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Halothane Decreases the Rate of Production of Cerebrospinal Fluid

Possible Role of Vasopressin V_1 Receptors

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Background: **Circulating vasoactive hormones (*e.g.*, vasopressin) play an important role in the regulation of blood flow to the choroid plexus and the rate of cerebrospinal fluid (CSF) production. We tested the hypothesis that halothane decreases CSF production through a vasopressin-related mechanism and examined the related changes in blood flow to the choroid plexus.**

Methods: **Using ventriculocisternal perfusion, CSF production was measured in chloralose anesthetized, normothermic rabbits whose lungs were mechanically ventilated. Rabbits received either 0.5 minimum alveolar concentration (MAC; end-tidal) of halothane (added to a preestablished chloralose anesthetic), 0.5 MAC of halothane in the presence of a vasopressin V_1 antagonist (iv), or the V_1 antagonist alone. In addition, we examined animals in which no intervention was made (time control) and animals subjected to a 25% decrease in mean blood pressure produced by hemorrhage, with and without the V_1-antagonist. In a separate series of rabbits, regional and total blood flows to the brain and the choroid plexus were measured using radioactive microspheres. These studies were carried out under similar conditions, except that the effects of end-tidal 0.25, 0.5, and 1 MAC of halothane were examined in each rabbit (each added to a preestablished chloralose anesthetic).**

Results: **Under control conditions, blood flow to the choroid plexus averaged 351 ± 198 ml · min^{-1} · 100 g^{-1} (mean ± SD) and CSF production averaged 10.1 ± 1.9 μl · min^{-1}. Halothane (0.25, 0.5, and 1 MAC) did not alter choroid plexus blood flow but decreased CSF production by 28 ± 6% at 0.5 MAC ($P < .05$). In contrast, 1 MAC of halothane increased total blood flow to the brain by 20 ± 25% ($P < .05$). The V_1 antagonist, which did not affect production of CSF when given alone, prevented the decrease in CSF production in response to halothane. Hemorrhage decreased blood flow to the choroid plexus but not to the brain, and the V_1 antagonist attenuated the decrease in the rate of CSF production by hemorrhage (34 ± 11% *vs.* 48 ± 18%, $P < .05$).**

Conclusions: **Halothane decreases CSF production with no net change in the blood flow to the choroid plexus. Decrease in CSF production appears to be mediated through a vasopressin-related mechanism and not to the blood pressure decrease seen during halothane anesthesia. (Key words: Anesthetics, volatile: halothane. Brain, choroid plexus: brain blood flow; cerebrospinal fluid. Hormones: vasopressin; V_1-antagonist. Measurement techniques: microspheres; rabbit.)**

THE choroid plexus is the major site of production of cerebrospinal fluid (CSF).[1] Unlike most blood vessels of the brain, in which a blood-brain barrier exists, vessels of the choroid plexus do not have tight junctions between endothelial cells.[2,3] As a result, the vascular muscle and interstitium of the choroid plexus are relatively accessible to blood-borne stimuli. Therefore it is not surprising that humoral mechanisms play a role in the regulation of blood flow to the choroid plexus and in the production of CSF. For example, our recent studies suggest that norepinephrine,[4] vasopressin,[5,6] and both angiotensin I and angiotensin II[7,8] selectively decrease the blood flow to the choroid plexus, without decreasing cerebral blood flow. Furthermore, vasopressin decreases the production of CSF through a vasopressin (V_1) receptor mechanism.[6]

Volatile anesthetics have profound and variable effects on the rate of production of CSF.[9,10] For example, halothane decreases the rate of production of CSF by approximately 30%.[9] The mechanisms that account for this effect have not been evaluated. However, halothane increases the levels of vasopressin in the CSF and the circulation.[11] Since the choroid plexus has a high density of V_1 receptors,[12,13] it is possible that halothane is acting through a vasopressin-related mechanism. Therefore, the first goal of the present study was to test

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the hypothesis that the effects of halothane on the rate of production of CSF are mediated through V_1 receptors. Since the blood flow to the choroid plexus may be an important determinant of the production of CSF, the second goal was to examine the influence of halothane on blood flow to the choroid plexus.

Methods

Preparation of Animals

The experimental protocol for this study was approved by the Animal Care and Use Committee of the authors' institution. Eighty-four New Zealand white rabbits (2–3.4 kg) were anesthetized with sodium thiopental, given *via* an ear vein catheter (25–30 mg/kg iv followed by intermittent boluses of 0.8–1.0 mg/kg). After infiltration with 0.5% bupivacaine, a tracheostomy was performed. The administration of sodium thiopental was stopped and chloralose was given intravenously (30–40 mg/kg followed by 10–20 mg hourly throughout the duration of the experiment). One dose of gallamine triethiodide (5 mg/kg iv) was administered after induction, but no further relaxant was given for the remainder of the experiment. Throughout each experiment, adequate depth of anesthesia was assessed by noting the response to periodic pressure on the tail. Additional chloralose (10–20 mg iv) was administered if movement or a change in blood pressure occurred. The animals' lungs were mechanically ventilated with air and supplemental oxygen ($F_{I_{O_2}} \sim 0.5$) to maintain a $Pa_{CO_2} \sim 35$–45 mmHg and Pa_{O_2} above 100 mmHg. Ventilation and $F_{I_{O_2}}$ adjustments were based on measurements of arterial blood gases. A catheter was inserted into a femoral artery (again after infiltration with bupivacaine) to monitor blood pressure and to sample arterial blood, and another catheter was inserted in a femoral vein for administration of fluids and drugs. Arterial blood gases were monitored two or three times during the surgical preparation and once before each measurement of blood flow. The partial pressures of inspired oxygen, end-tidal carbon dioxide, and end-tidal halothane also were monitored constantly during the studies (Datex 254 Airway Gas Monitor, Puritan Bennett). Rectal temperature was monitored and maintained at 37–38° C with a heating pad. Lactated Ringer's solution was infused at 7–10 $ml \cdot kg^{-1} \cdot h^{-1}$ to maintain hydration.

Rate of Production of CSF

To measure the rate of CSF production, we used the ventriculocisternal perfusion method.[6,14,15] After placement of arterial and venous catheters, the animal was placed in a stereotactic head-holder and a 25-G needle was placed stereotactically into one lateral ventricle. The location of the lateral ventricle was confirmed during the insertion of the needle when the pressure, measured through a side port, abruptly decreased and an appropriate CSF pressure wave pattern was identified (CSF pressure tracing is identified by the characteristic change in amplitude with ventilation and heartbeats). After exposure of the atlanto-occipital membrane, the tip of a 19-G "butterfly" needle was placed in the cisterna magna and the plastic hub of the catheter was adjusted to the level of the auditory meatus. To prevent leakage around the cisternal needle, Super Glue® was applied around the insertion site. Artificial CSF‖ was infused into the lateral ventricle at 30 μl/min using a glass gas-tight syringe while infusion pressure was measured through a side port. The perfusate contained 1 mg/ml of the nondiffusible indicator blue dextran (molecular weight = 2×10^6, Sigma). Success of ventriculocisternal perfusion was subsequently confirmed by the appearance of the blue dye and the absence of blood in the effluent that drained through the cisternal catheters. The effluent was collected continuously and divided into aliquots at 15-min intervals. If obstruction of the cisternal needle occurred, as evidenced by a gradual increase in CSF pressure and a pattern of decreased volume of the collected perfusate, the experiment was discontinued (n = 4). If leak of perfusate was noted around the cisternal needle (which was evidenced also by a decrease in the measured CSF pressure and a decrease in collection of cisternal perfusate), the experiment was discontinued (n = 5). The concentration of blue dextran in the infusate and effluent was measured with a spectrophotometer (wave length 610 nm) and the rate of production of CSF in μl/min was calculated from the dilution of blue dextran with the formula: $V_f = V_i(C_i - C_o)/C_o$, where V_f is the rate of production of CSF, V_i is the rate of infusion of perfusate, and C_i and C_o are

‖ The artificial cerebrospinal fluid (CSF) consisted of 0.44 g KCl, 0.26 g $MgCl_2 \cdot 6H_2O$, 0.50 g $CaCl_2 \cdot 2H_2O$, 1.33 g dextrose, 15.42 g NaCl, dissolved in 2 L of deionized water. Sodium bicarbonate (50 mEq) was then added to 50 ml of the solution, which was then bubbled with a 5% carbon dioxide in nitrogen gas mixture to produce normal values for pH, P_{CO_2}, and P_{O_2}.[16] The pH, P_{CO_2}, and P_{O_2} of artificial CSF averaged 7.27 ± 0.03, 43 ± 3 mmHg, 62 ± 15 mmHg, respectively.

the concentration of blue dextran in the inflow and outflow fluids, respectively.

Experimental Protocol of Studies of Production of CSF

In all CSF study animals, ventriculocisternal perfusion was performed for 4 h. The first 2 h provided adequate time for equilibration of the tracer to be reached. A timer was then reset to zero. If a stable value for the rate of production of CSF was not reached during the first 2 h of ventriculocisternal perfusion, the results of the experiment were discarded. In all groups, the cisternal outflow aliquots collected between 0 and 15 min and between 15 and 30 min after the end of the equilibration period were used to determine the first and second control measurements of CSF production. After the second control measurement, the agent (or intervention) to be tested was administered and ventriculocisternal perfusion continued for another 90 min. Cisternal perfusate collected at 60, 75, and 90 min following the start of each intervention were used to determine the effects of that intervention on the rate of CSF production.

Individual rabbits were assigned to one of six experimental groups:

In group 1 (n = 8), after two control measurements, halothane was added to the inspired gas mixture to achieve an end-tidal concentration of 0.5 minimum alveolar concentration (MAC). This concentration of halothane was maintained for 90 min to the end of the experiment (1 MAC of halothane for the rabbit is 1.4%).[17] The administration of chloralose continued as noted above.

In group 2 (n = 8), after the first control measurement, the vasopressin V_1 antagonist [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(O-methyl) tyrosine] arginine vasopressin (10 μg/kg, Peninsula Laboratories, Belmont, CA) was given, CSF production was measured again to establish a second control, then halothane was added to achieve an end-tidal concentration of 0.5 MAC as in the first group. The goal with this group was to test whether the effects of halothane on CSF production were mediated through stimulation of vasopressin V_1 receptors. Again, halothane was administered in the presence of chloralose anesthesia as described above.

In group 3 (n = 4), after the two control measurements, the V_1 antagonist (10 μg/kg) was given and the measurement of CSF production followed for 90 min. The purpose with this group was to rule out the possibility that the V_1 antagonist, by itself, affected CSF production.

In group 4 (n = 12), after the two control measurements, a 25% decrease in mean blood pressure from control values was produced by hemorrhage. This group was studied to rule out the possibility that any effect of halothane on rate of production of CSF was due to a decrease in blood pressure.

In group 5 (n = 9), a 25% decrease in mean blood pressure from control values was produced by hemorrhage in the presence of the V_1 antagonist.

Finally, in group 6 (n = 8), no intervention was made in the chloralose anesthetized animals. These animals served as time controls to verify the stability of CSF production measurements during the experimental time period.

We examined the effect of 0.5 MAC of halothane and 25% decrease of mean pressure on rate of CSF production because they are values at the middle of the dose-response relationship for effect of halothane and hemorrhagic hypotension on the blood flow to the choroid plexus (see below).

Blood Flow Studies

Animals used for blood flow studies were anesthetized and prepared as noted above, except that needles were not inserted in the lateral ventricle or cisterna magna. Instead, a pigtail polyethylene catheter was inserted into a femoral artery and advanced *via* the aorta into the left ventricle for injection of microspheres. Catheters also were inserted into both brachial arteries to withdraw reference blood samples during injection of microspheres. Blood flow was measured (see below) using, at random, one of 15-μm microspheres labelled with ^{46}Sc, ^{95}Nb, ^{153}Gd, ^{85}Sr, ^{113}Sn, or ^{141}Ce (New England Nuclear, Boston, MA). Microspheres ($0.5–1.3 \times 10^6$) were injected through the left ventricular catheter over 10 s. Reference blood samples were withdrawn at a rate of 0.5 ml/min from each brachial artery, using a Harvard pump, started 15 s before injection of microspheres and continuing for 1 min after the injection.

At the end of each experiment, the anesthetized animal was killed with intravenous KCl. The brain was removed, fixed in formalin, and later dissected into regional samples. The choroid plexuses from the lateral, third, and fourth ventricles were removed, weighed, and placed together in a separate plastic tube. Other regions of the brain that were counted included the caudate nucleus, thalamus, midbrain, pons, me-

dulla, and cerebellum. The remaining cortex was divided into six parts, each of which was weighed and put into a separate tube. Radioactivity of tissue samples and reference arterial blood samples was determined using a NaI well-type gamma counter. Isotope separation was performed using standard techniques. Blood flow (BF) was calculated from the formula: BF = CT × 100 × QR/CR, where QR is the reference sample withdrawal rate and CT and CR are counts in tissue and reference blood samples, respectively. The counts of the two reference blood samples were averaged.

Experimental Protocol of Blood Flow Studies

Four groups of animals were studied:

In group 1 (n = 9), we tested whether halothane affects blood vessels of the brain and the choroid plexus differentially. Blood flow was measured five times: twice under control conditions (20 min apart), and then during the administration of end-tidal 0.25, 0.5, and 1 MAC of halothane in an ascending fashion. At each stage, flow was measured at the end of a 20-min period of stable halothane concentration. No effort was made to support the blood pressure (see Discussion).

In group 2 (n = 7), we examined whether the response of choroid plexus blood flow to halothane is mediated by a vasopressin V_1 receptor mechanism. We measured blood flow five times as described in the first group with the following exception: between the two control measurements, the vasopressin V_1 antagonist (10 μg/kg) was given intravenously over 3 min.

In group 3 (n = 8), we decreased blood pressure by withdrawal of blood. Blood flow was measured five times: twice under control conditions, then after mean blood pressure had been reduced by 10%, 25%, and 50% of control values in a descending fashion (each for 20 min) by hemorrhage. Animals of this group were anesthetized with chloralose only. The goal with this group was to rule out the possibility that effects of halothane on blood flow to the choroid plexus were simply due to a to decrease in blood pressure.

In group 4 (n = 11), blood flow was measured during the administration of anesthesia with chloralose as described in the surgical preparation of animals. The measurements of blood flow were made at intervals of approximately 20 min. This group served as a time control for studies of halothane.

In all groups, before each blood flow measurement (approximately every 20 min), depth of anesthesia was checked as described above and chloralose (10–20 mg) was administered if needed. In all animals, the order of injection of isotope-labelled microspheres was randomized.

In four rabbits in each of groups 1, 3, and 4, plasma levels of vasopressin were measured by radioimmunoassay as described previously.[18] In group 1, the first blood sample was taken between measurements of control blood flow. The two subsequent blood samples were taken 20 min after the administration of 0.5 and 1 MAC of halothane, immediately before the respective measurements of blood flow. In group 3, the first blood sample was taken between measurements of control blood flow. The two subsequent blood samples were taken 20 min after decreasing the blood pressure by 25% and 50% from control values, immediately before the respective measurements of blood flow. In group 4, the first blood sample was taken between measurements of control blood flows. The two subsequent blood samples were taken immediately before the last two measurements of blood flow, respectively. To maximize the detection of vasopressin in the plasma by radioimmunoassay, the required volume of each blood sample was 5 ml.

Statistics

CSF Studies. Statistical analysis of the rates of CSF of production was performed using two-way analysis of variance (ANOVA) to compare CSF production at 90 min after the intervention to the second control observation. The experimental groups were treated as a "between-group" factor and measurement interval treated as a "within-group" factor. Pair-wise comparison between groups was performed with Student-Newman-Keul's test. $P < .05$ was considered significant. Repeated measures ANOVA followed, when indicated, by Newman-Keul's test was performed to compare values at 60, 75, and 90 min after interventions to each other and to the control observations.

Blood Flow. Statistical analysis of the blood flow studies was performed using repeated measures ANOVA. When indicated, it was followed by Dunnett's *t* test to compare values during interventions to the second control.

Plasma Levels of Vasopressin. Statistical analysis of the plasma levels of vasopressin was performed using two-way ANOVA. Repeated measures ANOVA also was performed. When indicated, this was followed by Dunnett's *t* test to compare values during control and intervention.

Results

Production of CSF

Under control conditions, the rate of production of CSF was similar in all groups, averaging 10.1 ± 1.9 μl/min (mean ± SD; fig. 1, table 1). There was no significant difference between the two control observations in any experimental group. Administration 0.5 MAC of halothane decreased the production of CSF by 28 ± 6% at the 90-min observation point ($P < .05$). The V_1 antagonist prevented the decrease in CSF production by halothane, but the V_1 antagonist, by itself, did not affect production of CSF. Cerebrospinal fluid production was stable throughout the experiment in the time control animals.

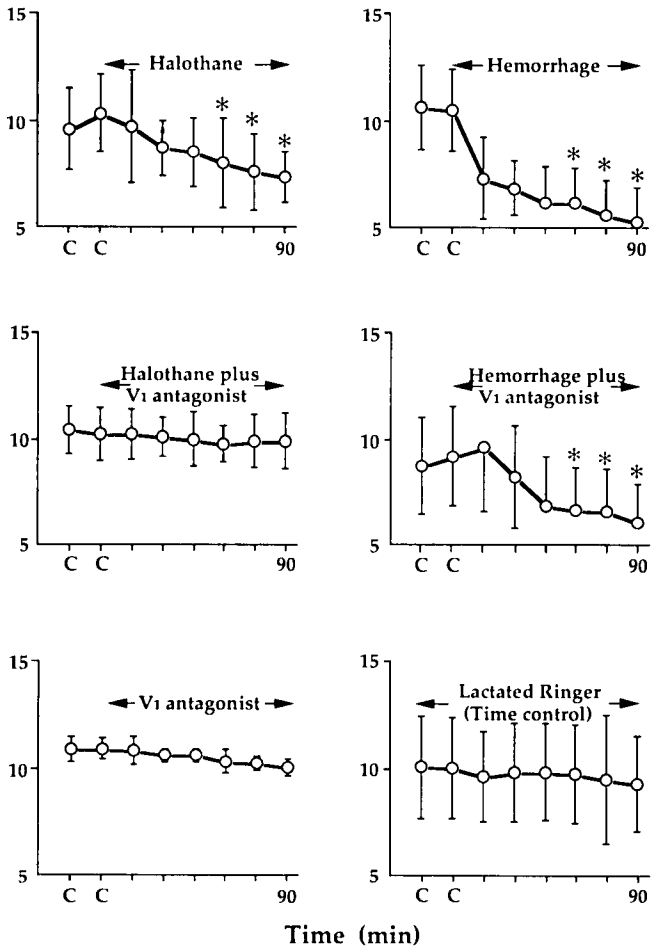

Fig. 1. Rate of formation of cerebrospinal fluid during administration of 0.5 MAC halothane (n = 8, *top left*), halothane in the presence of V_1 antagonist (n = 8, *middle left*), V_1 antagonist (n = 4, *bottom left*), hemorrhagic hypotension (n = 12, *top right*), hemorrhagic hypotension in the presence of V_1 antagonist (n = 9, *middle right*), time control (n = 8, *bottom right*). Each letter C on the x axis of the graphs refers to a control observation. In each group, there was no difference between the two control observations. *Significantly different from each of the controls. Values are mean ± SD.

Hemorrhagic hypotension decreased the rate of production of CSF by 48 ± 18% ($P < .05$) at the 90-min observation point. In the presence of the V_1 antagonist, hemorrhagic hypotension decreased production of CSF by 34 ± 11% ($P < .05$) at the 90-min observation point. This was significantly less than the value obtained with hemorrhage alone ($P < .05$).

Within each of the groups there was no significant difference between the rates of CSF production at 60, 75, and 90 min after the intervention(s), indicating that equilibrium had been achieved.

Blood Flow

Under control conditions, blood flow to the choroid plexus was much greater than total blood flow to the brain (the whole brain and brain stem, excluding the choroid plexus; tables 2–4). Halothane (1 MAC) increased total blood flow to the brain by 20 ± 25% ($P < .05$; table 2). Halothane also increased regional blood flows to the caudate nucleus, midbrain, pons, medulla, and cerebellum. In contrast, halothane did not affect the blood flow to the choroid plexus (table 2). The effect of halothane on the blood flow to the choroid plexus and the brain was not altered in the presence of the V_1 antagonist (table 3). Hemorrhagic hypotension did not affect total blood flow to the brain, but it did decrease the blood flow to the choroid plexus (table 4). In time control animals, blood flow to the choroid plexus and to the brain were 442 ± 123 and 58 ± 12 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, respectively, and did not change significantly throughout the experiment.

Plasma Levels of Vasopressin

Before the administration of halothane, plasma levels of vasopressin were 25 ± 33 pg/ml (mean ± SD). During administration of 0.5 and 1 MAC halothane, plasma vasopressin levels significantly increased to 109 ± 72 and 150 ± 94 pg/ml, respectively ($P < .05$). In group 3, prior to hemorrhage, plasma levels of vasopressin were 24 ± 26 pg/ml. When blood pressure was decreased by hemorrhage, plasma vasopressin levels were 108 ± 84 pg/ml ($P > .05$) when blood pressure decreased by 25% of control values and significantly increased to 495 ± 468 pg/ml when blood pressure decreased by 50% of control values ($P < .05$). In animals that received no intervention (time control),

Table 1. Effects of the Various Interventions on Rate of Formation of Cerebrospinal Fluid

Group	Control	90 min	Significance *versus* Other Groups
Group 1. Halothane (n = 8)			
V_f ($\mu l \cdot min^{-1}$)	10.30 ± 1.8	7.40 ± 1.2*	$P < .05$ *vs.* groups 2, 3, 4, 5, and 6
Mean blood pressure (mmHg)	93 ± 11	69 ± 6*	
*p*H	7.38 ± 0.03	7.35 ± 0.03	
P_{CO_2} (mmHg)	39 ± 4	39 ± 2	
P_{O_2} (mmHg)	239 ± 17	253 ± 22	
Group 2. Halothane + V_1 antagonist (n = 8)			
V_f ($\mu l \cdot min^{-1}$)	10.20 ± 1.3	9.90 ± 1.3	$P > .05$ *vs.* groups 3 and 6
Mean blood pressure (mmHg)	84 ± 10	69 ± 7*	
*p*H	7.40 ± 0.04	7.36 ± 0.04	
P_{CO_2} (mmHg)	38 ± 3	38 ± 2	
P_{O_2} (mmHg)	247 ± 42	243 ± 22	
Group 3. V_1 antagonist (n = 4)			
V_f ($\mu l \cdot min^{-1}$)	10.90 ± 0.5	10.03 ± 0.4	$P > .05$ *vs.* groups 2 and 6
Mean blood pressure (mmHg)	94 ± 12	85 ± 10*	
*p*H	7.37 ± 0.04	7.37 ± 0.01	
P_{CO_2} (mmHg)	39 ± 3	38 ± 2	
P_{O_2} (mmHg)	248 ± 18	237 ± 27	
Group 4. Hemorrhage (n = 12)			
V_f ($\mu l \cdot min^{-1}$)	10.5 ± 1.9	5.3 ± 1.6*	$P < .05$ *vs.* groups 1, 2, 3, 5, and 6
Mean blood pressure (mmHg)	92 ± 12	69 ± 6*	
*p*H	7.51 ± 0.04	7.29 ± 0.04*	
P_{CO_2} (mmHg)	39 ± 1	39 ± 2	
P_{O_2} (mmHg)	265 ± 30	255 ± 23	
Group 5. Hemorrhage + V_1 antagonist (n = 9)			
V_f ($\mu l \cdot min^{-1}$)	9.19 ± 2.3	6.05 ± 1.8*	$P < .05$ *vs.* groups 1, 2, 3, 4, and 6
Mean blood pressure (mmHg)	89 ± 9	68 ± 9*	
*p*H	7.40 ± 0.03	7.32 ± 0.02*	
P_{CO_2} (mmHg)	36 ± 4	38 ± 4	
P_{O_2} (mmHg)	236 ± 28	231 ± 29	
Group 6. Time control (n = 8)			
V_f ($\mu l \cdot min^{-1}$)	10.04 ± 2.4	9.31 ± 2.2	$P > .05$ *vs.* groups 2 and 3
Mean blood pressure (mmHg)	88 ± 12	80 ± 10*	
*p*H	7.42 ± 0.03	7.42 ± 0.05	
P_{CO_2} (mmHg)	36 ± 3	38 ± 3	
P_{O_2} (mmHg)	329 ± 66	306 ± 57	

Values are mean ± SD.

* Significantly different from own control at 90 min after intervention at $P < .05$. Between-group comparisons of cerebrospinal fluid production are under significance *versus* other groups column.

plasma levels of vasopressin were 8 ± 3, 10 ± 5, and 12 ± 5 pg/ml, respectively ($P > .05$). The control values of plasma vasopressin did not differ significantly among the three groups ($P > .05$).

Discussion

The principal finding of this study is that halothane decreases the rate of CSF production *via* a vasopressin-related mechanism. This effect is not related to choroid plexus blood flow or to any blood pressure decrease during the administration of halothane.

Halothane has been shown to decrease the rate of CSF production.[9] Circulating vasopressin also decreases CSF production.[6] Since halothane increases both plasma and CSF levels of vasopressin,[11] we anticipated that halothane may decrease the production of CSF *via* a vasopressin-related mechanism. However, before discussing our findings, we will comment on the methods we used to measure CSF production and blood flow to the choroid plexus.

Consideration of Methods

We measured the rate of production of CSF with the ventriculocisternal perfusion method. This entails the

Table 2. Effects of Halothane on Blood Flow to the Choroid Plexus and Other Brain Regions

			Halothane		
	First Control	Second Control	0.25 MAC	0.5 MAC	1 MAC
Blood flow (ml · min^{-1} · 100 g^{-1})					
Choroid plexus	351 ± 197	348 ± 114	346 ± 175	311 ± 174	321 ± 197
Total brain	55 ± 18	59 ± 13	59 ± 15	61 ± 19	71 ± 9*
Caudate nucleus	81 ± 27	71 ± 27	82 ± 35	84 ± 48	116 ± 61*
Thalamus	102 ± 43	114 ± 45	119 ± 49	110 ± 43	111 ± 41
Midbrain	57 ± 24	55 ± 18	58 ± 20	58 ± 21	69 ± 32*
Pons	42 ± 12	45 ± 10	45 ± 9	46 ± 16	57 ± 12*
Medulla	52 ± 14	55 ± 14	53 ± 13	60 ± 23	71 ± 32*
Cerebellum	60 ± 17	63 ± 14	61 ± 13	69 ± 21	84 ± 32*
Mean blood pressure (mmHg)	95 ± 12	95 ± 14	83 ± 14*	77 ± 18*	59 ± 21*
Arterial blood gases					
*p*H	7.41 ± 0.03	7.40 ± 0.04	7.39 ± 0.04	7.38 ± 0.03	7.34 ± 0.04
P_{CO_2} (mmHg)	36 ± 2	37 ± 4	36 ± 4	36 ± 4	35 ± 2
P_{O_2} (mmHg)	211 ± 83	221 ± 96	218 ± 95	215 ± 90	230 ± 68

Data belong to animals of group I in the blood flow studies. Values are mean ± SD (n = 9). Brain means all brain tissue down to and including the medulla oblongata (choroid plexus not included). Wet weights were used to calculate blood flows.

* Significantly different from the second control at $P < .05$.

use of a nondiffusible indicator and is used widely in the studies of CSF production.[9,10,14,15,19–21] Determination of the rate of production of CSF with this method is based on the dilution of blue dextran containing artificial CSF by CSF produced in the ventricular system of the brain. The rates of production of CSF in this study were stable after equilibration of the tracer in the time control animals or the animals that received the V_1 antagonist alone (fig. 1, table 1) and are similar to rates that were reported previously for rabbits.[1,14,15,19–21]

We measured the blood flow to the choroid plexus using radioactive microspheres. Radioactive microspheres have been used to measure blood flow to the choroid plexus in several studies,[4–8,22–28] and this method was validated by Faraci[4] for repeated measure-

Table 3. Effects of Halothane after V_1 Antagonist on Blood Flow to the Choroid Plexus and Other Brain Regions

			Halothane		
	First Control	V_1 Antagonist	0.25 MAC	0.5 MAC	1 MAC
Blood flow (ml · min^{-1} · 100 g^{-1})					
Choroid plexus	337 ± 135	494 ± 229*	511 ± 147*	454 ± 143*	346 ± 126†
Brain	60 ± 11	55 ± 13	62 ± 8	66 ± 11†	76 ± 9†
Caudate	68 ± 23	62 ± 10	63 ± 15	69 ± 18	81 ± 16†
Thalamus	91 ± 25	87 ± 28	120 ± 37	104 ± 23	124 ± 42†
Midbrain	59 ± 10	54 ± 6	60 ± 6	61 ± 4†	68 ± 10†
Pons	35 ± 7	34 ± 9	33 ± 9	38 ± 6	48 ± 7†
Medulla	47 ± 6	45 ± 6	46 ± 9	51 ± 6	69 ± 17†
Cerebellum	69 ± 9	55 ± 8	60 ± 7	64 ± 11†	77 ± 13†
Mean blood pressure (mmHg)	90 ± 8	89 ± 11	83 ± 12	75 ± 14†	69 ± 13†
Arterial blood gases					
*p*H	7.41 ± 0.04	7.39 ± 0.04	7.39 ± 0.04	7.37 ± 0.04	7.36 ± 0.04†
P_{CO_2} (mmHg)	36 ± 4	37 ± 4	37 ± 3	38 ± 3	39 ± 3
P_{O_2} (mmHg)	264 ± 202	204 ± 51	172 ± 11	172 ± 19	159 ± 15

Data belong to animals of group II in the blood flow studies. Values are mean ± SD (n = 7). Brain means all brain tissue down to and including the medullar oblongata (choroid plexus not included). Wet weights were used to calculate blood flows.

* Significantly different from first control at $P < .05$.

† Significantly different from V_1 antagonist at $P < .05$.

Table 4. Effects of Hemorrhagic Hypotension on Blood Flow to the Choroid Plexus and the Brain

	First Control	Second Control	Hemorrhagic Hypotension (% change from control) −10%	−25%	−50%
Blood flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)					
Choroid plexus	394 ± 208	354 ± 161	349 ± 207	150 ± 84*	99 ± 43*
Brain	71 ± 18	64 ± 17	70 ± 23	64 ± 16	62 ± 18
Caudate	73 ± 32	80 ± 25	89 ± 43	102 ± 22	78 ± 23
Thalamus	114 ± 101	101 ± 35	114 ± 45	107 ± 26	97 ± 19
Midbrain	71 ± 21	68 ± 18	69 ± 15	72 ± 18	76 ± 33
Pons	46 ± 18	38 ± 15	38 ± 14	40 ± 15	50 ± 20*
Medulla	68 ± 18	53 ± 14	56 ± 16	57 ± 21	62 ± 23
Cerebellum	75 ± 23	74 ± 20	82 ± 27	82 ± 20	86 ± 36*
Mean blood pressure (mmHg)	91 ± 10	89 ± 10	81 ± 8*	67 ± 9*	48 ± 7*
Arterial blood gases					
*p*H	7.41 ± 0.05	7.42 ± 0.05	7.42 ± 0.05	7.42 ± 0.03	7.36 ± 0.06*
P_{CO_2} (mmHg)	40 ± 1	37 ± 3	39 ± 3	37 ± 4	35 ± 4
P_{O_2} (mmHg)	165 ± 17	184 ± 28	193 ± 40	226 ± 47	229 ± 102

Data belong to animals of group III in the blood flow studies. Values are mean ± SD (n = 8). Brain means all brain tissue down to and including the medulla oblongata (choroid plexus not included). Wet weights were used to calculate blood flows.

* Significantly different from the second control at $P < .05$.

ments. The variability of repeated measurements of the blood flow to the choroid plexus under control conditions was slightly greater than that for the whole brain (11% *vs.* 6%), but despite this somewhat greater variability, the method is sufficiently sensitive to detect changes in blood flow to the choroid plexus in response to vasoactive stimuli.[4-8] Our values of blood flow to the choroid plexus are similar to values reported by other investigators using radioactive microspheres,[22-28] iodoantipyrine with freezing of the tissues,[29] and isopropyl iodoamphetamine.[30]

Volatile Agents and the Choroid Plexus

Potent volatile agents have important and variable effects on the dynamics of CSF and on intracranial pressure. For example, although halothane decreases CSF production by 30%,[9] enflurane increases the rate of CSF production by approximately 50%,[10] a change that is associated with a gradual increase in intracranial pressure unrelated to any effects on cerebral blood flow. It was suggested previously that the mechanism by which halothane decreases and enflurane increases the rate of production of CSF may be explained by an effect on choroid plexus metabolism, possibly a reduction in the choroid plexus metabolic rate for glucose by halothane and an increase by enflurane.[9]

In this study, halothane decreased CSF production by almost one third (fig. 1, table 1). This is similar to the value reported by Artru in dogs.[9] However, halothane did not affect the blood flow to the choroid plexus (table 2). We have reported previously that acetazolamide increases choroid plexus blood flow by 140% and decreases CSF production by 55%.[31] This suggests that the effects of acetazolamide and halothane on CSF production were due to effects on the secretory epithelium and were not related to changes in blood flow to the choroid plexus. This contrasts with the observation that norepinephrine, angiotensin II,[32] and vasopressin decrease both blood flow to the choroid plexus[5,6] and CSF production.[6,15] This also is compatible with the hypothesis that blood flow to the choroid plexus sets an upper limit on the levels of CSF production.

As expected, halothane increased total blood flow to the brain (tables 2 and 3). In contrast, it did not affect the blood flow to the choroid plexus (table 2). Since halothane increased plasma levels of vasopressin, the response of choroid plexus blood flow to halothane may be the net result of the constrictor effect of vasopressin and the dilator effect of halothane on vascular smooth muscles of the choroid plexus.

In pilot experiments, we could not prevent decreases in blood pressure during the administration of halothane by infusing hetastarch sodium or blood. The use of intravenous vasopressors, to support the blood pressure during administration of halothane, would not be

appropriate in this study because circulating pressors decrease the blood flow to the choroid plexus[4–8] and the production of CSF.[6,15,32] Therefore, no effort was made to support the blood pressure during the administration of halothane.

Receptor Mechanisms of Vasopressin

The vasopressin receptors are designated V_1 and V_2.[33,34] Stimulation of V_1 receptors results in contraction of vascular smooth muscle, whereas V_2 receptors influence epithelial functions such as changes in water permeability in the collecting tubules of the kidney.[33,34] However, the choroid plexus has a relatively high density of V_1 receptors and apparently no V_2 receptors,[12,13] with fluid secretion in the choroid plexus being mediated through a V_1 mechanism. This is supported by the observation that during vasopressin infusion, the V_1 antagonist prevented both the decrease in blood flow to the choroid plexus and the decrease in CSF production (plasma levels of vasopressin were 55 and ~350 pg/ml[5,6] respectively). The specificity of this antagonist for V_1 receptors has been established in various preparations and vascular beds,[35–38] including cerebral vessels.[37] We previously have examined the specificity of the V_1 antagonist in the choroid plexus and demonstrated that the V_1 receptor blocker prevented the constrictor effect of vasopressin whereas the constrictor effect of phenylephrine was preserved.[5]

In this study, the V_1 antagonist prevented the decrease in CSF production due to halothane (fig. 1, table 1) without either affecting the associated decrease in blood pressure (tables 1 and 3) or altering CSF production by itself (fig. 1, table 1). Despite the fact that blood pressure during hemorrhage and administration of halothane was similar, hemorrhage resulted in a greater decrease in CSF production. Furthermore, the influence of the V_1 antagonist on the decrease in CSF production during hemorrhage, albeit statistically significant, was modest. These findings suggest that a vasopressin-related mechanism but not the decrease in blood pressure *per se*, may account for the reduction in CSF production by halothane. However, we cannot exclude the possibility that this phenomenon is further mediated by a direct effect of halothane on the choroidal epithelium.

The V_1 antagonist did not completely block the effects of hemorrhagic hypotension on CSF production. This may be because the administration of the V_1 antagonist during hemorrhage blocks only effects of vasopressin on CSF production, without altering the influence of the sympathetic nervous system and the renin-angiotensin system.[32,39]

Effect of Hemorrhagic Hypotension

During hemorrhagic hypotension, total blood flow to the brain did not change significantly from control values (table 4) indicating that cerebral autoregulation was preserved. Our findings are similar to previously published reports that measured blood flow to the brain in rabbits[23,24] using radioactive microspheres. Effects of hemorrhage on cerebral blood flow also were examined in dogs with the microsphere technique[40,41] and more recently with the venous outflow method.[42] Blood flow to the brain was not maintained when cerebral perfusion pressure was decreased to ~45–55 mmHg. The use of different anesthetics and different species might have contributed to the difference between our results and the previous results in dogs.

Blood flow to the choroid plexus decreased drastically during hemorrhage in this study (table 4). Previous studies also reported large decreases in blood flow to the choroid plexus[23–25] and in CSF production[21,43] during hemorrhage. This suggests that, during hemorrhagic hypotension, circulating humoral stimuli readily reach vascular smooth muscles, and perhaps the secretory epithelium of the choroid plexus, and exert marked effects on blood flow. This also may suggest lack of autoregulation in blood vessels of the choroid plexus.

Plasma Levels of Vasopressin

The baseline concentrations of vasopressin produced in this study are within the range of previously reported values (0.3–30 pg/ml).[33,34] However, after the administration of halothane, plasma vasopressin levels were greater than previously reported values during the administration of halothane.[11,44] Animals of the blood flow studies received chloralose and underwent surgical preparation to insert the various intravascular catheters. Thus, it is possible that surgery and/or chloralose potentiated the release of vasopressin by halothane. Our data show large variability under the same conditions from one response to the other. This is consistent with vasopressin levels seen during hypotension in a variety of species.[45–49] The cause of this large variability is not clear.

In conclusion, we have confirmed in rabbits the previous findings in dogs that halothane decreases rate of production of CSF. Our findings suggest dissociation between blood flow to the choroid plexus and rate of production of CSF during halothane anesthesia and are

consistent with the hypothesis that halothane decreases the rate of production of CSF through a vasopressin-related mechanism.

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