Effect of acetazolamide and ouabain on CSF production rate in the newborn dog

L. S. HOLLOWAY, JR., AND S. CASSIN
Department of Physiology, College of Medicine, University of Florida, Gainesville, Florida 32601

HOLLOWAY, L. S., JR., AND S. CASSIN. Effect of acetazolamide and ouabain on CSF production rate in the newborn dog. Am. J. Physiol. 223(3): 503-506. 1972.—Brains of 31 newborn dogs (2.1 days old) were perfused from the lateral cerebral ventricle to the cisterna magna with an artificial cerebrospinal fluid (CSF) containing radioiodinated human serum albumin (RIHSA-125I). CSF production rate (Vf) was calculated from measurements of inflow and outflow rates and concentrations of RIHSA-125I. Vf was constant during 4 hr of perfusion. Intravenous acetazolamide reduced the Vf by 43%. Ouabain, given intravenously or by perfusion through the ventriculocisternal system, reduced the rate of CSF production by approximately 50%. From these data, we suggest that the production of CSF is, in part, an active process in the newborn dog.

ventriculocisternal perfusion; Na-K-ATPase; carbonic anhydrase

In 1962, HEISEY ET AL. (18) clearly demonstrated that net cerebrospinal fluid (CSF) production rate could be determined using the technique of ventriculocisternal perfusion. They showed that inulin is lost from the CSF by bulk absorption distal to the fourth ventricle and that a negligible amount is lost by diffusion across ventricular walls. Therefore, any dilution of inulin as it passes through the ventriculocisternal system is the result of newly formed CSF. Similar logic has been applied to the use of other large molecules such as radioiodinated human serum albumin (RIHSA) (6, 17) and dextrans (11).

CSF formation is, in part, due to an active secretion (1, 2, 3, 4, 9, 20, 31, 34) implying the metabolic energy is necessary for active transport processes and that metabolic poisons and specific inhibitors of active transport should affect CSF production rate (Vf). Many attempts have been made to control Vf and to elucidate the basic mechanisms involved in its formation.

Davson and Segal (14) summarized the effect of many inhibitors on CSF production rate in adult animals. In the present study we tested the effect of acetazolamide and ouabain on the Vf in newborn animals. Previous studies in rats (15) have suggested maturation with increasing age of a transport mechanism in the choroid plexus and increased CSF flow, but direct measurements of CSF flow have not been made in newborn animals. Knowledge of substances that reduce production rate of CSF in newborn animals could be of clinical value in treating obstructive hydrocephalus.

METHODS

The ventriculocisternal perfusion technique used in this investigation is a modification of that described by Pappenheimer et al. (30). Mongrel dogs, 2-4 days old (body wt = 255-440 g) were anesthetized with sodium pentobarbital (30 mg/kg in a 6 mg/ml solution) and prepared for ventricular perfusion as previously described (19). An artificial cerebrospinal fluid containing radioiodinated human serum albumin (RIHSA-125I) was perfused from the right lateral ventricle to the cisterna magna.

Diffusion of RIHSA from the ventricular system is assumed to be negligible, loss of RIHSA from CSF occurs by bulk absorption distal to the fourth ventricle. Any dilution of RIHSA during passage through the ventricular system must result from newly formed, RIHSA-free fluid. Rate of CSF formation (Vf) is calculated as previously described (equation 2, ref. 19).

The initial perfusion rate was rapid (96.6 μl/min) in order to quickly bring the ventricles into equilibrium with the perfusate. After 15 min of rapid perfusion, the rate was reduced to 38 μl/min for the remainder of the experiment. No measurements were made during the first 30-45 min because a steady-state outflow rate and concentration had not been established. Outflow rate and concentration were measured after approximately 90 min of perfusion for three consecutive 3-min periods, and Vf was calculated for each period. The mean of the three measurements is reported as the control production rate. Postdrug production-rate measurements are reported as the means of three similar 3-min-measurement periods at 30, 60, 90, and 120 min after giving ouabain or acetazolamide.

During control periods, animals were ventilated with room air using a Palmer respirator through a right-fitting endotracheal tube connected to a CO2 analyzer (Beckman CO2 analyzer model LB-1) for continuously monitoring expired CO2. Expired CO2 was not regulated during the administration of either acetazolamide or ouabain.

Acetazolamide was given intravenously in single doses of 1, 3, 10, 20, 30, and 200 mg/kg. Solutions were prepared by dissolving 120 mg of acetazolamide in equimolar NaOH and diluting to 40 ml with saline. The resulting solution (3 mg/ml) was titrated to pH 9.0 with 2.0 N HCl. A solution of 50 mg/ml (pH 10.5) was used in experiments in which animals received 200 mg/kg.

Ouabain was given intravenously in a single dose of 0.05
RESULTS

Cerebrospinal fluid production rate remained relatively constant in five pups perfused for 4 hr. The rates obtained for the 1st, 2nd, 3rd, and 4th hr were 5.22 ± 0.21, 5.04 ± 0.21, 5.20 ± 0.30 and 5.90 ± 0.44 μl/min, respectively.

Acetazolamide or ouabain intravenously. Data showing the effects of various doses of intravenous acetazolamide on CSF production are presented in Table 1. The minimal dose of acetazolamide that produced maximal depression of the CSF production rate was 10 mg/kg.

Ouabain (0.05 mg/kg) decreased the CSF production rate by 50% after 90 min as shown in Fig. 1.

The effects of ouabain and acetazolamide on expired CO₂ were recorded throughout each experiment. Expired CO₂ did not change after administration of ouabain, but end-expiratory CO₂ decreased from 5.9% during the control period to 4.6% 30 min after administration of acetazolamide. In contrast to the response generally seen in adult dogs (8), the expired CO₂ of the newborn remained depressed for 120 min.

Ouabain in perfusion fluid. Data illustrating the effect of perfusing the ventriculocisternal system with 10⁻⁷ M ouabain on CSF production rate are shown in Fig. 2. The values obtained were not significantly different from the values obtained with intravenous ouabain (Fig. 1). The V₁ was reduced by 51% after 90 min.

DISCUSSION

Woodbury (39) inferred, from ionic flux measurements, that ouabain and acetazolamide were relatively ineffective in reducing the CSF production rate in newborn rats because the carbonic anhydrase and Na−K-ATPase systems were not completely mature until after 9 days of age. In contrast, our study demonstrates that acetazolamide reduced CSF production rate in newborn dogs (24-72 hr old) just as it does in adult dogs (14, 29, 38) and adult cats (16, 36). The decreased production rate suggests that the newborn dog has a fully developed carbonic anhydrase system at 1-3 days after birth and that this is involved in CSF production.

Carbonic anhydrase is found in high concentrations in the choroid plexus as well as in other secretory tissues such as kidney, pancreas, stomach, and eye (25). Theories proposed to explain the mechanism of action of acetazolamide have related the ability of the drug to reduce the secretory rate to its ability to inhibit carbonic anhydrase (25). Recently, Macrì et al. (24) have suggested that acetazolamide produces vasoconstriction in the choroidal artery. The present investigation cannot distinguish between an effect on the carbonic anhydrase system and a direct effect on the vasculature.

In the newborn dog ouabain inhibits CSF production whether given intravenously or in the perfusion fluid. Davson et al. (13) and Oppelt et al. (29) found that ouabain had no effect on V₁ in adult dogs whether given intravenously (0.05-0.1 mg/kg) or in a ventricular perfusate (1.1 × 10⁻³ M to 6.8 × 10⁻⁴ M). In contrast, ouabain applied topically on the choroid plexus in rabbits (9), intrathecally
in cats (37), and intravenously in dogs (10) decreased CSF production. The mechanism of action of ouabain in reducing CSF production is not completely clear. The working assumption, as in other tissue that actively transport Na and K, is that ouabain inhibits the Na-K-ATPase (35) and therefore reduces the active component of CSF formation.

Ouabain causes severe cardiovascular effects at high doses and therefore has limited use both experimentally and therapeutically for reducing CSF production. When ouabain was given intravenously (0.05 mg/kg) the only cardiovascular change we noted was an increase in the pulse pressure. In contrast, when ouabain was perfused through the brain ventricles, dramatic cardiovascular abnormalities were induced. Thus, of necessity, all data for CSF production measurements were obtained during ventricular perfusion of $10^{-7}$ M ouabain in animals with both vagi cut. We have no explanation for the elimination by vagotomy of ouabain-induced cardiovascular abnormalities.

The effect of alterations of blood pressure and brain blood flow on CSF production must be considered when discussing changes in fluid formation rate. Blood pressure (usually between 40-50 mm Hg in the newborn dog) was constant throughout the experiments and therefore did not affect the formation rate. An increase in the blood flow to the brain could increase capillary hydrostatic pressure and therefore increase the passive component of CSF fluid production. Several investigators (22, 23) have shown an increased brain blood flow during respiratory acidosis. The respiratory acidosis observed in the present experiments with acetazolamide ($P_{\text{CO}_2}$ increased from 43 mm Hg (control period) to 60 mm Hg (after acetazolamide)) could have increased brain blood flow and therefore $V_b$. However, $V_b$ was reduced significantly indicating that the drug indeed worked on the active mechanisms of CSF formation and not on the passive hydrostatic pressure-related component.

The present investigation demonstrates that CSF production can be decreased significantly in newborn dogs. This fact invites application.

REFERENCES


