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THE EFFECT OF ANAESTHETIC AGENTS ON THE CEREBROSPINAL FLUID CLEARANCE OF ^{35}S-SULPHATE AND ^{125}I-IODIDE*†

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Abstract—In recent years, use of the ventriculocisternal perfusion technique for the study of cerebrospinal fluid transport of substances has become prevalent. This report compares the effect of three anesthetic agents, commonly used in the laboratory, on the clearance of ^{35}S-sulfate and ^{125}I-iodide out of the CSF. When compared with chloralose, pentobarbital and (to a lesser extent) urethane inhibit the CSF clearance of these anions. In studies with isolated choroid plexus incubated *in vitro*, the accumulation of ^{125}I-iodide was found to be significantly less in the choroid plexus obtained from the anesthetized than from the unanesthetized animal. These results suggest that the reduced CSF clearance observed *in vivo* was a result, at least in part, of inhibition of choroid plexus transport.

THE TECHNIQUE of ventriculocisternal perfusion has been widely used to study the active transport of substances out of the cerebrospinal fluid (CSF). With the exception of studies carried out on the unanesthetized goat,[1] most ventriculocisternal perfusions have been performed on animals anesthetized with pentobarbital.[2–4] The possible effect of pentobarbital and other anesthetic agents on CSF transport has not been examined. This report compares the CSF clearance of ^{35}S-sulfate and ^{125}I-iodide in cats anesthetized with pentobarbital, chloralose and urethane.

METHODS

Ventriculocisternal perfusions were carried out on 65 adult cats of either sex. The cats were anesthetized and placed in a stereotaxic apparatus. An inflow needle was inserted into the lateral ventricle at stereotaxic coordinates, 12·5 mm anterior and 2·5 mm lateral, and an outflow needle was inserted percutaneously in the cisterna magna. A 20-ml syringe driven by an infusion pump at a constant rate of 0·092 ml/min was connected with polyethylene tubing to the inflow needle and, in parallel, to a pressure transducer used to monitor the inflow pressure. The outflow needle was connected to another polyethylene tube with its opening 5·0 cm below the external auditory canal. In this manner the perfusion was performed at a slightly negative pressure preventing the loss of indicator by bulk flow. Artificial CSF as described by Merlis[5] was used as the perfusion medium. The fluid contained Blue Dextran 2000

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with a molecular weight of 2×10^6 (Pharmacia, Uppsala, Sweden) to determine the rate of CSF formation, and tracer amounts of ^{35}S-sodium sulfate (5 μc/ml) and ^{125}I-sodium iodide (0·3 μc/ml). Perfusions were usually run for 6 hr, making necessary a refill of the syringe at the end of 3 hr. Collection of the cisternal effluent was begun when the ^{125}I-iodide radioactivity of successive 10-min samples remained constant. Normally, the collection period lasted 30–45 min and occurred during the first 3-hr period at 2 or $2\frac{1}{2}$ hr, and at 5 or $5\frac{1}{2}$ hr during the second period. The concentration of isotope in inflow and outflow samples was determined by counting ^{125}I in a well scintillation counter at 40 per cent efficiency and ^{35}S in a thin-window Geiger-Mueller counter at 15 per cent efficiency. Blue Dextran 2000 concentration was determined by measuring the optical density of the perfusion medium and the effluent at 620 mμ. Inflow and outflow rates were determined gravimetrically. The clearance (K_0) for ^{35}S-sulfate and ^{125}I-iodide from the CSF, and the rate of CSF formation (V_f) were calculated from the equations of Heisey *et al.*[6] In a few experiments, $2{\cdot}5 \times 10^{-4}$ M pentobarbital was added to the perfusion medium of cats anesthetized with chloralose or urethane. Occasionally, in such experiments, the initial perfusion period conducted with medium containing pentobarbital was followed by an additional 3-hr perfusion with pentobarbital-free medium. As a methodologic control, two animals anesthetized with chloralose were perfused with CSF medium containing $5{\cdot}0 \times 10^{-4}$ M chloralose.

The ^{35}S-sulfate and ^{125}I-iodide content of 4 brains removed from animals which were perfused for 6 hr was determined by homogenizing the brain in 50 ml water and counting 1-ml aliquot samples for ^{125}I-iodide and ^{35}S-sulfate activity.

Since previous studies have shown an accumulation of iodide by the choroid plexus incubated *in vitro*,[7,8] it was decided to study the effect of chloralose and pentobarbital anesthesia on the uptake of iodide by the isolated choroid plexus. Choroid plexus from the lateral and fourth ventricles was removed from animals killed with an air embolus. The animals either (a) had received no previous anesthetic agent, or (b) had been previously anesthetized with chloralose (100 mg/kg) or pentobarbital (45 mg/kg). The plexus was inserted into a 25-ml Ehrlenmeyer flask containing 3 ml of artificial CSF with glucose (1 mg/ml) and tracer amounts of ^{125}I-sodium iodide (0·03 μc/ml). In addition, pentobarbital (4 mM) was added to the incubating media in which some of the choroid plexus obtained from unanesthetized animals was incubated. They were incubated for 30 min in a metabolic shaker at 37° under an atmosphere of 5% CO_2 and 95% O_2. The plexus was then removed, dipped into artificial CSF medium containing no isotope, and drawn across a glass slide to remove surface liquid. Finally, they were inserted into weighing bottles and weighed. The ^{125}I-iodide concentration in the choroid plexus and in duplicate 1-ml samples of medium was determined by counting in a well type scintillation counter. Data were expressed as a tissue (cpm/mg tissue water) to medium (cpm/μl medium) ratio, T:M.

RESULTS

CSF clearances of ^{35}S-sulfate and ^{125}I-iodide and the rate of CSF formation are shown in Table 1. Animals are grouped according to the anesthetic agent employed and compared statistically with the group anesthetized with pentobarbital. The clearance of both ions, previously reported to be actively transported from the CSF,[7,9] was significantly higher in cats anesthetized with chloralose than with pentobarbital. In cats anesthetized with urethane, only ^{35}S-sulfate clearance was significantly higher.

The addition of pentobarbital to the perfusate reduced the clearance of both ions in animals anesthetized with either chloralose or urethane. This effect was not reversed after an additional 3 hr of perfusion with pentobarbital-free medium. Fig. 1 graphically illustrates the reduction in removal of both anions from the CSF produced by the addition of pentobarbital to the perfusate in an animal anesthetized with chloralose.

TABLE 1. EFFECT OF ANESTHETIC AGENTS ON CSF CLEARANCE OF ^{125}I-IODIDE AND ^{35}S-SULPHATE

	Group I (n = 29) Pentobarbital (45 mg/kg i.p.)	Group II (n = 13) Chloralose (100 mg/kg i.p.)	Group III (n = 5) Urethane (1·5 g/kg i.p.)	Group IV (n = 9) Chloralose + Vent. Pentobarbital	Group V (n = 3) Urethane + Vent. Pentobarbital
K_{125_I}	0·059 ± 0·006*	0·074 ± 0·008 ($P < 0·02$)†	0·062 ± 0·007	0·049 ± 0·005 ($P < 0·01$)	0·057 ± 0·018
K_{35SO_4}	0·023 ± 0·002	0·043 ± 0·007 ($P < 0·001$)	0·032 ± 0·007 ($P < 0·01$)	0·025 ± 0·003 ($P < 0·05$)	0·016 ± 0·004 ($P \leqslant 0·05$)
V_f‡	0·014 ± 0·001	0·012 ± 0·002	0·011 ± 0·004	0·011 ± 0·002	0·016 ± 0·004

* Values given are in ml/min and represent the mean ± S.E. of each group.

† Means of groups II and III were compared with the mean of group I. Those of groups IV and V were compared with those of groups II and III respectively. Statistical analysis was performed with a modified *t* test,[10] and P values are given when the difference is statistically significant, i.e. $P \leqslant 0·05$.

‡ V_f = rate of CSF formation.

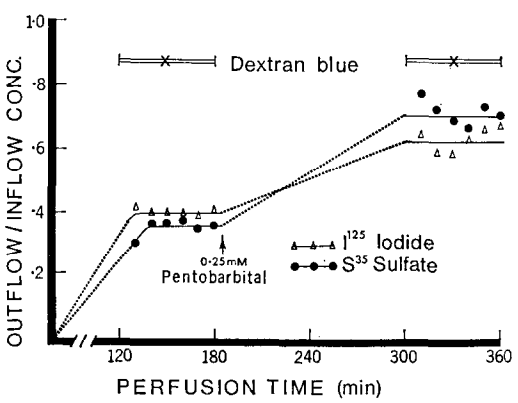

FIG. 1. Inhibition of ^{35}S-sulfate and ^{125}I-iodide CSF transport by pentobarbital and the lack of effect on CSF turnover of Dextran Blue in a chloralose-anesthetized cat. The collection period for ^{35}S-sulfate, ^{125}I-iodide and Dextran Blue during the 6-hr perfusion is indicated by solid lines. Arrow indicates when perfusion with pentobarbital-containing medium was begun.

In Table 1 as in Fig. 1, no change was noted in the removal of Blue Dextran 2000 with addition of pentobarbital to the perfusion medium, or in the rate of CSF formation between groups I, II and III.

In experiments where $5·0 \times 10^{-4}$ M chloralose was added to the perfusion media, no reduction occurred in the transport of either anion from the CSF.

The isotope content of brains removed from animals, expressed as a percentage of the total ^{35}S-sulfate or ^{125}I-iodide removed from the CSF during the 6-hr perfusion, is presented in Table 2. Little or no difference was noted between the isotope content of brains removed from pentobarbital- and chloralose-anesthetized animals.

The results obtained in the experiments *in vitro* are shown in Table 3. The accumulation of ^{125}I-iodide by the choroid plexus removed from unanesthetized animals was appreciably greater than that observed in choroid plexus removed from animals anesthetized with chloralose or pentobarbital. In addition, the accumulation of ^{125}I-iodide by the choroid plexus obtained from unanesthetized animals but incubated in media containing 4 mM pentobarbital was reduced by 68·5 ± 2·7 per cent (N = 4).

TABLE 2. PERCENT OF TOTAL ACTIVITY CLEARED FROM CSF AFTER 6 HR PERFUSION

	Found in brain of pentobarbital-anesthetized animals (%) (n = 2)	Found in brain of chloralose-anesthetized animals (%) (n = 2)
^{125}I	4·8 (4·7–4·9)	4·2 (4·0–4·4)
$^{35}SO_4$	15·1 (14·7–16·5)	18·1 (15·4–20·9)

TABLE 3. EFFECT OF PRIOR ANESTHESIA ON THE ABILITY OF THE ISOLATED CHOROID PLEXUS INCUBATED *IN VITRO* TO ACCUMULATE ^{125}I-IODIDE

	Air (n = 9)	Chloralose (n = 8)	Pentobarbital (n = 10)
Tissue to medium ratio after 30-min incubation	27·67 ± 3·40	18·95 ± 0·30* ($P < 0·05$)	15·31 ± 2·05* ($P < 0·01$)

* Means of anesthetized groups were compared with those of the air group.

DISCUSSION

The above results are in agreement with previous work showing that ^{125}I-iodide[4] and ^{35}S-sulfate[9] are removed from the CSF by routes other than bulk flow. Only a small portion of the total iodide and sulfate removed from the CSF during the 6-hr perfusion was detected in brain. Thus, it would appear that these anions pass from CSF to blood either directly or through the brain substance. Whatever the route, it is apparent that the CSF clearance was affected by the anesthetic agent employed. The effect produced by the anesthetic agents on CSF clearance did not appear to be related to variations in CSF formation, since no significant differences were noted in the rate of CSF formation among the five groups.

The following evidence suggests that the effect of the anesthetic agents employed occurred, at least in part, within the ventricular compartment: (1) The total amount of pentobarbital perfused through the ventricular compartment was approximately 1/100 of the systemic dose. (2) Similar levels of isotope were found in brains removed from animals anesthetized with chloralose or pentobarbital. Therefore, the higher CSF clearance observed with chloralose could not be attributed to greater accumulation of these ions in brain. (3) Tissue to medium ratios were significantly lower in choroid plexus removed from animals anesthetized with pentobarbital or incubated in medium containing pentobarbital. A similar observation concerning the inhibition of iodide uptake by amobarbital in rabbit choroid plexus has been reported.[8]

Thus, when compared with chloralose and to a lesser extent urethane, it would appear that pentobarbital has an inhibitory effect on the transport of ^{35}S-sulfate and ^{125}I-iodide from the CSF. Furthermore, the inhibitory effect was found to be irreversible during the period of time studied, suggesting the continuing presence of bound pentobarbital or a metabolite, or a metabolic alteration. A recent study comparing the effect of ether and pentobarbital anesthesia on the fate of intracisternally administered norepinephrine-H^3 showed greater retention of radioactivity in brains of rats anesthetized with pentobarbital.[11] The authors suggested that pentobarbital may have affected the relative distribution of catecholamines in the brain or decreased the loss to blood from the CSF. The fact that norepinephrine is known to be taken up by an active process into the choroid plexus incubated *in vitro*,[12] and that brain extracellular levels of substances can be affected by the concentration of the substance established in the CSF, lends support to the latter interpretation.[13,14] However, the retention of ^{14}C-urea, a substance not known to be accumulated by the isolated choroid plexus, was also prolonged in the brain of pentobarbital-anesthetized rats. It is possible, therefore, that other factors, such as altered brain permeability, may have affected the retention of norepinephrine in brains of pentobarbital-anesthetized rats. Whether these factors are of significance in the clearance of ionic substances from the CSF would require further investigation. Studies are now in progress to determine the nature of this inhibition and possibly the site of action of pentobarbital.

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