

Pressure-Dependent PAH and Creatinine Transport from the Brain Ventricles (38755)

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Perfusion of the brain ventricular system with an artificial cerebrospinal fluid (CSF) allows estimations of bulk absorption rates for CSF from subarachnoid spaces, diffusion of molecules through ventricular ependymal linings and active transport of molecules from CSF (1-3). The rate of bulk absorption is dependent on the difference between CSF pressure and that in the cerebral venous sinuses (4), whereas the rate of removal by diffusion or active transport is reported to be independent of this pressure gradient (1, 3, 4). Pappenheimer *et al.* (2) demonstrated that organic anions (Diodrast and phenolsulfonphthalein) were actively removed from the brain ventricular system in the area of the fourth ventricle, and we showed (5) that both the fourth and lateral ventricular choroid plexuses of the adult dog actively accumulate *p*-aminohippuric acid (PAH) *in vitro*. Data reported here suggest that in the adult dog, both PAH and creatinine are actively removed from CSF, and the efflux rate is dependent on intraventricular pressure.

Methods. Mongrel adult dogs (5-12 kg) of either sex were anesthetized with sodium pentobarbital (60 mg/kg; ip) or Dial (Ciba; 0.6 mg/kg; ip). The animal's ventilation was controlled by a respiratory pump, and femoral arterial pressure was monitored by a Statham P-23 DC pressure transducer and Grass polygraph. The dog's head was first secured in a stereotaxic frame, the right cerebral ventricle and cisterna magna were then cannulated with 22 g and 20 g needles, respectively, and brain ventricular perfusion was initiated (6). Intraventricular pressure was calculated as previously described (3, 4, 6).

The perfused artificial CSF (3) contained inulin (1.0 mg/ml), creatinine (2.0 mg/ml), mannitol (0.25 mg/ml), carbon-14 labeled mannitol (*d*-mannitol-1-¹⁴C; 0.01 μ c/ml;

New England Nuclear Corp., Boston, MA), *p*-aminohippuric acid (PAH; 2-10 μ g/ml), and tritiated PAH (glycyl-2-³H; 0.1 μ c/ml; New England Nuclear Corp., Boston, MA). This fluid was perfused into the lateral cerebral ventricle at 190 μ l/min. Inflow and outflow concentrations were determined on aliquots from the inflow syringe and from outflow vials, respectively.

Tritium and carbon-14 were counted differentially in a trichannel liquid scintillation spectrometer (Mark I, Nuclear Chicago Corp.). Nonradioactive inulin (7), PAH (8), and creatinine (7) concentrations were determined colorimetrically. Molecular clearance and efflux coefficients (K_o) were calculated using equations derived by Heisey *et al.* (1). The 0.05 level of probability was used as the criteria of significance in all statistical tests.

Results. Table I contains the equations (calculated by the method of linear regression) relating clearance and efflux coefficients to intraventricular pressure (P). The slopes of all clearance lines are significantly greater than zero ($P < 0.05$). Although mannitol clearance was usually greater than the clearance of inulin, the slope of the regression lines for mannitol and inulin were not significantly different ($P > 0.05$). Over the pressure range -15 to +12 cm H₂O, PAH and creatinine clearances were always greater than that of inulin or mannitol and the slopes of the lines relating clearance to P were significantly greater ($P < 0.05$) than those of inulin and mannitol. The slope of the line relating mannitol efflux to P is not significantly different from zero ($P > 0.05$). The slopes of lines relating PAH or creatinine efflux to P are significantly greater than zero ($P < 0.05$) but are not different from each other ($P > 0.05$). These data indicate that the nonbulk clearance of mannitol (a passively diffusing molecule) from CSF is independent of P whereas the nonbulk

TABLE I. EFFECT OF INTRAVENTRICULAR PRESSURE ON CLEARANCE AND OUTFLOW COEFFICIENT.^a

Molecule	Equation of line	N	r
Inulin	$C = 4.5 \pm 1.1P + 62.1$	13	0.76
Mannitol	$C = 3.9 \pm 0.7P + 61.5$	6	0.91
	$K_o = -0.03 \pm 0.8P + 13.7$	6	0.00
PAH	$C = 4.9 \pm 0.6P + 91.3$	10	0.94
	$K_o = 2.6 \pm 0.5P + 54.7$	10	0.87
Creatinine	$C = 5.4 \pm 1.0P + 100$	12	0.85
	$K_o = 2.8 \pm 0.9P + 57.6$	12	0.67

^a (C = clearance; K_o = efflux coefficient; P = intraventricular pressure; N = number of observations; r = correlation coefficient).

clearance of PAH in low concentrations (<10 $\mu\text{g}/\text{ml}$) and creatinine (2 mg/ml) is pressure dependent.

In five experiments (Table II) the ventricular system was perfused simultaneously with inulin, mannitol, creatinine and PAH at comparable intraventricular pressures. Calculated clearances for these molecules were always: Creatinine > PAH > mannitol > inulin. Although molecular weights of creatinine, PAH and mannitol are similar, the efflux coefficient for creatinine and PAH is significantly greater than that of mannitol ($P < 0.05$), suggesting some mechanism other than passive diffusion for the removal of PAH and creatinine from CSF.

Discussion. Clearance of inulin from CSF has been used as a measure of CSF bulk absorption at sites distal to the fourth ventricle (1, 3, 6). The clearance of inulin is pressure dependent and in the present experiments decreased linearly from 106 $\mu\text{l}/\text{min}$ at 12 cm H_2O intraventricular pressure (P) to 0 $\mu\text{l}/\text{min}$ at -15 cm H_2O P, confirming earlier findings (3, 4, 6). Molecules smaller than inulin may be expected to leave the ventricular system by bulk absorption at arachnoid villi as well as by diffusion and/or active transport at other sites along the ventricles. Heisey *et al.* showed that the total clearance of molecules from CSF could be divided into a pressure-dependent clearance and a pressure-independent component (K_o) which represents diffusional or active movement (1).

The slopes of the lines relating clearance to intraventricular pressure (P) for inulin and mannitol were equal and the non-bulk clearance of mannitol was independent of P

TABLE II. EFFLUX COEFFICIENTS (K_o) IN FIVE ANIMALS AT COMPARABLE INTRAVENTRICULAR PRESSURES OVER A RANGE -3 TO -12 cm H_2O .

Molecule	K_o ($\mu\text{l}/\text{min}$) ^a
Mannitol	16 \pm 8
p-Aminohippuric acid (PAH)	34 \pm 4
Creatinine	46 \pm 4

^a Values are means \pm SEM.

(Table I). Similarly, as observed by others, when the bulk absorption component was subtracted from the total clearance, molecules such as tritiated H_2O and mannitol had K_o 's which were independent of P (1, 3, 4, 9). In contrast, PAH non-bulk efflux (K_o) increased with intraventricular pressure (Table I). Pappenheimer *et al.* (2) demonstrated that low concentrations of organic anions were actively removed from CSF in the region of the fourth ventricle and we have shown (5) that the isolated fourth ventricular choroid plexus (FVCP) actively accumulates PAH. The FVCP *in situ* projects through the foramina of Luschka into the subarachnoid space. Conceivably at low intraventricular pressures perfusion fluid may flow to the outflow cannula in the cisterna magna bypassing much of the subarachnoid space and much of the FVCP's surface; at high pressures more fluid is reabsorbed distal to the fourth ventricle (as evidenced by the greater inulin clearance) and a larger area of the FVCP may be exposed to fluid enroute to more rostral portions of the subarachnoid space. At higher pressures, PAH would be presented to a greater surface

area of tissue capable of transporting it to blood.

It has been assumed that creatinine leaves CSF by passive diffusion (1) (3) (4), but no one has compared efflux coefficients of creatinine to those of mannitol in the same animal. Molecular weights of mannitol, creatinine, and PAH are similar, but K_o for both creatinine and PAH were significantly greater than that for mannitol (Table II). In other studies in dogs, K_o for creatinine was 27 $\mu\text{l}/\text{min}$. (3) and 47 $\mu\text{l}/\text{min}$ (4) which are greater than that for mannitol found in the present study (Table II). Since mannitol and creatinine are substances with different chemical properties, the data might be explained on the basis of passive diffusion, with rate of movement being a function of the interaction between the membrane and specific substance or the escaping tendency from an aqueous environment into the membrane. The larger K_o for creatinine and its dependence on intraventricular pressure suggest an active transport process at a location similar to that for PAH. Active transport of creatinine from chicken CSF has been suggested (9) and cells of the FVCP are structurally similar to those of the renal proximal tubule (10) where active secretion has been demonstrated (11–13).

Summary. The brain ventricular system of the adult dog was perfused with an artificial cerebrospinal fluid (CSF) containing inulin, creatinine and radioactively labeled *p*-aminohippuric acid (PAH) and mannitol. Inflow and outflow rates and concentrations of test molecules were measured at different intraventricular pressures, allowing calculation of their steady-state rates of removal from the ventricles. Clearance of inulin, a measure of CSF bulk absorption, varied linearly with intraventricular pressure (-15 to $+12$ cm H_2O relative to the external auditory meatus). The efflux coefficient (K_o ; representing clearance of a molecule by means other than bulk absorption) for mannitol was independent of intraventricu-

lar pressure. K_o 's for PAH and creatinine were pressure dependent. PAH and creatinine efflux may be related to the amount of fourth ventricular choroid plexus surface exposed to the perfusion fluid. K_o 's for creatinine and PAH ($46 \pm 4 \mu\text{l}/\text{min}$; $34 \pm 4 \mu\text{l}/\text{min}$, respectively) were significantly greater than mannitol ($16 \pm 8 \mu\text{l}/\text{min}$) at comparable intraventricular pressures, suggesting that both creatinine and PAH leave the CSF by an active process in addition to passive diffusion.

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