Further data on the acute effect of intravenous steroids on canine CSF secretion and absorption

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Considerable difference of opinion has arisen as to whether intravenously administered steroids affect cerebrospinal fluid (CSF) production in the acute laboratory animal undergoing ventriculocisternal perfusion. Our experiments with ventriculocisternal perfusion in dogs indicate that, when given intravenously, neither dexamethasone, methylprednisolone, hydrocortisone, nor aldosterone result in a significant, acute effect upon CSF production. Similarly, CSF absorption and outflow resistance mechanisms are not acutely affected by intravenous methylprednisolone, hydrocortisone, and aldosterone. Dexamethasone also probably does not produce an immediate effect upon CSF absorption.

Key Words • cerebrospinal fluid production • cerebrospinal fluid absorption • steroid • ventriculocisternal perfusion

Several prior studies have shown that, in the acute laboratory animal, steroid administration may apparently affect the rate of bulk cerebrospinal fluid (CSF) formation. Sato, Amano, and Weiss and Nulsen all have reported a decrease in canine CSF production following the intravenous injection of dexamethasone or methylprednisolone. Martins et al., however, in studying the immediate effect of dexamethasone on monkey CSF formation, observed that CSF production in both control and dexamethasone-treated animals gradually fell during a 4-hour experimental period. Final CSF production in steroid-treated monkeys was not significantly different from that of controls. Reasons for the differing effect of steroids on CSF formation between monkeys and dogs was unclear but species differences and the fact that dogs may drain CSF via nasal lymphatic systems were mentioned as possible factors. Considerable dialogue concerning the effect of intravenous dexamethasone upon CSF formation in laboratory animals then followed. Sato pointed out that in his dogs, CSF formation fell sharply and drastically after intravenous steroid administration, while Martins reiterated his observation that even in control monkeys that had received no steroids, CSF production fell gradually at about 1.5 µl/hr after 2 hours of ventriculocisternal perfusion (VCP).

These differences in experimental results indicated a lack of resolution concerning the acute effects of steroids upon CSF formation in laboratory animals. The purpose of the experiments discussed in this report was to further ascertain the effect of steroids upon the rate of CSF formation in dogs undergoing VCP. The canine experiments of Sato, Amano, and Weiss, indicating an immediate reduction of CSF formation with steroids, have been widely quoted. In view of Martins' data, however, we felt that this issue should be further clarified. Theoretically, it would be most important to ascertain whether CSF secretory mechanisms in the dog respond to steroids differently than do those in the monkey. Dogs are relatively inexpensive, accessible laboratory animals upon which some basic research in CSF physiology has been based. Should dogs prove to have major differences in CSF secretory mechanisms from other laboratory animals, their continued use in CSF physiological research might be questioned.

Our initial dexamethasone experiments were concerned solely with possible acute effects of steroids upon CSF production. In our later experiments, we measured the effect of various steroids upon CSF absorption as well.

Materials and Methods

Thirty-two, nonfasting, adult, mongrel dogs unselected as to sex and weighing 9 to 18 kg (mean 12.8 kg) were anesthetized with intravenous pentobarbital (30 mg/kg). Catheters were placed in a femoral artery for measurement of arterial pressure. The femoral
vein was cannulated for steroid and intermittent anesthetic administration as well as for a slow intravenous infusion of normal saline, 4 ml/kg/hr. A Harvard respirator* and endotracheal tube provided controlled respirations. The dogs were placed in a stereotaxic frame† (sphinx position) and the scalp incised for placement of two small, lateral twist-drill holes so that shortened No. 20 spinal needles could be inserted stereotaxically into each lateral ventricle. During insertion, the ventricular needles were attached by polyethylene tubing to a linear core pressure transducer connected to a Physiograph recorder.‡ Ventricular penetration was determined by the appearance of respiratory and pulse waves on the Physiograph tracing. For our control and dexamethasone experiments, once ventricular penetration had been assured, the transducer was disconnected and each needle was then attached to the CSF inflow syringe tubing in order to achieve biventricular perfusion. Ventricular pressures were not recorded in these animals.

In our later experiments, we used a double-lumen delivery needle in one ventricle so that ventricular pressures could be measured concomitant with perfusion. A No. 20 regular spinal needle was inserted into each animal's cisterna magna and taped to a stereotaxic arm allowing perfusate outflow. A polyethylene (PE 190) collecting cannula was connected to this cisternal needle and its end positioned 5 cm above the external auditory meatus. No attempt was made to maintain constant intraventricular pressure by altering the height of the cannula end during these experiments, nor were specific and variable outflow pressures created by cannula movement in order to purposely affect CSF absorption. A Harvard constant infusion pump§ delivered mock CSF containing blue dextran (2 mg/ml) to both ventricular needles at a mean rate of 0.233 µl/min. Arterial and ventricular pressures were measured with linear core pressure transducers and recorded on a multichannel direct-writing Physiograph recorder. Esophageal temperatures were obtained by a thermistor probe and maintained constant by a heating pad placed under the dog.

Each dog was perfused for 2.5 hours before the commencement of the experimental period for maximal equilibration of the mock CSF-blue dextran perfusate with endogenous CSF. Effluent samples from the cisternal needle were collected and weighed every 20 minutes throughout the ensuing 4-hour experimental period which consisted of three 80-minute periods.

Four 20-minute determinations of CSF-formation and absorption were made for each period. Measurements made during the first period (P1) served to establish control values for CSF formation and absorption. Immediately after the first period, animals received a single, selected steroid in an intravenous bolus. All calculations to ascertain the effects of steroid administration were based upon the four 20-minute CSF effluent collections during the final period (P3), 160 to 240 minutes after steroid administration.

Control dogs received no steroids. Experimental dogs received a single dose of one of the following steroids: dexamethasone (Decadron), 0.4 mg/kg; methylprednisolone (Solu-Medrol), 2 mg/kg; hydrocortisone (Solu-Cortef), 10 mg/kg; or d-aldosterone, 0.15 mg/kg. The d-aldosterone was prepared by dissolving 25 mg of the mineralocorticoid in 20 ml of 70% alcohol, and passing the solution through a sterile millipore filter. The dexamethasone dose was selected with reference to Martins' experiments in monkeys. The other glucocorticoids were given in equivalent anti-inflammatory doses; the d-aldosterone dose was extrapolated from doses utilized in humans.14

The concentration of blue dextran in a sample inflow and all outflow aliquots was determined by duplicate optical density determinations at 490 mµ in a photoelectric colorimeter. Cerebrospinal fluid production (Vf) was calculated according to the formula:

$$V_f = V_i \left( \frac{C_i - C_o}{C_o} \right).$$

Cerebrospinal fluid absorption (Va) occurred distal to the cisternal outflow cannula,17 and, therefore, was determined by the formula:

$$V_a = \frac{V_i C_i - V_o C_o}{C_o}.$$

In both formulas, V is the rate of volume flow, C is the blue dextran concentration, the subscripts i and o are inflow and outflow, and f and a are production and absorption, respectively.

All data are presented as the mean ± standard deviation. Regression lines were determined by the least-squares method. The statistical significance of our tabular data and regression lines has been determined by the Student’s t-test for difference between means and regressions.18

Results

Cerebrospinal fluid production and absorption rates for control and steroid-treated groups of dogs are

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*A Respirator manufactured by Harvard Apparatus, Millis, Massachusetts.
†Stereotaxic frame manufactured by David Kopf Instruments, Tijunga, California.
‡Linear core transducer and Physiograph recorder manufactured by Narco Bio-systems Inc., Houston, Texas.
§Infusion pump manufactured by Harvard Apparatus, Millis, Massachusetts.
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Fig. 1. Poststeroid (Period 3) mean cerebrospinal fluid production rates (Vf) plotted against control rates (Period 1) for each dog in the control and steroid-treated groups. None of the steroid-treated dogs manifested regression line slopes significantly different from the control group (p > 0.05).

given in Table 1. For the controls, CSF formation decreased from $46.7 \pm 26.2 \mu l/min$ to $42.5 \pm 22.8 \mu l/min$ over the 4-hour experimental interval, but this was not significant (p > 0.05). Three of the four groups of dogs given steroid preparations also tended to show a decrease in CSF formation from control period P1 to final period P3, 160 to 240 minutes later, but in no instance were these decreases significant (p > 0.05). The animals treated with hydrocortisone showed an insignificant rise in CSF production (p > 0.05).

The various groups of dogs exhibited significant differences in CSF production rates during their control periods. Normalized data, therefore, must be used to compare CSF formation rates between control and experimental dogs during the final period P3. Cerebrospinal fluid formation during the control period (P1) is defined as 100% for all groups of animals. The percent production during the final interval (P3) for the various groups was as follows:

control dogs: $95.6 \pm 18.3 \mu l/min$

dexamethasone-treated dogs: $87.3 \pm 40.5 \mu l/min$

methylprednisolone-treated dogs: $94.6 \pm 34.6 \mu l/min$

hydrocortisone-treated dogs: $104.8 \pm 100.1 \mu l/min$

aldosterone-treated dogs: $86.2 \pm 66.1 \mu l/min$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vf (µl/min)</th>
<th>Va (µl/min)</th>
<th>Vp (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P3</td>
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<tr>
<td>controls (6)</td>
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<td>42.5</td>
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<tr>
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<td>27.9</td>
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<td>SD</td>
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*Vf = cerebrospinal fluid (CSF) production; Va = CSF absorption; Vp = CSF pressure; P1 = period 1 (control), P3 = poststeroid period 3.
None of these final percent production rates among the steroid-treated dogs are significantly different from the percent CSF production observed for the control dogs during the final P3 interval.

Analysis of any alterations in CSF formation rates between control and steroid-treated animals is enhanced by plotting final (P3) against initial (P1) CSF formation rates, and fitting a regression line to these data as shown in Fig. 1. The control animal regression slope is slightly less than unity because of the somewhat lower CSF production during the P3 interval. None of the dogs treated with steroids manifested regression line slopes significantly different from those of the control group (p > 0.05) and in fact, the slope of the dexamethasone-treated animals is exactly equal to the slope in the control dogs. We conclude, therefore, that in dogs, CSF formation does tend to fall somewhat during VCP, and that steroid administration does not decrease CSF production beyond that which may occur normally with prolonged perfusion times.

Although most experimental evidence indicates that CSF production is independent of ventricular pressure, CSF absorption is pressure-dependent. The acute effects of steroid administration upon CSF absorption can only be delineated with reference to simultaneous ventricular pressure measurements both before and after drug administration (Fig. 2). Analysis of ventricular pressure-CSF absorption regression lines for the steroid groups where these parameters were measured indicates that pre- and posttreatment slopes are not significantly different (p > 0.05). No reason is apparent for the large rise in intraventricular pressure consequent to hydrocortisone administration. Faulty effluent drainage and consequent intracranial pressure rise in the dogs treated with hydrocortisone probably cannot explain the effect because mean CSF outflows during P1 and P3 were quite similar (0.235

Fig. 2. Mean cerebrospinal fluid absorption rates (Va) plotted against mean ventricular pressures (Vp) before and after steroid administration for each dog in the indicated groups. The posttreatment slopes are not significantly different from the pretreatment slopes (p > 0.05). Black dot = control Period 1; white dot = poststeroid Period 3.
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and 0.230 μl/min, respectively) in these animals. Blood gases were not monitored.

We conclude that within the limits of these acute VCP experiments in which systemic alterations of ventricular pressure were not undertaken to purposefully alter CSF absorption, intravenous methylprednisolone, hydrocortisone, and aldosterone did not significantly alter canine CSF absorptive mechanisms. Our data also suggest that dexamethasone was also ineffective in acutely altering CSF absorption.

Discussion

During the 240-minute interval of our study, CSF formation in the control dogs decreased by an average of 9%. The large standard deviations of our data preclude this decrease from being statistically significant but, nevertheless, the tendency for CSF production to decrease with time in normal dogs undergoing VCP accords with the observations of Martins, et al., who noted an apparent 12% decrease in CSF formation in control monkeys over a 240-minute perfusion period. They attributed this diminution either to further equilibration of CSF perfusate marker with endogenous CSF or to preparation deterioration and actual decrease in fluid formation.

Beginning (P1) and terminal (P3) production rates for all groups of dogs are plotted and expressed as regression lines (Fig. 1). The regression-line slopes of the steroid-treated dogs do not differ significantly from those of the control dogs. These data indicate that in the acute, canine VCP experiment, neither intravenous glucocorticoids (dexamethasone, hydrocortisone, and methylprednisolone) nor the mineralocorticoid aldosterone significantly alter CSF production. Our experiments in the dog, therefore, accord with the VCP data of Martins, et al., in the monkey, wherein dexamethasone also was observed to have no immediate effect upon CSF production. Thus, the dog and monkey appear similar in their lack of immediate response to intravenous steroids. The fact that dogs may drain CSF via nasal lymphatic systems appears not to be a critical factor. In theory, this possible alternate absorptive pathway should not affect CSF formation as determined by indicator dilution because production is determined from the mock CSF inflow rate and the mock CSF marker concentrations only.

Our results in the dog stand in direct contrast to those of Amano and Sato, who also utilized VCP to study the effect of dexamethasone upon canine CSF formation. Both of these investigators reported a 50% decrease in production consequent to steroid administration, but apparently their data were not subject to statistical analysis and any conclusions drawn from these experiments must be regarded with some reservations. Weiss and Nulsen used a gravimetric technique for the measurement of CSF formation and also concluded that steroids decreased CSF production in the dog by 50%. The pitfalls of this method as a measure of CSF production have been discussed by Martins, et al., and basically result from transient alterations in CSF compartment size in the experimental animal, which may cause greater or lesser amounts of fluid to exit from the cisternal needle. Effluent increases or decreases may be interpreted as alterations in CSF production. We have observed periodic changes in effluent volumes in many dogs undergoing VCP where CSF formation itself was constant when determined by indicator dilution.

The rate of CSF absorption has been calculated for all animal groups. Because absorption has been shown to vary with ventricular pressure, the most meaningful CSF absorption data are for the dogs treated with methylprednisolone, hydrocortisone, and aldosterone, wherein CSF absorption could be plotted as a function of ventricular pressure both before and after steroid administration. Because the pre- and poststeroid treatment regression slopes are virtually the same in all groups, we conclude that in the acute canine experiment, neither intravenous methylprednisolone, hydrocortisone, nor aldosterone significantly alter the rate of CSF absorption or outflow resistance (the reciprocal of the CSF absorption regression slope). Our data also suggest that absorption and outflow resistance are unaffected by dexamethasone.

These acute results are in direct contrast to those of Johnston, et al., who demonstrated that canine CSF absorption was markedly decreased and outflow resistance increased when steroids were withdrawn after having been administered for 2 weeks. Longer-term treatment with steroids presumably affected CSF absorption by causing membrane changes in the dogs' arachnoid villi which enhanced absorption. Our data suggest that no acute changes in CSF absorptive membranes occur consequent to steroid administration in the dog.

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References


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