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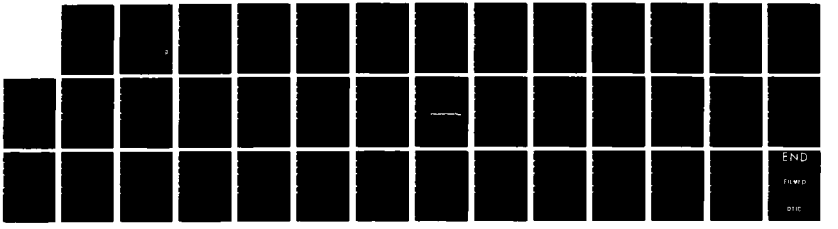

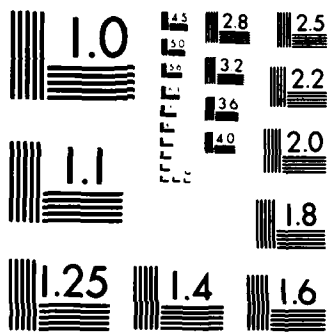

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AD-A148 644

Altitude Exposure and the Role of Hypoxia and Arginine Vasopressin in Cerebral Fluid Dynamics

Annual and Final Report

Leo C. Senay, Jr.
Daniel L. Tolbert

May 31, 1983

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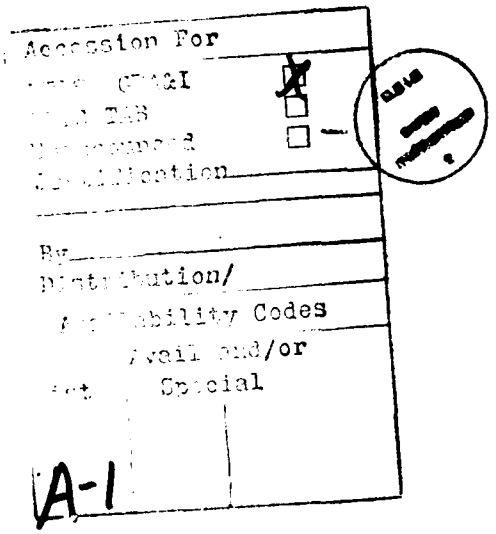
Through exposure of rabbits (V=10) to barometric pressures equivalent to that encountered at 18,000 ft for 6-8 hr, we found no increase in water content of various parts of the brain (temporal, parietal, frontal cortices; dien cephalon, mid-brain, hindbrain, cerebellum). Repeated exposure of rabbits (N=57) to hypoxia equivalent to 8,000 to 16,000 ft generally lead to an increase in CSF pressure. However, we found no influence of hypoxia upon CSF arginine vasopressin contents.

When injected into the lateral ventricles of rabbits, arginine vasopressin, norepinephrine, prostaglandin E_2 and prostaglandin $F_{1\alpha}$ did not influence CSF pressures. Angiotensin II generally caused an increase in CSF pressure while saralasin, an AII blocker generally caused a decrease in CSF pressure when given by itself and prevented a rise in CSF pressure if given before AII.

Injection of acetazolamide into the lateral ventricles did not consistently alter CSF pressures but when given systemically, acetazolamide caused a decrease in CSF pressure in two thirds of the treated animals.

In Brattleboro rats genetically deficient of AVP delivery of AVP into the lateral ventricles was generally followed by a decrease in CSF pressure when animals were at ambient pressure and an increase in CSF pressure when animals were in a hypobaric environment.



SUMMARY

The overall objective of this research was to gain insight as to the influence of hypoxia upon cerebrospinal fluid dynamics of rabbits and rats. The hypotheses to be tested were: a) does hypoxia lead to an increase in brain water and/or an increase in cerebrospinal fluid pressure; b) does arginine vasopressin (AVP) play a central role in the changes in cerebrospinal fluid (CSF) dynamics when animals were exposed to reduced barometric pressures.

1. Compared to controls, we found no increase in the water content of various parts of the brain (temporal, parietal, frontal lobes; diencephalon mid-brain, hindbrain, cerebellum) of ten rabbits following 6-8 hr exposure to barometric pressures equivalent to that encountered at 18,000 feet.

2. Exposure of rabbits (N=57) to hypoxia equivalent to 8,000 to 16,000 feet generally lead to increases in CSF pressures. These increases ranged from 0.5 mmH_2O to 38 mmH_2O and repeat exposure to the same barometric pressures did not necessarily result in the same pressure rise for individual rabbits.

3. We found no influence of hypoxia upon CSF AVP contents.

4. When injected into the lateral ventricles of rabbits. the following did not influence CSF pressures; arginine vasopressin, norepinephrine, prostaglandins E_2 and $F_{1\alpha}$.

5. When injected into the lateral ventricles of rabbits the following did influence CSF pressures both at ambient and reduced barometric pressures: angiotensin II resulted in uniform and consistent increases in CSF pressures. A blocker of angiotensin II (trivial name Saralasin) uniformly caused decreases in CSF pressure when given by itself and when given prior to angiotensin II prevented the rise in CSF pressures.

6. Injection of acetazolamide, an inhibitor of carbonic anhydrase, into the lateral ventricles lead to mixed responses - about one-half the animals so treated showed an increase in CSF pressure while one-half showed a decrease. When given systemically, acetazolamide rarely caused an increase in CSF

pressure. Two-thirds of the animals so treated showed decreases in CSF pressures, while one-third showed no changes in CSF pressures.

7. In Brattleboro rats genetically deficient of AVP the delivery of AVP into the lateral ventricles of these AVP deficient animals was generally followed by a decrease in CSF pressure when animals were at ambient pressure and an increase in CSF pressure when the animals were in a hypobaric environment.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication NO (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

Statement of the Problem

Upon exposure to altitude, certain individuals rapidly present signs of acute mountain sickness. The symptoms of this illness are suspected to be caused by an increase in intracranial volume and/or pressure. One portion of our research is aimed at revealing whether either or both of these events accompany exposure to hypoxia. There is evidence in the literature that arginine vasopressin (AVP) within the cerebrospinal fluid may play a role in intracranial fluid dynamics. We investigated the role of hypoxia and AVP upon intracranial fluid dynamics in rabbits and attempted to separate out the roles of hypoxia and AVP through the use of AVP deficient animals (Brattleboro rats). We have also examined the influence of norepinephrine, acetazolamide (an inhibitor of carbonic anhydrase), Angiotensin II, an inhibitor of Angiotensin II (Saralasin), and Prostaglandins E_2 and $F_{1\alpha}$ on CSF pressure at ambient barometric pressure and at several levels of hypoxia (reduced barometric pressure).

Background

When man rapidly ascends from low (<5000 ft.) to high altitude (>10,000 ft.), arrival at altitude is shortly followed by onset of acute mountain sickness (AMS) (1,2,3,4,5). Not all who ascend contract AMS and in those who do, the severity of symptoms appear to vary from individual to individual. If men with severe AMS cannot be returned to lower altitudes the course of the disability can end in death (1,2,4). In other subjects, AMS appears transient and the severity of the symptoms rather rapidly disappear with continued existence at altitude (6 and a host of others). Of concern in all cases whether reversible or irreversible is the degree of mental and physical incapacity suffered by those men contracting AMS. Slow ascension appears to prevent such symptoms (7), but in situations where slow ascent is not possible AMS may be as debilitating to certain populations (i.e., the military) as the shortage of water has proven to be in desert combat.

There is one general hypothesis as to the cause of AMS which appears to be supported by accumulated data. This is the hypothesis put forth by Hansen and Evans (2) wherein they postulated that the AMS symptomatology is probably the result of either an increase in brain volume and/or an increase in intracranial pressure. Further, the cerebral edema (and associated symptomatology) occurs in spite of an overall reduction in total body water (3,6,8,9,10,11). We are then presented with the curious state wherein total body water is depleted but simultaneously there may be an increase in CNS intracellular water. Since Hansen and Evans proposed their hypothesis, information has accumulated that suggests (a) how these shifts in body water at altitude may occur; and (b) a possible cause for the cerebral edema.

The hypotheses we proposed to explore was as follows: Hypoxic exposure results in cerebral edema and/or increased intracranial pressure. The edema and pressure changes may be the result of:

1. A central action of arginine vasopressin (AVP) upon the function of the choroid plexus and, in combination with the lowered PO_2, alters cell membrane permeability in the CNS thus causing CSF pressure to increase.

2. Alternatively - instead of triggering an increase in CNS AVP, hypoxia may suppress intraventricular AVP such that the transfer of CSF out of the intracranial compartment is reduced. With no change in production, intraventricular pressures will rise. Hypoxia in concert with the increased intracranial pressure will cause changes in cell membrane permeability resulting in cerebral edema.

The overall aim of the proposed study was to investigate the role of arginine vasopressin in the cerebral events accompanying exposure to lowered partial pressures of oxygen. The study also included several other chemical compounds thought to influence CSF dynamics or events related to changes in CSF dynamics.

The general hypothesis was based on the following evidence. Improved methods of tagging various peptide hormones has supplied evidence for concentrations of AVP at places within the CNS at some distance from the supraoptic and paraventricular nuclei as well as being remote from the posterior pituitary (12,13,14,15,16,17).

More specifically, information now suggests a role for certain structures bordering the IIIrd ventricle in body fluid homeostasis. Chief among these structures is the subfornical organ (SFO). Summy-Long et al. (15, 16) have shown that the SFO of the rat not only contains a considerable amount of AVP (44.5 pg/mg dry protein) but that the concentration of AVP doubled after 48 hours of water deprivation. How the AVP comes to be present in the SFO is not known.

Intraventricular (IVC) injection of AII has been shown to increase plasma AVP levels (18,19,20,21,22,23). AII cannot cross the blood brain barrier and therefore the target of such IVC injections would have to lie outside this barrier. At least two logical choices exist: The SFO and the organum vasculosum of the lamina terminalis. Assuming a response to AII, SFO response to such stimulation could be either a direct release of AVP from local cells and/or possible neural influence on other nuclei containing AVP (such as the suprachiasmatic nucleus). It must be said that the response of periventricular tissue to AII is far from settled. However, it does appear that the periventricular organs may play a role in body fluid balance (24,25,26).

Finally, there appears to be a relationship between acute mountain sickness and elevations in both plasma and urinary concentrations of AVP (1,3,27). Whether these increases are effects of the illness or are a causative factor in man awaits further experiments. Based on our diverse citations it appears plausible to suggest that the central changes in AVP may not necessarily reflect systemic changes in AVP (and vice versa).

Approach to the Problem

1. Animals. To gather information upon CSF pressure and AVP concentrations in CSF, indwelling tubes had to be placed in the third and lateral ventricles of the brain and, therefore, some consideration was given to the choice of experimental animals. The most crucial item was to obtain a sufficient amount of cerebrospinal fluid for accurate radioimmunoassay for AVP. Cost and ease of handling were also considerations, and we concluded that rabbits were the animals of choice for the studies wherein CSF-AVP must be known, while an AVP deficient strain of rats should be used to separate the effects of hypoxia from AVP.

As subjects of high altitude research, there is much less known about rabbits than rats. However, two publications have appeared that caused us select the rabbit (28,29). Jain et al. (29) found that the plasma volume of rabbits was significantly reduced at 6100 M but the other body water compartments were minimally affected.

Next Noto et al. (28) found that when injected into the lateral ventricles of normal rabbits, vasopressin lowered intracranial pressure. Intravenous acetazolamide also had a similar effect, and most interesting to us was that the effects of AVP and acetazolamide were additive. Suggestions have been made that acetazolamide affects production of CSF and now Noto et al. (28) feel that AVP may influence passage of CSF from ventricles to cerebral veins via the arachnoid villi in rats. Evidence for the influence of AVP upon cell membranes and CNS capillary endothelium in monkeys has been reported (30,31). Therefore, based upon the reports of Noto et al. and of Jain et al. and other considerations, the rabbit seemed to be an appropriate choice to investigate the relationship between hypoxia and intracranial fluid dynamics.

The choice of the AVP-deficient rats (Brattleboro strain) to sort out the separate and combined effects of hypoxia and AVP on CNS fluid dynamics appears self-evident.

2. Methods

a. Hypobaric chamber: The hypobaric chamber has been described by Devine (32). Briefly, it is box with a removable top. The box was made of 1 inch cast acrylic plexiglass bolted to a framework of aluminum angle. A cross brace in the open side of the box (36" x 28" x 20") helped support a removable top also made of 1" plexiglass. The end caps and base plate of the box were cut from 1/4" aluminum plate. Threaded openings were drilled through both end plates. Through these openings various tubes and electrical leads could be passed and sealed in place using Swagelock fittings and/or a silicone sealant. Our instrumentation of the chamber was simple and effective. High pressure tubing led from the chamber to a ballast vessel ($0.78M^3$) which in turn was connected to a vacuum line. Our house vacuum line could reduce chamber barometric pressure to 250 mmHg. We adjusted our chamber barometric pressure such that at any pressure used (390 to 565) mmHg) air flow through the chamber was 6 liters/min, a flow equivalent to approximately 2% of the chamber volume. A circulating fan was placed in the chamber (32), and a vacuum gage was fitted to one of the end plate openings. The opposite end plate openings were used to pass two pressure transducer leads and several fluid delivery tubes into the chamber. These latter tubes were used to deliver microliter quantities of fluid into the lateral ventricles of hypobaric rabbits and rats.

b. Transducer modification: Statham pressure transducers (P23 ID) were used to record CSF and intra-arterial pressures. These transducers have within their attached cables, small plastic tubing that allows for pressure equilization between the cable end and the transducer diaphragm. In other words, the reference pressure ("0") is always atmospheric. To record within the hypobaric chamber, the reference pressure must be that within the chamber. Therefore, the open tubing running in the connector cable must be sealed and provision made for the adequate pressure equilization within the chambers. The transducers were calibrated with appropriate water and mercury manometers. Recordings were made using a Grass Instrument Recorder.

c. Cannula Implantation (Rabbits): Male New Zealand rabbits (2-3 kg) were used in these experiments. Prior to surgery all animals were tranquilized with Rompum (9 mg/kg Haver-Lockhart) and anesthetized with Ketamine hydrochloride (Bristol-Myers; 40 mg/kg). The animals were mounted in a modified rabbit stereotaxic head holder and secured in a Kopf sterotaxic apparatus. Lidocaine hydrochloride (2%) was injected subdermally at all pressure points. The juncture of the coronal and sagittal bony sutures (bregma) was aseptically localized through a midline scalp incision. Four mm lateral to the bregma, a 3 mm trephination was performed bilaterally exposing the underlying dura mater and cerebral cortex. Small (0-80 x 3/16") self-tapping stainless steel screws were implanted in the calvarium in front of and behind these openings. Stainless steel cannulae (21 gage, Plastic Products Co., Roanoke, VA) were perpendicularly mounted into the cortex, 4 mm lateral to the bregma. The entrance of the cannula tip into the lateral ventricle was indicated by the flow of sterile, mock CSF from a reservoir into the cannula. The roof of the lateral ventricle varied from 4 to 4.8 mm below the surface of the cerebral cortex. The bilaterally implanted cannulae were secured to the calvarium with cranio-plastic cement and the hollow cannulae were closed with a screw-down stylet. The skin flaps were drawn about the cement islet and sutured in place. All implanted animals were closely monitored over a 4-7 day post-operative period.

d. Cannula Implantation (Rats): Homo- and heterozygous Brattelboro rats (275gm - 300gm, Blue Spruce Farms, Altamount, NY) were chronically implanted with cannulae in the lateral ventricle. All animals were anesthetized with sodium pentobarbital (65 mg/kg) and mounted into a Kopf rat stereotaxic head holder. Bregma was identified through a midline scalp incision using aseptic surgical techniques and six to eight stainless steel self-tapping screws (0-80- 1/8") were implanted in the exposed calvarium. Two mm lateral to

bregma a 2 mm trephination was performed unilaterally in some animals or bilaterally in others. Stainless steel 21-gauge cannula (Plastic Products, Roanoke, VA) were perpendicularly inserted into the exposed cortex 2 mm lateral to bregma. These cannulae were attached to a small reservoir of mock CSF. As the cannulae were inserted into the brain, the level of mock CSF was monitored and when the roof of the lateral ventricle was penetrated CSF would flow through the cannulae into the ventricle. At 2 mm lateral to bregma, the distance from the surface of the cortex to the roof of the lateral ventricle varied from 2.5 to 2.9 mm. The implanted cannulae were secured to the calvarium and anchoring screws using cranio-plastic cement around which the skin flaps were approximated and sutured. All cannulae were secured with a screw-down stylet. The animals implanted at the beginning of these experiments were perfused transcardially with 10% formol-saline at the time of sacrifice and the brains histologically processed. Microscopic examination of stained sections of the brains from these animals confirmed the localization of the cannulae within the lateral ventricle.

e. Drug Delivery: Provision was made to inject drugs either into the lateral ventricle or intra-arterially (rabbits only). The injections into the lateral ventricles did not exceed 25 μl in volume for rabbits and 5 μl for rats while intra-arterial injections were 1-2 ml in volume. In order to accomplish the injection, the dead space of the cannulae and attached plastic tubing was filled with the desired drug. When two drugs were sequentially used, a known volume of the first drug was separated from the second drug in the plastic tubing by a small air bubble. The appropriate volume could then be displaced from outside the hypobaric chamber. Extreme care had to be taken to avoid leaks in this system.

f. Drugs: Angiotensin II and [Sar; Ala^8] angiotensin II were obtained from Beckman; Arginine vasopressin in a saline solution (Park-Davis, 10 pressor units in 0.5 ml) as well as the crystalline product (Sigma) were the source of AVP. Angiotensin II (crystalline), [Sar; Ala^8] Angiotensin II (crystalline) as well as the arginine vasopressin were dissolved in either normal saline or artificial CSF and diluted to a final concentration of 5 pg/μl.

Acetazolamide sodium was purchased from Lederle and l-arterenol bitartrate from Winthrop. Both intra-arterial and lateral ventricle injections were made using a solution containing 50 mg/ml of acetazolamide. Two ml of the solution was given intra-arterially while 25μl were given intraventricularly. Dilution of arterenol was similar to that of AII and AVP. The artificial CSF contained the following (gm/l) $NaHCO_3$, 2.1; NaCl, 6.7206; $MgCl_2$, 0.4483; KCl, 0.2089; and $CaCl_2$, 0.2664. Cold sterilizations of this solution was done using a Nalgene ® 0.45 micron filter unit.

g. Radioimmunoassay for AVP: The method of Robertson et al. (41) was used. ^{125}I arginine vasopressin was obtained from New England Nuclear while AVP antibody and other necessary chemicals were purchased from Cal Biochem. Our extracts were dried in a N_2 stream.

3. Procedures:

a. Brain water content: A group of 20 rabbits became accustomed to the laboratory environment. After anesthetization with Rompum and Ketamine, one half of the group was sacrificed and water content of gross regions of the brain was obtained. The brain areas selected were frontal cortex, parietal cortex, temporal cortex, diencephalon, midbrain, cerebellum and hindbrain. Water content was determined by drying to constant weight at 60°C. The remaining ten rabbits were sacrificed following a 6-8 hr hypobaric exposure

(barometric pressure = 390 mm Hg) and similarly processed for determination of brain water content.

b. General procedures (Rabbits): Three to five days after cannula implantation, rabbits were placed in standard restraint boxes in the laboratory for 2-4 hrs on two to three consecutive days in order to accustom them to the laboratory environment. During this time, the head wounds were inspected for proper healing and the patency of the cannulae was assessed. On the day designated as control, the rabbit was brought to the laboratory, placed in stocks and a sample of cerebrospinal fluid was obtained. Originally, we attempted withdrawing the fluid using a Harvard Apparatus withdrawal/infusion pump but found that if the cannulae were patent, ordinary production rate and pressure gradients were sufficient to supply the necessary sample within 1 hr. Following sample collection, an ear surface was shaved and coated with a 2% Lidocaine jelly. The central ear artery was cannulated using a 23 gage butterfly whose dead space was filled with a heparinize saline solution (48 units/ml). The rabbit was then placed in the hypobaric chamber and using care as to maintain sterility of all tubing and transducer domes, one of the ventricular cannulas was connected to a Statham transducer as was the arterial cannula. The top of the chamber was partially put in place, the air circulating fan was started and control CSF pressures were obtained for at least one hour before any drug was introduced.

The procedures were identical preceeding hypobaric exposure. Following the 1 hr control period at ambient pressure, the top of the hypobaric chamber was locked in place and the chamber slowly evacuated until the desired barometric pressure was reached. Reduction of chamber pressure never took less than 20 min and occasionally took as long as 45 min. After stabilization of

the chamber pressure, a 1 hr control period was again observed before any manipulations were done to the animal. If the experiment was considered a control study, the rabbit remained in the chamber, undisturbed for at least 6 hours.

If an agent was to be injected into the lateral ventricle, initial preparations were as above. It should be noted that all injections into the lateral ventricle or arterial blood were preceded by an injection of an equal volume of the solvent.

The delivery of agents into the lateral ventricle was straightforward for one ventricular cannula was connected externally to the chamber. Padded hemostats were used to pinch off the tubing leading to this cannula and when injection of solvent or solvent-plus agent was desired, the appropriate microliter syringe filled with solvent was placed at the end of the tubing and the hemostat removed. In many instances, the syringe then spontaneously emptied. After fluid delivery into the lateral ventricle, the tubing was again clamped.

A similar arrangement was made for the ear artery cannula. However, this connection was made through the dome of the transducer in the chamber (volume of dome, 3 ml).

Usually, the influence of more than one chemical agent could be examined during a single hypobaric exposure. The sequence of recording then became: 1) control period for at least 1 hr; 2) injection of solvent (saline or artificial CSF) and record for 30 min; 3) if pressure was stable inject first agent - record for at least 2 hr; 4) inject solvent, record for 30 min and then inject final agent and record for at least 2 hr. This sequence was

particularly useful when the agents had been shown to have minimum effects on CSF pressures.

c. General Procedures (Rats): In order to record for prolonged periods from both the arginine vasopressin deficient and control rats, the animals' legs were lightly taped. If this were not done, the animals injured themselves while attempting to escape from the wire mesh restraint cages. Water was placed within reach of all animals in order to avoid dehydration over the span of the experiments. Again, preliminary exposure of the rats to the restraints and laboratory environment took place for 2-3 days preceding any experimental procedures and some 2-3 days after ventricular cannulation.

On experimental days, the rats were lightly anesthetized with Metaphane®, their limbs bound and the animals were placed in a rat restraint cage. Sterile plastic tubing filled with artificial CSF was attached to one of the ventricular cannulae and connected to a pressure transducer. The appropriate sequence of solutions was placed in a second sterile plastic tubing, attached to the contralateral cannula and the tubing, in turn, was connected to fluid filled tubing exiting the hypobaric chamber. We did not attempt to record arterial blood pressures nor did we attempt to collect CSF for AVP analysis. The remaining experimental procedures were similar to those noted above for the rabbits.

RESULTS

Brain Water:

Exposure to hypoxia for 7-8 hrs did not appear to influence water content of rabbit brain (Table 1).

Rabbit CSF Pressure at Ambient Barometric Pressure:

Pressures recorded from the lateral ventricle were only accepted if respiratory artifacts were discerned in the recording (Fig. 1). CSF pressures

Table 1. Water Content (%) of Various Brain Regions and of Hemi-brains in Control Rabbits and Rabbits Exposed to a Barometric Pressure of 390 mm Hg for 7 - 8 hrs.

CONTROL

Region of Brain	Frontal	Parietal	Temporal	Diencephalon	Mid-brain	Cerebellum	Hindbrain	Hemi-brain
$\bar{X}$	79.59	79.42	79.51	76.28	75.46	78.43	73.74	76.62
SD	1.3	1.79	1.76	1.53	1.68	1.01	3.27	.71
N	10	10	10	10	9	10	7	10

EXPERIMENTAL

$\bar{X}$	79.46	80.23	80.79	76.98	75.84	78.81	74.16	79.87
SD	.68	.41	.77	.75	.87	1.21	.93	.42
N	9	9	9	9	8	9	7	9

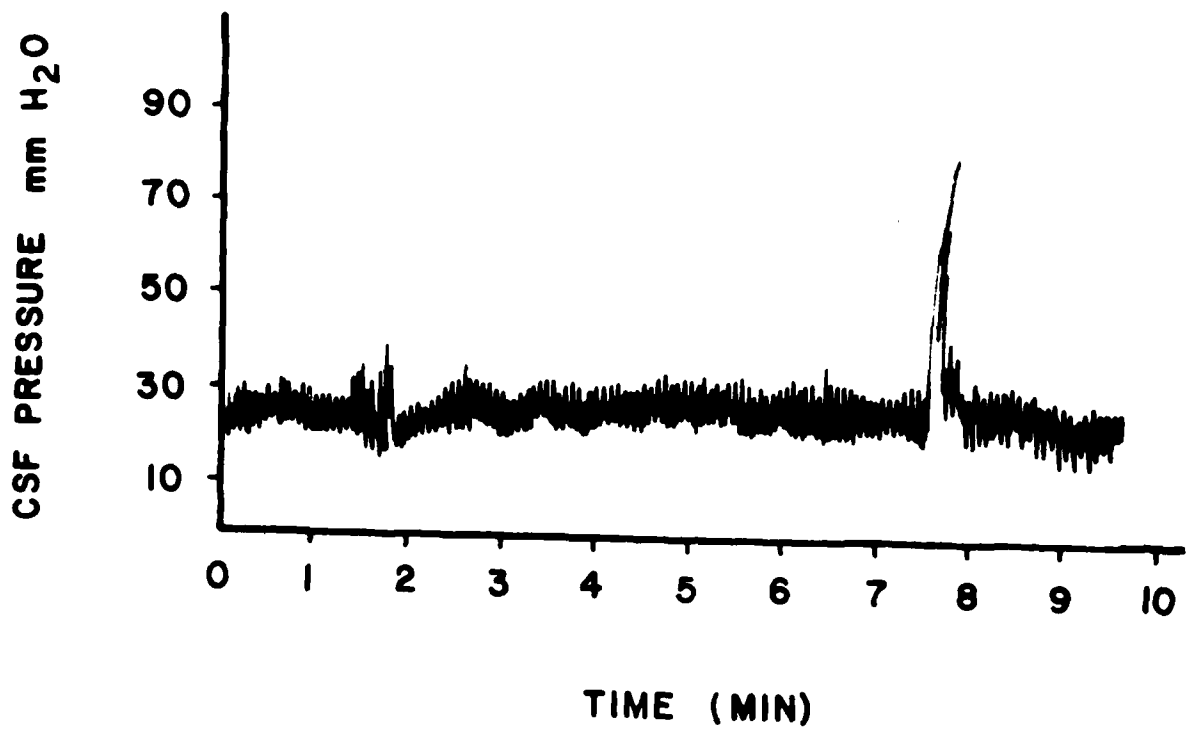


Figure 1. CSF pressures recorded from the 73rd to 82nd minute of an experiment conducted at ambient barometric pressure (747 Torr, T_A=22°C). The CSF pressures clearly reflect respiratory movements. The large displacement was due to head movement. Note that the respiratory movements are superimposed upon a longer cyclical pressure change.

showed considerable variability from animal to animal (mean ± SD) averaging 16.7 ± 12 mmH_2O for 48 rabbits. Day to day variations were also noted for each individual rabbit but variations (S.D.) for any particular rabbit seldom exceeded 5 mmH_2O. CSF pressure recordings were acutely effected by animal movement and more subtle changes were noted when an occasional stranger entered the laboratory.

Table 2 contains the results of 159 experiments at ambient barometric pressure than were technically acceptable. Several general conclusions can be drawn from this table.

1. Neither intraventricular or intra-arterial delivery of arginine vasopressin caused consistent changes in rabbit CSF pressure.

2. Intra-arterial acetazolamide lowered CSF pressure but this was not true in all cases (Fig. 2).

3. Intraventricular injection of acetazolamide (25μl) either raised, lowered or had no effect on CSF pressure.

4. Delivery of angiotensin II consistently caused an increase in CSF pressures (Fig. 3). This could be prevented if AII was preceded by saralasin, an AII blocker (Fig. 3).

5. By itself, the AII blocker saralasin did not consistently alter CSF pressure at ambient pressure. As will be seen below, saralasin was more consistent in its action when delivered with the animal under reduced barometric pressure.

6. Prostaglandins $F_{1\alpha}$ and E_2 did not effect CSF pressure when given intraventricularly.

CSF Arginine Vasopressin:

As noted in an earlier progress report, we had experienced technical difficulties with our assay. The chief problem was not using nitrogen to dry our extracts. When properly done, our values for CSF arginine vasopressin

Table 2. Summary of 159 experiments on the influence of various agents in rabbit cerebrospinal fluid pressure. All experiments were done at ambient pressure (744-755 Torr)

AGENT	CHANGE IN CSF PRESSURE		
	↑	±	↓
AVP (IC)	4	30	6
	one ADH, initial	↑ then ↓	
NOR-EPI (IC)	4	10	
ACETAZOLAMIDE (IC)	7	15	7
ACETAZOLAMIDE (IA)	2	9	22
ANGIOTENSIN II (IC)	8	1	1
	one AII, initial	↑ then ↓	
SARALASIN (IC)	2	4	7
+ ADH (IC)	1	9	
ACETAZOLAMIDE (IA)		1	4
+ NOR-EPI (IC)	1	2	2
ACETAZOLAMIDE (IA)			4
+ ANGIOTENSIN II (IC)	3	1	
ACETAZOLAMIDE (IA)		1	5
+ SARALASIN (IC)	2	1	3
PROSTAGLANDIN E_2 (IC)	1	8	1
PROSTAGLANDIN $F_{1\alpha}$ (IC)	1	7	2

AVP, arginine vasopressin; saralasin, [Sar^1, ALA^8] angiotensin II; NOR-EPI, nor-epinephrine. IA, intra-arterial; IC, intracerebral

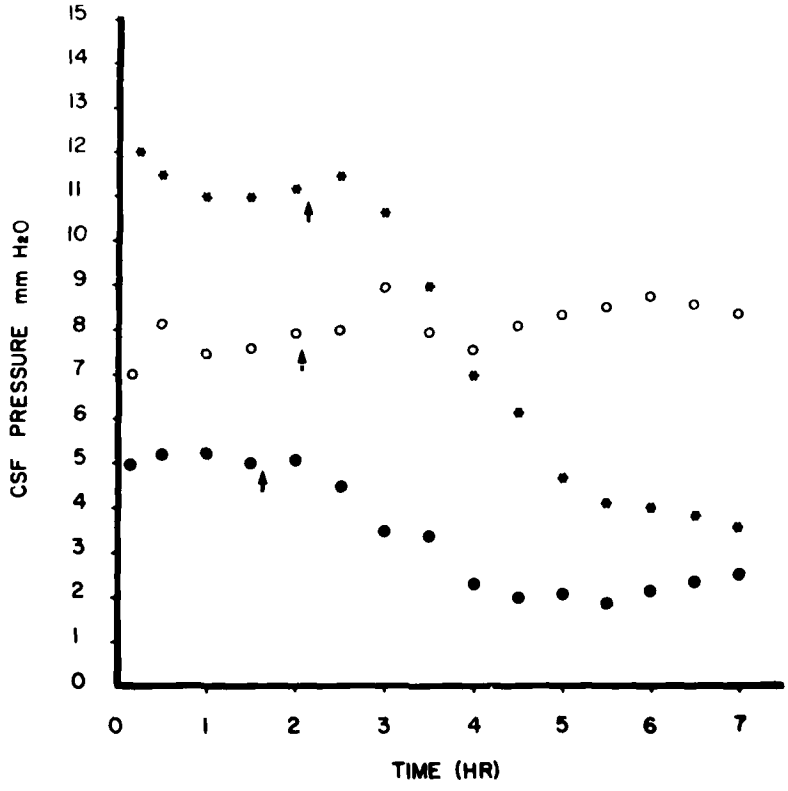


Figure 2. The influence of intra-arterial acetazolamide upon CSF pressure of 3 rabbits at ambient barometric pressure. Arrows indicate time of injection of 100 mg of acetazolamide. * = rabbit 31, O = rabbit 30, ● = rabbit 37.

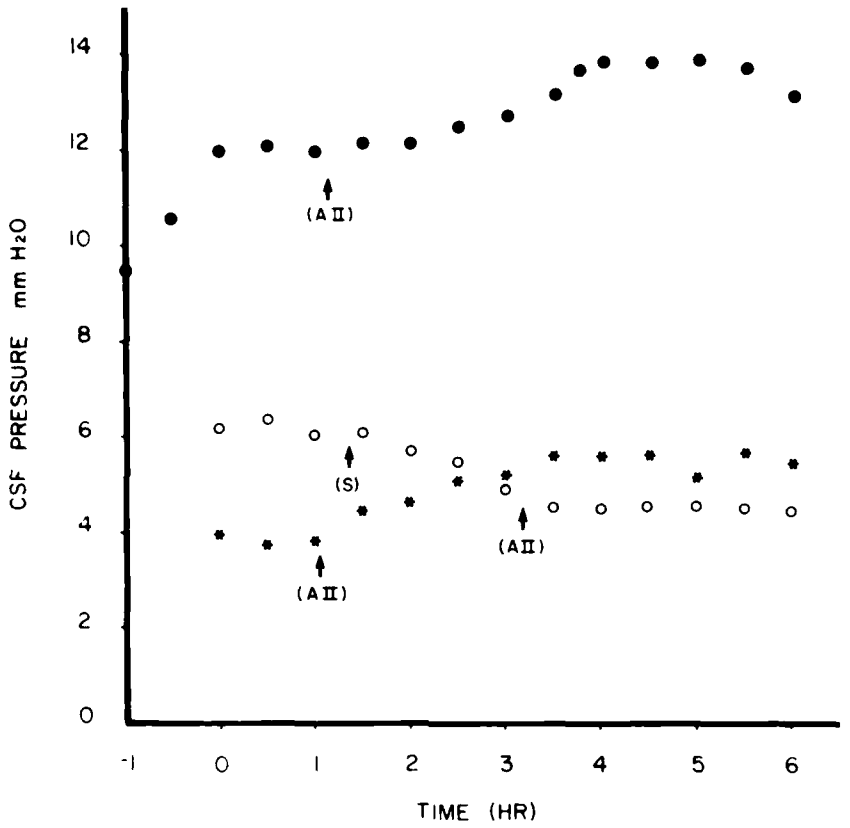


Figure 3. Angiotensin II (25 pg) only was intraventricularly injected in rabbit #53 (✱) at ambient barometric pressure and rabbit #54 (●) at 495 Torr. The CSF pressure in both animals increased. In rabbit #44 (▲), the same dose of angiotensin II was delivered after an equal weight of saralasin (AII blocker) had been injected into the lateral ventricle. Rabbit #44 was at ambient barometric pressure. Note the decrease in CSF pressure after saralasin and the lack of response of CSF pressure to AII injection in rabbit #44.

averaged 8 pg/ml with a wide range (2-30 pg/ml) giving us a standard deviation of 8 pg/ml for 15 determinations, at ambient barometric pressure. We did not find any significant changes following any of the manipulations listed in Table 2.

Rabbit CSF Pressures at Altitude:

The general response of the exposure of rabbits to reduced barometric pressure was an increase in CSF pressure. The increase in CSF pressure was approximately the same for animals exposed to simulated altitudes from 8,000 to 18,000 feet. Figure 4 shows the responses of one rabbit to 3 different reduced pressures. On average, the increase in CSF pressures for 53 technically acceptable experiments was 15 mm $\pm$ 10 mmH_2O. While the general response was one of pressure increase it was not unusual for an animal to possess a lower CSF pressure following reduction of barometric pressure (Fig. 5). Indeed, some animals gave varied responses to the same reduction in barometric pressure (Fig. 5).

Table 3 is a qualitative summary of 106 experiments done to modify CSF pressures at reduced ambient pressures. The results were similar to those seen at ambient pressures for arginine vasopressin, intra-arterial and intra-ventricular acetazolamide and intraventricular angiotensin II. The greatest change in frequency and direction of response was seen for the angiotensin II blocker, saralasin. Intraventricular delivery was shortly followed by a reduction in CSF pressure (Fig. 6). This reduction averaged 6 mmH_2O and appeared to last for the duration of the hypobaric exposure (6-7 hr). It should be noted that all changes at altitudes were usually superimposed upon an already elevated CSF pressures.

CSF Arginine Vasopressin:

For 15 rabbits 35 determinations for AVP were done for samples collected at ambient pressures and 35 for CSF samples were collected after 6-8 hrs of

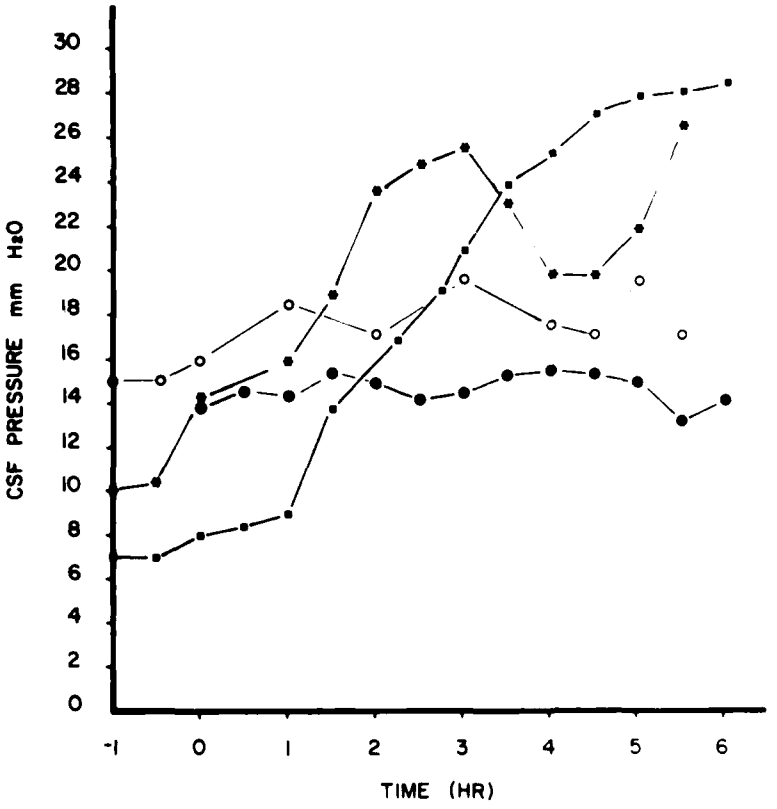


Figure 4. The CSF pressure responses of one rabbit (#14) to 4 different barometric pressures. ● = ambient pressure; ✱ = 525 Torr; 0 = 495 Torr; ■ = 463 Torr. These responses emphasize the CSF pressure variability seen in individual rabbits.

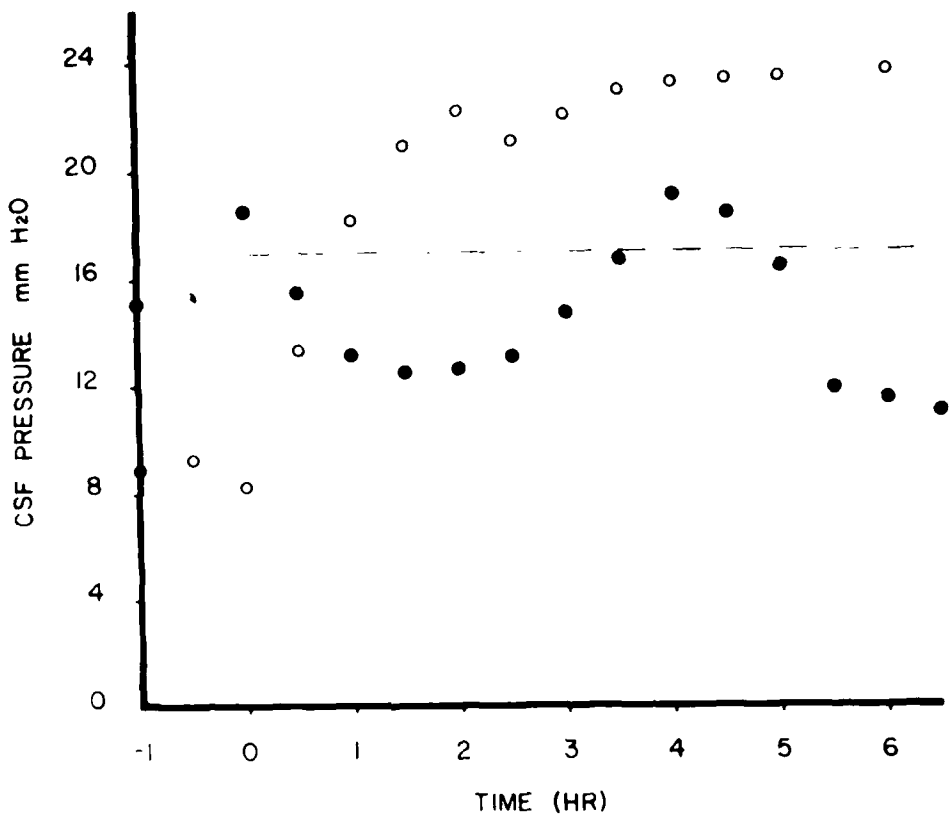


Figure 5. CSF pressure responses of rabbit #26 to environments of ● = 525 Torr and ○ = 390 Torr. The horizontal line indicates mean CSF pressure measured at ambient barometric pressure. While the usual finding was increased CSF pressures at simulated altitudes, depressions in CSF pressure were sometimes seen.

Table 3. Summary of 106 experiments on the influence of various agents on rabbit cerebrospinal fluid pressures. Experiments were done at barometric pressures ranging from 390-565 Torr (equivalent to 8,000 - 18,000 feet)

AGENT	CHANGE IN CSF PRESSURE		
	↑	±	↓
AVP (IC)	2	16	4
NOREPI (IC)	1	6	2
ACETAZOLAMIDE (IC)		4	1
ACETAZOLAMIDE (IA)		2	6
ANGIOTENSIN II (IC)	18	2	1
SARALASIN (IC)		3	12
SARALASIN (IC) + ANGIOTENSIN II (IC)	1	4 10	7
PROSTAGLANDIN E_2 (IC)		6	1
PROSTAGLANDIN $F_{1\alpha}$ (IC)		8	

Abbreviations as in Table 2

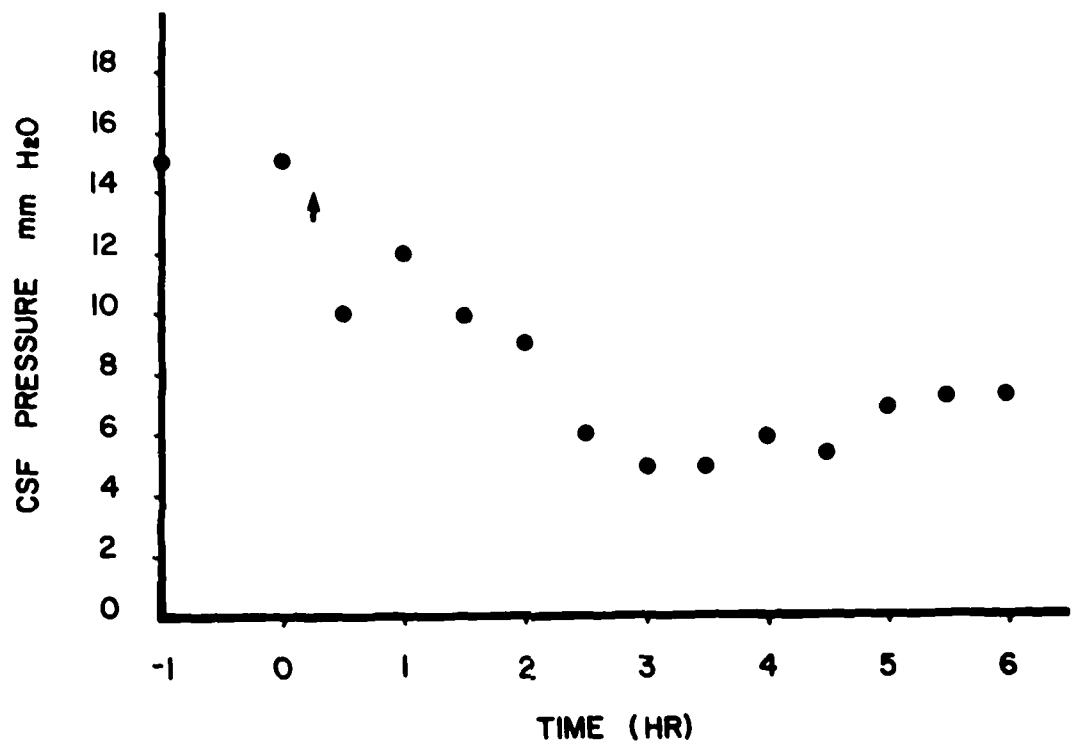


Figure 6. The CSF pressure response of rabbit #52 to intraventricular injection of saralasin. Barometric pressure = 463 Torr.

exposure to simulated altitudes of 10,000 to 18,000 ft. For this group of animals control (ambient barometric pressure) arginine vasopressin levels for CSF was 16 ± 4 pg/ml. After hypoxic exposure the values were 12 ± 5 pg/ml. These differences were not significant.

Blood Pressure:

As noted above (Methods) we did record arterial blood pressure from the central ear artery. Since the rabbit partially regulates body temperature by vasomotor activity of the ear vascular bed, our recordings not only reflected arterial blood pressure, but also reflected changes in vessel resistance downstream from our recording point. To test the viability of our system, AII was systemically injected and in all instances we recorded increases in ear arterial pressure. When rabbits were exposed to hypoxia, there were no remarkable changes seen in ear artery pressure. Therefore, we feel that changes in CSF pressure, particularly upon exposure to hypoxia and after AII and saralasin are not due to changes in blood pressure.

Arginine Vasopressin-deficient Rats:

When arginine vasopressin (5 pg/μl; total dose, 5μl) was injected into the lateral ventricles of AVP deficient rats or their heterozygous controls, the pressure response appeared to depend upon the ambient pressure surrounding the animals. The CSF pressure of both homozygous and heterzygous Brattleboro rats generally decreased following intraventricular delivery of AVP when the animals were at ambient pressure. It should be noted here that homozygous Brattleboro rats have a congenital lack of AVP while there heterozygous controls, while not lacking AVP, are not entirely the same as other rats as far as water metabolism is concerned (33).

When the rats were exposed in a hypobaric environment, the usual response to intraventricular AVP delivery was an increase in CSF pressure. Figure 7 shows the responses for one animal at ambient and reduced atmospheric pressure. Because of time constraints only 3 homozyous and 3 heterozyous rats were used in these experiments. Repeat testing in these animals gave the general results noted above.

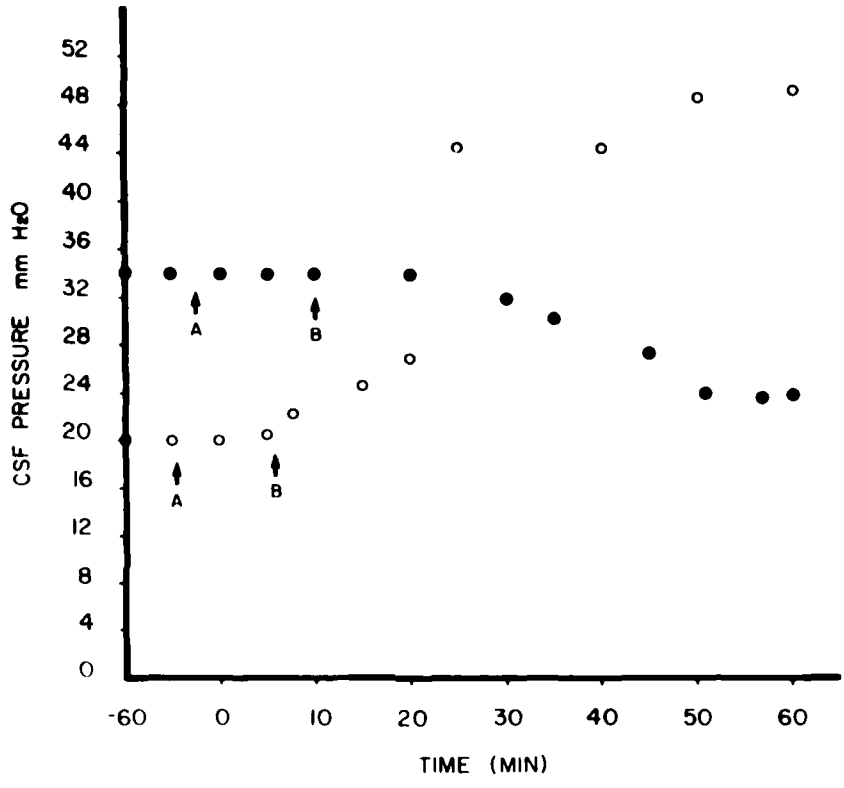


Figure 7. Brattleboro Rat. CSF pressure response to artificial CSF (A) and to 25 pg of AVP (B) at ambient pressure (●) and at 490 Torr (O). The same AVP-deficient rat was used at both pressures.

DISCUSSION

Based on the work of Noto et al. (28) and upon evidence that the subfornical organ contained arginine vasopressin (15, 17), we had hypothesized that this peptide hormone may play a role in changes in CSF dynamics when rabbits were exposed to hypoxia. In our extensive series of rabbit experiments, we were unable to find evidence to support the hypothesis.

In Brattleboro rats, however, our results at ambient barometric pressure did resemble those of Noto et al. for rabbits (28) i.e. intraventricular AVP was generally followed by a decrease in CSF pressure. Surprisingly, the general finding at reduced barometric pressure was just the opposite i.e. intraventricular AVP was followed by an increase in CSF pressure.

Though our original hypothesis was not supported, several of our results may have implications for individuals undergoing acute hypoxic exposure.

First, was the general finding that the CSF pressure in rabbits increased upon hypoxic exposure. Though the increase was variable from rabbit to rabbit and indeed, even in the same rabbit, CSF pressure generally increased. While the increase in CSF pressure was related to lowering of the ambient pressure, we were unable to show any relationship of the changes in CSF pressure to the changes in barometric pressure. We feel that emphasis must be placed upon how one records pressures from animals placed under reduced barometric pressure in chambers of various size. The reference pressure must be that of the environment surrounding the rabbit and care must be taken that the instruments chosen for such measurements do indeed have as their reference pressure that surrounding the animal.

While acute exposure to reduced barometric pressures did result in elevated CSF pressures, we were unable to record any change in water content of various portions of the rabbit brain. Therefore, our rabbits partially supported the hypothesis of Hansen and Evans who suggested that the symptoms of

acute mountain sickness were probably the result of either an increase in brain volume and/or an increase in intracranial pressure (2). It does appear that our rabbits did have increases in intracranial pressure. It is possible that increases in CSF pressure is a general result of exposure to hypoxia but only those individuals that suffer from an increase in brain water exhibit symptoms of acute mountain sickness.

Responses of rabbit CSF pressure to intravenous and intraventricular acetazolamide were of interest. Acetazolamide has been shown to be useful in combating acute mountain sickness (34, 35, 36). Ingestion by humans has been shown to alleviate symptoms and possibly prevents their occurrence. Noto et al. (28) did record decreases in CSF pressure when acetazolamide was given intravenously. We also observed the same result. However, acetazolamide appeared to be relatively ineffective in lowering CSF pressure following intraventricular delivery. Apparently the enzyme carbonic anhydrase is vulnerable to blood-borne inhibitors but not to inhibitors present in the ventricular fluid. We failed to confirm the results of Noto et al. (28) wherein the lowering of CSF pressure by acetazolamide and arginine vasopressin was additive.

Delivery of 0.1 - 1.0 picograms of angiotensin II (AII) directly into the subfornical organ has been shown to cause drinking and the drinking seems to be dose responsive (37). Following the delivery of AII into the subfornical organ there is a brief, transient rise in the systemic concentration of arginine vasopressin and arterial blood pressure also has been known to increase. Indeed, when the subfornical organ was electrically stimulated (38) mean arterial blood pressure immediately increased without any apparent change in pulse pressure. Following intraventricular delivery of AII to our rabbits we did not see any increases in ear artery blood pressure. It may be that this artery is unsuitable for recording such changes but systemic AII always increased ear

artery mean pressure. We did consistently record increases in CSF pressure both at ambient and reduced barometric pressures and this response could be blocked by saralasin. Further, when the animals were hypoxic (reduced barometric pressures) introduction of saralasin into the lateral ventricles was generally followed by a reduction in CSF pressure. It would appear, then that angiotensin II may play a role in increasing CSF pressure when our rabbits were exposed to reduced barometric pressure. Since we did not record any notable changes in ear artery blood pressure with either intraventricular AII and/or saralasin we do not feel that the influence of these agents upon CSF pressure was via systemic blood pressure.

At the present time we do not know for certain that AII is produced in or around the subfornical organ upon exposure of the animal to hypoxia. Since exposure of humans to reduced ambient barometric pressures (high altitude) is rather quickly followed by alterations in water balance (39, 40), it may well be that increased production of AII may occur both within the systemic circulation and within the central nervous system.

Conclusions:

1. Hypoxic exposure did not cause an increase in brain water content.

2. Hypoxic exposure was generally accompanied by an increase in CSF pressures.

3. Hypoxic exposure did not cause any apparent change in CSF AVP content.

4. Intravenous delivery of a carbonic anhydrase inhibitor lowered CSF pressure in two-thirds of tested rabbits both at ambient and reduced barometric pressures.

5. Delivery of angiotensin II into the lateral ventricle of rabbits always caused an increase in CSF pressures. Delivery of a blocker of angiotensin II activity (Saralasin) usually reduced CSF pressure when delivered

alone. The blocker, when given prior to AII CSF delivery also prevented the response to AII.

6. Injections of arginine vasopressin into the arterial ventricles of rabbits had little or no influence on CSF pressures whether the animals were at ambient or reduced barometric pressures.

7. In rats with congenital lack of arginine vasopressin, (Brattleboro rats) response to delivery of arginine vasopressin into the CSF depended on the ambient barometric pressure.

8. In rabbits, CSF pressures were not consistently altered by systemic delivery of Angiotensin II, angiotensin II blocker, arginine vasopressin, and norepinephrine.

9. In rabbits, prostaglandins E_2 and $F_{1\alpha}$ when injected into the CSF had no consistent effect on CSF pressures.

Hypothesis based on these results: Exposure of humans to altitude generally results in fluid shifts and loss of body water. This loss may trigger central production of angiotensin II thus increasing CSF pressures. In combination with hypoxia such a response could contribute to the syndrome of acute mountain sickness.

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