1 h and rapidly progressed after 3 h. In our opinion two factors could contribute to the dilation of the isolated ventricles in their case. The filling of the Foley catheter with saline could have increased the ICP and impaired venous drainage of periventricular capillaries (Hanner et al., 1988), which may have caused a rise in the pressure and filtration of fluid from these vessels into isolated ventricles, an increase in the ventricular pressure and ventricular dilation. Furthermore, the opening of the atlantooccipital membrane and thereafter its reconstitution could have permitted the leakage of CSF from the CM and so artificially decreased the CSF pressure in the subarachnoid space, thus creating the pressure gradient between the isolated ventricles and subarachnoid space which could have caused the dilation of the isolated ventricles. However, the authors did not measure the CSF pressure in either the isolated ventricles or the subarachnoid space, and so their results should be taken with caution. In our model, all the experimental problems in the approach adopted by Milhorat et al., 1970 were avoided, since neither CSF pressure changes in the isolated ventricles and the subarachnoid space were observed (Fig. 3A and B), nor was the CSF leakage present. In our cat model, when we injected mock CSF (800 μL) with the contrast as a bolus (similar to the fast filling of the balloon of Foley catheter in the Milhorat et al. model) into the isolated ventricles in a volume closely matching the volume which is supposed to occur during the period of observation (see Experimental Procedures and Results), the lateral ventricle evidently dilated. This suggests that

the size of the BV could increase very quickly under similar conditions.

The absence of the ventricular dilation and the absence of CSF pressure increase (Fig. 3) in the isolated BV in our model cannot be easily incorporated into the classic hypothesis, but are rather consistent with our recently established observations which indicate that the CSF volume accumulation does not take place inside the BV under physiological pressure (Orešković et al., 2001, 2002). Thus, the results observed in this study, as well as the results of some clinical and experimental studies (Holtzer and de Lange, 1973; Stephensen et al., 2002; Orešković et al., 1991) suggest that CSF volume under physiological pressure is constant within isolated ventricles, i.e. that the CSF formation and absorption in the ventricles are in balance.

The pressure gradient is often associated with the occurrence of hydrocephalus, particularly the acute one, and some authors view it as the fundamental mechanism of hydrocephalus development regardless of whether a low gradient (Conner et al., 1984; Hakim and Hakim, 1984; Penn et al., 2005; Levine, 2008) or a high gradient is in question (Nagashima et al., 1987; Kaczmarek et al., 1997; Smillic et al., 2005). There are, nevertheless, some other authors who believe that CSF pressure gradient is not possible within the cranium firmly enclosed by bones, and more so because they did not observe such a gradient either in experiments involving animals (Shapiro et al., 1987) or in patients with communicating or non-communicating hydrocephalus (Stephensen et al., 2002).

Since the data about the gradient-related results in literature are so contradictory, the question arises as to whether the transmantle pressure gradient is necessary for the development of hydrocephalus or some other factors may play an important role in such a process with occlusion or the stenosis of CSF pathways. It was shown in cats that 3 weeks after the application of kaolin into the CM with an obstruction of cervical subarachnoid space, or the stenosis of the aqueduct with a plastic screw, a dilation of ventricles is developed without a rise in the ventricular CSF pressure (Miše et al., 1996). Our acute experiments show that the occlusion of the aqueduct by itself does not cause the rise of CSF pressure in isolated ventricles and their dilation. As has already been mentioned, we did not prolong our experiments because we wanted to avoid tissue reaction to cyanoacrylate glue on the tip of the aqueductal catheter or a potential development of CSF communication between the isolated ventricles and the fourth ventricle (see Experimental Procedures). However, during a prolonged occlusion or stenosis of the aqueduct, we would expect the development of a ventricular dilation, probably without an increase in the ventricular pressure. This idea is supported by the observation that in patients with communicating and non-communicating hydrocephalus the transmantle pressure is absent (Stephensen et al., 2002). Furthermore, Holtzer and de Lange (1973) observed that after the shunt obstruction the hydrocephalus did not progress in some children with communicating and non-communicating hydrocephalus, suggesting that this pathological process was compensated. All of this evidence supports the idea that the transmantle pressure gradient may not be necessary or instrumental for the development of hydrocephalus, and that some other factors, such as an increase in the ventricular CSF pulse pressure (Di Rocco et al., 1978), an impairment of systolic-diastolic displacement of the CSF with the development of periventricular ischemia (Miše et al., 1996), changes in the arterial pulsations (Greitz, 2004, 2007) and venous compliance (Bateman, 2000, 2003) may play an important role in the development of that pathological process.

All of these potential mechanisms indicate that hydrocephalus develops over a prolonged period. We assume that hydrocephalus is essentially a chronic process which may change into its acute form under certain conditions (ventricular dilation with a high CSF pressure) due to the appearance of the transmantle pressure gradient. Namely, in our model it was clearly shown that the transmantle pressure gradient (and potentially quick dilation of the ventricle) could indeed be developed. Our experiments indicate that the transmantle pressure can be generated only when the CSF accumulation is increased by infusing mock CSF into isolated ventricles (Fig. 4 and Table 1). This suggests that, in hydrocephalus, if a significant shift of the brain mass, with the stenosis or blockage of communication (e.g. in the aqueduct) occurs during the slow ventricular dilation, it could lead to a biophysical condition similar to the one in our model.

Such observation of an interruption of communication in the CSF system, due to the occurrence of the brain mass shift, was described in detail by other authors (Williams, 1973; Masters et al., 1977). If pathological changes take place, along with an interruption of communication before the obstruction, and they result in a CSF pressure increase in the ventricles (e.g. bleeding, infection, a tumor, a cysticercus cyst), this should lead to appearance of the pressure gradient, an accelerated ventricular dilation and the occurrence of the acute hydrocephalus phase. Previously, Zülch (1958) described many cases of arrested hydrocephalus that remained dormant for years, with the aggravation occurring only when some other pathological process (infection, bleeding, trauma, etc.) took place within the cranium. Finally, our results also proffer an explanation of the aforementioned contradictory data, i.e. they explain why some authors have observed a normal pressure before and after the occlusion in the so-called obstructive hydrocephalus, while others have noted the occurrence of hydrocephalus with the transmantle pressure gradient.

All of the presented results of our study can hardly be fitted within the classic hypothesis of secretion, unidirectional circulation and absorption of CSF outside of ventricles. However, our results can be easily explained by the recent hypothesis (Bulat and Klarica, 2005; Bulat et al., 2008), suggesting that during the filtration of water from arterial capillaries under a high hydrostatic pressure, plasma osmolytes are sieved (retained) since their permeability across the capillary wall is very poor, and so an osmotic counter-pressure is generated opposing water filtration. When such hyperosmolar plasma reaches venous

capillaries and postcapillary venules where the hydrostatic pressure is low, it is instrumental in water reabsorption from interstitial fluid (ISF) and CSF (Bulat and Klarica, 2005; Bulat et al., 2008). Thus, a rapid turnover of water, which constitutes 99% of ISF–CSF volume, continuously takes place between plasma and ISF–CSF (Bulat et al., 2008). This hypothesis is supported by the observation that when ^{3}H-water in physiological saline was slowly infused into the lateral ventricle of cats, it was not delivered to CM but rather locally absorbed into the periventricular capillaries and drained via the great cerebral vein of Galeni into the confluence of the sinuses (Bulat, 1993; Bulat et al., 2008). Furthermore, when the aqueduct of Sylvius was cannulated the same way as in the presented experiments, and the outflow of the cannula positioned at a normal CSF pressure, no outflow of CSF from the isolated ventricles was observed indicating that the CSF formation and absorption in those ventricles were in balance (Orešković et al., 2001, 2002). A lot of data related to dynamics of CSF have been obtained from experiments on cats. However, we should be careful with generalization and transmission of those data to other species, despite the fact that the physiology of CSF in cats is generally explained the same way as in other mammals. Thus, we expect that our results obtained on cats will initiate similar experiments on other mammals.

CONCLUSIONS

Our new model of acute aqueductal blockade is sensitive enough to detect a small increase of CSF volume in isolated ventricles and in the subarachnoid space. Namely, the infusion of mock CSF at rates lower than the previously determined formation rate of CSF in cats leads to an increase of CSF pressure in isolated ventricles and the development of the transmantle pressure gradient. Since after the occlusion of the aqueduct no increase in CSF pressure and the transmantle pressure gradient was developed over 2 h, this indicates that the CSF formation and absorption are in balance, i.e. there is no net formation of CSF in isolated ventricles, as has been shown previously in our laboratory. Our observation that an aqueductal occlusion by itself does not lead to either an increase in CSF pressure or the development of a transmantle pressure is in accordance with previous observations in patients with non-communicating hydrocephalus.

The transmantle gradient can be developed only during the infusion of mock CSF into isolated ventricles. Namely, an increase in the CSF volume and pressure in the subarachnoid space does not lead to the development of the pressure gradient in case of an aqueductal occlusion. Thus, our results suggest that, besides occlusion, a pathological process should take place in the ventricles causing the CSF volume accumulation and transmantle pressure gradient and eventually leading to the acute dilation of BV. Finally, all of this suggests that an occlusion or a major stenosis of CSF pathways by itself cannot cause a sudden onset of hydrocephalus, but that during a prolonged period of time either one can lead to the development of hydrocephalus without an increase in CSF pressure or the transmantle pressure gradient.

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